Intakte polare Membranlipide als Biomarker zur Charakterisierung mikrobieller Lebensgemeinschaften in Wattsedimenten

Intact polar membrane lipids as biomarkers for characterization of microbial communities in Wadden Sea sediments

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Seidel M., Gittel A., Bischof K., Köster J., Sass H., Rullkötter J., 2007. Diverse active microbial communities in a tidal flat sediment as deciphered by a multidisciplinary approach. International Conference and 97th Annual Meeting of the Geologische Vereinigung e.V. (GV), October 1–5, Bremen, Germany. (POSTER)

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Freese, E., Rütters, H., **Seidel, M.**, Köster, J., Rullkötter, J., Sass, H., 2007. Eicosapentaenoic acid in bacterial isolates and tidal flat sediments (Wadden Sea, North Sea, Germany). 23rd International Meeting on Organic Geochemistry (IMOG), September 9–14, Torquay, UK. (POSTER)

Seidel, M., Gittel, A., Bischof, K., Köster, J., Sass, H., Rullkötter, J., 2007. Diverse active microbial communities in a tidal flat sediment as deciphered by a multidisciplinary approach. 17th Annual V.M. Goldschmidt Conference, August 20–24, Cologne, Germany. (POSTER)

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Süß, J., Herrmann, K., **Seidel, M**., Engelen, B., Cypionka, H., Sass, H., 2006. Diversity of subsurface *Photobacterium* sp. as a response to specific *in situ* conditions? Annual Conference of the Association for General and Applied Microbiology (VAAM), March 19-22, Jena, Germany. (POSTER)

Seidel, M., Süß, J., Hermann, K., Sass, H., Rullkötter, J., 2005. Molecular analysis of intact phospholipids of bacteria from Mediterranean sapropels. 22nd International Meeting on Organic Geochemistry (IMOG), September 12–16, Sevilla, Spain. (POSTER)

Kurzfassung

Im Rahmen der vorliegenden Arbeit wurden intakte polare Membranlipide mittels HPLC-Massenspektrometrie-Kopplung in bakteriellen Reinkulturen und in einem Sedimentkern aus dem Rückseitenwatt der Insel Spiekeroog biogeochemisch untersucht.

Der erste Teil der Arbeit beschreibt die chemotaxonomische und physiologische Charakterisierung verschiedener bakterieller Reinkulturen, die aus Wattsedimenten und der tiefen Biosphäre isoliert wurden. Zunächst wurden acht sulfatreduzierende Stämme von *Desulfovibrio acrylicus*, die als abundante Vertreter von verschieden Standorten des Wattenmeers stammen, hinsichtlich ihrer temperaturabhängigen Veränderungen in Wachstum und metabolischer Aktivität genauer charakterisiert. Ein besonderer Fokus lag außerdem auf der Analyse der temperaturabhängigen Anpassung ihrer Membranlipide. Unerwartet war, dass alle Stämme neben Phospholipiden auch hohe Anteile von phosphatfreien Ornithinlipiden enthielten, die bisher nur in einem anderen Vertreter der *Deltaproteobacteria* nachgewiesen wurden. Der relative Anteil der Ornithinlipide nahm mit der Wachstumstemperatur stark zu. Die molekulare Analyse der intakten polaren Lipide hat jedoch gezeigt, dass die temperaturabhängige Anpassung der Seitenketten in Phospholipiden und Ornithinlipiden gleich war und somit vermutlich der Hauptmechanismus zur Anpassung der Membranviskosität ist. Dagegen scheint die Änderung der Anteile der verschiedenen Lipidklassen in der Zellmembran und der zunehmende Anteil von Ornithinlipiden eine wichtige Rolle bei der Regulierung membrangebundener Enzyme zu spielen.

Im zweiten Teil der vorliegenden Arbeit werden die Ergebnisse der geochemischen Analyse eines fünf Meter langen Sedimentkerns vom Standort Janssand aus dem Rückseitenwatt der Insel Spiekeroog im norddeutschen Wattenmeer diskutiert. Die Analyse der intakten polaren Lipide als Biomarker für lebende Mikroorganismen ließ auf eine hohe Zahl vegetativer Mikroorganismen selbst in fünf Metern Sedimenttiefe schließen, da die Gehalte der Membranlipide kaum mit der Tiefe abnahmen.

In den oberen sanddominierten 130 cm des Sedimentkerns wurden hohe Gehalte von phosphatfreien eukaryotischen Membranlipiden wie Sulfochinovo-

syldiacylglycerol, Betainlipiden und Glycolipiden detektiert, die auf einen Eintrag von relativ frischem organischen Material in Form von intakten Algenzellen hinwiesen. Das hat gezeigt, dass intensiver advektiver Porenwasserfluss am Platenrand des Janssands nicht nur gelöstes, sondern offensichtlich auch frisches partikuläres organisches Material in die tieferen permeablen Sedimentschichten transportiert. Die Kohlenstoffisotopenverhältnisse der hydrolisierten polaren Membranlipide zeigten außerdem, dass dieses organische Material auch in mehr als einem Meter Tiefe von den autochthonen Mikroorganismen bevorzugt als Kohlenstoffquelle verwendet wird.

Insgesamt dominierten Bakterien den untersuchten Standort. Im gesamten Sedimentkern lag der Gehalt von Archaeenlipiden um mindestens eine Größenordnung unter dem der bakteriellen Membranlipide. Die detektierten Archaeenlipide waren vergleichbar mit denen der methanogenen *Methanosarcinales* aus dem Subphylum *Euryarchaeota* und lassen den Schluss zu, dass diese die Archaeengemeinschaft dominieren. Eine starke Korrelation der Gehalte an Bakterien- und Archaeenlipiden in den Sedimentschichten unterhalb von 50 cm Tiefe lassen vermuten, dass Archaeen und Bakterien eine syntrophe Gemeinschaft bilden. Bemerkenswert war der Nachweis bakterieller Phospholipide mit Etherglycerolgerüsten sowie die Detektion phosphatfreier bakterieller Membranlipide im gesamten Sedimentkern. Die Quellorganismen dieser Lipide sind jedoch noch weitestgehend unbekannt.

Summary

In the present work intact polar membrane lipids of pure bacterial cultures and a sediment core from the backbarrier tidal flat of Spiekeroog Island were analyzed biogeochemically using HPLC-mass spectrometry.

The first part of this thesis describes the chemotaxonomical and physiological characterization of several bacterial isolates of Wadden Sea sediments and the deep biosphere. Eight isolates of sulfate reducing bacteria of *Desulfovibrio acrylicus* were found to be abundant in sediments at different locations of the Wadden Sea. They were further analyzed for temperature-dependent changes in growth and activities. The analysis especially focused on their intact polar membrane lipids with respect to changes in composition in response to different growth temperatures. Besides phospholipids, all strains unexpectedly contained high amounts of phosphate-free ornithine lipids that have so far been detected in only one other member of the *Deltaproteobacteria*. The relative amount of ornithine lipids strongly increased with growth temperatures. Molecular analysis of intact polar lipids revealed that temperature-dependent adaptation of the side chain composition was similar in phospholipids and ornithine lipids. Therefore, this modification seems to be the main mechanism for adaptation of membrane viscosity to changing growth temperatures. In contrast, changes in the composition of polar lipid types in the cell membranes and the increase in ornithine lipid contents seem to play an important role in the regulation of membrane-bound enzymes.

The second part of this thesis discusses the results of the geochemical analysis of a five-meter long sediment core from site Janssand in the backbarrier tidal flats of Spiekeroog Island in the North German Wadden Sea. The contents of intact polar lipids as biomarkers for living microorganisms did not decrease significantly with depth indicating high numbers of vegetative microbial cells down to five meters depth. In the upper sand-dominated 130 cm of the sediment core high contents of phosphate-free sulfoquinovosyldiacylglycerol, betaine lipids and glycolipids of eukaryotic origin demonstrated an input of relatively fresh organic matter, *i.e.* intact benthic algae. This result showed that the intense advective pore water flow at the tidal flat margin of site Janssand apparently does not

only transport dissolved but also particulate organic matter into the deeper permeable sediments. The isotopic composition of the hydrolyzed polar membrane lipids revealed that the autochthonous microorganisms preferentially use this marine organic matter as a carbon source.

Bacteria were found to be the dominating microorganisms at the investigated sampling site. In all sediment layers the contents of archaeal lipids were at least one order of magnitude lower than those of bacterial lipids. The detected archaeal lipids were typical for methanogenic *Methanosarcinales* of the subphylum *Euryarchaeota* indicating their dominance within the archaeal community. In the sediments below 50 cm depth, the strong correlation between bacterial and archaeal lipid contents suggests a syntrophic association between archaea and bacteria. Furthermore, unusual bacterial phospholipids with ether-linked glycerol core lipids and phosphate-free bacterial membrane lipids of so far uncultured anaerobic bacteria were detected throughout the sediment core.

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Abkürzungen

AEG	*Acyl-ether glycerol*
ANME	*Anaerobic methanotrophic archaea*
AOM	*Anaerobic oxidation of methane*
BCFA	*Branched-chain fatty acid*
BL	*Betaine lipid*
CARD-FISH	*Catalyzed reporter deposition-fluorescence in-situ hybridization*
DAG	*Diacylglycerol*
DEG	*Diether glycerol*
DGGE	*Denaturing gradient gel electrophoresis*
DNA	*Deoxyribonucleic acid*
DOC	*Dissolved organic carbon*
DPG	*Diphosphatidylglycerol*
ELSD	*Evaporative light scattering detector*
ERIC	*Enterobacterial repetitive intergenic consensus*
ESI	*Electrospray ionization*
FAME	*Fatty acid methyl ester*
GC-FID	*Gas chromatography flame ionization detection*
GC-IRMS	*Gas chromatography combustion isotope ratio mass spectrometry*
GC-MS	*Gas chromatography mass spectrometry*
GDGT	*Glyceroldialkylglyceroltetraether*
HPLC	*High-performance liquid chromatography*
HPLC-MS	*High-performance liquid chromatography mass spectrometry*
IPL	*Intact polar lipid*
MPN	*Most probable number*
MUFA	*Monounsaturated straight-chain fatty acid*
OL	*Ornithine lipid*
PA	*Phosphatidic acid*
PC	*Phosphatidylcholine*
PCR	*Polymerase chain reaction*
PDME	*Phosphatidyl-(N,N-dimethyl)-ethanolamine*
PE	*Phosphatidylethanolamine*
PG	*Phosphatidylglycerol*
PI	*Phosphatidylinositol*
PLFA	*Polar lipid fatty acid*
PMME	*Phosphatidyl-(N-monomethyl)-ethanolamine*
PS	*Phosphatidylserine*
PUFA	*Polyunsaturated fatty acid*
RNA	*Ribonucleic acid*
rRNA	*Ribosomal ribonucleic acid*
SCFA	*Saturated straight-chain fatty acid*
SQDG	*Sulfoquinovosyldiacylglycerol*
SRB	*Sulfate-reducing bacterium*
TOC	*Total organic carbon*

1. Einleitung

1.1 Mikrobielle Prozesse in intertidalen Wattsedimenten

Die Küste der südlichen Nordsee ist eines der größten zusammenhängenden Wattgebiete der Erde, das sich über ca. 500 km von den Niederlanden bis nach Dänemark erstreckt (Abb. 1). Das Wattenmeer ist aufgrund des Nährstoffeintrags vom Land durch eine hohe Primärproduktion in der Wassersäule geprägt, die Grundlage bietet für hohe mikrobielle Aktivitäten und Umsatzraten (Poremba et al., 1999; van Beusekom & de Jonge, 2002). Bedingt durch den hohen Eintrag organischen Materials und die geringe Wassertiefe können bis zu 50 % der Primärproduktion die Sedimentoberfläche erreichen. Das absinkende und das von benthischen Algen produzierte organische Material bilden eine wichtige und leicht verfügbare Substratquelle für die Mikroorganismen im Oberflächensediment (MacIntyre et al., 1996; Stal, 2003).

Der Abbau von organischem Material ist eine Abfolge von Prozessen innerhalb einer mikrobiellen Gemeinschaft, bei dem die Abbauprodukte des einen Prozesses als Substrat für den nächsten Abbauschritt dienen (Sørensen et al., 1981; Laanbroek et al., 1985; Schink, 1997). Für die Mikroorganismen leicht verwertbare Substrate und organisches Material marinen Ursprungs werden in schlickigen und diffusionsdominierten Wattsedimenten jedoch schnell aufgebraucht (Böttcher et al., 1998; Volkman et al., 2000; Rütters et al., 2002b). In den tieferen Sedimentschichten steht daher häufig nur noch refraktäres, schwer abbaubares organisches Material vorwiegend terrestrischen Ursprungs zur Verfügung (Böttcher et al., 1998; Freese et al., 2008a).

Die ablaufenden Stoffkreisläufe hängen aber nicht nur stark von der Verfügbarkeit und Qualität des organischen Materials ab, sondern auch davon, ob ausreichend reaktive Elektronenakzeptoren zur Verfügung stehen. Die Oxidation von organischem Material geht mit einem Verbrauch von Elektronenakzeptoren einher, deren vertikale Verteilung den abnehmenden Redoxpotenzialen und dem damit verbundenem potenziellen Energiegewinn folgt. Der größte Energiegewinn wird durch Sauerstoff erzielt, weshalb dieser bereits in den obersten Millimetern

der meisten Wattsedimente verbraucht wird (z.B. Böttcher et al., 2000; Llobet-Brossa et al., 2002). Anoxische Bedingungen entstehen, wenn der Sauerstoffeintrag durch molekulare Diffusion für den Verbrauch nicht mehr ausreicht. Der Abbau organischen Materials erfolgt dann ausschließlich durch mikrobielle Remineralisierungsprozesse wie Gärung und die Reduktion von Nitrat, Mangan(IV), Eisen(III) oder Sulfat. In tieferen Sedimentschichten spielt die von Archaeen durchgeführte Methanogenese eine zunehmend wichtige Rolle (Froelich et al., 1979; Sørensen et al., 1979). Sulfat ist in hohen Konzentrationen von ca. 28 mM im Meerwasser verfügbar und diffundiert in tiefere Sedimentschichten, in denen andere Elektronenakzeptoren bereits verbraucht sind (Jørgensen, 1977). In anoxischen Sedimenten ist Sulfat daher der vorherrschende terminale Elektronenakzeptor, und Sulfatreduktion macht bis zu 50 % der gesamten Remineralisierung aus (Jørgensen, 1982). Sulfatreduzierende Bakterien bilden daher in Wattsedimenten häufig einen wesentlichen Bestandteil der mikrobiellen Gemeinschaft (Llobet-Brossa et al., 2002; Mußmann et al., 2005). In den tiefen Wattsedimenten nehmen die Zellzahlen, vermutlich wegen des Nährstoffmangels und schlechter Substratverfügbarkeit stark ab, und es werden vorrangig endosporenbildende Bakterien der Gattung *Firmicutes* (Köpke et al., 2005) und ein erhöhter Anteil inaktiver Endosporen gefunden (Fichtel et al., 2008). Molekularbiologische Untersuchungen zeigen außerdem einen hohen Anteil nicht kultivierbarer Bakterien, die typisch sind für die nährstoffarmen Sedimente der tiefen Biosphäre (Parkes et al., 2005; Wilms et al., 2006a; Batzke et al., 2007; Webster et al., 2007). Daher stellt sich die Frage, ob Nährstofflimitierung und Qualität des organischen Materials in tiefen Wattschichten nur geringe mikrobielle Aktivitäten zulassen, die mit den nährstoffarmen Sedimenten der tiefen Biosphäre vergleichbar sind (Engelen & Cypionka, 2009).

1.1.1 Porenwasserflüsse als Antrieb für den ‚Bioreaktor' Janssand

Sandige Sedimente weisen im Vergleich zu schlickigen Sedimenten eine geringere Adsorptionskapazität und größere Sedimentpartikel auf und zeichnen sich häufig durch einen geringen Gehalt an organischem Material aus (Bergamaschi et al., 1997; Volkman et al., 2000; Rusch et al., 2003; Freese et al., 2008a). In früheren Studien wurden an sanddominierten Standorten oft geringere Gesamtzellzahlen nachgewiesen als an schlickigen (Llobet-Brossa et al., 1998; Wieringa et al., 2000; Rusch et al., 2001). Es wurde daher angenommen, dass diese Standorte durch geringere mikrobielle Aktivität charakterisiert sind. Neue Untersuchungen haben jedoch gezeigt, dass permeable Sandsedimente durch einen verstärkten advektiven Porenwasserfluss geprägt sind. Folglich können sie trotz ihres geringen Gehalts an organischem Material eine vielfältige Mikrobengemeinschaft beherbergen. Dadurch können sich ähnlich hohe Umsatzraten wie in Schlicksedimenten entwickeln, die reich an organischem Material sind (Huettel & Rusch, 2000; Ishii et al., 2004; Billerbeck et al., 2006a; Musat et al., 2006; Rusch et al., 2006). Dies beruht im Wesentlichen auf zwei Prozessen, die im Folgenden kurz erläutert werden.

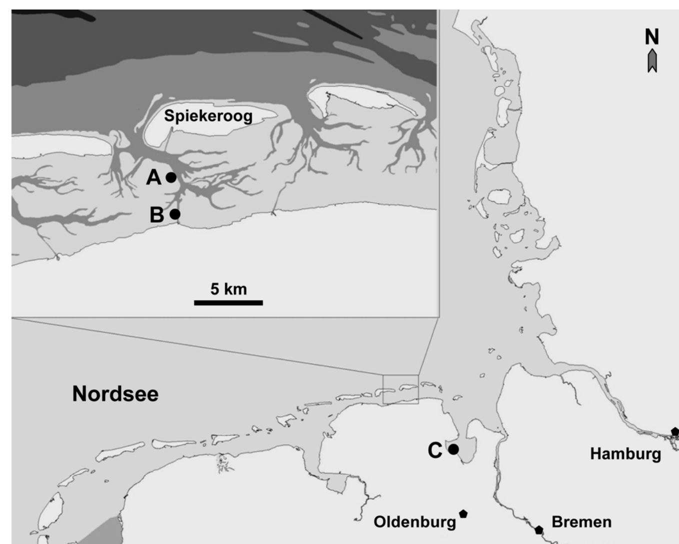

Abb. 1: Karte des Wattenmeers der südlichen Nordsee. Die Markierungen kennzeichnen die Standorte der Probennahmen der vorliegenden Arbeit. Die Sandplate Janssand (**A**) und der Standort (**B**) auf dem Neuharlingersieler Nacken liegen im Rückseitenwatt der Insel Spiekeroog; **C**: Jadebusen bei Dangast.

Schnelle Austauschprozesse an der Sedimentoberfläche

Schnelle advektive Austauschprozesse zwischen Sediment und Wassersäule finden insbesondere an der Sedimentoberfläche statt. Dieser Austausch wird durch die Hydrodynamik und Beschaffenheit der Sedimentoberfläche (Sedimentrippel) bestimmt (Huettel & Gust, 1992; Huettel et al., 1998; Røy et al., 2002). Er erfolgt innerhalb weniger Minuten und reicht nur einige Zentimeter tief ins Sediment (Precht & Huettel, 2004; de Beer et al., 2005; Franke et al., 2006; Werner et al., 2006). Dabei wird partikuläres organisches Material aus dem Meerwasser im Oberflächensediment filtriert, und Stoffwechselabbauprodukte gelangen nach dem Durchfließen des Sediments wieder in die Wassersäule (Huettel & Rusch, 2000; Rusch & Huettel, 2000). Als Konsequenz gelangen relativ frisches und mikrobiell leicht verwertbares organisches Material und Elektronenakzeptoren wie Sulfat aus der Wassersäule ins Sediment.

Advektiver Porenwassertransport in tieferen Sedimenten

Eine tiefgreifende advektive Zirkulation von Porenwasser findet in den tieferen Sedimentschichten der Sandplate Janssand im Rückseitenwatt der Insel Spiekeroog statt (Abb. 1, Standort **A**). Die Niedrigwasserlinie befindet sich ca. ein bis zwei Meter unterhalb der Platenoberfläche (Beck et al., 2008a). Bei ablaufendem Wasser fließt durch den hydraulischen Druckgradienten das Porenwasser von der Platenoberfläche in Richtung Niedrigwasserlinie. Das austretende Porenwasser ist an Sulfat abgereichert, an Methan gesättigt und enthält hohe Konzentrationen von gelöstem organischem Material (Billerbeck et al., 2006a,b; Beck et al., 2008b). Der Porenwasserfluss in der Janssandplate ist stark von der Position abhängig. In der Platenmitte ist er sehr schwach, aufgrund der überwiegenden diffusiven Prozesse herrschen dort nur geringe mikrobielle Aktivitäten (Beck et al., 2008b). Im Gegensatz dazu deuten die Porenwasserprofile und hohen Zellzahlen am Platenrand bis in 5 Metern Sedimenttiefe darauf hin, dass durch den advektiven Porenwasserfluss Substrate in tiefe, durchlässige Sandschichten transportiert und die Abbauprodukte der Mikroorganismen ausgespült werden (Billerbeck et al., 2006b; Beck et al., 2008b; Gittel et al., 2008; Røy et al., 2008). Entgegen früheren Annahmen sind sanddominierte Platenränder im Wattenmeer also durch hohe

mikrobielle Aktivitäten geprägt. Folglich besteht ein großes Interesse daran, diese Standorte biogeochemisch genauer zu untersuchen, da sie als „Bioreaktoren“ grundlegenden Einfluss auf die Umsatzprozesse im gesamten Wattgebiet haben (Boudreau et al., 2001; Billerbeck et al., 2006a; Beck et al., 2009).

1.2 Methoden für die Analyse mikrobieller Gemeinschaften

Geochemische Profile in marinen Habitaten lassen häufig auf mikrobielle Stoffumsatzprozesse schließen. Eine Abschätzung der Diversität und Aktivität der *in situ* aktiven Mikroorganismen kann jedoch nur durch den Einsatz kultivierungsabhängiger und kultivierungsunabhängiger Analysemethoden erfolgen, die im Folgenden kurz beschrieben werden.

1.2.1 Methoden der mikrobiellen Ökologie

Von grundlegender Bedeutung ist die Kultivierung von Mikroorganismen, um ihr physiologisches Potenzial und ihre Rolle in der mikrobiellen Gemeinschaft verstehen zu können (Leadbetter, 2003; Giovannoni & Stingl, 2007). Niedrige Wachstumsraten und das Fehlen syntropher Partner erschweren jedoch häufig die Isolierung von Mikroorganismen aus ihrem natürlichen Habitat, sodass weit weniger als 1 % aller Mikroorganismen in Reinkultur vorliegen (Amann et al., 1995; D'Hondt et al., 2004).

Eine kultivierungsunabhängige Möglichkeit, die Gesamtzellzahlen bakterieller Lebensgemeinschaften in Sedimenten abzuschätzen, ist die Zellfärbung mit Fluoreszenzfarbstoffen wie Acridinorange oder DAPI (4’-6-Diamino-2-phenylindol). Über diese Färbungstechniken sollen nur metabolisch aktive Zellen mit intaktem Chromosom erfasst werden, die anschließend mittels Epifluoreszenzmikroskopie gezählt werden. Mit dieser Methode kann jedoch nicht zwischen metabolisch aktiven und toten Zellen unterschieden werden (Kepner & Pratt, 1994; Luna et al., 2002). Weiterhin wird vermutet, dass auch inaktive Endosporen mit erfasst werden (Fichtel et al., 2007). Diese Methode kann daher zu einer deutlichen Überschätzung der Anzahl metabolisch aktiver Mikroorganismen führen.

Eine weitere Möglichkeit, die gesamte aktive Biomasse abzuschätzen, besteht in der Messung der im Sediment enthaltenen Nukleinsäuren. Probleme können jedoch bei der quantitativen Gewinnung von DNA auftreten, da z.B. die Extraktionseffizienz von der Zellwandbeschaffenheit der Mikroorganismen und auch der Sedimentmatrix abhängt (Head et al., 1998; Frostegård et al., 1999). Die anschließende Amplifizierung von Genen über PCR kann problematisch sein, wenn die verwendeten *primer* ungewollt selektiv wirken (Suzuki & Giovannoni, 1996; Teske & Sørensen, 2008) oder Artefakte im PCR-Produkt entstehen (von Wintzingerode et al., 1997). Offen ist z.B., ob die Selektivität einiger verwendeter *primer* dazu führt, dass die Abundanz von Archaeen systematisch unterschätzt wird, wie es von Teske und Sørensen (2008) postuliert wird. Außerdem wurde gezeigt, dass extrazelluläre DNA außerhalb intakter Zellen über einen längeren Zeitraum im Sediment erhalten bleibt (Dell'Anno et al., 1998) und über einige tausend (Coolen & Overmann, 2007) bis möglicherweise Millionen Jahre (Inagaki et al., 2005) nachweisbar ist. Die Identifizierung der tatsächlich *in situ* aktiven Mikroorganismen wird dadurch extrem erschwert. Die Analyse von 16S rDNA zeigt außerdem nicht die physiologische Diversität der tatsächlich aktiven Mikroorganismen (z.B. Sass et al., 1998c). Um neben der phylogenetischen auch die physiologische Vielfalt eines Habitats zu erfassen, werden daher auch funktionelle Gene wie das der dissimilatorischen Sulfitreduktase, ein Schlüsselenzym aller sulfatreduzierenden Bakterien, quantifiziert (z.B. Minz et al., 1999; Wilms et al., 2006b).

Eine empfindliche Methode zum Nachweis aktiver Mikroorganismen bietet die Untersuchung von ribosomaler RNA, die nur in metabolisch aktiven Zellen vorhanden ist. Mit Hilfe von spezifischen 16S rRNA Gensequenzen können Oligonukleotidsonden entwickelt werden, die mit einem Fluoreszenzfarbstoff markiert sind (*fluorescence in-situ hybridization*; FISH). Diese binden spezifisch an die 16S rRNA innerhalb der Zellen und können anschließend fluoreszenzmikroskopisch direkt und mit hoher räumlicher Auflösung quantifiziert werden (DeLong et al., 1989; Amann et al., 1990). Die Empfindlichkeit dieser Methode ist jedoch vom Ribosomengehalt der Mikroorganismen abhängig, der insbesondere in oligotrophen Habitaten auf Grund der verringerten metabolischen Aktivität

der Mikroorganismen unterhalb der Nachweisgrenze liegen kann (Amann et al., 1995). Durch die Weiterentwicklung CARD-FISH (*catalyzed reporter deposition-fluorescence in-situ hybridization*) können jedoch auch Mikroorganismen mit geringem rRNA-Gehalt detektiert werden (Pernthaler et al., 2002; Ishii et al., 2004).

Die Verwendung von verschiedenen molekularbiologischen Untersuchungsmethoden wird kontrovers diskutiert, da sie teilweise zu unterschiedlichen Resultaten geführt haben: In Tiefseesedimenten vor Peru wurden unter Verwendung von CARD-FISH und quantitativer PCR in einer Studie von Schippers et al. (2005) ein dominierender Anteil von Bakterien und nur geringe Mengen von Archaeen gefunden. Dahingegen wurden von Biddle et al. (2006) am gleichen Standort mittels FISH Archaeen als dominierende Mikroorganismen nachgewiesen. Die scheinbar einfache Frage nach der Dominanz von Archaeen oder Bakterien in der tiefen Biosphäre stellt deshalb nach wie vor eine große Herausforderung an die Wissenschaft dar. Das verdeutlicht die Notwendigkeit einer kritischen Betrachtung und Weiterentwicklung etablierter Methoden sowie der Verwendung komplementärer Methoden, wie der Analyse intakter polarer Membranlipide.

1.2.2 Intakte polare Lipide für die Analyse mikrobieller Gemeinschaften

Als kultivierungsunabhängige Methode wurde die Analyse intakter polarer Lipide (IPL) bereits in einer Vielzahl biogeochemischer Studien von aquatischen Habitaten bis hin zu Sedimenten der tiefen Biosphäre verwendet (z.B. Rütters et al., 2002b; Zink et al., 2003; Van Mooy et al., 2006; Ertefai et al., 2008; Lipp et al., 2008). IPL sind ideale Biomarker für lebende Organismen, da sie in allen lebenden Zellen enthalten sind (Fritsche & Laplace, 1990) und innerhalb von Tagen nach der Zelllyse abgebaut werden (White et al., 1979; Harvey et al., 1986). Für die Analyse mikrobieller Gemeinschaften ist dabei von entscheidender Bedeutung, dass diese Biomarker die Unterscheidung zwischen metabolisch aktiven Organismen und inaktiven Ruhestadien oder Zelldetritus ermöglichen.

Die Analyse von Membranlipiden in Umweltproben war bis vor wenigen Jahren auf deren apolare Abbauprodukte (z.B. Hinrichs et al., 2000; Jahnke et al., 2001; Pancost et al., 2001; Blumenberg et al., 2004; Elvert et al., 2005) oder die

derivatisierten Fettsäuren der polaren Lipide (*polar lipid fatty acids*, PLFA) beschränkt (z.B. Guckert et al., 1986; Rajendran et al., 1995; Guezennec & Fiala-Medioni, 1996; White, 1997; Green & Scow, 2000). Durch die Aufspaltung der IPL gehen allerdings wertvolle Informationen über Kopfgruppen oder Seitenkettenkombinationen verloren. Dadurch wird eine genaue Unterscheidung, z.B. zwischen eukaryotischen und prokaryotischen Ursprungsorganismen, erschwert (Rütters et al., 2002b; Freese et al., 2008a). Außerdem können während der chemischen Hydrolyse auch Fettsäuren aus anderen polaren Komponenten freigesetzt werden, die ein ähnliches Fettsäuremuster aufweisen können wie Bakterien (Aries et al., 2001). Häufig ähneln sich die Fettsäuremuster von verschiedenen Bakteriengruppen sogar stark, da es kaum Fettsäuren gibt, die spezifisch für eine bestimmte Gruppe von Mikroorganismen sind. Interpretationen von PLFA-Profilen im Hinblick auf die Veränderung der Zusammensetzung einer mikrobiellen Gemeinschaft, z.B. in Folge von Änderungen der Umweltbedingungen, werden dadurch erschwert (Haack et al., 1994; Zelles, 1999; Hill et al., 2000).

Daten über Fettsäureverteilungen werden mittlerweile routinemäßig genutzt, um Reinkulturen isolierter Mikroorganismen chemotaxonomisch zu klassifizieren (Vainshtein et al., 1992; Kohring et al., 1994; Sasser, 2001). In der Literatur sind diese Daten daher weit verbreitet (Ratledge & Wilkinson, 1988). Dagegen ist der Datensatz über IPL noch unvollständig, da es bis vor wenigen Jahren noch vergleichsweise aufwendig war, sie als intakte Moleküle zu analysieren (Makula & Finnerty, 1974; Nunes et al., 1992). Die direkte molekulare Analyse komplexer Moleküle wurde erst mit der Entwicklung schonender Ionisierungsverfahren wie der Elektrospray-Ionisierung (ESI; *electrospray ionization*) in der Massenspektrometrie (MS) möglich. In Kombination mit gekoppelten MS-Techniken standen somit erstmals hochempfindliche Untersuchungsverfahren zur Verfügung, die auch die Strukturaufklärung intakter Moleküle erleichterte (Kayganich & Murphy, 1992; Cole & Enke, 1994; Han & Gross, 1995). Die relativ schnelle und quantitative Analyse wurde entscheidend vereinfacht, als die Hochleistungsflüssigkeitschromatographie (HPLC) in Verbindung mit der Massenspektrometrie die Auftrennung komplexer Phospholipidgemische nach Kopfgruppen ermöglichte (Fang & Barcelona, 1998).

Die taxonomische Auflösung, die durch die direkte Analyse intakter Phospholipide erreicht wird, ist deutlich höher als die einzelner hydrolisierter Phospholipidfettsäuren (Fang et al., 2000a; Rütters, 2001; Sturt et al., 2004). Während für die Charakterisierung einer mikrobiellen Gemeinschaft nur 20 bis 50 verschiedene Fettsäuren zur Verfügung stehen (Zelles, 1999), ergibt sich die deutlich höhere chemotaxonomische Auflösung der IPL aus der Zahl der möglichen Kombinationen der Seitenketten in Verbindung mit den verschiedenen Kopfgruppen. Zusätzlich können sowohl ester- als auch ethergebundene Seitenketten mittels ESI-MS analysiert werden (Han & Gross, 1995; Rütters et al., 2001) wodurch der analytische Fokus auch auf die in Umweltproben enthaltenen intakten Archaeenlipide erweitert werden kann (Sturt et al., 2004; Biddle et al., 2006; Lipp et al., 2008). Ethergebundene Seitenketten werden bei der für die PLFA-Analyse verwendeten milden alkalischen Hydrolyse nicht abgespalten. Eine Lipidanalyse, die sich in Umweltproben ausschließlich auf PLFA beschränkt, könnte bewirken, dass die Menge und Diversität lebender Mikroorganismen unterschätzt wird. Anhand der Menge der extrahierten intakten Phospholipide einer Sedimentprobe lassen sich Zahlen vegetativer Zellen grob abschätzen (Rütters et al., 2002b). Inaktive Endosporen werden bei dem für die Phospholipidanalytik verwendeten Extraktionsverfahren vermutlich nicht aufgeschlossen (Macnaughton et al., 1997; Rütters et al., 2002b). In dieser Hinsicht bietet die Messung intakter Phospholipide gegenüber der Epifluoreszenzmikroskopie, aber auch der Extraktion von Nukleinsäuren Vorteile bei der Quantifizierung von Mikroorganismen.

Die Analyse intakter Phospholipide mittels ESI-MS hat sich für die chemotaxonomische Klassifizierung mikrobieller Reinkulturen als sehr nützlich erwiesen (z.B. Smith et al., 1995; Black et al., 1997; Fang & Barcelona, 1998; Rütters, 2001). Tiefenprofile intakter Phospholipide wurden außerdem erfolgreich in marinen und limnischen Sedimenten untersucht, um die Größe und Struktur der vorhandenen mikrobiellen Gemeinschaften im natürlichen Habitat abzuschätzen (Rütters et al., 2002a,b; Zink & Mangelsdorf, 2004; Zink et al., 2008). Sogar auf marine Tiefseesedimente, die durch sehr geringe Biomassen gekennzeichnet sind,

wurde die IPL-Analyse erfolgreich angewendet (Zink et al., 2003; Sturt et al., 2004; Biddle et al., 2006; Lipp et al., 2008).

1.3 Strukturen und Vorkommen polarer Membranlipide

Polare Lipide tragen zwischen 5 % und 9 % zum Trockengewicht prokaryotischer Zellen bei (Fritsche & Laplace, 1990; Madigan et al., 2006). Phospholipide bilden die Lipiddoppelschicht in mikrobiellen Zellmembranen (Abb. 2). Sie sind ein Hauptbestandteil der Zellen und stellen den Großteil der meisten Bakterienlipide dar (White, 1997).

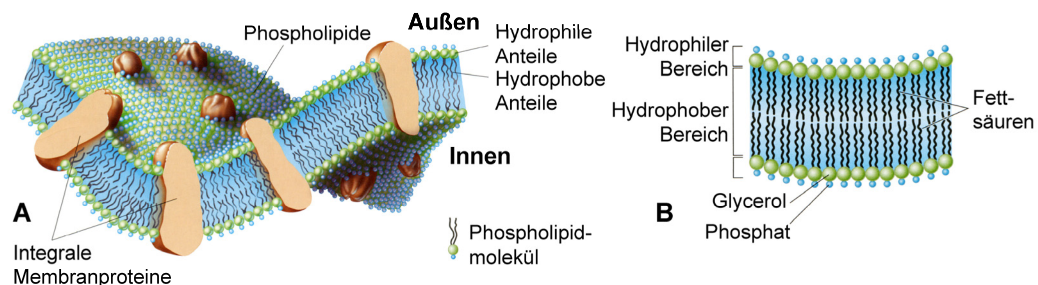

Abb. 2: Strukturen bakterieller Phospholipid-Doppelschichten. **A:** Cytoplasmamembran mit eingelagerten Membranproteinen. Die inneren hydrophilen Kopfgruppen zeigen in Richtung des Cytoplasmas, während die hydrophoben Seitenketten in die Membran weisen. **B:** Detaillierter Querschnitt durch die Lipiddoppelschicht. Abbildung modifiziert nach Madigan et al. (2006)

1.3.1 Ausgewählte bakterielle und eukaryotische polare Lipide

Grundgerüst

Ein Großteil der polaren Lipide besteht aus einem hydrophoben Glycerolgerüst, an das zwei Seitenketten gebunden sind. Die häufigsten bakteriellen Phospholipide mit einem Diacylglycerolgerüst enthalten zwei estergebundene Fettsäuren an der *sn*-1- und *sn*-2-Position des Glycerols (DAG in Abb. 3, unten).

Typische bakterielle Phospholipide sind mit zwei Fettsäuren substituiert, deren Kettenlänge zwischen C_{14} und C_{20} liegt. Häufig kommen einfach ungesättigte, verzweigte oder Fettsäuren mit einem Cyclopropylring vor (Lechevalier & Lechevalier, 1988). Mehrfach ungesättigte Fettsäuren (*polyunsaturated fatty acids*) wurden noch bis vor wenigen Jahren hauptsächlich Eukaryoten (z.B.

Lechevalier & Lechevalier, 1988; Green & Scow, 2000), später aber auch marinen psychrophilen und piezophilen Bakterien zugeordnet (z.B. DeLong & Yayanos, 1986; Hamamoto et al., 1994; Yayanos, 1995; Nichols et al., 1999; Russell & Nichols, 1999). Ihre Entdeckung in mesophilen Bakterienisolaten aus dem Flachwasser und sogar anoxischen Sedimenten in gemäßigten Breiten lassen jedoch auf ihre weite Verbreitung innerhalb der *Gammaproteobacteria* schließen (Ivanova et al., 2001; Skerratt et al., 2002; Frolova et al., 2005; Freese et al., 2008a; Freese et al., 2009).

Einige anaerobe Bakterien enthalten neben einer Fettsäure ein Aldehyd als Seitenkette, das in Form einer O-Alk-1-enylgruppe an der *sn*-1-Position ethergebunden ist. Diese Phospholipide werden auch Plasmalogene genannt (Ratledge & Wilkinson, 1988). Sie wurden bisher in wenigen aeroben und fakultativ anaeroben Bakterien nachgewiesen (Zelles, 1999). Signifikante Mengen von Phospholipiden mit gemischten Acyletherglycerolen (AEG in Abb. 3, unten) wurden erst vor wenigen Jahren in mesophilen sulfatreduzierenden Bakterien nachgewiesen (Rütters et al., 2001; Sturt et al., 2004). Dietherglycerole (DEG in Abb. 3, unten) mit ethergebundenen Substituenten wurden bisher vorwiegend in Archaeen und in einigen thermophilen Bakterien nachgewiesen (Langworthy et al., 1983; Sturt et al., 2004). Bakterielle DEG enthalten vor allem geradkettige oder einfach verzweigte Alkylseitenketten (Langworthy et al., 1983).

Phospholipide

Die polaren Kopfgruppen der Phospholipide enthalten eine mit dem C-3-Atom des Glycerols veresterte Phosphorsäure, die mit weiteren polaren Molekülen verbunden sein kann (Abb. 3, oben). Aufgrund der Vielzahl möglicher Kombinationen von Kopfgruppen und Seitenketten kann sich die Phospholipidzusammensetzung von Bakterien erheblich unterscheiden. So enthalten z.B. Gram-positive Bakterien oft große Mengen von Phosphatidylglycerol (PG), aber nur wenig oder gar kein Phosphatidylethanolamin (PE; O'Leary & Wilkinson, 1988; Dowhan, 1997). Dagegen ist PE der Hauptbestandteil vieler Gram-negativer Bakterien, wobei auch PG und Diphosphatidylglycerol (DPG) weit verbreitet sind

(Wilkinson, 1988; Dowhan, 1997). PG ist essenziell in allen photosynthetischen Organismen, da es ein wichtiger Bestandteil in der Thylakoidmembran ist (Wada & Murata, 2007). Phosphatidylcholin (PC) ist eines der wichtigsten membranformenden Lipide in Eukaryoten (Raetz, 1986). Basierend auf genomischen Untersuchungen wird jedoch vermutet, dass auch mehr als 10 % aller Bakterien PC enthalten (Sohlenkamp et al., 2003). Bei der Biosynthese von PC aus PE können Phosphatidyl-(*N*-monomethyl)-ethanolamin (PMME) und Phosphatidyl-(*N*,*N*-dimethyl)-ethanolamin (PDME) als methylierte Zwischenprodukte auftreten (Dowhan, 1997; Sohlenkamp et al., 2003). Phosphatidylsäure (PA) ist ein Zwischenprodukt der Biosynthese von Phospholipiden und wird daher in geringeren Mengen in vielen Mikroorganismen detektiert (Lechevalier, 1977). Phosphatidylserin (PS) ist ein relativ weit verbreiteter Bestandteil bakterieller Zellmembranen, der dort aber meist nur in geringen Mengen enthalten ist (Christie, 2003).

Phosphatfreie polare Lipide

Phosphatfreie polare Membranlipide sind z.B. die Glycolipide. Sie enthalten im polaren Teil des Lipids eine oder mehrere Hexosen, die direkt an das C-3-Atom des Glycerols gebunden sind (Mono- und Digalactosyldiacylglycerol in Abb. 3). Eine Besonderheit der Glycolipide ist Sulfochinovosyldiacylglycerol (SQDG, Abb. 3). Es ist in phototrophen Prokaryoten und Eukaryoten weit verbreitet (Benning, 1998; Hölzl & Dörmann, 2007), wurde aber auch schon in einigen photosynthetisch inaktiven Bakterien gefunden (Langworthy et al., 1976; Cedergren & Hollingsworth, 1994; Abraham et al., 1997; Sprott et al., 2006). Zusammen mit Digalactosyldiacylglycerol (DGDG) und Monogalactosyldiacylglycerol (MGDG) bildet es den Hauptbestandteil der IPLs von Phytoplankton einschließlich der Cyanobakterien (Sanina et al., 2004; Van Mooy et al., 2006; Hölzl & Dörmann, 2007).

Betainlipide (BL) enthalten als polare Kopfgruppe eine permethylierte Hydroxyaminosäure, die über eine Etherbindung direkt mit dem Diacylglycerol verbunden ist (Abb. 3). BL sind weit verbreitet in nicht-blühenden Pflanzen und Algen, aber auch in Protozoen und Pilzen (Dembitsky, 1993; Kato et al., 1996;

Sanina et al., 2004). Bisher wurden sie nur in wenigen photosynthetischen oder pflanzenassoziierten Bakterien gefunden (Benning et al., 1995; Geiger et al., 1999).

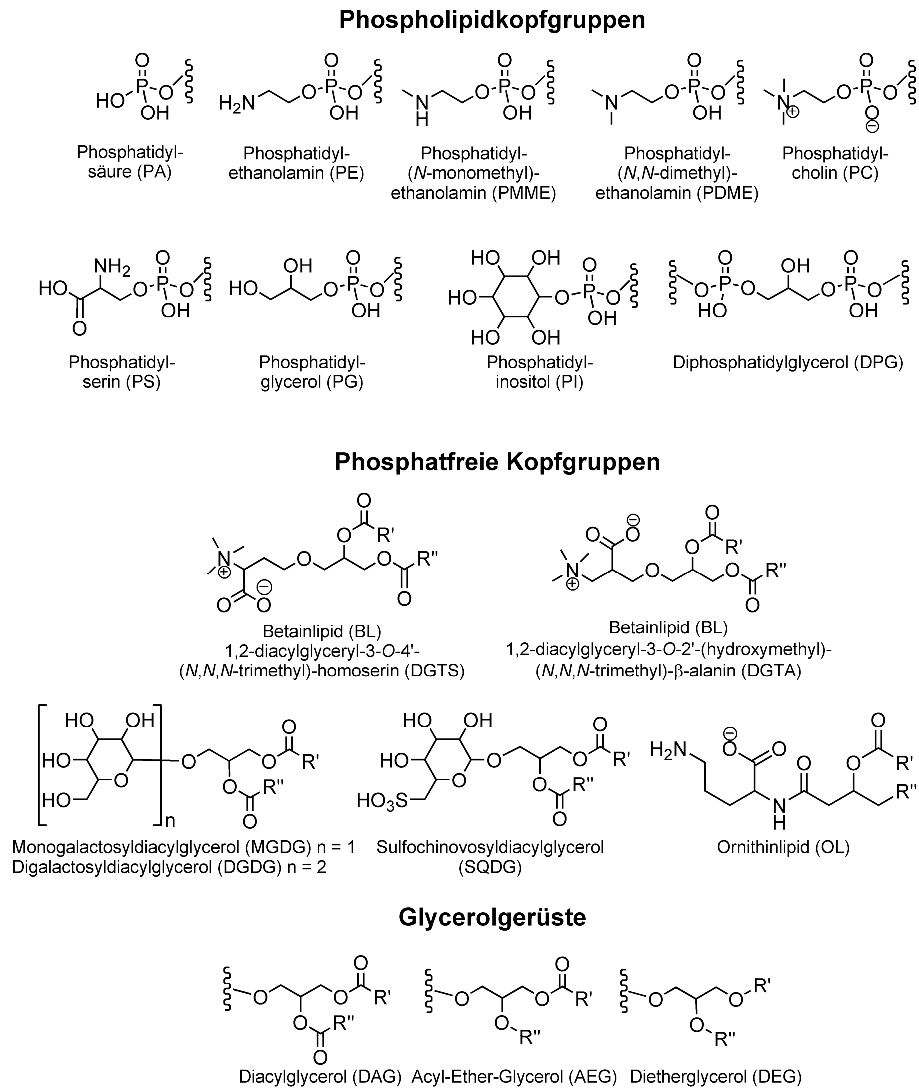

Abb. 3: Ausgewählte Strukturen bakterieller und eukaryotischer polarer Membranlipide.

Ornithinlipide (OL) unterscheiden sich grundsätzlich im Aufbau von den glycerolbasierten IPL (Abb. 3). Der polare Teil besteht aus der Aminosäure Ornithin, an dessen α-Aminogruppe eine 3-Hydroxyfettsäure amidgebunden ist. Sie bildet zusammen mit der an der 3-Hydroxygruppe veresterten zweiten Fettsäure den unpolaren Teil des Lipids (Knoche & Shively, 1972). OL sind wahrscheinlich auf die Domäne der Bakterien beschränkt (López-Lara et al., 2003), in der sie jedoch nicht weit verbreitet sind (Ratledge & Wilkinson, 1988; Asselineau, 1991).

1.3.2 Membranlipide von Archaeen

Archaeenlipide besitzen im Gegensatz zu Bakterien Membranlipide mit einem unpolaren Teil, bei dem die an das Glycerol gebundenen Seitenketten aus Isoprenoideinheiten aufgebaut sind. Die Zellmembranen bestehen entweder aus Diethern mit zwei Phytanylseitenketten (Archaeol in Abb. 4) in einer Lipiddoppelschicht, aus membranübergreifenden Glyceroldibiphytanyltetraethern (*Glyceroldialkylglyceroltetraether*, GDGT in Abb. 4) in einer Lipideinfachschicht oder einer Mischung aus beidem (Valentine, 2007). Zur Anpassung der Membranfluidität bei unterschiedlichen Wachstumstemperaturen können die Anzahl der Ringe in den Biphytanylseitenketten und das Diether- zu Tetraether-Verhältnis variiert werden (Gliozzi et al., 1983; De Rosa & Gambacorta, 1988; Uda et al., 2001; Wuchter et al., 2004; Lai et al., 2008). Die polaren Kopfgruppen können sowohl aus einer oder mehreren Hexose(n) als auch aus Phosphatidylkopfgruppen oder einer Kombination von beiden bestehen (Koga et al., 1993; Sturt et al., 2004; Koga & Nakano, 2008; Schouten et al., 2008). In marinen Sedimenten der tiefen Biosphäre wurden bisher vorwiegend Archaeenlipide aus Biphytanyltetraethern mit Kopfgruppen gefunden, die hauptsächlich Hexosen enthielten (Biddle et al., 2006; Lipp et al., 2008). Viele *Euryarchaeota* synthetisieren gemischte Phosphatidyl-Glycolipide auf Archaeol- und Tetraether-Basis. Allerdings gibt es z.B. einige Vertreter der *Methanosarcinales* und *Methanococcales*, die ausschließlich Phosphatidylarchaeole enthalten (Koga & Nakano, 2008).

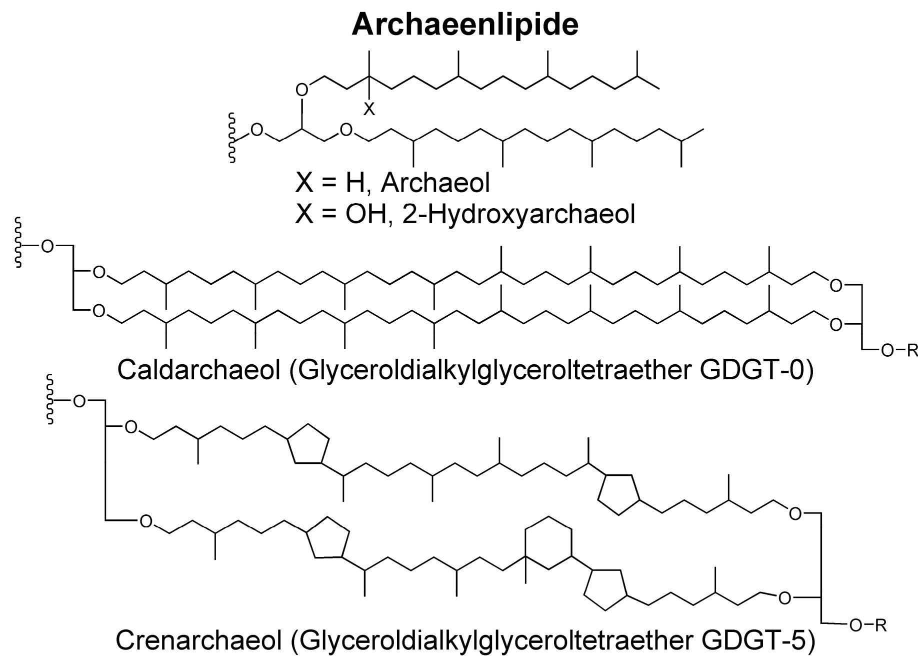

Abb. 4: Grundgerüste ausgewählter Archaeenlipide. Die Anzahl der Ringe in der Biphytanyltetraether-Struktur kann je nach Wachstumstemperatur variiert werden (Null Ringe: GDGT-0, fünf Ringe: GDGT-5). Häufige Kopfgruppen sind z.B. Hexosen. R = H oder R = Phosphatidylkopfgruppe (Kopfgruppen in Abb. 3, oben).

1.4 Anpassungsmechanismen in der Cytoplasmamembran

Zellmembranen dienen nicht nur der Abgrenzung des Mikroorganismus von seiner Umwelt. Über sie laufen wichtige physiologische Prozesse wie Respiration, Chemotaxis und Substrataufnahme ab (Hazel, 1995). Für diese Prozesse ist es essenziell, den flüssigkristallinen Zustand der Membran aufrecht zu erhalten (Kell, 1984). Durch die Veränderung von externen Umweltparametern wie Temperatur, Salinität, pH-Wert oder Druck wird jedoch die Membranfluidität stark gestört (Cronan & Gelmann, 1975; Hazel, 1995; Dowhan, 1997). Sinkende Temperaturen führen z.B. dazu, dass die Zellmembran von ihrem flüssigkristallinen in einen gelartigen oder kristallinen Zustand übergeht, der die Mobilität von Makromolekülen (z.B. Enzymen) in der Zellmembran einschränkt (Quinn, 1981; de Mendoza & Cronan, 1983). Steigende Temperaturen führen zu einer Unordnung in den Fettsäureseitenketten, so dass die Membran in einen nicht-lamellaren Zustand übergeht und sogar schmelzen kann (Hazel & Williams, 1990).

Bakterien sind jedoch durch Veränderungen der Zusammensetzung ihrer Zellmembran in der Lage, die strukturelle Integrität und die Funktion aufrecht zu erhalten (homoviskose Adaption; Sinensky, 1974). Dies geschieht durch die Anpassung des Hopan- oder Proteingehalts (Kannenberg & Poralla, 1999; Denich et al., 2003), Veränderung der Kopfgruppen der Phospholipide (Hasegawa et al., 1980; Donato et al., 2000) oder insbesondere durch Veränderungen in den Phospholipidseitenketten (Russell, 1984; Suutari & Laakso, 1994). Diese Anpassungsprozesse resultieren entweder aus der *de-novo*-Synthese von polaren Lipiden oder aus enzymatischen Veränderungen bestehender Phospholipidfettsäuren in der Zellmembran (Abb. 5; Zhang & Rock, 2008). In der Literatur häufig beschrieben ist die Anpassung des Grads der Unsättigung oder der Seitenkettenlänge (Zhang & Rock, 2008). Mit sinkender Temperatur werden mehr kurzkettige und ungesättigte Fettsäuren in die Membran eingebaut, um die Membranfluidität zu erhöhen (Donato et al., 2000; Männistö & Puhakka, 2001). Vergleichbare Auswirkungen werden auch durch die Erhöhung des relativen Gehalts von verzweigten Fettsäuren (Haque & Russell, 2004), durch Erhöhung des Anteils von *cis*- zu *trans*-Doppelbindungen (Heipieper et al., 1992; Henderson et al., 1995) oder durch die Erhöhung des Verhältnisses von *anteiso*- zu *iso*-verzweigten Fettsäuren erzielt (Haque & Russell, 2004; Unell et al., 2007). Die Anpassungsmechanismen sind nicht auf den hydrophoben Teil von polaren Lipiden beschränkt, sondern können auch in den polaren Kopfgruppen auftreten (Hasegawa et al., 1980; Pluschke & Overath, 1981; Donato et al., 2000). Um die Fluidität zu erhöhen, wird bei sinkenden Temperaturen in die Zellmembran z.B. vermehrt Phosphatidylglycerol (PG) anstelle von Phosphatidylethanolamin (PE) eingebaut, da PG-Lipide einen niedrigeren Schmelzpunkt haben (Hasegawa et al., 1980). Ein vergleichbarer Prozess wurde auch in Bakterienkulturen beobachtet, die unter erhöhtem Druck kultiviert wurden (Fang et al., 2000b; Mangelsdorf et al., 2005). Insbesondere in piezophilen und psychrophilen Bakterien ist dieser Anpassungsprozess auch an den vermehrten Einbau mehrfach ungesättigter Fettsäuren wie Eicosapentaensäure (*n*-20:5ω3) oder Docosahexaensäure (*n*-22:6ω3) gekoppelt. Diese sind aufgrund ihrer niedrigen Schmelzpunkte besonders effektiv bei der

Anpassung der Membranfluidität (z.B. DeLong & Yayanos, 1986; Gounot, 1991; Nichols et al., 1997; Yano et al., 1998).

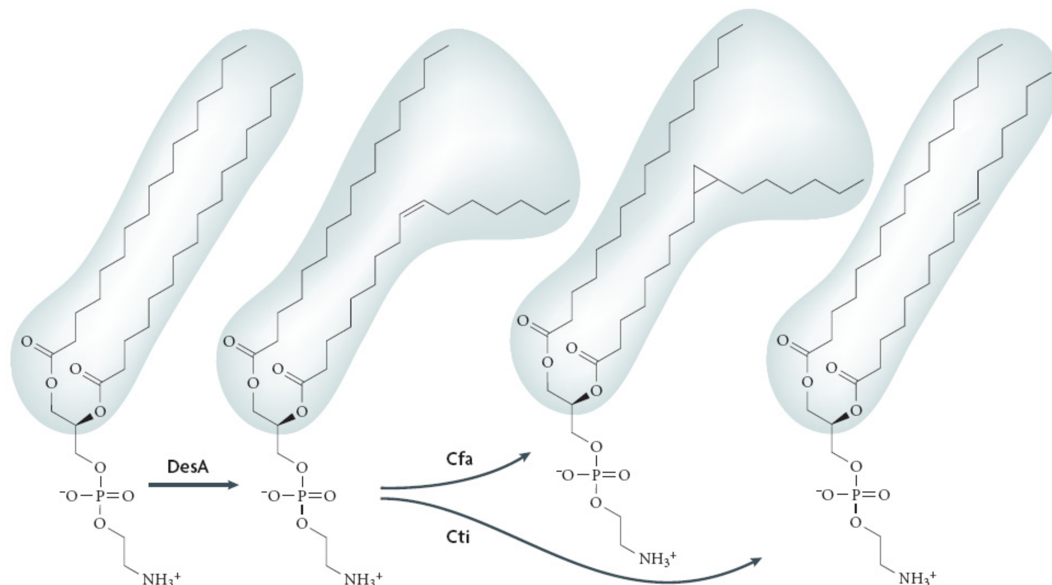

Abb. 5: Modifizierung der bestehenden Seitenketten eines Phospholipids (PE) innerhalb der Cytoplasmamembran. Die Desaturase (DesA) baut eine Doppelbindung ein, die die Membranfluidität erhöht. Die Cyclopropanfettsäuresynthase (Cfa) kann die *cis*-Doppelbindung in einen Cyclopropylring umwandeln, der die Membranstabilität unter Säurestress erhöht. Cis-Trans-Isomerasen (Cti) können *cis*- in *trans*-Doppelbindungen umwandeln, die sich ähnlich wie gesättigte Fettsäuren verhalten, aber gleichzeitig die Membranfluidität erhöhen. Abbildung entnommen aus Zhang & Rock (2008).

Der Einbau mehrfach ungesättigter Fettsäuren bei sinkenden Wachstumstemperaturen wurde erst vor kurzem auch in anaeroben mesophilen Bakterien festgestellt (Freese et al., 2008b).

Ein interessanter Aspekt ist die Fähigkeit einiger Bakterien, Phospholipide unter Phosphatlimitierung gegen phosphatfreie Lipide auszutauschen (z.B. Wilkinson, 1972; Minnikin & Abdolrahimzadeh, 1974; Benning et al., 1995). Diese Mikroorganismen sind in der Lage, vermehrt OL, BL, SQDG oder Glycolipide in ihre Zellmembran einzubauen. Es wird vermutet, dass diese Lipide die Rolle von Phospholipiden in Zellen übernehmen, die an Phosphat verarmt sind (Makula & Finnerty, 1975; Batrakov, 1982; Benning et al., 1995; Geiger et al., 1999). Somit könnten in oligotrophen marinen Habitaten Organismen, die unter Phosphatlimitierung phosphatfreie Lipide synthetisieren, einen signifikanten Wachstumsvorteil

gegenüber Bakterien mit hohem Phospholipidgehalt erlangen (Van Mooy et al., 2006, 2009). Einige Bakterien können wiederum unter Nährstoffmangel den relativen Gehalt von Diphosphatidylglycerol (DPG) stark erhöhen. Es wird vermutet, dass die damit einhergehende Verringerung der Membranfluidität der Aufrechterhaltung der morphologischen Integrität der Zellen unter Substratmangel und physischem oder chemischem Stress dient (Oliver & Stringer, 1984; Wanner & Egli, 1990; Mukamolova et al., 1995).

Die Anpassungsstrategien verschiedener Bakterien können sich deutlich unterscheiden, da die meisten Mechanismen sowohl einzeln als auch in Kombination zur Anwendung kommen (White et al., 2000). Daher sind vergleichende physiologische Studien an Reinkulturen unbedingt erforderlich, um die unterschiedlichen Anpassungsmechanismen zu verstehen und Biomarker-Profile aus Umweltproben entsprechend interpretieren zu können.

1.5 Zielsetzung und Gliederung der vorliegenden Arbeit

Die vorliegende Arbeit ist in der interdisziplinären Forschergruppe „BioGeo-Chemie des Watts" entstanden, in deren Rahmen insbesondere das Rückseitenwatt der Insel Spiekeroog untersucht wurde. Schwerpunkt der zweiten Phase des Teilprojekts 6, „Gradienten und mikrobielle Stoffumsetzungen in der anoxischen Zone", war die Aufklärung mikrobieller und geochemischer Prozesse in den tieferen anoxischen Schichten von Wattsedimenten. Im Übergang zur dritten Projektphase wurde ein besonderer Fokus auf die Sedimente des „Bioreaktors Janssand" gelegt.

Ziel der vorliegenden Arbeit war die qualitative und quantitative Analyse von intakten polaren Lipiden (IPL) als Biomarker für lebende Mikroorganismen. Dazu wurde eine empfindliche und hochauflösende HPLC-ESI-MS-Methode entwickelt, deren entscheidender Vorteil die Verwendung eines Quadrupol-Flugzeit-Massenspektrometers (Q-TOF; *hybrid quadrupole-time-of-flight*) ist. Seine hohe Empfindlichkeit, die Fähigkeit, akkurate Massen mit hoher Genauigkeit zu bestimmen, und die Kombination mit MS/MS-Studien machten auch die strukturelle Aufklärung unbekannter Substanzen möglich.

Ein wesentlicher Bestandteil der vorliegenden Arbeit waren die Untersuchungen der charakteristischen Zusammensetzung der IPL von Isolaten abundanter Mikroorganismen aus dem Watt. Der Datensatz über IPL bakterieller Reinkulturen, insbesondere von bakteriellen Isolaten aus dem Watt, ist noch unvollständig. Aufgrund der wichtigen Rolle der sulfatreduzierenden Bakterien in Wattsedimenten wurde ein besonderer analytischer Fokus auf diese Organismen gelegt. Vorherige Studien hatten gezeigt, dass sulfatreduzierende Bakterien einen wichtigen Anteil an der gesamten mikrobiellen Gemeinschaft ausmachen (Llobet-Brossa et al., 2002; Mußmann et al., 2005). Selbst in tiefen sulfatfreien Schichten wurden noch erhöhte prozentuale Anteile von bis zu 7 % an der mikrobiellen Gemeinschaft detektiert (Wilms et al., 2007; Gittel et al., 2008). Ein weiteres Ziel war es, die Funktion der polaren Membranlipide im Hinblick auf ihre Rolle bei der Anpassung der Membranfluidität bei unterschiedlichen Wachstumstemperaturen zu untersuchen. Dies ist in intertidalen Wattsedimenten von besonderem

Interesse, da Mikroorganismen starken Temperaturänderungen ausgesetzt sind. Jahreszeitlich können innerhalb von zwei Metern Sedimenttiefe die Temperaturen um mehr als 10 °C variieren (Beck et al., 2008b). An der Sedimentoberfläche kann der Unterschied im Tagesverlauf bis zu 15 °C betragen (Harrison & Phizacklea, 1987; Sass et al., 2003b). Die Temperaturänderungen haben starke Auswirkungen auf die metabolischen Prozesse im Sediment (Beck et al., 2008b). Daher wurde dieser Einfluss auf die Wachstumserträge in die Untersuchungen der Reinkulturen mit einbezogen.

Ein weiteres Ziel war die Analyse der IPL eines Sedimentkerns des Janssands, um Aufschluss über die Zusammensetzung der mikrobiellen Gemeinschaft zu erhalten. Dieser Standort im Rückseitenwatt der Insel Spiekeroog zeichnet sich durch eine tiefreichende Porenwasserzirkulation in den permeablen sandigen Schichten aus. Sie versorgt die mikrobielle Gemeinschaft anscheinend mit frischen Nährstoffen und Substraten aus der Wassersäule (Beck et al., 2008b). Infolgedessen nehmen die Gesamtzellzahlen selbst in fünf Metern Tiefe kaum ab (Gittel et al., 2008). Bisher war die Analyse auf bakterielle IPL aus oberflächennahen Wattsedimenten beschränkt (Rütters et al., 2002a,b; Freese et al., 2009). Die verwendete Quantifizierungsmethode der IPL über einen analogen Lichtstreudetektor war stark eingeschränkt. Insbesondere in den tieferen Sedimentschichten wurde die Analyse durch Koelutionen und hohes Untergrundrauschen anderer polarer Komponenten verschlechtert. Daher musste die HPLC-ESI-MS-Methode an die genauere und empfindlichere Quantifizierung der IPL über das Massenspektrometer angepasst werden. Mit dieser Methodenanpassung wurde außerdem der analytische Fokus auf die IPL von Archaeen erweitert. Der Vorteil der Analyse intakter polarer Membranlipide ist der große analytische Rahmen, der die drei Domänen Archaeen, Bakterien und Eukaryoten einschließt. Die Kombination mit parallelen Studien über Porenwasserzusammensetzung (Beck et al., 2009), Endosporenanteil (Fichtel et al., 2008) und sulfatreduzierende Bakterien (Gittel et al., 2008) gewährte detaillierte Einblicke in die mikrobiellen und geochemischen Prozesse am Standort Janssand.

Gliederung der vorliegenden Arbeit

Die Beiträge der Autoren zu den Manuskripten sind in der Publikationsliste am Anfang dieser Arbeit aufgeführt.

Kapitel 2

Ziel dieser Studie war es, die Zusammensetzung der IPL von acht verschiedenen, aber sehr eng miteinander verwandten Stämmen von *Desulfovibrio acrylicus* zu charakterisieren. Da alle untersuchten Stämme aus Sedimenten von unterschiedlichen Standorten des Wattenmeers der südlichen Nordsee isoliert wurden, scheinen sie in diesem Habitat weit verbreitet zu sein. Dieses Manuskript zeigt die ersten IPL-Daten für diese relativ häufig nachgewiesene Art. Die Verwendung der neuen HPLC-ESI-MS-Methode ermöglichte die Identifizierung signifikanter Anteile eines polaren Lipids, das bisher nur in einem anderen Vertreter der *Deltaproteobacteria* gefunden worden war (Makula & Finnerty, 1975). Die Isolate wurden außerdem phänotypisch und im Hinblick auf ihre metabolischen Fähigkeiten analysiert. Die hohe analytische Auflösung auf der Artebene verdeutlichte eine hohe Mikrodiversität, die für sulfatreduzierende Bakterien so kaum bekannt war.

Kapitel 3

Ziel dieser Studie war es, die acht sulfatreduzierenden Stämme von *Desulfovibrio acrylicus* im Hinblick auf temperaturabhängige Veränderungen in Wachstumsrate und Wachstumsertrag und metabolischer Aktivität zu untersuchen. Ein besonderer Fokus lag auf der Untersuchung der Anpassung der Membranfluidität über die molekulare Analyse der intakten polaren Membranlipide. Die Standorte im Wattenmeer, aus denen die Stämme isoliert wurden, zeichnen sich durch gemäßigte Temperaturen aus, und die Isolierungstemperaturen der acht Stämme lagen zwischen 10 und 30 °C (van der Maarel et al., 1996; Sass et al., 2003b). Daher wurde auch untersucht, ob die breite Spanne von Wachstumstemperaturen zu unterschiedlichen Isolaten geführt hatte, die sich durch unterschiedliche Temperaturanpassungen auszeichnen.

Kapitel 4 und Kapitel 5

Ein weiteres Beispiel für die Anwendung von Membranfettsäuren zur chemotaxonomischen Charakterisierung bakterieller Isolate aus Sedimenten des Wattenmeers veranschaulicht die Studie aus Kapitel 4. Basierend auf der Kombination von phylogenetischen, physiologischen und chemotaxonomischen Untersuchungen wird eine neue Gattung sulfatreduzierender Bakterien vorgeschlagen. Die HPLC-ESI-MS-Methode wurde darüber hinaus erfolgreich für die Analyse der intakten Phospholipide bakterieller Reinkulturen aus Sedimenten der tiefen Biosphäre angewandt (Kapitel 5).

Kapitel 6

Ziel dieser Studie war es, durch die lithologiebezogene Analyse der IPL aus einem 5 m langen Sedimentkern die mikrobielle Gemeinschaft zu charakterisieren. Die unterschiedlichen eukaryotischen und prokaryotischen IPL werden im Hinblick auf ihre potenziellen Quellorganismen und im Zusammenhang mit den geochemischen und lithologischen Profilen diskutiert. Da die Analytik auch IPL eukaryotischen Ursprungs einschließt, war es ein Ziel zu untersuchen, ob anhand der IPL benthischer Algen nachweisbar ist, wie tief der Porenwassertransport ins Sediment reicht. Die Kohlenstoffisotopenverhältnisse des gesamten organischen Kohlenstoffs wurden in Relation zu denen der hydrolisierten Archaeen- und Bakterienlipide untersucht, um zu klären, welche Kohlenstoffquellen von den Mikroorganismen verwendet werden. Durch die Verwendung der hochauflösenden Massenspektrometrie sollte auch ein Fokus auf IPL gelegt werden, deren Strukturen in vorangegangen Studien nicht eindeutig geklärt worden waren (Rütters et al., 2002a,b).

2 A combined phenotypical and physiological study of *Desulfovibrio acrylicus* sp. emend. a sulfate-reducing bacterium with ornithine lipids

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2.1 Abstract

Seven strains of sulfate-reducing bacteria (SRBs) were isolated from intertidal sediments of the German North Sea coast. The isolates were obtained from the highest positive dilutions of most-probable-number series. Phylogenetic analysis based on 16S rRNA gene analysis revealed that they affiliated with the species *Desulfovibrio acrylicus*. The type strain of the species (DSM 10141) was included in further characterization. All strains grew with hydrogen, lactate, pyruvate, succinate, malate, fumarate, glycerol and ethanol. Other substrates were less frequently utilized. All isolates showed highest growth yield when grown with thiosulfate as electron acceptor. Although sulfite reduction was observed, growth was reduced or inhibited in some strains (including the type strain). Only two of the strains grew by sulfur reduction. Although all strains reduced $Fe(OH)_3$ this did not support growth.

The strains showed a diverse polar lipid fatty acid (PLFA) pattern with up to 26 different compounds dominated by monounsaturated and *iso*- and *anteiso*-branched fatty acids. Main phospholipids were phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). Diphosphatidylglycerol (DPG, cardiolipin) was found in all strains but in much lower concentrations. Phosphate-free ornithine lipids (OLs) contributed significantly to the polar lipids of all strains (12 to 60 %). The dominant molecular species of PE and PG were similar with respect to their fatty acid side chains, *i.e.* dominated by fatty acids with 17 or 18 carbon atoms, while fatty acids with 15 carbon atoms were found predominantly in OL. The physiological role of the rarely detected ornithine lipids is still unknown.

2.2 Introduction

In coastal marine sediments sulfate reduction is responsible for over 50 % of the terminal remineralization of organic matter (Jørgensen, 1977). Molecular biological studies revealed that sulfate-reducing bacteria (SRBs) in intertidal sediments may represent up to 15 % of the total prokaryotic population (Mußmann et al., 2005) and that elevated numbers may be present even in sulfate-depleted layers (Wilms et al., 2007; Gittel et al., 2008). Although sulfate reducers as terminal oxidizers generally rely on metabolic products provided by fermenting microorganisms, they are metabolically more versatile and diverse than expected. Some of them were found to utilize a range of electron donors like amino acids and even carbohydrates (van der Maarel et al., 1996; Sass et al., 2002) or can grow in syntrophic association with methanogens (McInerney & Bryant, 1981). Besides sulfate they can reduce a range of oxidized sulfur compounds like thiosulfate, sulfite, elemental sulfur, or may reduce nitrate to ammonium (Widdel, 1988).

SRB represent a number of genera and species with most of them affiliating with the *Deltaproteobacteria* and the *Firmicutes*. However, despite the large number of species for example in the genus *Desulfovibrio* only a very limited number of strains of most species is available in culture. This means that only very limited knowledge about phylogenetic diversity, variability of phenotype features and physiological capacities within a single species is available and it was criticized that a small number of strains used for species description may lead to inaccurate results (Rosselló-Mora & Amann, 2001).

In the present work we analyzed eight strains of *Desulfovibrio acrylicus*, including the type strain of the species, which were all isolated from intertidal sediments of the southern North Sea. The isolates were analyzed for phenotype variations and for their metabolic capacities including utilization of electron acceptors and donors. In addition, polar lipid fatty acid (PLFA) patterns and intact polar lipids were determined.

2.3 Materials and Methods

2.3.1 Source of organisms

Sediment was sampled from an intertidal mud flat off the German North Sea coast close to the village of Neuharlingersiel (53°42.5’ N, 7°42.05’ E, Rütters et al., 2002b). Sediment cores were taken in perspex cylinders and processed within two hours after sampling. The temperature at the sediment surface ranged from 3ºC (January 2000) to 20ºC (August 2000, Sass et al., 2003b). Isolates were obtained from the highest positive dilutions of MPN series. Origin depth, isolation substrate, temperature and the respective MPN counts of the isolates are given in Tab. 1. The type strain *Desulfovibrio acrylicus* W218^T (DSM 10141) was purchased from DSMZ (Braunschweig, Germany) and was isolated from intertidal sediments close to the town of Westernieland (The Netherlands, van der Maarel et al., 1996). Strain D1 was isolated from mudflat surface sediments taken close the village of Dangast on the German North Sea coast (53°27.9’ N, 8°7.9’ E) and was kindly provided by A. Sass.

2.3.2 Viable counts, cultivation, isolation, phenotype characterization

For cultivation bicarbonate-buffered artificial seawater medium was used which contained the following components (in $g \cdot l^{-1}$): NaCl (24.3), $MgCl_2 \cdot 6\,H_2O$ (10), $CaCl_2 \cdot 2\,H_2O$ (1.5), KCl (0.66), Na_2SO_4 (4.0), KBr (0.1), H_3BO_3 (0.025), $SrCl_2 \cdot 6\,H_2O$ (0.04), NH_4Cl (0.021), KH_2PO_4 (0.0054), NaF (0.003). The medium was supplemented with $1\,ml \cdot l^{-1}$ of nonchelated trace element solution, $0.2\,ml \cdot l^{-1}$ of a selenite and tungstate solution (Widdel & Bak, 1992) and $0.5\,mg \cdot l^{-1}$ of the redox indicator resazurin. After autoclaving, the medium was cooled under N_2/CO_2 (80/20, v/v). To the cold medium 10 ml of a solution of ten vitamins (Balch et al., 1979) and $30\,ml \cdot l^{-1}$ of a 1 M $NaHCO_3$ solution were added from sterile stocks. Media used for MPN series and isolation were reduced by adding sodium sulfide ($1\,mmol \cdot l^{-1}$), whereas media for pure culture experiments were reduced by the aseptic addition of a few crystals of sodium dithionite until the resazurin turned colorless.

Tab. 1: Isolation details, phenotypical and physiological properties of eight strains of *Desulfovibrio acrylicus*. Concentrations (in mM) in the culture medium are given in parentheses. +: growth, (+): poor growth or only in part of the experiments, -: no growth, n.a.: data not available. YE: yeast extract, AA: amino acids, SRBKS: substrate mixture.

	Strain							
	W218^T	**D1**	**NA81**	**NA202**	**NA302**	**NB21**	**NB62**	**NC301**
Isolation								
Date	n.a.	Feb 1998	May 2000	Jan 2000	Jan 2000	Sep 1999	Jan 2000	Sep 1999
Origin depth (cm)	n.a.	0.5	0.5	0.5	0.5	5	5	50
Isolation substrate	YE	Lactate	Serine	SRBKS	SRBKS	AA	Ethanol	SRBKS
Isolation temp (°C)	30	10	20	20	30	20	20	30
MPN counts (cm^{-3} sediment)	n.a.	n.a.	$6.7 \cdot 10^5$	$3.1 \cdot 10^6$	$2.4 \cdot 10^5$	$1.4 \cdot 10^5$	$4.5 \cdot 10^5$	650
Length (µm)	1.8-4.5	2.7-4.8	2.1-3.8	2.0-5.6	0.5-1	2.1-3.9	0.4-0.7	2.8-6.5
Width (µm)	0.4-1	0.7-1.2	0.4-0.8	0.5-0.9	2.9-5	0.4-0.8	2.2-4.1	0.5-1.1
Temp. range (°C)	7-35	7-32	7-32	7-35	4-35	7-32	4-35	4-40
Electron donors								
H_2 [a)]	+	+	+	-	+	+	+	+
Formate (10) [a)]	+	+	+	-	+	+	+	+
Succinate (10)	+	+	+	+	+	+	+	+
Fumarate (10)	+	+	+	+	+	+	+	+
Malate (10)	+	+	+	+	+	+	+	+
Pyruvate (10)	+	+	+	+	+	+	+	+
Lactate (10)	+	+	+	+	+	+	+	+
Ethanol (10)	+	+	+	+	+	+	+	-
Propanol (5)	+	+	+	+	+	-	+	-
Butanol (5)	-	-	+	-	-	-	-	-
1,2-Propanediol (5)	+	-	+	-	-	-	-	-
Glycerol (5)	+	+	+	+	+	+	+	+
Alanine (10)	+	-	+	-	-	+	+	+
Cysteine	+	-	-	-	-	-	-	-
Choline (10)	-	-	+	-	+	+	+	-

[a)] Acetate (2 mmol · l^{-1}) served as carbon source.
None of the strains disproportionated thiosulfate. The following compound were tested but utilized by none of the strains: Acetate, propionate, butyrate, valerate, caproate, caprylate, 2-methyl butyrate, 3-methyl butyrate, malonate, glycolate, citrate, methanol, pentanol, isopropanol, 1,2-butanediol, 2,3-butanediol, arginine, asparagine, glutamine, isoleucine, tyrosine, tryptophane, proline, benzoate, salicylate, nicotinate, acetone.

For the MPN series and the subsequent isolation four different substrate combinations were used: 1) Serine (5 mmol · l^{-1}), 2) ethanol (10 mmol · l^{-1}), 3) amino acids (alanine 2 mmol · l^{-1}, asparagine 1 mmol · l^{-1}, arginine 1 mmol · l^{-1}, and cysteine 1 mmol · l^{-1}) and 4) the substrate mixture SRBKS containing sodium lactate (5 mmol · l^{-1}) alanine (1.2 mmol · l^{-1}), asparagine, arginine, cysteine (0.6 mmol · l^{-1} each), sodium acetate (2.5 mmol · l^{-1}), sodium propionate (1 mmol · l^{-1}), sodium butyrate (1 mmol · l^{-1}), sodium isobutyrate (0.5 mmol · l^{-1}),

sodium benzoate and sodium salicylate (0.5 mmol · l^{-1} each). Pure cultures were obtained using deep-agar shake tubes.

2.3.3 Morphology and pigments

Cell dimensions were determined using a Leitz DMRB microscope (Wetzlar, Germany) equipped with a digital image analysis system (H & K, Berlin, Germany). Flagella and Gram-staining were done according to standard procedures (Gerhardt et al., 1994). Cytochromes were analyzed by recording the spectra (Lambda 2S spectrophotometer, Perkin Elmer, Überlingen, Germany) of cell-free extracts obtained by use of a French press and subsequent centrifugation (40,000 × g, 30 min).

2.3.4 Physiological tests

Substrates were tested in completely filled screw-capped tubes. Growth tests with hydrogen as electron donor were carried out in 100 ml serum bottles sealed with butyl rubber stoppers and filled with 20 ml medium and a gas phase consisting of H_2/CO_2 (80/20, v/v). Electron acceptors were tested with lactate (10 mmol · l^{-1}) as electron donor in the sulfate-free medium. Electron acceptor tests were analyzed as described by Köpke et al. (2005). Growth rates were determined in batch cultures by monitoring turbidity using a turbidimeter (Hach, Loveland, CO, USA). Sulfide was analyzed as described by Cline (1969). Thiosulfate, sulfate, sulfite, nitrate, acetate, formate, and lactate were analyzed on an ICS-2000 ion chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a self-regenerating suppressor unit (Dionex ASRS-Ultra II 4-mm) and a conductivity detector (Webster et al., 2009).

Growth yields were determined as total carbon content of freeze-dried samples determined by combustion using a CS 500 IR analyzer (Eltra, Neuss, Germany) as described previously (Sass et al., 2002). Cultures were grown with sodium lactate (10 mmol · l^{-1}) as electron donor. Growth was limited by the electron donor in all experiments. At the end of the exponential phase prior to harvest, samples were taken and cell densities were analyzed by fluorimetry after

addition of SybrGreenI (Martens-Habbena & Sass, 2006) and converted into dry mass.

2.3.5 Phylogenetic analysis and genomic fingerprinting using ERIC-PCR

The phylogenetic affiliation of the seven new strains (including strain D1) was determined by analysis of almost complete 16S rRNA genes. Nucleic acid extraction, sequence and phylogenetic analysis were done as described previously (Sass et al., 2002). The partial 16S rRNA gene sequences of the seven new strains are available at GenBank under the accession numbers AJ318380 (strain D1), AJ866930-AJ866932 (strains NA81, NB21, NB62), and AJ866943-AJ866945 (strains NA202, NA302, NC301).

To investigate intraspecies diversity of the *Desulfovibrio acrylicus* strains genomic fingerprints using the primers ERIC1R and ERIC2 (Versalovic et al., 1991) was performed as described by Sass et al. (1998b). Fragments were separated on 1 % (w/v) agarose gels (90 V for 3 h). Gels were stained for 20 min with ethidium bromide and documented by means of a digital imaging system and the respective software (BioDoc Analyze Biometra, Göttingen, Germany). The resulting band patterns were analyzed using the software package GelCompar II Version 2.5 (Applied Maths, St-Martens-Latem, Belgium). The densometric curves were compared using the Pearson coefficient. Dendograms were generated by the UPGMA method (unweighted-pair group method with arithmetic averages).

2.3.6 Column-chromatographic separation of lipid fractions

For lipid analysis cells were grown with lactate (20 mmol $\cdot$ l^{-1}) and sodium sulfate (10 mmol $\cdot$ l^{-1}). Cells were harvested at the end of their exponential growth phase. Cultures were sparged with CO_2 to remove hydrogen sulfide, centrifuged (20,000 × g, 15 min) and resuspended in PBS. After two washing steps cultures were freeze dried and stored at -20 °C. Lipids were extracted and separated into compound classes of different polarity by liquid chromatography on a silica gel

column (2 g silica 60, 63 - 200 µm, Merck, Darmstadt, Germany) as described by Rütters et al. (2002a).

2.3.7 Transesterification and gas chromatography-mass spectrometry (GC-MS) analysis

Aliquots of the phospholipid fractions were transesterified with trimethylsulfoniumhydroxide as described by Müller et al. (1993). The methyl esters obtained were quantified by GC (Hewlett Packard HP 5890 Series II gas chromatograph, Hewlett Packard, Waldbronn, Germany), equipped with a DB-5HT capillary column (30 m × 0.25 mm, 0.1 µm film thickness, J&W, Folsom, CA, USA), a flame ionization detector (FID), and a cold injection system (Gerstel KAS3, Gerstel, Mühlheim a. d. Ruhr, Germany). Fatty acid methyl esters were identified by GC-MS using a Finnigan MAT SSQ 710B mass spectrometer (Finnigan-Thermoquest, San Jose, CA, USA). The carrier gas was helium (constant pressure of 12 psi). The oven temperature was raised from 60 °C (isothermal for 2 min) to 360 °C at rate of 3 °C min^{-1} and held for 5 min. Mass spectra were collected in full scan mode (*m/z* 50 - 650, ionization energy 70 eV). Positions of double bonds were tentatively assigned by comparison with retention times of standards (Bacterial Acid Methyl Esters CP Mix; Supelco, Bellefonte, PA, USA).

2.3.8 HPLC-MS and –MS/MS

An aliquot of the most polar fraction was analyzed using an HPLC instrument (Waters 2695 Separations Module, Waters, Manchester, UK) coupled to a hybrid-quadrupole/time-of-flight mass spectrometer (Micromass Q-TOF micro, Waters, Manchester, UK) equipped with an electrospray source and coupled to an evaporative light scattering detector used for qualitative and quantitative analysis, respectively. HPLC separation was achieved on a diol phase (Lichrospher 100 Diol 5µ, Merck, Germany) using a 2 × 125 mm column and a flow rate of 0.40 ml min^{-1}. The following linear solvent gradient (modified after Rütters et al., 2002b) was used: 1 min 100 % A, increasing over 20 min to 35 % A and 65 % B, followed by 40 min reconditioning with Eluent A. Eluent A was a

78:20:1.2:0.04 (v/v) mixture of *n*-hexane/*i*-propanol/formic acid/ammonia (25 % solution in water), Eluent B was 88:10:1.2:0.04 (v/v) *i*-propanol/water/formic acid/ammonia (25 % solution in water). Quantification was done after external calibration of ELSD and MS signals with phospholipid standards representing major phospholipids types (purchased from Avanti Polar Lipids, Alabaster, USA and Sigma Aldrich, München, Germany). Due to the lack of commercially available standards for quantification of ornithine lipids the response factor of phosphatidylglycerol was used, because its response factor is an average of the other intact polar lipids used in the HPLC-ESI-MS application.

The mass spectrometer was operated in negative ion mode with the capillary voltage set to 2800 V and a sample cone voltage of 35 V. The source temperature was 110 °C with the desolvation temperature being 220 °C. The desolvation gas used was nitrogen. During full scan mode (*m/z* 500 - 2000) the voltage in the collision cell was set to 7 V. To identify structures of the intact polar lipids MS/MS experiments were carried out by running a profile in the collision cell from 30 - 40 V (in 5 V steps) with argon as collision gas.

All MS and MS/MS measurements were performed at high resolution by using the lock spray channel of the Q-TOF and a 10 mmol l^{-1} sodium formate solution (in acetonitrile) as calibration standard.

2.4 Results

2.4.1 Phenotype and physiological characteristics

All eight strains were Gram-negative vibrios, motile by one or two terminally inserted flagella. They were catalase and oxidase-negative, contained desulfoviridin and *c*-type cytochromes. All strains utilized a range of electron donors typically used by members of the genus *Desulfovibrio* (Tab. 1), including lactate, pyruvate, succinate, malate, fumarate, glycerol and ethanol. Other alcohols, alanine, choline, or formate were less efficiently utilized.

The strains grew well with sulfate and thiosulfate as electron acceptors (Tab. 2). Growth yields with thiosulfate were generally highest (1.9 to 2.7 times the yield obtained with sulfate). Although $Fe(OH)_3$ was reduced by all isolates it

allowed only little growth. Strains NA81, NA202, NA302, and NC301 grew well with sulfite, whereas the others achieved only little cell yields despite oxidizing between 7 and 12 mmol lactate per liter in these assays. Similarly, reduction of DMSO without significant growth was observed for three strains (*D. acrylicus*^{T}, NA81, NB62). Only strains NB21 and NB62 grew with elemental sulfur as electron acceptor. Among the eight strains some variation in their growth yields was found. For example growth yield with sulfate was 7.1 ± 0.7 g dry weight (mol lactate)$^{-1}$ in strain NB62, but only 4.1 ± 0.3 g dry weight (mol lactate)$^{-1}$ in strain NC301.

Tab. 2: Growth of *Desulfovibrio acrylicus* with lactate as electron donor and different electron acceptors. Concentrations of metabolites were determined at the beginning of the experiment and at the end of the exponential growth phase. Yields were corrected against growth in assays with lactate and without an electron acceptor and are given as g dry weight · (mol lactate)$^{-1}$. n.d.: not determined. 0: no sulfide formation or lactate oxidation.

	Strain							
e^--acceptor	**W218^T**	**D1**	**NA81**	**NA202**	**NA302**	**NB21**	**NB62**	**NC301**
Sulfate	5.9 ± 0.6	4.6 ± 0.2	5.4 ± 1.1	4.6 ± 0.7	4.5 ± 0.1	5.3 ± 0.5	7.1 ± 0.7	4.1 ± 0.3
Thiosulfate	12.1 ± 0.3	12.6 ± 3.9	11.3 ± 0.6	9.5 ± 3.6	11.8 ± 2.6	13.3 ± 0.4	13.2 ± 3.7	n.d.
Sulfite	0.4 ± 0.3	1.9 ± 0.1	8.7± 0.3	5.7 ± 0.6	9.6 ± 2.4	2.0 ± 0.8	1.9 ± 0.08	9.7 ± 0.2
Sulfur	0	0	0	0	0	4.5 ± 1.7	6.3 ± 2.0	0
$Fe(OH)_3$	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.3	0.7 ± 0.01	2.1 ± 1.0	0.6 ± 0.01	0.1 ± 0.04	1.8 ± 0.8

None of the strains reduced nitrate, nitrite or manganese oxides.

2.4.2 Molecular characterization

Phylogenetic analysis revealed a close relationship among the isolates. Their partial 16S rRNA genes showed more than 99 % similarity (Fig. 1).

To evaluate the relationship of the strains on the subspecies level they were analyzed by ERIC-PCR. Cluster analysis of banding patterns obtained in this way revealed three clusters (Fig. 2): 1) *Desulfovibrio acrylicus*^{T} and strain D1, 2) strains NA202, NB21, NA81 and NB62, and 3) strains NA302 and NC301. This clustering reflected the isolation temperature as all cluster 2 strains were isolated at 20 °C and all cluster 3 strains at 30 °C. Cluster one comprised *D. acrylicus*^{T} and strain D1 that differed with respect to their isolation temperatures but were the only two isolates that were not from site Neuharlingersiel.

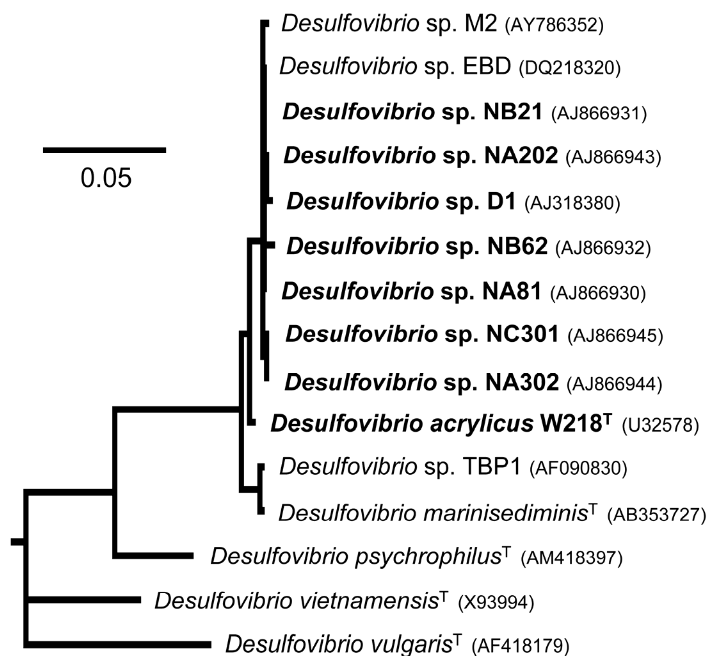

Fig. 1: Maximum-likelihood tree based on 16S rRNA sequences showing the phylogenetic position of the strains investigated in this study within the genus *Desulfovibrio*. GenBank accession numbers are given in parentheses. The bar represents 0.05 substitutions per nucleotide position.

2.4.3 Fatty acid patterns

In general, patterns of the analyzed polar lipid ester-linked fatty acids (PLFAs) of the different *Desulfovibrio acrylicus* strains were similar (Tab. 3). They contained a large number (12 to 26) of different fatty acids. The isolates possessed saturated (4 - 28 %) and monounsaturated (15 - 60 %), *iso*- and *anteiso*-branched (36 - 56 %) fatty acids. Eight fatty acids were generally dominant (more than 5 %): *i*-15:0 (< 1 - 21 %), *i*-16:0 (2 - 9 %), *n*-16:0 (2 - 19 %), *n*-16:1ω7 (7 - 21 %), *i*-17:1ω7 (6 - 24 %), *ai*-17:1ω7 (3 - 8 %), *n*-18:1ω7 (4 - 39 %), *n*-18:0 (5 - 10 %). All others were present in lower abundances (up to 5 %).

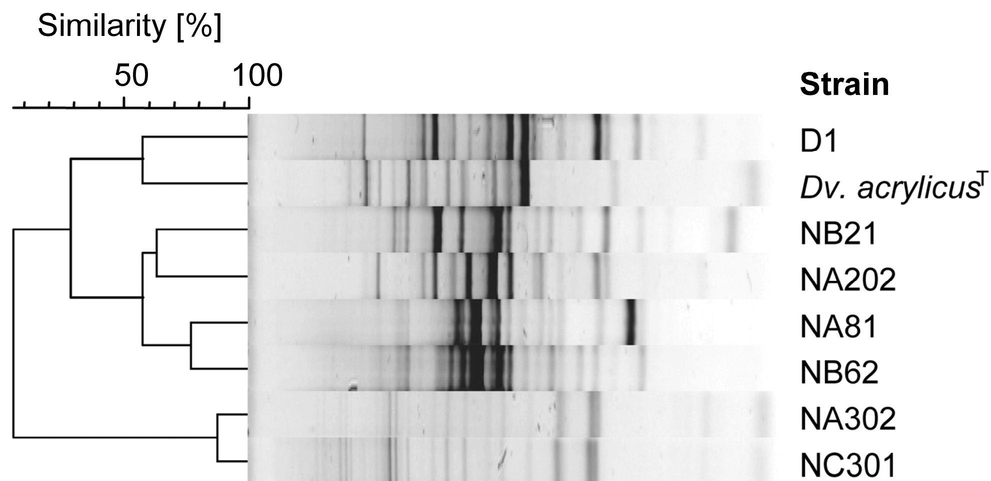

Fig. 2: Cluster analysis of ERIC-PCR fingerprinting band patterns of the *Desulfovibrio acrylicus* strains. The dendrogram was calculated using Pearson correlation and UPGMA and is based on computer-generated densitometric curves obtained by analysis of negative images of ethidium bromide stained agarose gels.

2.4.4 Polar lipids in *Desulfovibrio acrylicus*

The eight *D. acrylicus* strains exhibited a phospholipid profile dominated by phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), and with lesser amounts of diphosphatidylglycerol (DPG). In addition to these phospholipids, a class of phosphorus-free ornithine lipids (OL) was representing a major portion of the polar lipids (12 – 60 %). Phosphatidylserine (PS) and phosphatidic acid (PA) were less abundant and were found only in four and seven strains, respectively. One unidentified polar lipid (U1) was present in six strains and accounted for up to 11 % of the total polar lipid pool in strain NA302, whereas traces of a second unidentified polar lipid (U2) were found only in strain D1 (Tab. 4).

Tab. 3: Polar lipid fatty acids of the eight *Desulfovibrio acrylicus* strains grown at 20 °C.

	Strain							
Fatty acid	**W218^T**	**D1**	**NA81**	**NA202[a)]**	**NA302**	**NB21**	**NB62**	**NC301**
***i*-14:0**	0.2	-	0.4	0.2	0.2	0.1	0.1	0.5
***n*-14:1ω5**	0.5	-	0.5	0.6	0.7	0.9	1.3	0.7
***n*-14:0**	0.5	0.3	1.4	0.6	1.1	0.4	0.5	1.9
***i*-15:1ω7**	2.0	0.2	0.2	3.5	1.7	1.6	2.6	2.7
***ai*-15:1ω7**	0.6	0.1	-	0.4	0.7	0.3	0.5	-
***i*-15:0**	4.2	3.2	13.7	6.5	1.7	1.8	2.4	15.3
***ai*-15:0**	2.1	1.3	4.2	3.8	1.5	1.2	1.4	2.4
***n*-15:1ω8**	-	0.3	-	-	0.4	-	0.1	1.4
***n*-15:0**	0.2	0.1	0.5	0.8	0.6	0.1	0.1	1.8
***i*-16:1ω6**	6.5	6.5	4.0	4.8	8.4	6.2	6.3	1.9
***i*-16:0**	3.8	3.1	8.7	4.5	0.7	2.2	2.4	3.0
***n*-16:1ω9**	0.4	0.2	0.3	-	2.3	0.3	0.3	1.5
***n*-16:1ω7**	14.3	12.2	9.4	11.2	17.6	18.4	19.2	6.1
***n*-16:1ω5**	0.7	0.4	0.4	-	0.8	0.9	0.7	0.4
***n*-16:0**	16.8	3.5	18.8	13.1	5.0	11.2	14.2	18.7
***i*-17:1ω7**	13.2	16.1	7.1	15.4	15.6	10.7	12.0	6.5
***ai*-17:1ω7**	5.2	3.9	3.2	6.5	7.2	4.5	4.9	3.3
***i*-17:0**	2.1	2.1	2.0	3.6	0.5	1.1	1.2	2.3
***ai*-17:0**	2.9	3.5	0.2	4.5	2.4	2.5	2.8	3.3
***n*-17:1**	0.4	0.5	5.4	0.6	2.1	0.3	0.4	0.6
***n*-17:0**	1.3	0.5	1.2	3.5	0.5	0.9	1.0	3.3
***i*-18:1ω6**	2.1	1.4	1.9	1.7	3.4	3.6	3.0	0.5
***i*-18:0**	0.2	1.1	0.3	-	0.7	0.4	0.2	1.0
***n*-18:1ω9**	0.4	1.5	0.5	0.3	2.8	0.5	0.3	1.4
***n*-18:1ω7**	12.2	20.4	9.1	9.2	15.3	19.9	16.1	5.1
***n*-18:1ω5**	0.7	0.7	0.4	0.3	0.6	1.0	0.6	0.4
***n*-18:0**	6.2	9.7	6.3	3.8	3.0	7.8	4.6	12.7
∑*n*-19:0-*n*-20:0	0.3	2.9	-	0.8	1.0	0.9	0.7	0.6

[a)] Whole cell fatty acid patterns

Tab. 4: Major polar lipid types in *Desulfovibrio acrylicus* isolates grown at 20 °C (in % of total polar lipids). PA: phosphatidic acid, OL: ornithine lipid, PG: phosphatidylglycerol, DPG: diphosphatidylglycerol, PE: phosphatidylethanolamine, PS: phosphatidylserine (all phospholipids were detected as diacylglycerols). The unknown lipids U1 and U2 are characterized by the following most intense signals in their averaged mass spectra: U1: *m/z* 730, 742, 756; U2: *m/z* 840, 854, 868. tr = traces.

	Strain							
Lipid	**W218^T**	**D1**	**NA81**	**NA202**	**NA302**	**NB21**	**NB62**	**NC301**
PA	1	tr	1	-	7	tr	tr	5
U1	-	tr	2	-	11	tr	tr	7
OL	20	30	60	26	17	14	12	43
PG	38	30	13	20	16	28	17	26
DPG	8	12	5	7	6	4	2	5
PE	33	27	18	47	42	54	68	14
PS	1	tr	-	-	-	tr	tr	-
U2	-	tr	-	-	-	-	-	-

The most abundant phospholipid species in PE and PG were substituted with 16:0, 16:1, 17:0, 17:1, 18:0 and 18:1 fatty acids (Tab. 6 and Tab. 7). Although most of the fatty acid combinations were present in PE and PG, there were some minor differences in their substitution patterns. For example, the fatty acid combinations 17:1/16:0 and 17:0/17:1 were found in PE but not in PG. The 15:0 fatty acids were rare in PE and PG, but were highly abundant in OL. In most OL species the amide-bound 3-hydroxy (3-OH) fatty acids were two to three carbon atoms longer than the hydroxy-bound acyl side chain (Tab. 8).

2.4.5 The structure of ornithine lipids in *Desulfovibrio acrylicus*

Ornithine lipids were identified by comparison with published MS/MS spectra (Geiger et al., 1999; Aygun-Sunar et al., 2006; Zhang et al., 2009). To elucidate the molecular structure of OL species, MS/MS experiments were performed using high resolution tandem mass spectrometry which allowed the determination of the elemtental composition of the individual fragments (Tab. 5).

Tab. 5: Mass spectral fragments of two isobaric ornithine lipid species of *Desulfovibrio acrylicus* W218^T with quasi-molecular ions $[M-H]^-$ at *m/z* 651 (side chain combinations 3-OH-18:0/16:0 and 3-OH-17:0/17:0; see Fig. 3 for structures). Accurate masses of fragments were determined using high resolution mass spectrometry in negative-ion mode. Molecular formulas of ions were calculated using the Mass-Lynx V4.1 software (Waters, Manchester, UK). Peak labels refer to ESI-MS/MS spectrum in Fig. 3.

Peak label	Measured mass (Da)	Calculated mass (Da)	Difference (mDa)	Formula
$[M-H]^-$	651.5630	651.5676	-4.6	$C_{39}H_{75}N_2O_5$
'ornithine'	131.0821	131.0821	0	$C_5H_{11}N_2O_2$
3-OH-18:0/16:0				
I	395.3273	395.3274	-0.1	$C_{23}H_{43}N_2O_3$
II	351.3359	351.3375	-1.6	$C_{22}H_{43}N_2O$
III	255.2299	255.2324	-2.5	$C_{16}H_{31}O_2$
3-OH-17:0/17:0				
A	381.3202	381.3243	-4.1	$C_{22}H_{41}N_2O_3$
B	337.3164	337.3219	-5.5	$C_{21}H_{41}N_2O$
C	269.2481	269.2483	-0.2	$C_{17}H_{33}O_2$

In MS/MS experiments in the negative-ion mode, OL species were readily identified by their characteristic head group fragments ('ornithine' in Fig. 3). The most prominent fragments were lyso-ornithine lipids resulting from the elimination of the fatty acid bound to the 3-hydroxy (3-OH) group of the amide-linked fatty acid (fragments 'A' and 'I' in Fig. 3). Lyso-fragments with a neutral loss of the carboxyl group of the ornithine amino acid were also detected (fragments 'B' and 'II' in Fig. 3). Fatty acids bound to the 3-hydroxy group of the amide-linked fatty acid were identified by their carboxylate anions (fragments 'C' and 'III' in Fig. 3). Some of the investigated ornithine lipid species contained also

a second isobaric isomer with a different side chain combination but similar quasi-molecular ion, *i.e.* 3-OH-18:0/16:0 and 3-OH-17:0/17:0 in Tab. 5 and Fig. 3. However, in most cases one isomer dominated the MS/MS spectrum (isomer 3-OH-18:0/16:0 in Fig. 3). Therefore, only the dominating isomers of each OL species are shown in Tab. 8.

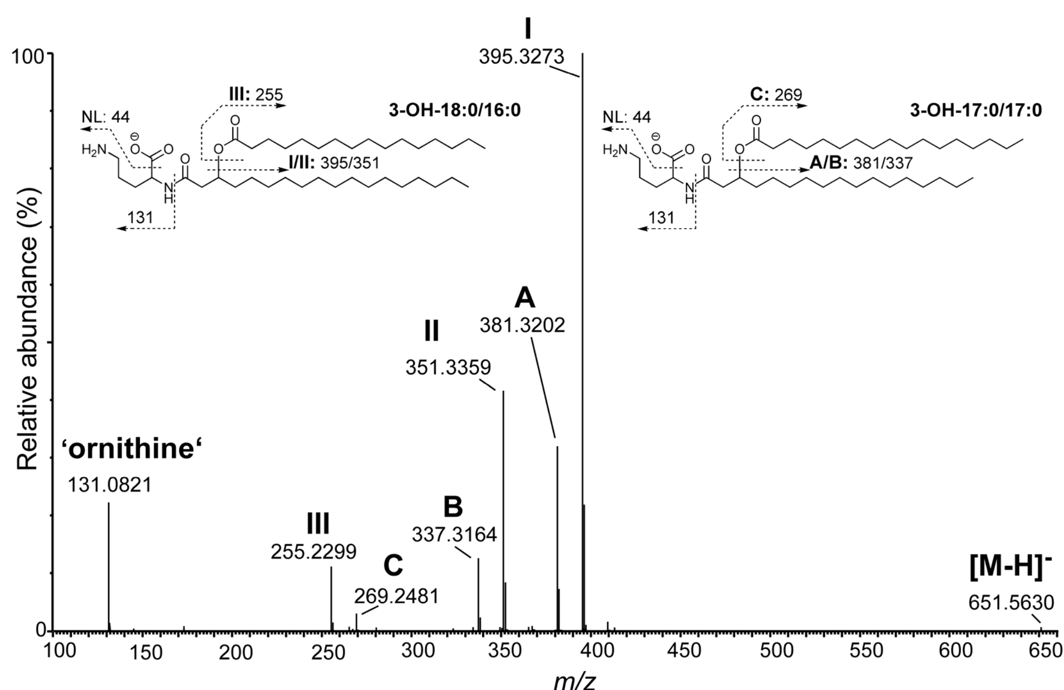

Fig. 3: Structures and ESI-MS/MS spectrum of two isobaric ornithine lipids (OL) of *Desulfovibrio acrylicus* W218^{T} with quasi-molecular ions $[M-H]^-$ at *m/z* 651 (negative-ion mode). Peaks are labeled according to the fragments of the respective OL structures (cf. Tab. 5). The peak labeled 'ornithine' refers to the characteristic ornithine amino acid head group fragment of all OL species identified in MS/MS experiments of this study. NL: neutral loss

Tab. 6: Diacyl phosphatidylethanolamine (PE) molecular species of *Desulfovibrio acrylicus* strains grown at 20 °C (abundances (%) are given relative to total ion intensities in the averaged base peak). $[M-H]^-$: quasi-molecular ion.

		Strain							
[M-H]⁻	**PE species**	**W218ᵀ**	**D1**	**NA81**	**NA202**	**NA302**	**NB21**	**NB62**	**NC301**
672.3	**16:1/15:1**	3.1	0.3	0.8	2.9	2.2	2.2	3.8	4.2
674.5	**16:0/15:1**	2.7	0.2	1.2	1.7	0.4	1.2	2.3	3.8
676.5	**16:0/15:0**	1.6	0.1	1.1	0.7	0.1	0.5	0.8	1.0
686.5	**16:1/16:1**	7.2	2.2	3.3	11.9	9.1	6.7	8.6	9.0
688.5	**16:0/16:1**	13.9	2.0	13.3	10.4	2.1	10.2	13.5	12.9
690.5	**16:0/16:0**	3.5	0.5	3.9	1.5	0.3	1.9	2.6	3.8
700.5	**16:1/17:1**	8.7	5.7	5.5	10.6	17.2	8.4	9.0	10.4
702.5	**17:1/16:0**	12.2	4.2	12.7	12.4	3.7	8.8	10.4	13.1
704.5	**17:0/16:0**	3.0	1.2	3.2	1.6	0.6	1.8	2.1	3.2
714.5	**16:1/18:1**	12.1	18.5	12.8	14.4	30.6	18.6	14.1	11.9
716.5	**17:0/17:1**	11.0	9.5	18.7	12.0	5.2	14.6	12.1	10.3
718.5	**18:0/16:0**	2.9	1.8	3.9	1.3	0.8	2.6	2.3	2.3
728.5	**18:1/17:1**	6.5	18.8	6.5	6.6	15.6	8.5	6.2	5.0
730.5	**18:0/17:1**	4.8	8.6	6.2	3.7	3.5	5.6	4.3	2.9
732.5	**18:0/17:0**	1.2	1.6	1.0	0.7	0.8	0.9	1.0	0.0
742.5	**18:1/18:1**	1.1	11.5	1.9	1.3	2.6	2.8	1.6	0.8
744.5	**18:0/18:1**	0.8	4.9	1.6	0.8	1.3	1.5	0.9	0.4
746.5	**18:0/18:0**	0.5	1.3	0.4	0.3	0.6	0.4	0.4	0.2

2.5 Discussion

2.5.1 Abundance of *Desulfovibrio acrylicus* in coastal sediments

Desulfovibrio acrylicus appears to be a common sulfate-reducing bacterium of temperate coastal sediments. The strains analyzed in this study originated from three different sites along the southern North Sea coast (van der Maarel et al., 1996, this study), and *D. acrylicus*-related strains or close relatives were repeatedly obtained from other temperate coastal sediments (Boyle et al., 1999; Köpke et al., 2005; Suzuki et al., 2007b; Villanueva et al., 2007; Takii et al., 2008). The

strains used in this study were obtained from the highest positive dilutions of MPN series indicating that they were among the most abundant cultureable organisms. Inferring *in situ* numbers from the respective MPN counts indicates abundances of up to 10^5 to 10^6 cells per cm^3 sediment (Tab. 1), this is in the range of 0.1 to 1 % of the total cell counts (Sass et al., 2003b). These numbers agree well with CARD-FISH data by Gittel et al. (2008) who found *Desulfovibrio* spp. only in the surface layers (1.1 % of the DAPI count) of a tidal sand flat close to the site of origin of strains NA81 to NC301.

Tab. 7: Diacyl phosphatidylglycerol (PG) molecular species of *Desulfovibrio acrylicus* strains grown at 20 °C (abundances (%) are given relative to total ion intensities in the averaged base peak). $[M-H]^-$: quasi-molecular ion.

		Strain							
[M-H]$^-$	**PG species**	**W218^T**	**D1**	**NA81**	**NA202**	**NA302**	**NB21**	**NB62**	**NC301**
703.3	**16:1/15:1**	1.0	0.1	0.4	2.2	1.0	1.2	3.0	1.8
705.4	**16:0/15:1**	2.1	0.1	1.0	2.2	0.3	0.9	1.9	4.3
707.5	**16:0/15:0**	2.2	0.4	1.1	0.9	0.1	0.5	0.7	1.0
717.5	**16:1/16:1**	4.8	1.3	2.6	12.0	6.7	5.2	8.8	7.3
719.5	**16:0/16:1**	15.4	2.8	13.9	13.1	2.1	10.4	14.1	13.5
721.5	**16:0/16:0**	5.4	1.6	4.0	2.4	0.4	2.3	2.8	2.6
731.5	**16:1/17:1**	8.0	5.2	6.1	13.1	16.3	8.0	10.2	11.0
733.5	**17:0/16:1**	14.5	6.4	14.5	13.7	3.8	9.6	11.1	15.0
735.5	**17:0/16:0**	4.4	3.5	3.2	2.0	0.7	2.1	2.0	2.4
745.5	**16:1/18:1**	13.0	23.4	16.7	18.3	33.6	20.3	16.8	15.7
747.5	**16:0/18:1**	12.5	15.0	17.9	7.7	5.7	16.2	12.5	11.7
749.5	**18:0/16:0**	3.1	3.3	2.9	0.6	1.0	2.9	2.0	1.5
759.5	**18:1/17:1**	6.4	17.8	7.5	5.6	16.3	8.7	6.7	5.8
761.5	**18:0/17:1**	4.1	7.8	3.8	1.3	2.7	5.1	3.1	2.8
763.5	**18:0/17:0**	0.7	1.3	0.5	0.2	0.3	0.7	0.4	0.4
773.5	**18:1/18:1**	0.8	6.0	1.6	1.0	2.9	2.6	1.3	1.2
775.5	**18:0/18:1**	0.4	2.4	0.5	0.3	0.5	1.1	0.4	0.4

Tab. 8: Ornithine lipid (OL) molecular species in *Desulfovibrio acrylicus* strains grown at 20 °C (abundances (%) are given relative to total ion intensities in the averaged base peak). $[M-H]^-$: quasi-molecular ion.

		Strain							
[M-H]$^-$	**OL species**	**W218^T**	**D1**	**NA81**	**NA202**	**NA302**	**NB21**	**NB62**	**NC301**
595.5	**3-OH-15:0/15:0**	0.3	-	0.9	0.6	-	0.3	0.7	1.0
607.5	**3-OH-16:0/15:1**	0.1	-	0.3	0.4	0.6	-	0.2	0.2
609.5	**3-OH-16:0/15:0**	7.1	1.9	8.9	6.0	1.2	5.9	9.6	7.3
619.5	**3-OH-17:1/15:1**	-	-	-	0.1	0.9	-	-	0.0
621.5	**3-OH-17:1/15:0**	1.8	0.4	2.5	2.6	4.2	1.6	2.4	3.6
623.5	**3-OH-17:0/15:0**	20.7	8.5	17.9	18.8	3.7	17.0	21.4	27.3
633.5	**3-OH-18:1/15:1**	0.2	0.1	0.2	0.3	5.0	0.1	0.1	0.1
635.5	**3-OH-18:1/15:0**	5.5	2.6	6.8	6.2	12.2	6.8	7.6	5.1
637.5	**3-OH-17:0/16:0**	26.4	16.2	22.8	24.0	4.2	23.1	24.1	21.0
647.5	**3-OH-18:1/16:1**	0.5	0.6	0.7	0.6	15.7	0.7	0.6	0.5
649.5	**3-OH-18:1/16:0**	5.3	5.9	6.9	5.8	9.3	8.2	7.1	6.3
651.5	**3-OH-18:0/16:0**	20.7	22.6	18.5	14.0	3.4	18.0	16.2	14.0
661.5	**3-OH-18:1/17:1**	0.4	1.6	0.7	0.7	17.3	0.8	0.5	0.6
663.5	**3-OH-18:1/17:0**	2.3	6.0	3.7	3.9	6.0	3.9	3.1	3.4
665.5	**3-OH-19:0/16:0**	6.5	13.9	6.0	7.1	1.7	5.0	3.9	5.4
675.5	**3-OH-18:1/18:1**	0.2	2.5	0.3	0.4	8.4	0.4	0.2	0.4
677.5	**3-OH-18:1/18:0**	0.6	4.3	1.3	1.5	1.5	1.7	0.7	1.3
679.5	**3-OH-20:0/16:0**	0.9	9.0	1.0	1.5	0.4	0.7	0.3	1.1

2.5.2 Physiology of *Desulfovibrio acrylicus*

With respect to its substrates used the type strain *Desulfovibrio acylicus* does not differ significantly from other representatives of its genus (Widdel & Bak, 1992). Its growth yields (Y) were in a range also observed for other *Desulfovibrio* spp. (Traore et al., 1982; Sass et al., 2003a). A higher growth yield with thiosulfate than with sulfate as electron acceptor was reported for other sulfate reducers as well: *Desulfovibrio vulgaris* (Badziong & Thauer, 1978) and *Desulfobacter vibrioforme* (Lien & Beeder, 1997). In both species the yield obtained with thiosulfate ($Y_{thiosulfate}$) was twice as high as with sulfate ($Y_{sulfate}$) as electron acceptor. However, in *D. vibrioforme* the highest growth yield was obtained with

sulfite (2.8 × $Y_{sulfate}$). Similarly high yields with sulfite as electron acceptor were obtained only with strains NC301 (2.4 × $Y_{sulfate}$) and NA302 (2.1 × $Y_{sulfate}$), but not with the other strains. Hence, our results support the original description of *D. acrylicus* W218^T by van der Maarel et al. (1996) who also found no growth in assays with sulfite as electron donor. We can only speculate about the reason why some strains apparently reduce sulfite but achieve only very low growth yields. But it appears likely that high concentrations of sulfite as they were present in these experiments have an inhibitory effect on growth.

2.5.3 Fatty acid and phospholipid patterns of *Desulfovibrio acrylicus*

The eight strains of *D. acrylicus* exhibited a phospholipid profile dominated by phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and diphosphatidyl-glycerol (DPG). These are typical phospholipids of Gram-negative bacteria and have been found in other *Desulfovibrio* spp. before (Makula & Finnerty, 1974). Regarding their PLFAs, all strains were characterized by a very high level of branched-chain fatty acids (BCFAs). This is a typical feature of the genus *Desulfovibrio* (Boon et al., 1977; Ueki & Suto, 1979; Vainshtein et al., 1992) that differ from other BCFA-rich bacteria (*e.g. Bacillus* spp.) by the predominance of monounsaturated BCFAs (*e.g.* *ai*-15:1ω7, *i*-15:1ω7, *i*-16:1ω6, *i*-17:1ω7, *ai*-17:1ω7). The *i*-17:1ω7 fatty acid (between 6 % and 24 % in our strains) was considered characteristic for *Desulfovibrio* spp. (Boon et al., 1977; Edlund et al., 1985).

Generally, our PLFA results agree very well with the results obtained after whole-cell hydrolysis for the same strains by Freese et al. (2008b). However, a few differences became obvious for strains NA302, NA81 and NC301. For example, in these strains *n*-16:1ω7 contents were lower in the PLFA fraction analyzed in this study (between 13 and 65 %) whereas the *i*-15:0 fatty acid was elevated by a factor of six to seven in strains NA81 and NC301. We assume that these changes are due to non-polar lipid sources in the whole cell fatty acid patterns.

2.5.4 Presence of ornithine lipids in *Desulfovibrio acrylicus*

Ornithine lipids have so far only been detected in a small number of bacteria from the *Proteobacteria*, *Actinobacteria, Firmicutes* and *Bacteroidetes*. Many of these were purple non-sulfur bacteria (*e.g. Rhodomicrobium* spp., Park & Berger, 1967), but OL was also found in chemotrophic bacteria like *Bacillus*, *Pseudomonas, Thiobacillus*, *Flavobacterium* and some rhizobia (Knoche & Shively, 1972; Minnikin & Abdolrahimzadeh, 1974; Asselineau et al., 1988; Galbraith et al., 1999; Choma & Komaniecka, 2002; Weissenmayer et al., 2002; Haque & Russell, 2004). The role of OL in these organisms is only poorly understood. Although it was shown that OL increases at the expense of PE under phosphate limitation (Minnikin & Abdolrahimzadeh, 1974; Weissenmayer et al., 2002), only subtle physiological and phenotype changes were found in OL-deficient mutants of *Sinorhizobium meliloti* (Weissenmayer et al., 2002). The fact that our artificial seawater medium is particularly poor in phosphorous may explain the high OL levels in our strains although they grew well and did not show any abnormities under the microscope.

At present we can only speculate about the distribution of ornithine lipids in the genus *Desulfovibrio* as only very few studies on the phospholipid composition are available. OL species were found in *D. gigas*, but not in *D. vulgaris* (Makula & Finnerty, 1974, 1975; Rütters, 2001). However, a reason for the few reports of ornithine lipids may be related to the analytical procedures commonly used. For the investigation of PLFA patterns polar lipid fatty acids are usually transesterified under mild alkaline conditions as described by White et al. (1979). This method does not cleave the amide bond of the 3-OH-fatty acid. The use of differential hydrolysis protocols for ester and (ester plus amide)-bound fatty acids may yield different PLFA patterns. For example in *Anaerosinus glycerini* (Strömpl et al., 1999) the amount of hydroxy fatty acids was much higher after the ester plus amide hydrolysis, although after alkaline transesterification hydroxy fatty acids were already detected in small concentration in contrast to our study. However, an indication for a wider distribution of ornithine (or other amino acid-containing) lipids among sulfate-reducing bacteria may be the detection of hydroxy fatty acids in several other *Desulfovibrio* spp. (Boon et al., 1977; Edlund

et al., 1985). Whether these are indicative of the presence of ornithine lipids can only be revealed by future research.

2.5.5 Emended description of *Desulfovibrio acrylicus* van der Maarel et al. 1996

The species description of *Desulfovibrio acrylicus* is as given by van der Maarel et al. (1996) with the following additions and changes. Major membrane lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol (cardiolipin) and ornithine lipids. Some strains contain minor amounts of phosphatidic acid, phosphatidylserine and an unidentified lipid. The dominant polar lipid fatty acids (> 5 %) are *i*-15:0, *ai*-15:0, *i*-16:1ω6, *i*-16:0, *n*-16:1ω7, *n*-16:0, *i*-17:1ω7, *ai*-17:1ω7, *n*-18:1ω7, and *n*-18:0. Some strains grow with choline, *n*-butanol, or 1,2-propanediol. Sulfite and elemental sulfur are used as an electron acceptor for growth by some strains. $Fe(OH)_3$ is reduced but allows only little growth. DMSO is reduced by some strains but does not support growth. Nitrate, nitrite and MnO_2 are not used as electron acceptors.

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3 Temperature-related changes of phenotype, physiology, phospholipids and ornithine lipids in strains of *Desulfovibrio acrylicus*

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3.1 Abstract

Eight strains of *Desulfovibrio acrylicus*, including the type strain, were analyzed for temperature-related changes in phenotype, growth, activity and intact polar lipids. The strains grew from 4 or 7 °C to 32 or 37 °C. One isolate grew at 40 °C. Maximum growth rates ranged from 1.75 to 3.44 d^{-1}, maximum growth yields from 4.4 to 5.7 g dw $(mol\ lactate)^{-1}$. Five isolates showed a strong decrease in growth yield close to the minimum and maximum temperatures, as is typical for mesophiles, whereas three strains showed constant yields from the minimum to the optimum temperature, as is typical for psychrotrophs. Sulfide production was found at temperatures above the temperature range for growth. Changes in polar lipid fatty acid (PLFA) patterns were similar in all strains: branched fatty acids and the *anteiso/iso* ratio increased in abundance with temperature and unsaturated PLFAs decreased. Analysis of polar lipid molecular species indicated that between 10 and 20 °C the main temperature adaptation is an increase in branched side chains. Between 20 and 30 °C a strong decrease of unsaturation (including branched unsaturated fatty acids) was found in the mesophilic strains, whereas in the psychrotrophs no net decrease of unsaturation was found. Ornithine lipids (OLs) replaced phosphatidylethanolamines (PEs) at higher growth temperatures. Phosphatidylglycerol (PG) showed inconsistent results. As the temperature-related side chain variations were similar in all polar lipid types, the observed changes in lipid headgroups may not be involved in the maintenance of membrane fluidity. An alternative role in the regulation of membrane-bound proteins will be discussed.

3.2 Introduction

Microbial communities are influenced by a multitude of physical factors with temperature being one of the most prominent ones. Whereas large parts of the ocean floor are characterized by constantly low temperatures, coastal sediments can experience significant temperature fluctuations, particularly in the temperate and polar regions (Austin, 1988). Intertidal sediments are characterized not only by annual but also by diurnal and tidal temperature cycles (Harrison & Phizacklea, 1987). Sediment temperature gradients can change quickly during a tidal cycle, in North Sea sediments by as much as 15 °C (Harrison & Phizacklea, 1987).

Microorganisms usually grow over a specific temperature range and with respect to their optimal growth temperature can be subdivided into different categories (Morita, 1975; Isaksen & Jørgensen, 1996): Psychrophiles and psychrotrophs grow at temperatures below or around 0 °C and show an upper temperature limit for growth below 20 °C or of up to 35 °C, respectively. Mesophiles, in turn, rarely grow below 10 °C but still grow at temperatures between 35 and 45 °C. Although Sieburth (1967) found temperature-related seasonal fluctuations of psychrophilic and mesophilic bacteria in temperate coastal bacterioplankton, psychrophilic bacteria appear to be mostly present in permanently cold environments like the Arctic Ocean or in the deep sea (Morita, 1975). At a few centigrades above 0 °C or if relatively fast temperature fluctuations occur, psychrotrophs generally outcompete psychrophiles (Harder & Veldkamp, 1971; Nedwell, 1999), whereas at higher temperatures (> 10 – 15 °C) psychrotrophs are, in turn, outcompeted by mesophiles. It appears that the competitive advantage of a particular group is not only related to its growth rate but rather to its energetic efficiency, *i.e.* its growth yield (Harder & Veldkamp, 1971; Isaksen & Jørgensen, 1996; Nedwell, 1999).

Furthermore, maintaining appropriate membrane fluidity with changing growth temperature is of particular importance, as membrane proteins are involved in energy conservation, chemotaxis and the uptake of nutrients (Kell, 1984). Free diffusion of membrane proteins may be strongly restricted at low temperatures if the membrane changes from a ‘liquid-crystalline’ to a gel-like

state or even to a crystalline array of fatty acids (Quinn, 1981; de Mendoza & Cronan, 1983). In contrast, at elevated temperatures fatty acid side chains become more disordered and the membrane may change into a non-lamellar state or even melt (Hazel & Williams, 1990). Most studies on temperature adaptation in bacterial membranes have focused on modification in fatty acid side chains. Most common are changes in the average chain length, the degree of unsaturation, the relative contribution of branched chain fatty acids or the ratio of *anteiso* to *iso* branched fatty acids (Donato et al., 2000; Männistö & Puhakka, 2001; Haque & Russell, 2004; Unell et al., 2007; Freese et al., 2008b). Temperature-related changes in polar lipid headgroups have been less frequently investigated (Bhakoo & Herbert, 1980; Donato et al., 2000).

In the present work eight strains of the sulfate-reducing bacterium *Desulfovibrio acrylicus* were investigated for temperature-related changes in growth rate and yield, metabolic activity and of fatty acid and polar lipid composition. All eight strains were isolated from tidal flat sediment of the southern North Sea coast, a temperate environment with annual temperatures ranging from below the freezing point to up to 25 °C (Sass et al., 2003b; Beck et al., 2008b). The isolation temperatures of the eight isolates strains ranged from 10 to 30 °C (van der Maarel et al., 1996; Sass et al., 2003b) and it was analyzed whether different isolation temperatures yielded organisms with different temperature adaptations, *i.e.* psychrotrophs and mesophiles, and how these respond to different growth temperatures.

3.3 Materials and Methods

3.3.1 Source and Cultivation of Organisms

Eight strains of *Desulfovibrio acrylicus* were isolated from intertidal sediments of the southern North Sea coast. The type strain *Desulfovibrio acrylicus* DSM 10141^T (van der Maarel et al., 1996) was purchased from DSMZ (Braunschweig, Germany), strain D1 was kindly provided by A. Sass and the other six strains (NA81, NA202, NA302, NB21, NB62, and NC301) were isolated from sediment taken near the village of Neuharlingersiel (Sass et al., 2003b). *Desulfovibrio acrylicus* DSM 10141^T and strains NA302 and NC301 were isolated at 30 °C, whereas strains NA202, NA81, NB21, NB62 were isolated at 20 °C and strain D1 at 10 °C, respectively (van der Maarel et al., 1996; Sass et al., 2003b; Freese et al., 2008b). The different isolates shared at least 99 % similarity in their 16S rRNA genes.

A bicarbonate-buffered artificial seawater medium was used for cultivation (Süß et al., 2004) containing (in g · l^{-1}): NaCl (24.3), $MgCl_2 \cdot 6\,H_2O$ (10), $CaCl_2 \cdot H_2O$ (1.5), KCl (0.66), Na_2SO_4 (4), KBr (0.1), H_3BO_3 (0.025), $SrCl_2 \cdot 6\,H_2O$ (0.04), NH_4Cl (0.021), KH_2PO_4 (0.0054), NaF (0.003), resazurin (0.25 mg · l^{-1}), trace element solution SL10 (1 ml · l^{-1}), selenite and tungstate solution (0.2 ml · l^{-1}, Widdel & Bak 1992). After autoclaving, the medium was cooled under a N_2/CO_2 (80/20, v/v) atmosphere and supplemented with 10 ml of a vitamin solution (Balch et al., 1979) and 30 ml · l^{-1} of a 1 M $NaHCO_3$ solution. The medium was reduced by adding sterile sodium dithionite crystals until the resazurin turned colorless. The pH of reduced medium was adjusted to 7.2 - 7.4 with sterile HCl or Na_2CO_3 if necessary.

Cells for lipid analysis were grown with sodium lactate (20 mmol · l^{-1}) and sodium sulfate (10 mmol · l^{-1}) as substrates, harvested at the end of their exponential growth phase by centrifugation, washed with saline phosphate buffer (9 g · l^{-1} NaCl, 7.5 mmol · l^{-1} NaH_2PO_4, 7.5 mmol · l^{-1} Na_2HPO_4, pH 7.2), freeze dried and stored at -20 °C.

3.3.2 Growth rate and yield determination

Growth was studied in completely filled 22-ml screw cap tubes with sodium lactate (10 mmol · l^{-1}) and sodium sulfate as substrates (10 mmol · l^{-1}), ensuring electron donor limitation in all experiments. Growth rates were determined in batch cultures by monitoring the increase of turbidity using a turbidimeter (Hach, Loveland, CO, USA). Samples for measuring cell density, lactate, acetate, sulfate and sulfide were taken at the start and the end of the experiment. Cell densities were analyzed by fluorimetry after addition of SybrGreenI (Martens-Habbena & Sass, 2006). Cell densities were converted into dry mass using conversion factors determined for 1-l batch cultures. Cells were harvested at the end of the exponential phase, washed twice with bicarbonate-free PBS buffer (130 mmol NaCl l^{-1}, 10 mmol sodium phosphate buffer l^{-1}, pH 7.2) and freeze-dried. Growth yields were determined as total carbon content of freeze-dried samples determined by combustion using a CS 500 IR analyzer (Eltra, Neuss, Germany) as described previously (Sass et al., 2002).

3.3.3 Chemical and phenotype determinations

Sulfide was analyzed by the methylene blue method described by Cline (1969). Short-chain organic acids, sulfate, thiosulfate and nitrate were analyzed on an ICS-2000 ion chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a self-regenerating suppressor unit (Dionex ASRS-Ultra II 4-mm) and a conductivity detector (Webster et al., 2009). Cell size and shape were analyzed using a Leitz DMRB microscope (Wetzlar, Germany) equipped with a digital image analysis system (H & K, Berlin, Germany).

3.3.4 Anaerobic respiration

Anaerobic respiration was determined in a multielectrode chamber (Cypionka, 1994). Cells were grown with lactate and sulfite or thiosulfate to the late exponential phase. Cultures were sparged with CO_2 to remove H_2S and washed with HEPES-buffered (10 mM, pH 7.2) artificial anoxic seawater. Respiration rates

were determined with hydrogen or formate as electron donor and sulfate or thiosulfate as electron acceptor (Sass et al., 1996).

3.3.5 Polar lipid and PLFA analysis

Freeze-dried cells (30 - 100 mg) were ultrasonically extracted up to ten times for 10 minutes using a solvent mixture of methanol/dichloromethane/ammonium acetate buffer (50 mM, pH 7.6), 2:1:0.8 by volume, in centrifuge tubes (Bligh & Dyer 1959, modified after Vancanneyt et al., 1996). After centrifugation at 2200 × g for 10 minutes at 15 °C the supernatants were removed and collected in a separating funnel. Dichloromethane and ammonium acetate buffer (pH 7.6) were added to the combined extracts to achieve a final ratio of methanol/dichloromethane/ammonium acetate buffer of 1:1:0.9 (by volume). After phase separation, the organic phase was removed and the aqueous phase reextracted five times with dichloromethane. Combined extracts were dried over anhydrous sodium sulfate, evaporated to dryness and stored at -20 °C.

Lipids were separated into compound classes of different polarity by liquid chromatography on a silica gel column (2 g silica 60, 63 - 200 µm, Merck, Darmstadt, Germany) as described by Rütters et al. (2002b). Aliquots of the polar lipid fractions were transesterified with trimethylsulfoniumhydroxide as described by Müller et al. (1993, 1998). The methyl esters obtained were quantified by gas chromatography (GC; Hewlett Packard HP 5890 Series II gas chromatograph, Hewlett Packard, Waldbronn, Germany) equipped with a DB-5HT capillary column (30 m × 0.25 mm, 0.1 µm film thickness, J&W, Folsom, CA, USA) and a flame ionization detector (FID). Fatty acid methyl esters were identified by gas chromatography-mass spectrometry (GC-MS) analysis using a Finnigan MAT SSQ 710B mass spectrometer (Finnigan-Thermoquest, San Jose, CA, USA). The carrier gas was helium (constant pressure of 12 psi). The oven temperature was run from 60 °C (isothermal for 2 min) to 360 °C at rate of 3 °C min^{-1} and held for 5 min. Mass spectra were collected in full scan mode (*m/z* 50 - 650, ionization energy 70 eV). Positions of double bonds were tentatively assigned by comparison with retention times of standards (Bacterial Acid Methyl Esters CP Mix; Supelco, Bellefonte, PA, USA).

An aliquot of the most polar lipid fraction was analyzed on an HPLC instrument (Waters 2695 Separations Module, Waters, Manchester, UK) coupled to a hybrid-quadrupole/time-of-flight mass spectrometer (Micromass Q-TOF micro) equipped with an electrospray source and to an evaporative light scattering detector (ELSD) used both for qualitative and quantitative analysis, respectively. HPLC separation was achieved on a diol phase (Lichrospher 100 Diol 5µ, Merck, Germany) using a 2 × 125 mm column. A flow rate of 0.40 ml min^{-1} was employed with the following linear solvent gradient: 1 min 100 % A, increasing over 20 min to 35 % A and 65 % B, followed by 40 min reconditioning. Eluent A was a 78:20:1.2:0.04 (v/v) mixture of *n*-hexane/*i*-propanol/formic acid/ammonia (25 % solution in water), Eluent B was 88:10:1.2:0.04 (v/v) *i*-propanol/water/formic acid/ammonia (25 % solution in water). Quantification was done after external calibration of ELSD and MS signals with phospholipid standards representing major phospholipids types (purchased from Avanti Polar Lipids, Alabaster, USA and Sigma Aldrich, München, Germany). Due to the lack of commercially available standards for quantification of ornithine lipids the response factor of phosphatidylglycerol was used, because its response factor is an average of the other intact polar lipids used in the HPLC-ESI-MS application. Another aliquot of the polar lipid fraction was used for further high resolution tandem mass measurements to confirm molecular structures. The mass spectrometer was operated in negative-ion mode with the capillary voltage set to 2800 V and a sample cone voltage of 35 V. The source temperature was 110 °C and the desolvation temperature 220 °C. The desolvation gas used was nitrogen. During full scan mode (*m/z* 500 - 2000) the voltage in the collision cell was set to 7 V. To identify structures of the intact polar lipids MS/MS experiments were carried out by running a profile in the collision cell from 30 - 40 V (in 5 V steps) with argon as collision gas. All MS and MS/MS measurements were performed at high resolution by using the lock spray channel of the Q-TOF and a 10 mmol l^{-1} sodium formate solution (in acetonitrile) as calibration standard.

3.4 Results

3.4.1 Temperature-related changes in cell size

Changes in cell size with growth temperature (10, 20 and 30 °C) were analyzed for four strains (*D. acrylicus*^{T}, NA202, NA302, and NC301). Strains NA302 and NC301 showed only little changes in average length with temperature (maximum 12 and 21 %, respectively), whereas cells of strains NA202 became larger with increasing temperature. Cells of strain NA202 grown at 10 °C and 20 °C measured 3.73 ± 1.15 µm ($n = 53$) and 5.68 ± 1.34 µm ($n = 56$) on average, respectively. Cells of the type strain were generally smaller than those of the other three strains and in contrast to these became smaller with increasing growth temperature. *D. acrylicus*^{T} cells measured 3.49 ± 0.96 µm ($n = 102$) on average when grown at 10 °C, but only 2.0 ± 0.55 µm ($n = 51$) if grown at 30 °C. Cell diameters changed at the extent as length in all four strains.

3.4.2 Growth rate and yield

Three of the strains (NA302, NB62, and NC301) grew at 4 °C, whereas the others showed a minimum growth temperature of 7 °C (Fig. 4). The upper temperature limit for growth ranged from 32 °C (strains D1, NA81, and NA202) to 37 °C (*D. acrylicus*^{T}, strains NA302, NB21, and NB62) and 40 °C (strain NC301). Fastest growth (T_{opt}) was observed at 25 °C (strains D1, NA81, NA202, NB21 and NC301), 30 °C (strains NA302 and NB62) or 35 °C (*D. acrylicus*^{T}). *D. acrylicus*^{T} grew fastest ($\mu_{max} = 3.44$ d^{-1}) whereas strain D1 showed the lowest μ_{max} with 1.75 d^{-1} (Tab. 9). For strains D1, NA81, NA202, and NB21 the linear section of the Arrhenius plot covered the range from 10 °C to T_{opt} before ln µ decreased again (Fig. 4), whereas in *D. acrylicus*^{T} the Arrhenius plot deviated from linearity already 10 °C below T_{max}. The other three strains (NA302, NB62, and NC301) showed two linear sections at temperatures below T_{opt} (Fig. 4).

Maximum growth yields (Y_{max}) were in the range from 4.4 to 5.7 g dw (mol lactate)$^{-1}$ (Tab. 9). The highest yields were generally obtained at temperatures 5 to 10 °C below T_{opt}. For five strains (*D. acrylicus*^{T}, D1, NA81, NA202, and NB21) strongly decreasing growth yields were obtained at temperatures close

to T_{min} and T_{max}, whereas the highest yield was obtained approximately half way between the extremes (Fig. 4) as is typical for mesophilic bacteria (Isaksen and Jørgensen 1996). The yield-temperature plot for the other three strains (NA302, NB21 and NC301) showed a high yield already at T_{min} and yields remaining more or less constant with increasing temperatures. A strong decrease was found at temperatures above 25 °C (Fig. 4). This pattern is typical for psychrotrophic bacteria (Isaksen & Jørgensen, 1996). Interestingly, these three strains were the only ones which grew at 4 °C and showed two linear sections in the Arrhenius plot of growth.

Tab. 9 : Growth parameters of the different *Desulfovibrio acrylicus* isolates. Lin. Arrh.: Temperature of the linear domains in the Arrhenius plot.

	Strain							
	D. acr. ^{T}	**D1**	**NA81**	**NA202**	**NA302**	**NB21**	**NB62**	**NC301**
Temp. range (°C)	7-37	7-32	7-32	7-32	4-37	7-37	4-37	4-40
μ_{max} (d^{-1})	3.44	1.75	3.05	2.46	2.6	2.75	2.39	2.73
Lin. Arrh. 1 (°C)	10-23	10-25	10-25	10-25	4-15	10-25	10-25	4-15
Lin. Arrh. 2 (°C)					15-30			15-25
Y_{max} (g dw (mol lac)$^{-1}$)	5.26±1.04	4.4±0.99	5.7±0.51	4.73±0.82	5.59±1.31	5.46±0.32	5.47±0.41	5.4±0.75

3.4.3 Influence of temperature on thiosulfate reduction

Sulfide production was analyzed for five strains: *D. acrylicus*^{T}, D1, NA81, NA202 and NB21. The other three strains were tested but did not yield reliable results. This was at least partly due to slimy material (*i.e.* lysed cells) that stuck to the electrodes. Whereas sulfate reduction was generally very slow and could hardly be analyzed by means of the electrode setup, thiosulfate as electron acceptor was reduced at significantly higher rate and delivered measurable rates even at low temperatures.

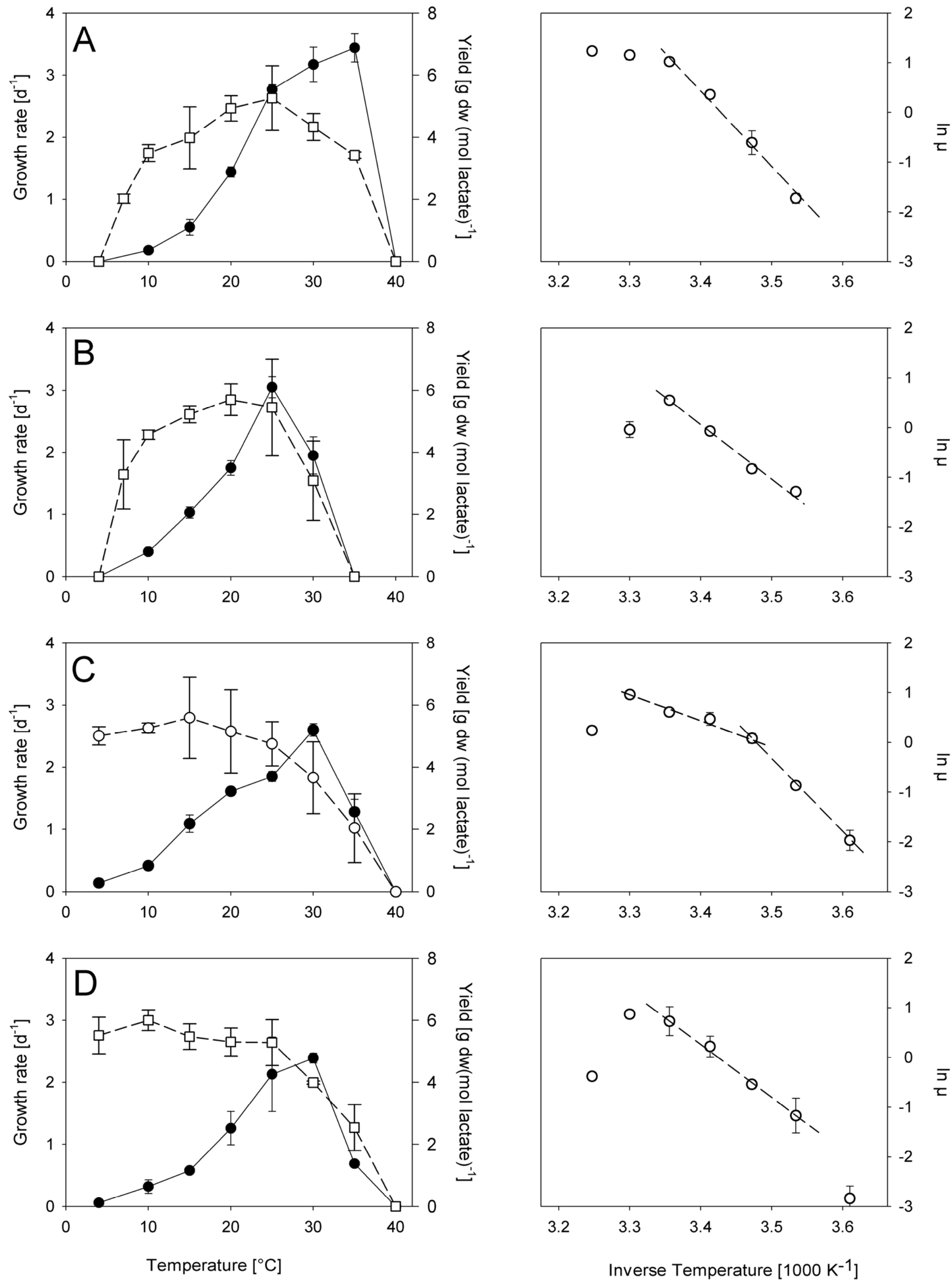

Fig. 4: Growth rates (filled circles) and growth yields (open squares) versus temperature and the Arrhenius plot of the growth rates (open circles). Data points represent averages of two to three parallels. **A:** *D. acrylicus*^{T}, **B:** Strain NA81 (representative of strains D1, NA202, NB21), **C:** Strain NA302 (representative of strain NC301), **D:** Strain NB62.

All strains showed significant sulfide production from thiosulfate at temperatures above the maximum growth temperature (Fig. 5). However, in *D. acrylicus*^{T} and strain D1 thiosulfate reduction rates (TRR) decreased at temperatures above the T_{opt} for growth. In strains NA81, NA202 and NB21, in contrast, sulfide production was fastest at 30 °C, whereas fastest growth was observed at 25 °C. Strain D1 showed the lowest sulfide production rates and was also the strain with the lowest growth rates. In the Arrhenius plot strains D1, NA81, NA202 and NB21 exhibited a linear increase of ln TRR from 5 to 20 °C, whereas *D. acrylicus*^{T} showed a strong decrease at temperatures below 10 °C (Fig. 5).

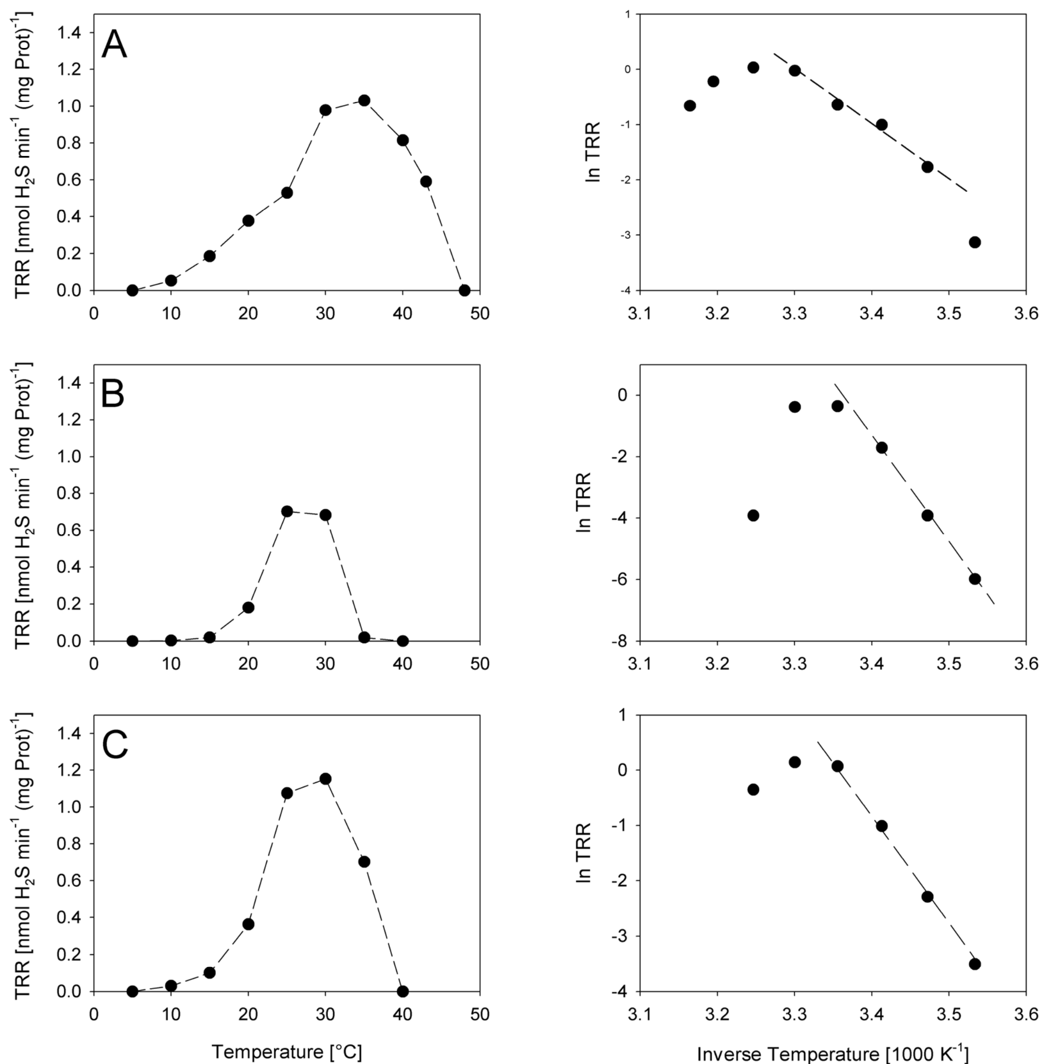

Fig. 5: Thiosulfate reduction rates versus temperature (left panel) and the respective Arrhenius plot (right panel). **A:** *D. acrylicus*^{T}, **B:** Strain D1 (representative of strains NA202, NB21), **C:** Strain NA81.

3.4.4 Temperature modulation of polar lipid fatty acid (PLFA) patterns

The eight strains showed similar temperature-related trends in their PLFA patterns: With increasing growth temperature contents of monounsaturated straight-chain fatty acids (MUFAs) decreased, whereas more saturated and monounsaturated branched-chain fatty acids (BCFAs) were formed. The *anteiso*/*iso* ratio of the BCFAs generally increased with growth temperature (Tab. 10). Saturated straight-chain fatty acids (SCFAs) appeared to be less influenced by growth temperatures. Whereas SCFA abundances decreased with increasing temperatures in *D. acrylicus*^{T} and strain NA81, they increased in strains D1, NA202 and NB21 or remained more or less constant in the other three strains. But even if SCFA contents changed, these changes were less pronounced than those of BCFAs and MUFAs. Only in three strains changes of the average chain length (ACL) were found: In strains NA81 and NC301 the ACL decreased by 0.4 carbon atoms with increasing growth temperature, whereas for strain D1 a decrease by 0.3 carbon atoms was found.

3.4.5 Changes of polar lipid species with growth temperatures

All eight strains possessed up to eight different polar lipid types, with phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and ornithine lipid (OL) being the dominant ones (Tab. 11). In all strains the amount of ornithine lipids increased with temperature, in most cases at the expense of PE. Exceptions were *D. acrylicus*^{T} and strain NB62 in which no or only a moderate decrease in the PE content was found. In these two strains the increase in OL was mostly compensated by a decrease in PG. The effect of temperature on PG was not as pronounced as on PE. In four strains (*D. acrylicus*^{T}, D1, NA81, and NB62) PG contents clearly decreased with temperature, whereas in the other strains there was only little change (strains NA202, NA302 and NC301) or even an increase (strain NB21). In strain NA302, the decrease in PG between cells grown at 10 and 20 °C was reversed if the growth temperature was further increased to 30 °C. DPG showed only minor temperature effects in most strains.

Tab. 10: Polar lipid fatty acids of the eight *Desulfovibrio acrylicus* strains grown at 10, 20, and 30 ºC (% of total FAs).

	D. acrylicus[T]			D1			NA81			NA202		NA302			NB21			NB62			NC301			
	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	40°C
***n*-12:0**	-	-	-	-	-	-	-	-	-	-	0.1	0.2	0.8	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.8	-	0.2
***i*-14:0**	0.1	0.2	-	-	-	-	0.5	0.4	0.2	0.2	0.2	0.2	0.2	0.5	0.1	0.1	0.2	0.1	0.1	0.2	0.3	0.5	-	0.5
***n*-14:1ω5**	1.7	0.5	-	0.7	-	-	1.5	0.5	0.1	0.6	0.3	1.8	0.7	0.3	2.8	0.9	0.2	4.1	1.3	0.1	1.8	0.7	0.2	0.2
***n*-14:0**	1.0	0.5	0.3	0.6	0.3	0.4	2.5	1.4	0.8	0.6	0.6	0.8	1.1	0.8	0.8	0.4	0.4	1.2	0.5	0.3	1.4	1.9	0.4	2.3
***i*-15:1ω7**	6.8	2.0	1	2.3	0.2	0.2	0.1	0.2	0.5	3.5	1.6	7.0	1.7	0.4	3.8	1.6	0.6	4.9	2.6	0.5	4.4	2.7	1.9	0.9
***ai*-15:1ω7**	1.1	0.6	-	0	0.1	0.1	-	-	-	0.4	0.3	0.8	0.7	0.5	0.4	0.3	0.2	0.6	0.5	0.1	-	-	-	-
***i*-15:0**	5.1	4.2	12	2.5	3.2	5.6	10.2	13.7	17.5	6.5	8.5	10.5	1.7	10.2	1.9	1.8	4.6	2.4	2.4	5.4	10.7	15.3	3.9	21.1
***ai*-15:0**	1.0	2.1	3.2	2	1.3	2.6	2.0	4.2	4.1	3.8	3.5	2.7	1.5	2.9	0.7	1.2	3.6	0.9	1.4	3.8	2.2	2.4	1.9	6.7
***n*-15:1ω6**	-	-	-	-	0.3	0.5	0	0	0	-	-	0.1	0.4	0.2	0.1	-	-	0.1	0.1	-	0.7	1.4	0.3	1.3
***n*-15:0**	0.1	0.2	0.1	0.4	0.1	0.1	0.7	0.5	0	0.8	0.3	0.2	0.6	0.4	0.1	0.1	0.2	0.2	0.1	0.2	0.9	1.8	0.8	3.4
***i*-16:1ω6**	3.9	6.5	1.5	4.9	6.5	5.2	3.3	4.0	2.0	4.8	3.2	7.0	8.4	9.0	4.9	6.2	4.8	4.5	6.3	4.9	3.5	1.9	3.2	3.1
***i*-16:0**	1.8	3.8	3	2.3	3.1	5.0	4.2	8.7	13.7	4.5	5.4	3.0	0.7	9.6	1.5	2.2	4.3	1.7	2.4	5.4	2.6	3.0	2.0	5.2
***n*-16:1ω9**	0.6	0.4	-	1.1	0.2	0.1	1.0	0.3	0.1	-	0.2	3.3	2.3	0.4	0.8	0.3	0.1	0.7	0.3	0.1	0.9	1.5	-	0.8
***n*-16:1ω7**	15.3	14.3	4	15	12.2	10.3	14.5	9.4	4.3	11.2	7.0	15.1	17.6	9.1	22.6	18.4	10.3	24.0	19.2	9.4	17.7	6.1	17.0	6.7
***n*-16:1ω5**	1.2	0.7	-	0.8	0.4	0.4	0.7	0.4	0.2	-	0.3	0.6	0.8	0.4	1.0	0.9	0.4	0.9	0.7	0.3	0.6	0.4	-	0.4
***n*-16:0**	20.8	16.8	9	8.3	3.5	8.3	27.1	18.8	15.1	13.1	14.5	7.4	5.0	7.1	17.5	11.2	9.7	23.0	14.2	9.8	20.1	18.7	13.0	17.9
***i*-17:1ω7**	14.9	13.2	22	16	16.1	15.9	4.8	7.1	5.8	15.4	12.8	11.4	15.6	11.3	9.3	10.7	12.3	7.8	12.0	13.7	8.4	6.5	15.0	6.2
***ai*-17:1ω7**	3.1	5.2	7.8	2.9	3.9	3.8	1.5	3.2	2.3	6.5	3.6	2.5	7.2	4.9	2.4	4.5	4.4	2.4	4.9	4.2	2.7	3.3	6.2	5.4
***i*-17:0**	2.4	2.1	13	2.4	2.1	5.3	1.1	2.0	5.6	3.6	6.7	1.5	0.5	2.3	0.8	1.1	4.5	0.9	1.2	5.1	1.8	2.3	2.7	1.2
***ai*-17:0**	1.5	2.9	6.5	2.5	3.5	4.3	2.2	0.2	10.5	4.5	5.8	2.6	2.4	4.5	1.2	2.5	5.2	1.3	2.8	5.6	2.9	3.3	4.8	4.8
***n*-17:1**	0.2	0.4	-	0.3	0.5	0.2	0.2	5.4	0.1	0.6	0.1	0.6	2.1	1.8	0.3	0.3	0.2	0.3	0.4	0.1	0.9	0.6	2.3	1.0
***n*-17:0**	0.5	1.3	1.1	0.4	0.5	1.7	1.1	1.2	2.2	3.5	1.7	0.4	0.5	0.7	0.5	0.9	2.3	0.6	1.0	2.3	1.9	3.3	5.3	2.5
***i*-18:1ω6**	1.1	2.1	-	3.3	1.4	0.4	1.5	1.9	0.9	1.7	1.5	2.4	3.4	4.0	2.4	3.6	2.3	1.6	3.0	2.2	0.8	0.5	1.3	0.6
***i*-18:0**	0.2	0.2	-	-	1.1	1.2	0.3	0.3	0.6	-	1.0	0.6	0.7	0.7	0.2	0.4	1.3	0.1	0.2	1.2	0.1	1.0	-	0
***n*-18:1ω9**	0.3	0.4	-	2.1	1.5	0.5	0.9	0.5	0.2	0.3	0.4	1.4	2.8	1.5	0.5	0.5	0.2	0.3	0.3	0.2	0.4	1.4	0.4	0.3
***n*-18:1ω7**	11.5	12.2	5.8	23	20.4	8.5	12.0	9.1	2.6	9.2	6.4	11.9	15.3	10.8	18.7	19.9	7.8	12.4	16.1	6.8	8.5	5.1	12.0	4.5
***n*-18:1ω5**	1.0	0.7	-	-	0.7	0.4	0.6	0.4	0.2	0.3	0.3	0.4	0.6	0.5	0.8	1.0	0.3	0.5	0.6	0.3	0.3	0.4	-	0.3
***n*-18:0**	2.7	6.2	10	4.9	9.7	14.7	5.5	6.3	9.7	3.8	12.7	2.9	3.0	3.4	3.6	7.8	18.2	2.2	4.6	16.4	3.2	12.7	5.4	2.1
∑ *n*-19:0-*n*-20:0	0.2	0.3	-	-	2.9	1.7	-	-	0.3	0.8	0.7	0.4	1.0	1.3	0.6	0.9	1.4	0.3	0.7	1.3	0.1	0.6	-	0.3
∑ SCFAs	25	25	21	15	14	25	37	28	28	22	30	12	11	13	22	21	31	27	21	29	28	39	25	28
∑ BCFAs	43	45	70	41	42	50	32	46	64	55	54	52	45	61	30	36	48	29	40	52	40	43	43	56
∑ MUFAs	32	30	10	43	36	21	31	26	8	22	15	35	42	25	47	42	19	43	39	17	32	18	32	16
Anteiso/iso	0.18	0.31	0.33	0.22	0.26	0.28	0.22	0.20	0.36	0.38	0.32	0.20	0.36	0.27	0.19	0.31	0.38	0.22	0.31	0.36	0.24	0.27	0.43	0.44

It became more abundant with temperature in strains NA202 and NA302, whereas the opposite was found in strain NA81. Phosphatidic acid (PA) was present in significant amounts only in strains NA81, NA302 and NC301. In these strains PA contents generally decreased with temperature.

3.4.6 Temperature-related variation in PE and PG molecular species

The number of carbon atoms of the fatty acid side chains of the three major polar lipids generally increased with growth temperature (Tab. 12 to Tab. 14). This trend was similar in PE and PG. For example, the average number of carbon atoms in the fatty acid side chains of PE in *D. acrylicus*^{T} grown at 10 and 30 °C was 15.2 and 16.6, respectively.

Because the ACL in the PLFA fraction was more or less constant with changing temperatures, the increase in the lengths of intact polar lipid side chains must have been due to the increase in branched fatty acids. In the calculation of the ACL from PLFA data only the number of carbon atoms in the longest “straight” fatty acid chain is taken into account. However, a distinction of straight-chain and branched isomers is not possible by HPLC-ESI-MS/MS analysis of side chains of intact polar lipids.

Fatty acid side chain combinations were relatively similar in PE and PG. Only a few combinations seemed to be restricted to one of the head groups: *e.g.* 17:0/17:1, 18:0/18:0, and 17:1/16:0 to PE, or 17:0/16:1 and 16:0/18:1 to PG (Tab. 12 and Tab. 13).

Phospholipids with two unsaturated side chains generally decreased in abundance with increasing temperature. The amounts of PE and PG with the relatively short side chains 16:0/15:0 increased with temperature in strains D1, NA81, NA202, NA302, NB21 and NB62, whereas the contents PG and PE with side chain combinations 16:1/15:1, 16:0/15:1 or 16:1/16:1 declined with increasing temperature.

Tab. 11: Major polar lipid types in *Desulfovibrio acrylicus* isolates grown at 10, 20, and 30 °C (% of total polar lipids). PA: phosphatidic acid, OL: ornithine lipid, PG: phosphatidylglycerol, DPG: diphosphatidylglycerol, PE: phosphatidylethanolamine, PS: phosphatidylserine (all phospholipids were detected as diacylglycerols). The unknown lipids U1 and U2 are characterized by the following most intense signals in their averaged mass spectra: U1: *m/z* 730, 742, 756; U2: *m/z* 840, 854, 868. tr = traces.

	D. acrylicus^{T}			**D1**			**NA81**			**NA202**		**NA302**			**NB21**			**NB62**			**NC301**			
Lipid	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**40°C**
PA	1	1	-	-	tr	tr	5	1	-	-	tr	15	7	2	tr	tr	tr	2	tr	tr	4	5	-	2
U1	-	-	-	-	tr	tr	8	2	1	tr	tr	-	11	3	tr	tr	tr	3	tr	tr	4	7	-	2
OL	15	20	39	8	30	37	26	60	76	26	33	23	17	36	9	14	18	13	12	34	26	43	22	60
PG	36	38	19	35	30	25	21	13	10	20	17	23	16	25	17	28	41	31	17	19	21	26	29	26
DPG	6	8	6	7	12	11	10	5	2	7	15	-	6	9	5	4	6	7	2	5	6	5	4	3
PE	42	33	36	50	27	27	30	18	8	47	35	39	42	26	69	54	36	45	68	43	39	14	44	7
PS	1	1	-	-	tr	tr	-	-	-	tr	tr	-	-	-	-	tr	tr	-	tr	tr	-	-	-	-
U2	-	-	-	-	tr	tr	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Tab. 12: Diacyl phosphatidylglycerol (PG) molecular species of *Desulfovibrio acrylicus* strains grown at 10, 20, and 30 °C (abundances (%) are given relative to total ion intensities in averaged base peak). $[M-H]^-$: quasi-molecular ion.

		D. acrylicus[T]			D1			NA81			NA202		NA302			NB21			NB62			NC301			
$[M-H]^-$	**PG species**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**40°C**
703.3	**16:1/15:1**	3.6	1.0	0.1	1.5	0.1	-	1.0	0.4	0.4	2.2	0.5	3.6	1.0	0.9	3.8	1.2	0.2	4.6	3.0	0.3	1.7	1.8		1.2
705.4	**16:0/15:1**	6.3	2.1	1.1	0.6	0.1	0.4	1.5	1.0	0.9	2.2	1.9	2.4	0.3	0.9	2.8	0.9	1.0	4.8	1.9	1.0	3.8	4.3	1.5	2.0
707.5	**16:0/15:0**	2.5	2.2	2.6	0.1	0.4	3.0	0.9	1.1	2.6	0.9	3.8	0.7	0.1	1.7	0.6	0.5	3.4	1.4	0.7	3.9	1.4	1.0	0.3	0.9
717.5	**16:1/16:1**	8.8	4.8	1.7	7.1	1.3	0.8	4.8	2.6	0.9	12.0	2.0	8.8	6.7	3.7	10.1	5.2	1.4	9.4	8.8	1.6	6.6	7.3	4.1	5.7
719.5	**16:0/16:1**	18.3	15.4	8.1	3.0	2.8	6.0	22.9	13.9	4.9	13.1	8.3	7.4	2.1	4.7	17.0	10.4	7.9	21.1	14.1	7.8	22.7	13.5	8.6	15.2
721.5	**16:0/16:0**	6.1	5.4	5.5	0.3	1.6	5.1	6.4	4.0	9.3	2.4	8.3	1.5	0.4	3.0	3.5	2.3	5.8	5.9	2.8	6.4	5.4	2.6	0.9	2.7
731.5	**16:1/17:1**	10.1	8.0	2.4	10.7	5.2	3.3	5.7	6.1	1.9	13.1	3.0	12.4	16.3	11.3	9.1	8.0	3.0	7.7	10.2	3.5	7.5	11.0	10.5	11.6
733.5	**17:0/16:1**	13.3	14.5	17.4	4.5	6.4	11.3	10.0	14.5	10.6	13.7	12.5	8.2	3.8	7.4	8.3	9.6	13.0	7.8	11.1	12.6	12.9	15.0	12.5	15.4
735.5	**17:0/16:0**	3.0	4.4	5.6	0.6	3.5	5.8	2.0	3.2	11.8	2.0	9.3	1.6	0.7	3.1	1.6	2.1	8.1	1.7	2.0	8.2	2.4	2.4	0.9	2.1
745.5	**16:1/18:1**	8.9	13.0	11.3	28.0	23.4	12.2	16.8	16.7	5.7	18.3	4.6	23.1	33.6	28.5	16.4	20.3	7.4	11.2	16.8	8.2	12.2	15.7	25.1	19.0
747.5	**16:0/18:1**	7.9	12.5	25.2	8.6	15.0	20.7	15.3	17.9	19.9	7.7	15.1	7.7	5.7	8.5	12.2	16.2	19.6	8.5	12.5	18.9	11.0	11.7	12.2	11.0
749.5	**18:0/16:0**	1.6	3.1	2.7	0.8	3.3	4.6	2.3	2.9	8.5	0.6	8.1	1.1	1.0	1.8	2.1	2.9	5.5	1.5	2.0	5.4	1.7	1.5	1.0	1.2
759.5	**18:1/17:1**	3.0	6.4	6.8	17.5	17.8	9.3	3.6	7.5	4.9	5.6	2.7	7.6	16.3	15.4	4.1	8.7	5.2	3.2	6.7	5.6	3.4	5.8	12.6	7.0
761.5	**18:0/17:1**	1.2	4.1	6.7	4.1	7.8	10.0	1.3	3.8	8.5	1.3	7.8	1.8	2.7	3.4	2.0	5.1	11.2	1.3	3.1	10.2	1.9	2.8	4.5	2.5
763.5	**18:0/17:0**	0.2	0.7	0.7	0.3	1.3	1.7	0.1	0.5	1.7	0.2	2.7	0.2	0.3	0.5	0.2	0.7	2.1	0.2	0.4	1.8	0.2	0.4	0.5	0.3
773.5	**18:1/18:1**	0.2	0.8	0.5	5.8	6.0	2.4	0.8	1.6	1.2	1.0	0.9	1.7	2.9	3.1	1.4	2.6	1.2	0.8	1.3	1.1	0.8	1.2	2.2	0.9
775.5	**18:0/18:1**	0.1	0.4		1.6	2.4	1.8	0.3	0.5	1.2	0.3	1.9	0.4	0.5	0.7	0.4	1.1	2.0	0.2	0.4	1.5	0.3	0.4	0.4	0.3

Tab. 13: Diacyl phosphatidylethanolamine (PE) molecular species of *Desulfovibrio acrylicus* strains grown at 10, 20, and 30 °C (abundances (%) are given relative to total ion intensities in averaged base peak). $[M-H]^-$: quasi-molecular ion.

		D. acrylicus[T]			D1			NA81			NA202		NA302			NB21			NB62			NC301			
$[M-H]^-$	**PE species**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**40°C**
672.3	**16:1/15:1**	5.6	3.1	0.1	1.3	0.3	0.4	1.2	0.8	0.1	2.9	0.8	6.5	2.2	0.8	3.9	2.2	0.5	4.3	3.8	0.4	4.3	4.2	0.9	2.1
674.5	**16:0/15:1**	6.0	2.7	0.4	0.5	0.2	0.8	1.7	1.2	0.4	1.7	1.9	2.2	0.4	0.4	2.8	1.2	1.0	4.5	2.3	0.9	4.5	3.8	1.1	1.9
676.5	**16:0/15:0**	1.7	1.6	0.9	0.2	0.1	3.1	0.7	1.1	2.5	0.7	3.0	0.5	0.1	0.6	0.6	0.5	2.6	1.5	0.8	3.0	1.5	1.0	0.4	0.8
686.5	**16:1/16:1**	9.6	7.2	2.7	5.4	2.2	2.0	5.2	3.3	0.6	11.9	3.0	13.0	9.1	4.4	9.3	6.7	2.2	8.9	8.6	1.7	8.9	9.0	4.8	6.6
688.5	**16:0/16:1**	14.2	13.9	4.2	3.6	2.0	6.7	22.5	13.3	4.6	10.4	8.8	8.3	2.1	3.7	15.7	10.2	8.6	21.3	13.5	7.4	21.3	12.9	9.1	13.7
690.5	**16:0/16:0**	4.1	3.5	2.6	0.4	0.5	4.1	7.6	3.9	7.1	1.5	5.9	1.5	0.3	1.2	3.2	1.9	5.1	5.2	2.6	4.8	5.2	3.8	1.0	2.8
700.5	**16:1/17:1**	9.4	8.7	2.3	9.9	5.7	4.6	4.8	5.5	1.6	10.6	4.2	13.0	17.2	10.7	8.6	8.4	4.6	7.4	9.0	3.6	7.4	10.4	11.1	10.9
702.5	**17:1/16:0**	11.4	12.2	12.8	5.0	4.2	9.7	9.0	12.7	8.9	12.4	13.1	7.7	3.7	6.4	8.1	8.8	14.6	10.8	10.4	12.1	10.8	13.1	12.3	14.5
704.5	**17:0/16:0**	2.7	3.0	3.2	0.5	1.2	4.8	2.0	3.2	11.1	1.6	6.5	1.2	0.6	1.8	1.6	1.8	7.9	2.3	2.1	6.7	2.3	3.2	1.2	2.5
714.5	**16:1/18:1**	8.5	12.1	12.7	28.2	18.5	11.3	13.0	12.8	4.0	14.4	6.1	19.5	30.6	24.9	16.0	18.6	10.6	10.1	14.1	7.9	10.1	11.9	23.3	16.4
716.5	**17:0/17:1**	8.7	11.0	27.7	8.3	9.5	17.9	17.8	18.7	18.5	12.0	15.6	7.5	5.2	8.3	12.3	14.6	-	9.3	12.1	18.6	9.3	10.3	12.0	11.6
718.5	**18:0/16:0**	2.1	2.9	3.1	0.7	1.8	4.3	3.7	3.9	11.9	1.3	5.2	1.2	0.8	2.2	2.3	2.6	6.5	1.7	2.3	5.3	1.7	2.3	1.0	1.8
728.5	**18:1/17:1**	3.9	6.5	8.6	18.5	18.8	8.2	2.9	6.5	3.2	6.6	4.0	6.7	15.6	15.8	4.6	8.5	7.8	2.7	6.2	5.7	2.7	5.0	10.8	7.6
730.5	**18:0/17:1**	2.5	4.8	12.3	4.1	8.6	10.9	2.1	6.2	12.4	3.7	10.0	1.8	3.5	5.4	2.7	5.6	16.3	1.8	4.3	13.3	1.8	2.9	4.6	3.5
732.5	**18:0/17:0**	0.8	1.2	1.1	0.5	1.6	2.1	0.4	1.0	4.0	0.7	2.4	0.3	0.8	1.4	0.5	0.9	3.2	0.4	1.0	2.5	0.4	-	0.4	0.5
742.5	**18:1/18:1**	0.5	1.1	1.4	6.4	11.5	2.6	1.0	1.9	0.9	1.3	1.5	1.4	2.6	4.0	1.6	2.8	1.9	0.5	1.6	1.3	0.5	0.8	1.8	1.0
744.5	**18:0/18:1**	0.4	0.8	1.2	1.9	4.9	2.9	0.5	1.6	3.9	0.8	3.0	0.3	1.3	2.0	0.7	1.5	3.9	0.3	0.9	2.7	0.3	0.4	0.7	0.3
746.5	**18:0/18:0**	0.3	0.5	0.3	0.5	1.3	0.8	0.2	0.4	2.3	0.3	1.1	0.2	0.6	1.0	0.3	0.4	1.1	0.1	0.4	0.8	0.1	0.2	0.2	0.1

3.4.7 Temperature-related changes in ornithine lipid molecular species

PE and PG were quite similar with respect to their fatty acid side chain compositions, but ornithine lipids differed significantly (Tab. 14). Ornithine lipids consist of the amino acid ornithine as a headgroup amide-bound 3-hydroxy (3-OH) fatty acid. A second fatty acid is ester-bound to the hydroxy group of the 3-OH fatty acid (Knoche & Shively, 1972; Wilkinson, 1972). These two different fatty acids were both characterized by lower degrees of unsaturation than those of PG and PE. Whereas 6 to 50 % of the 3-OH fatty acids were unsaturated, OL contained very little unsaturated fatty acids (1 to 5 %). An exception was strain NA302. The abundance of unsaturated 3-OH fatty acids strongly decreased with increasing temperature, except for strains NA302 and NC301, in which the opposite effect was found (Tab. 14). The OL fatty acids also differed with respect to the length of their chains: The average 3-OH fatty acid was by 1.6 to 2 carbon atoms longer than the ester-bound fatty acid. Like in PE and PG the number of carbon atoms in the side chains of OL increased with growth temperature, although the aliphatic fatty acid side chains were generally shorter than those of PE and PG. Only in *D. acrylicus*^{T} the fatty acids of OL remained more or less constant. Despite the differences in chain length and degree of unsaturation the temperature effects were similar for OL and phospholipid side chains.

3.4.8 Differences between mesophilic and psychrotrophic strains

Three of the *Desulfovibrio* strains were classified as psychrotrophs (strains NA302, NC301 and NB62). Interestingly, two of these isolates (strains NA302 and NC301) differed from the other isolates not only with respect to their growth behavior but also in the way they changed their lipids with temperature. In both strains unsaturation of side chains (including branched fatty acids) increased and only at the highest temperature a decrease was found. In strain NA302 unsaturated fatty acids (UFAs) represented 71 % of the side chains in PG at 10 °C, 84 % at 20 °C, and 76 % at 30 °C, respectively (Tab. 12). Similar values were found for PE and OL. In strain NC301 the absolute level of unsaturation was a bit lower, but

Tab. 14: Molecular species of ornithine lipids (OL) in *Desulfovibrio acrylicus* strains grown at 10, 20, and 30 °C (abundances (%) are given relative to total ion intensities in averaged base peak). $[M-H]^-$: quasi-molecular ion.

		D. acrylicus[T]			D1			NA81			NA202		NA302			NB21			NB62			NC301			
$[M-H]^-$	**OL species**	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	40°C
595.5	**3-OH-15:0/15:0**	0.6	0.3	0.6	0.3	-	-	2.3	0.9	0.7	0.6	0.4	1.2	-	0.4	0.5	0.3	-	1.2	0.7	0.1	1.5	1.0	0.6	2.2
605.5	**3-OH-16:1/15:1**	-	-	-	0.1	-	-	-	-	-	0.1	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-
607.5	**3-OH-16:0/15:1**	0.2	0.1	-	0.1	-	-	0.7	0.3	0.1	0.4	0.1	1.3	0.6	0.8	0.1	-	-	0.2	0.2	-	0.5	0.2	0.6	1.3
609.5	**3-OH-16:0/15:0**	11.9	7.1	3.8	2.9	1.9	1.2	14.0	8.9	6.9	6.0	5.9	10.9	1.2	3.9	11.1	5.9	1.9	18.5	9.6	3.6	14.5	7.3	4.9	11.2
619.5	**3-OH-17:1/15:1**	0.1	-	-	0.2	-	-	0.1	-	-	0.1	-	0.2	0.9	0.6	-	-	-	-	-	-	-	-	0.1	0.2
621.5	**3-OH-17:1/15:0**	3.5	1.8	1.2	2.8	0.4	0.2	4.5	2.5	0.8	2.6	0.9	7.8	4.2	4.5	2.3	1.6	0.1	2.8	2.4	0.4	4.6	3.6	3.7	6.2
623.5	**3-OH-17:0/15:0**	31.1	20.7	32.8	8.2	8.5	11.3	19.9	17.9	15.8	18.8	21.6	21.9	3.7	9.9	21.0	17.0	12.2	25.5	21.4	15.1	28.8	27.3	13.5	19.3
633.5	**3-OH-18:1/15:1**	0.2	0.2	-	0.6	0.1	-	0.3	0.2	0.1	0.3	0.1	0.9	5.0	2.7	0.1	0.1	-	0.1	0.1	-	0.2	0.1	0.4	0.7
635.5	**3-OH-18:1/15:0**	7.2	5.5	0.6	11.9	2.6	1.4	9.3	6.8	1.9	6.2	2.2	15.8	12.2	10.6	9.1	6.8	1.1	9.0	7.6	1.5	7.5	5.1	9.3	9.0
637.5	**3-OH-17:0/16:0**	22.2	26.4	26.1	13.8	16.2	22.2	18.3	22.8	23.7	24.0	26.6	11.1	4.2	13.3	21.3	23.1	28.0	20.7	24.1	26.2	19.6	21.0	20.2	19.0
647.5	**3-OH-18:1/16:1**	0.4	0.5	0.1	1.9	0.6	0.2	1.0	0.7	0.2	0.6	0.2	2.3	15.7	5.8	0.7	0.7	0.1	0.5	0.6	0.1	0.5	0.5	1.2	1.3
649.5	**3-OH-18:1/16:0**	3.6	5.3	2.2	12.2	5.9	3.3	7.6	6.9	2.2	5.8	2.5	6.9	9.3	10.1	7.7	8.2	2.3	5.2	7.1	2.4	5.2	6.3	10.0	7.4
651.5	**3-OH-18:0/16:0**	12.3	20.7	20.8	13.2	22.6	29.0	13.7	18.5	23.1	14.0	21.8	6.4	3.4	11.9	14.8	18.0	29.1	8.9	16.2	27.1	9.3	14.0	11.8	11.4
661.5	**3-OH-18:1/17:1**	0.3	0.4	0.1	3.1	1.6	0.5	0.6	0.7	0.2	0.7	0.2	1.7	17.3	5.7	0.6	0.8	0.1	0.4	0.5	0.1	0.5	0.6	1.9	1.2
663.5	**3-OH-18:1/17:0**	1.1	2.3	1.0	8.3	6.0	3.3	2.2	3.7	1.8	3.9	1.5	2.8	6.0	5.5	2.6	3.9	2.1	1.8	3.1	2.0	1.7	3.4	6.9	3.6
665.5	**3-OH-19:0/16:0**	3.9	6.5	6.5	5.2	13.9	17.8	2.7	6.0	14.8	7.1	10.5	1.8	1.7	5.3	3.3	5.0	16.4	1.5	3.9	15.2	2.3	5.4	5.9	3.6
675.5	**3-OH-18:1/18:1**	0.1	0.2	0.1	3.6	2.5	0.5	0.4	0.3	0.1	0.4	0.1	1.2	8.4	3.6	0.4	0.4	0.1	0.2	0.2	0.1	0.2	0.4	1.0	0.4
677.5	**3-OH-18:1/18:0**	0.3	0.6	0.5	4.2	4.3	1.9	0.9	1.3	0.9	1.5	0.7	1.0	1.5	1.6	1.2	1.7	0.9	0.5	0.7	1.0	0.7	1.3	2.4	0.9
679.5	**3-OH-20:0/16:0**	0.7	0.9	0.9	1.5	9.0	5.9	0.4	1.0	5.5	1.5	2.9	0.4	0.4	1.6	0.5	0.7	4.7	0.1	0.3	4.0	0.3	1.1	1.5	0.5

like in strain NA302 a net increase in unsaturated acids was found. In contrast, little change was found in the five mesophilic strains between 10 and 20 °C, but a strong decrease of UFAs between 20 and 30 °C. The behavior of strain NB62 was in between, showing an increase of UFAs between 10 and 20 °C (59 to 68 % in PG), but a net decrease at 30 °C (46 %).

3.5 Discussion

The eight strains of *Desulfovibrio* analyzed in the present study share at least 99 % sequence similarity in their 16S rRNA genes and hence can be considered to belong to a single species (Stackebrandt & Goebel, 1994). This is supported by a number of phenotype data, including the use of electron donors and acceptors (data not shown). However, with respect to their temperature range, their growth yield-temperature relationship and temperature-related changes in their polar lipids some significant differences were found.

3.5.1 Phenotypical and physiological responses to temperature changes

Cells of the investigated strains were in the size range described previously (van der Maarel et al., 1996). Whereas the two psychrotrophic strains of *Desulfovibrio acrylicus* did not change their cell sizes significantly, one isolate shrunk and the other one increased in size with temperature. It may be surprising to find these differences within a single phylotype, but all of these responses have previously been reported for other bacteria. Constant cell sizes were observed in *Salmonella* spp. (Schaechter et al., 1958), whereas in *Shewanella oneidensis* cells doubled in length when growth temperature was lowered from 22 to 3 °C (Abboud et al., 2005) and cells of *Acetobacterium tundrae* elongated by a factor of five close to the upper temperature limit (Simankova et al., 2000). The elongated cell shapes were explained by disequilibrium between growth and septation caused by temperature stress and, if correct, this explanation may account for the fact that the two strains with the largest temperature range for growth showed the smallest effects.

Five of the strains (*D. acrylicus*^{T}, strains D1, NA81, NA202, and NB21) did not grow at temperatures below 7 °C and can be classified as mesophiles (Morita, 1975) despite a T_{max} of 32 °C in three isolates (strains D1, NA81, and NA202). This is confirmed by their 'bell-shaped' growth yield-temperature curves typical for mesophilic bacteria (Isaksen & Jørgensen, 1996). Similar patterns were found for other *Desulfovibrio* spp., although these grew even at 4 °C (Sass et al., 1998a). Three of the isolates investigated here (strains NA302, NB62, and NC301) also grew at 4 °C but showed a more or less constant growth yield between T_{min} and 25 °C, a behavior found before for psychrophilic and psychrotrophic bacteria (Isaksen & Jørgensen, 1996; Knoblauch & Jørgensen, 1999; Tarpgaard et al., 2006). These isolates showed an Arrhenius plot for growth with two linear domains. A similar plot was found for the psychrotrophic *Desulfobacter psychrotolerans* (Tarpgaard et al., 2006), although the critical temperature (T_{crit}) that separates the two domains was significantly higher in our strains (12 - 13 °C) than in *D. psychrotolerans* (6.3 °C). A linear domain in the Arrhenius plot has been interpreted as a good temperature adaptation to this temperature interval. Also, metabolic processes seem lesser influenced by temperature if the Q_{10} value is lower (Harder & Veldkamp, 1971; Isaksen & Jørgensen, 1996; Tarpgaard et al., 2006). In our mesophilic strains, there appears to be only one linear domain. However, growth at 7 °C was very slow and would have resulted in a strongly negative ln μ (similar to strain NB62, Fig. 4).

To find mesophilic and psychrotrophic strains within a single phylotype is surprising at first sight. Similar results were obtained with freshwater *Actinobacteria* and this was interpreted as an adaptation to different thermal niches (Hahn & Pöckl, 2005). However, whereas the *Actinobacteria* were isolated from a number of freshwater lakes from the Alps to the tropics characterized by different temperature regimes, six of the *Desulfovibrio* strains including the three psychrotrophic isolates in this study were obtained from a single site. On the other hand, temperate tidal flat sediments experience relatively strong temperature fluctuations, particularly at the sediment surface where temperatures can change by as much as 15 °C within a tidal cycle (Harrison & Phizacklea, 1987). Such short-term temperature fluctuations can be expected to favor mesophilic bacteria

(Harder & Veldkamp, 1971; Nedwell & Rutter, 1994). However, the extent of these temperature fluctuations varies, depending on season and meteorological conditions like solar irradiation or exposure to wind. In addition, seasonal population changes seem to be less pronounced in sediments than in the water column. Tison et al. (1980) investigated bacterial populations in a temperate lake and found a shift from predominantly psychrotrophic to mesophilic bacteria from winter to summer. In sediments, in contrast, high numbers of psychrotrophs were also found in summer.

The *Desulfovibrio isolates* (except *D. acrylicus*^{T}) investigated in this study were obtained from the highest positive dilutions of MPN series. As MPN counts obtained at 20 °C were generally one to two orders of magnitude higher than those at 30 °C (Sass et al., 2003b), this indicates that the mesophilic strains isolated at 20 °C were more abundant *in situ* than the psychrotrophic strains NA302 and NC301 which were isolated at 30 °C. Unexpectedly, the psychrotrophic isolates were obtained at higher temperatures than the mesophilic isolates, but at 20 °C the mesophilic strains generally grew faster than strains NA302 and NC301. However, the latter have a wider temperature range for growth and grew faster at 30 °C (Fig. 4). Therefore, they were paradoxically able to outcompete the more abundant mesophiles only at elevated temperatures.

Thiosulfate reduction by our *Desulfovibrio* isolates was observed a few degrees centigrade above the maximum temperature for growth (Fig. 5). Metabolic activity at temperatures exceeding the growth range was found in a number of sulfate-reducing bacteria (Isaksen & Jørgensen, 1996; Knoblauch & Jørgensen, 1999; Rabus et al., 2002; Tarpgaard et al., 2006). In these SRBs, however, maximum sulfate reduction rates were measured several degrees above T_{opt} for growth. This deviation was explained by partially uncoupling of sulfate reduction from growth, as indicated by a decrease in growth yield, and by some of the increased energy yield to be spent for cell maintenance and repair. This was considered typical for cold-adapted organisms (Isaksen & Jørgensen, 1996; Tarpgaard et al., 2006). In our mesophilic strains maximum sulfide production was generally found at the same temperature as fastest growth as described for mesophiles and thermophiles (Isaksen & Jørgensen, 1996).

For *Desulfobacterium autotrophicum* it was shown that the growth temperature of the cells used for activity measurements influences the activity-temperature plot (Rabus et al., 2002). At temperatures above 10 °C, cells of *D. autotrophicum* grown at 28 °C showed higher sulfate reduction rates than those grown at 4 or 10 °C, whereas below 10 °C rates were very similar. Our cells were grown at 20 °C and it may be that cells grown closer to T_{opt} would have performed differently in the experiments. The differences in sulfate reduction rates among cells of *D. autotrophicum* acclimated to different temperatures may be due to differences in membrane composition (Rabus et al., 2002).

3.5.2 Temperature-related changes in polar lipid patterns

It has been suggested that the decrease of growth yield observed at lower growth temperatures (Fig. 4) can be explained by an enhanced substrate requirement for growth due to lower substrate affinity and decreased transport efficiency of membrane proteins (Nedwell, 1999). Membrane-bound enzymes rely on a narrow range of membrane fluidity that can be maintained by changes in the polar lipid fatty acid side chains but probably also by changing the polar lipid types. In *D. autotrophicum* modest changes in fatty acid side chains had a significant effect on sulfate reduction activity (Rabus et al., 2002). The major temperature effects observed by these authors (increase in BCFAs, decrease in MUFAs) were similar to those observed in our *Desulfovibrio* strains. However, *D. autotrophicum* showed a significant decrease of the average chain length at low growth temperatures (Rabus et al., 2002), whereas the average chain length remained relatively constant in *Desulfobacter psychrotolerans* (Tarpgaard et al., 2006) and in our isolates (Tab. 10).

In the *Desulfovibrio* isolates changes in polar lipids types with growth temperatures were significant and it may be that they are as important as those of the fatty acid side chains. In all strains contents of ornithine lipids strongly increased with growth temperature. The only SRB reported to contain ornithine lipids so far is *Desulfovibrio gigas* (Makula & Finnerty, 1975) but unfortunately no data about the amounts synthesized at different temperatures are available. In *Burkholderia cepacia* ornithine lipids and PE contents did not change much with

temperature, whereas a significant increase in abundances of hydroxy fatty acid side chains was found at elevated temperatures (Taylor et al., 1998). There are only very few reports on the role of these amide lipids in microbial physiology. The biosynthesis of ornithine lipids appear to be an adaptation to acidic conditions (Yuasa et al., 2002), but in most cases the presence of these phosphorous-free polar lipids was related to phosphorous limitation during growth (Wilkinson et al., 1982; Taylor et al., 1998; Choma & Komaniecka, 2002; Yuasa et al., 2002). This explanation, however, does not hold for our cultures as they were grown at all temperatures in the same medium at neutral pH. However, ornithine lipids appear to play an essential role in the presence of extracytoplasmatic proteins (*e.g.* cytochromes) in the purple non-sulfur bacterium *Rhodobacter capsulatus* (Aygun-Sunar et al., 2006), and this influence appeared to be particularly strong at elevated growth temperatures. Whereas OL-deficient mutants of *R. capsulatus* grown in rich media at 25 °C contained *c*-type cytochromes, these disappeared within 24 hours after the incubation temperature was changed to 35 °C. The OL-possessing wild type, in turn, contained *c*-type cytochromes also at elevated growth temperatures (Aygun-Sunar et al., 2006). Hence, ornithine lipids appear to be crucial for the presence of *c*-type cytochromes and thus to have a strong influence on the performance of the membrane-bound electron transport chain. A similar role of OLs in *Desulfovibrio* spp. may be feasible. Furthermore, PE showed only little temperature-related changes in other OL-free bacteria like *Pseudomonas* and *Clostridium* spp. (Bhakoo & Herbert, 1980; Wada et al., 1987; Evans et al., 1998), whereas in our study the abundance of PE strongly decreased with increasing temperature. However, PE and ornithine lipid appear to be functionally interchangeable with each other (Wilkinson, 1972). Hence, a strong influence of OL on the performance of the membrane-bound enzymes may explain why the increase of OL portions was mostly at the expense of PE and why in our study PE strongly decreased with increasing temperature.

3.6 Conclusions

The analysis of temperature-related changes of the physiology of eight very closely related strains of *Desulfovibrio acrylicus* yielded some unexpected results. Firstly, it revealed the presence of bacteria with different temperature adaptation in a single phylotype. This means that inferring the temperature adaptation solely from phylogenetic analysis, *i.e.* 16S rRNA gene analysis like it is done in some molecular ecological studies, may be misleading. Secondly, psychrotrophic and mesophilic bacteria can have similar temperature ranges for growth. Hence, the assignment to a temperature group should include the cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for growth, but also the growth yields as suggested by Isaksen & Jørgensen (1996). The results also showed that mesophilic and psychrotrophic bacteria use similar mechanisms for maintaining membrane fluidity. As the strains belong to a single phylotype, modifications of their polar lipids and fatty acid side chains must be due to the change of growth conditions and not due to phylogenetic differences.

The analysis of polar lipid molecular species enabled us to show that the temperature-induced modifications of the side chains in the three dominant polar lipid classes were similar. This suggests that variations in polar lipid types (*e.g.* OL replacing PE) may not necessarily influence membrane viscosity but may rather play a crucial role in maintaining the presence and performance of membrane-bound enzymes. In turn, the variation of fatty acid side chains appears to be the main mechanism for the modulation of membrane viscosity.

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4 Description of *Desulfocucumis infernus* gen. nov., sp. nov., a sulfate-reducing bacterium isolated from the subsurface of a tidal sandflat

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4.1 Abstract

A novel, mesophilic sulfate-reducing bacterium (strain JS_SRB250Lac^T) was isolated from a tidal sand flat in the German Wadden Sea. The cells were Gram-negative, non-motile, short rods. Strain JS_SRB250Lac^T utilized a variety of short-chain organic acids (formate, *n*-butyrate, valerate, caproate, caprate, lactate, fumarate, pyruvate, succinate), several alcohols (ethanol, 1-propanol, 1-butanol, glycerol), the amino acid proline, and hydrogen as electron donors for sulfate reduction. Lactate was incompletely oxidized to acetate. Beside sulfate, sulfite was utilized as electron acceptor. Growth with thiosulfate and nitrate was not observed. In the absence of an electron acceptor, fumarate and pyruvate were utilized for fermentative growth. Strain JS_SRB250Lac^T grew autotrophically with hydrogen and carbon dioxide/bicarbonate as sole carbon sources. CO dehydrogenase activity was observed, indicating the operation of the reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) for CO_2 fixation. Maximum growth rates were obtained at 28 °C and at marine NaCl concentrations (20 ‰ – 30 ‰). The major cellular fatty acids were *n*-16:0, *n*-17:0, *n*-16:1*c*9, and cyc17:0. Based on 16S rRNA gene sequence analysis, strain JS_SRB250Lac^T belonged to the family of the *Desulfobulbaceae* within the *Deltaproteobacteria* and shared 94.2 % sequence identity with its closest, validly described relative *Desulfopila aestuarii*. The closest cultured, but not validly described relative was *'Desulfobacterium corrodens'* strain IS4 (96.6 % sequence identity). Based on phenotypic and phylogenetic analyses, the establishment of a novel genus and species, *Desulfocucumis infernus* gen. nov., sp. nov., is proposed with strain JS_SRB250Lac^T (= DSM 19738^T = NBRC 103921^T) as the type strain. It is furthermore suggested to include *'Desulfobacterium corrodens'* strain IS4 in this novel genus.

4.2 Introduction

Sandy surface sediments of tidal flats in the Wadden Sea are densely populated by microorganisms and characterized by high remineralization rates (Ishii et al., 2004; de Beer et al., 2005; Billerbeck et al., 2006b; Musat et al., 2006). Due to their high permeability, seawater drains into the sediment and organic matter is filtered and enriched in the sediment surface fuelling microbial activities (Huettel & Rusch, 2000; D'Andrea et al., 2002). Besides acting as "biofilters", tidal sand flats are characterized by enhanced pore water exchange through a tide-driven hydraulic pressure gradient. This phenomenon facilitates the transport of pore water down to sediments of several meters depth and provides microbial communities in the subsurface with utilizable substrates (Billerbeck et al., 2006a; Røy et al., 2008). This hypothesis was recently supported by high total prokaryotic cell numbers and the presence of a highly abundant and potentially active community of sulfate-reducing bacteria (SRB) in several meters depth of a tidal sand flat in the German Wadden Sea (Gittel et al., 2008). This study furthermore demonstrated the dominance of members of the *Desulfobacteraceae* and the *Desulfobulbaceae* using *in situ* quantification via CARD-FISH (catalyzed reporter deposition *in situ* fluorescence hybridization) and selective cultivation. Amongst others, strain JS_SRB250Lac^T and four closely related strains were isolated from highly diluted sediment samples of different depths and formed a distinct cluster within the *Desulfobulbaceae*. These newly isolated strains appeared to be representative for an abundant and active fraction of SRB providing evidence that subsurface prokaryotic communities contributed significantly to the degradation of organic matter and element cycling within the tidal flat ecosystem.

Strain JS_SRB250Lac^T exhibited less than 95 % 16S rRNA sequence identity to its closest validly described relative, *Desulfopila aestuarii* (*Dp. aestuarii*), that was isolated from an estuarine sediment in Japan (Suzuki et al., 2007a). However, strain JS_SRB250Lac^T shared more than 96.6 % 16S rRNA sequence identity with the marine sulfate reducer *'Desulfobacterium corrodens'* (*Db. corrodens*) strain IS4 (Dinh et al., 2004). *'Db. corrodens'* strain IS4 was isolated from a surface sediment in the German Wadden Sea, with elemental iron

serving as electron donor for sulfate reduction. This strain has not been validly described taxonomically yet, but it is indicated from 16S rRNA gene analysis that it should not remain within the genus *Desulfobacterium*. The present study compares phylogenetic, physiological and chemotaxonomical characteristics of strain JS_SRB250Lac^T and *'Db. corrodens'* strain IS4 and suggests the establishment of a novel genus within the *Desulfobulbaceae* distinct from *Desulfopila* with strain JS_SRB250Lac^T as the type strain.

4.3 Methods

Strain JS_SRB250Lac^T was isolated from 2.5 meter sediment depth of a tidal sand flat in the German Wadden Sea ('Janssand', 53°44.177' N, 007°41.970' E). Details of the sampling site were described elsewhere (Beck et al., 2007; Røy et al., 2008).

Desulfopila aestuarii was kindly provided by Katsuji Ueki (Yamagata University, Japan). A culture of *'Desulfobacterium corrodens'* strain IS4 was provided by Dennis Enning (Max Planck Institute for Marine Microbiology, Bremen, Germany).

4.3.1 Enrichment, isolation and cultivation

Enrichment, isolation and strain maintenance were performed in an anoxic, carbonate-buffered, mineral medium consisting of (l^{-1} distilled water): 26.0 g NaCl, 11.2 g $MgCl_2 \cdot 6\ H_2O$, 1.4 g $CaCl_2 \cdot 2\ H_2O$, 4.0 g Na_2SO_4, 0.7 g KCl, 0.1 g KBr, 0.16 g NH_4Cl, 0.2 g KH_2PO_4, 1.0 ml trace element solution SL10 (Widdel & Bak, 1992), 1.0 ml resazurin solution (50 g l^{-1}), 1.0 ml selenite-tungstate solution (Widdel & Bak, 1992), 1.0 ml vitamine solution (Balch et al., 1979), 30 ml $NaHCO_3$ (1 M) and 1.0 ml Na_2S solution (1 M) as reducing agent. The pH was adjusted to 7.2 - 7.4. Unless otherwise noted, incubations were carried out with an inoculum volume of 5 % (v/v) and at 20 °C in the dark. Strain JS_SRB250Lac^T was enriched with lactate (20 mM) as the electron donor and sulfate (28 mM) as the electron acceptor and isolated by repeated application of the deep-agar dilution method (Widdel & Bak, 1992). The purity of the culture was repeatedly checked

by microscopy and by the transfer into a complex medium containing yeast extract (1 %, w/v), glucose (10 mM) and peptone (1 %, w/v). Gram-staining of heat-fixed cells was carried out as described by Murray et al. (1994).

4.3.2 Physiology and metabolism

Growth experiments were performed in duplicate or triplicate in Hungate culture tubes or 250 ml glass flasks sealed with air-tight rubber septa. Growth was monitored by phase-contrast microscopy combined with photometric sulfide measurement (Cord-Ruwisch, 1985), if sulfate was amended as the electron acceptor, and/or by determination of cellular protein content (Bradford, 1976). Growth rates were calculated from the linear regression of formed cellular protein as a function of time. The salinity requirement for growth was determined in media with eight different NaCl concentrations between 1 g l^{-1} and 50 g l^{-1}. The temperature range for growth was determined in triplicates between 4 °C and 35 °C. Substrate utilization was tested by substituting lactate with potential electron donors including various organic acids, alcohols, amino acids and aromatic compounds (Tab. 16) Substrates were added from sterile stock solutions at final concentrations between 2 and 10 mM. Lactate and acetate were quantified with a HPLC system equipped with an Aminex HPX-87H ion exclusion column (Bio-Rad, Munich, Germany) and analyzed at 60 °C, with 5 mM H_2SO_4 as the mobile phase and UV detection at 210 nm (UVIS 204, Linear Instruments Corporation, Reno, Nevada, USA). Lithoautotrophic growth was tested by repeated transfers into organic-free medium with a headspace of H_2/CO_2 (90:10, v/v). Growth tests on different electron acceptors were prepared in sulfate-free medium which was amended with lactate (20 mM) as electron donor in combination with either nitrate (5 mM), sulfite (2 mM) or thiosulfate (10 mM) as electron acceptor. Fermentative growth was tested in medium without an additional electron acceptor supplemented with lactate, malate, pyruvate or fumarate at a final concentration of 10 mM. Growth was determined microscopically and defined as positive after the third successful subcultivation.

4.3.3 Enzyme assays

Cell-free extract of strain JS_SRB250Lac^T was prepared from a late exponential growth phase culture grown on H_2/CO_2 (90:10, v/v) as described by Galushko & Schink (2000). All enzyme assays were done under strictly anoxic conditions at 30 °C in glass cuvettes (1 ml assay mixture) sealed with butyl rubber stoppers. Anoxic conditions were achieved by flushing cuvettes with N_2 or CO corresponding to the tested enzyme. Activity of CO dehydrogenase was determined following the reduction of benzyl viologen (2 mM) at 578 nm in the presence of CO (Zeikus et al., 1977). The presence of 2-oxoglutarate:electron acceptor oxidoreductase was assessed by following the reduction of benzyl viologen (2 mM) in the presence of 2-oxoglutarate (3 mM) and CoA (0.2 mM). Protein content of the cell-free extract was determined using the bicinchoninic acid method (BCA protein assay kit, Sigma).

4.3.4 Fatty acid analysis

For cellular fatty acid analysis, strain JS_SRB250Lac^T and *'Db. corrodens'* strain IS4 were cultivated in 250 ml glass flasks sealed with air-tight rubber septa containing 150 ml seawater medium amended with lactate (20 mM) and sulfate (28 mM). Cells from the late exponential phase were harvested by centrifugation. Fatty acid methyl esters (FAME) were obtained by saponification, methylation and extraction (Sasser, 2001). Positions of double bonds in FAME were determined by analysis of their dimethyl disulfide (DMDS) adducts according to the method of Dunkelblum et al. (1985). FAME were quantified by a gas chromatograph (Hewlett Packard HP 5890 Series II gas chromatograph, Hewlett Packard, Waldbronn, Germany) equipped with a DB-5HT capillary column (30 m × 0.25 mm, 0.1 µm film thickness, J&W, Folsom, CA, USA), coupled to a flame ionization detector (FID) and identified with gas chromatography-mass spectrometry using a Finnigan MAT SSQ 710B mass spectrometer (Finnigan-Thermoquest, San Jose, CA, USA). The carrier gas was helium (constant pressure of 12 psi). The oven temperature was run from 60 °C (isothermal for 2 min) to

360 °C at a rate of 3 °C min^{-1} and hold for 5 min. Mass spectra were collected in full scan mode (*m/z* 50 - 650, ionization energy 70 eV).

4.3.5 G + C content of genomic DNA

The G + C content of the genomic DNA was determined by means of HPLC at the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany).

4.3.6 Nucleic acid extraction, PCR amplification and sequencing

Nucleic acid extraction, amplification, cloning and sequencing of the 16S rRNA gene of JS_SRB250Lac^T were performed as described previously (Gittel et al., 2008). Additionally, approximately 2000 basepairs of the *dsr*AB gene sequence (genes encoding the α- and β-subunits of the dissimilatory sulfite reductase) for strain JS_SRB250Lac^T were amplified using the primers DSR1F and DSR4R (Wagner et al., 1998), cloned and plasmid inserts sequenced using the primers M13F, M13R and a custom-designed internal primer (‘5-GTGCCTTTGATCTGCAG-3’). The *apr*BA gene sequences (genes encoding the adenosine-5’-phosphosulfate reductase α- and β-subunits) of strain JS_SRB250Lac^T and *D. aestuarii* were determined using two sets of degenerate primers (AprB-1-FW/AprA-5-RV and AprA-1-FW/Apr-10-RV), a RED*Taq* DNA Polymerase kit and thermal cycling according to Meyer & Kuever (2007). PCR products of the expected size (1.2 - 1.35 kb and 1.4 kb, respectively) were cut out of agarose gels, purified and sequenced directly as described previously (Meyer & Kuever, 2007).

4.3.7 Phylogenetic analysis

Phylogenetic trees based on 16S rRNA gene sequence datasets (> 1400 nucleotides) were constructed using the neighbour-joining and the maximum-likelihood algorithms implemented in the ARB program package (Ludwig et al., 2004). *Desulfovibrio vulgaris* subsp. (a total of 20 sequences) were used as the outgroup. To simplify the trees, species not closely related to strain JS_SRB250Lac^T were

removed after calculation. DsrAB and AprBA amino acid sequence-based phylogenetic trees were inferred using the phyML program (maximum-likelihood method; http://atgc.lirmm.fr/phyml). Maximum-likelihood trees were constructed using the WAG amino acid substitution model matrices. The robustness of inferred trees was tested by bootstrap analysis with 100 resamplings. Datasets (deduced from *dsr*AB and aprBA gene sequences) including all available unambiguously aligned DsrAB and AprBA amino acid sequence positions of members of the family *Desulfobulbaceae* were analyzed.

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences reported in this study are AM774321 (16S rRNA gene sequence of JS_SRB250Lac^T), FJ548990 (*dsr*AB gene sequence of strain JS_SRB25Lac^T) and FJ548989 and FJ548988 (*apr*BA gene sequences of strain JS_SRB25Lac^T and *Desulfopila aestuarii*, respectively).

4.4 Results and Discussion

The cells of strain JS_SRB250Lac^T were straight rods with rounded ends, 0.3 - 0.5 µm wide and 1.0 - 2.0 µm long (Fig. 6). Longer cells of up to 5 µm developed at low temperatures (< 15 °C). Cells of strain JS_SRB250Lac^T formed light-brown colonies in agar tubes and aggregated during growth in liquid medium. The cells were Gram-negative and non-motile. Formation of endospores was not observed.

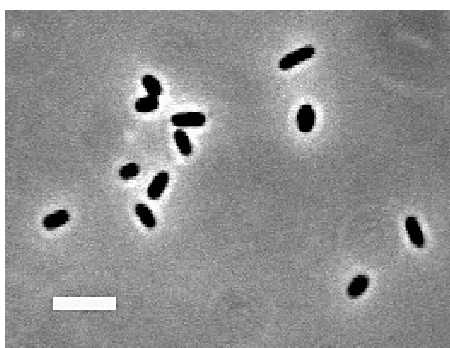

Fig. 6: Phase contrast micrograph of strain JS_SRB250Lac^T. The scale bar corresponds to 2 µm.

Strain JS_SRB250Lac^T grew in the presence of NaCl at concentrations from 5 ‰ to 50 ‰ (w/v). Optimum growth occurred between 20 ‰ and 30 ‰ NaCl (w/v). The temperature range for growth was 10 °C to 35 °C with an optimum at 28 °C. Strain JS_SRB250Lac^T utilized a variety of different classes of organic compounds as electron donors for sulfate reduction, including lactate, formate, butyrate, ethanol, glycerol, proline as well as hydrogen (see Tab. 16 for more details). Cultivation with lactate and sulfate at optimum temperature and salinity yielded a maximum growth rate of 0.198 day^{-1}. These values were in the same range as reported for *'Desulfobacterium corrodens'* strain IS4 (Dinh et al., 2004). In contrast, *Desulfopila aestuarii* grew ten times faster under the same conditions (Suzuki et al., 2007a). All three strains oxidized lactate incompletely to acetate. In cell-free extract of strain JS_SRB250Lac^T grown on H_2/CO_2, active CO dehydrogenase was found (205 nmol (mg protein^{-1}) min^{-1}), whereas 2-oxoglutarate:electron acceptor oxidoreductase (a key enzyme in the citric acid cycle) was not detected. This indicates the operation of the reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) for CO_2 fixation. Fermentative growth was observed with pyruvate and fumarate. In addition to sulfate, sulfite was utilized as electron acceptor in the presence of lactate as the electron donor. Thiosulfate and nitrate were not utilized.

The main fatty acids of both JS_SRB250Lac^T and *'Db. corrodens'* strain IS4 were the saturated straight-chained *n*-16:0 and *n*-17:0, the unsaturated straight-chained *n*-16:1*c*9 and the cyclopropane fatty acid cyc17:0 (Tab. 15). Thus, these chemotaxonomical signatures support the close phylogenetic relationship of both strains and the distinctness to *Dp. aestuarii* that lacked the cyclopropane fatty acid cyc17:0.

Tab. 15: Cellular fatty acid composition (weight %) of strain JS_SRB250Lac^T (1), *'Desulfobacterium corrodens'* strain IS4 (2, data from this study) and *Desulfopila aestuarii* strain MSL86T (3) (Suzuki et al., 2007a).

Fatty acid	1	2	3
***n*-14:0**	1.1	1.2	1.4
***n*-15:0**	1.6	1.3	-
***n*-15:1**	-	1.2	-
***n*-16:0**	23.3	15.6	33.6
***n*-16:1*c*7**	0.7	-	-
***n*-16:1*c*9**	18.3	11.1	6.0
***n*-16:1*c*11**	11.4	21.9	17.1
***n*-17:0**	8.3	5.9	3.4
***n*-17:1*c*9**	-	1.6	13.7
cyc17:0	15.3	22.5	-
***n*-18:0**	11.5	3.9	2.5
***n*-18:1*c*11**	8.6	7.6	5.7
***n*-18:1*c*13**	-	3.9	-

Phylogenetic analyses based on 16S rRNA gene sequence datasets showed that strain JS_SRB250Lac^T is grouped within the deltaproteobacterial family of the *Desulfobulbaceae* (Fig. 7) and formed a distinct cluster with four other strains isolated from the same habitat (Gittel et al., 2008). Members of this cluster shared more than 99 % 16S rRNA gene sequence identity with each other and are therefore assumed to represent the same species. The closest related and validly described species to strain JS_SRB250Lac^T was *Desulfopila aestuarii* isolated from an estuarine sediment in Japan (Suzuki et al., 2007a). As both strains shared only 94.2 % 16S rRNA gene sequence identity, strain JS_SRB250Lac^T is proposed to represent a novel genus within the *Desulfobulbaceae* that is distinct from the genus *Desulfopila*. '*Desulfobacterium corrodens*' strain IS4 was identified to be the closest cultured relative to strain JS_SRB250Lac^T sharing 96.6 % 16S rRNA sequence identity (Dinh et al., 2004).

Tab. 16: Comparison of selected characteristics of strain JS_SRB250Lac^T (1), *'Desulfobacterium corrodens'* strain IS4 (2) (Dinh, 2003; Dinh et al., 2004) and *Desulfopila aestuarii* strain MSL86T (3) (Suzuki et al., 2007a). Temperature and salinity range of growth are given in parentheses. +, positive; - negative; NA no data available. ND not determined.

Characteristic	**1**	**2**	**3**
Isolation source	Marine sediment	Marine sediment	Estuarine sediment
Morphology	Rod	Rod	Rod
G+C content (mol %)	50.3	51.9	54.5
Optimum salinity (g NaCl l^{-1})	20-30 (5-50)	10-15 (NA)	10 (<50)
Optimum temperature (°C)	28 (10-35)	28-30 (NA)	35 (10-40)
Utilization of electron donors (final concentration, mM) *			
Formate (10)	+	+	ND
Acetate (10)	-	-	-
Propionate (10)	-	-	-
n-Butyrate (5)	+	-	-
Lactate (10)	+	+	+
Fumarate (10)	+	-	+
Pyruvate (10)	+	+	+
Malate (10)	-	-	-
Succinate (10)	+	-	-
Methanol (10)	-	ND	-
Ethanol (10)	+	-	+
1-Propanol (10)	+	ND	+
1-Butanol (10)	+	ND	+
Glycerol (10)	+	ND	+
Glycine (10)	-	ND	-
Alanine (10)	-	-	-
Serine (10)	-	ND	-
Benzoate (10)	-	-	ND
H_2	+	+	-
H_2 + Acetate (2)	+	+	-
Utilization of electron acceptors (final concentration, mM)			
Nitrate (5)	-	ND	-
Sulfite (2)	+	-	+
Thiosulfate (10)	-	-	+
Fermentative growth	pyruvate, fumarate	pyruvate	pyruvate, fumarate

*Strain JS_SRB250Lac additionally utilized valerate (5), caproate (3), caprate (2) and proline (10), but not betaine (10), choline (10), sorbitol (5) and mannitol (5). These compounds were not tested for any of the two cultured relatives.

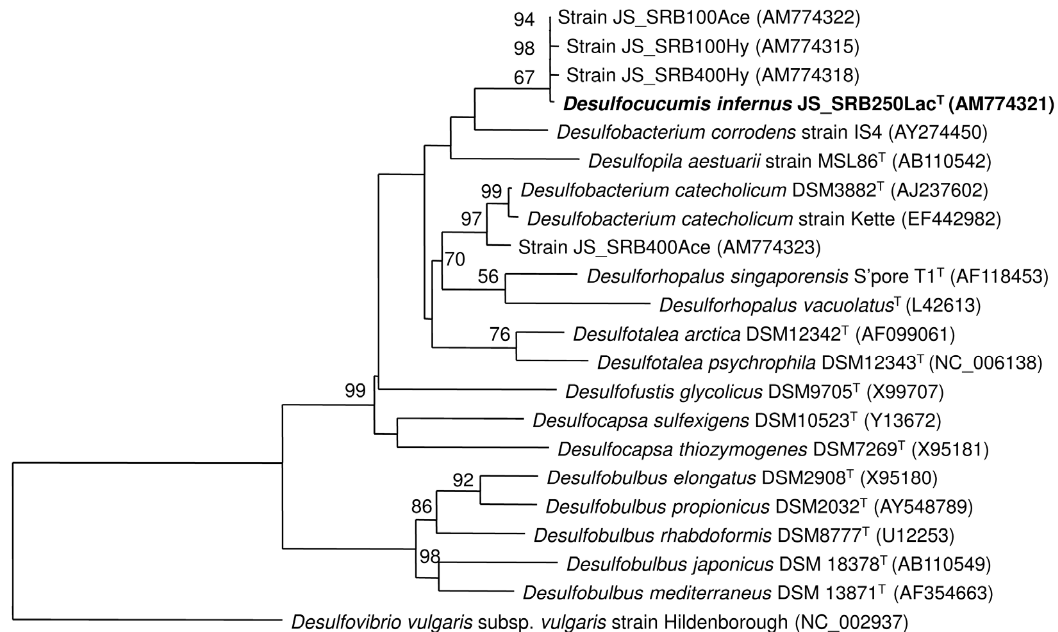

Fig. 7: Neighbour-joining tree showing the affiliation of the 16S rRNA gene sequence of strain JS_SRB250Lac^{T} to selected reference sequences from members of the *Deltaproteobacteria.* Bootstrap values are based on analysis of 1000 replicates, only values > 50 % are indicated near the nodes. The tree topology inferred from maximum-likelihood analysis was almost similar to that obtained from the neighbour-joining method. The scale bar represents 10 % sequence divergence.

This strain is not validly described so far, but its phylogenetic affiliation to strain JS_SRB250Lac^{T} appeared to meet the criterion for the incorporation into the proposed novel genus. The affiliation of strain JS_SRB250Lac^{T} to the *Desulfobulbaceae* was confirmed by DsrAB and AprBA amino acid sequence analyses (Fig. 8). In addition, AprBA amino acid sequence analyses supported its close relationship to *'Db. corrodens'* and *Dp. aestuarii* .

Although *'Db. corrodens'* strain IS4 and JS_ SRB250Lac^{T} are phylogenetically closely related and assumed to belong to one genus (> 95 % 16S rRNA sequence identity; Stackebrandt, 1994), they clearly differ with respect to their physiological properties. The strains were isolated from either surface or subsurface sediments in the German Wadden Sea. The lower salinity optimum of *'Db. corrodens'* strain IS4 (10 ‰ – 15 ‰) might be inferred from its exposure to freshwater in surface sediments, whereas JS_ SRB250Lac^{T} tolerated salt concentrations of up to 50 ‰. Besides its striking property to anaerobically oxidize

elemental iron, *'Db. corrodens'* was shown to utilize only few other electron donors, *e.g.* lactate and pyruvate. However, cultivation with these alternative electron donors yielded only poor growth compared to the utilization of elemental iron (Dinh et al., 2004). In contrast, strain JS_SRB250Lac^T is nutritionally versatile utilizing a variety of short-chain organic acids, alcohols as well as hydrogen as electron donors (Tab. 16), but did not utilize elemental iron as electron donor (Dennis Enning, personal communication). In addition, sulfate was found to be the only electron acceptor for candidatus *'Db. corrodens'*, whereas strain JS_SRB250Lac^T both reduced sulfate and sulfite.

4.4.1 Description of *Desulfocucumis* gen. nov.

On the basis of its phylogenetic and phenotypic properties, it is proposed that strain JS_SRB250Lac^T represents the type strain of a novel genus and species in the class *Deltaproteobacteria*, for which the name *Desulfocucumis infernus* gen. nov., sp. nov. is proposed.

Desulfocucumis (De.sul.fo.cu.'cu.mis. L. pref. *de* from; L. n. *sulfur* sulfur; L. m. n. *cucumis* cucumber; sulfate reducer shaped like a cucumber).
Mesophilic, strictly anaerobic, marine. Cells are straight rods, Gram-negative and non-spore-forming. Lactate is oxidized incompletely to acetate. Chemolithoautotrophic growth with H_2 plus CO_2/HCO_3^-. The type strain is *Desulfocucumis infernus*.

4.4.2 Description of *Desulfocucumis infernus* sp. nov.

Desulfocucumis infernus (in.'fer.nus. from lower site/underground/subsurface).
Cells are straight rods with rounded ends, 0.3 - 0.5 µm in width and 1.0 - 2.0 µm in length. Non-motile. The NaCl range for growth is 5 ‰ to 50 ‰ (w/v) with an optimum between 20 ‰ and 30 ‰ (w/v). The temperature range for growth is 10 to 35 °C with an optimum at 28 °C. Utilizes lactate, formate, fumarate, pyruvate, ethanol and hydrogen as electron donors for sulfate reduction. Does not utilize acetate, propionate and malate. Sulfate and sulfite serve as electron acceptors.

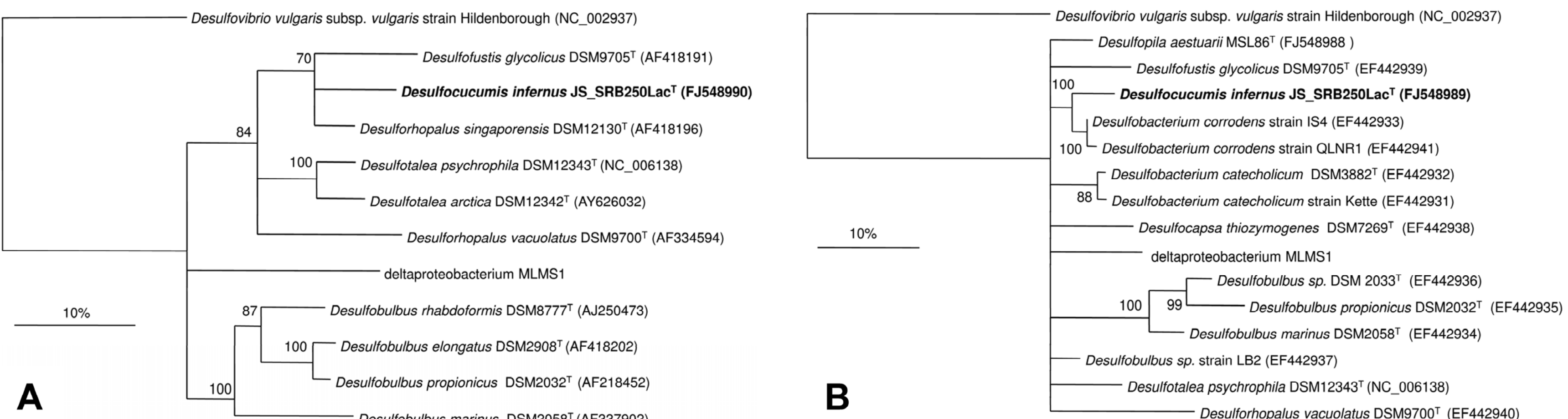

Fig. 8: Phylogenetic trees based on DsrAB **(A)** and AprBA amino acid sequences **(B)**. The sequences were deduced from *dsr*AB and *apr*BA sequences. Trees were inferred using phyML (maximum-likelihood method). *Desulfovibrio vulgaris* was used as outgroup. Percentages greater than 70 % of bootstrap resampling (100 replicates) that support each topological element are indicated near the nodes. The scale bar represents 10 % sequence divergence.

Pyruvate and fumarate are fermented in the absence of electron acceptors. The genomic DNA G + C content is 50.3 mol%. The major cellular fatty acids are *n*-16:0, *n*-17:0, *n*-16:1*c*9, and cyc17:0. The type strain is JS_SRB250Lac^T (= DSM 19738 = NBRC 103921).

4.5 Acknowledgements

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5 Two distinct *Photobacterium* populations thrive in ancient Mediterranean sapropels

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5.1 Abstract

Eastern Mediterranean sediments are characterised by the periodic occurrence of conspicuous, organic matter-rich sapropel layers. Phylogenetic analysis of a large culture collection isolated from these sediments revealed that about one third of the isolates belonged to the genus *Photobacterium*. In the present study 22 of these strains were examined with respect to their phylogenetic and metabolic diversity. The strains belonged two distinct *Photobacterium* populations (Mediterranean cluster I and II). Strains of cluster I were isolated almost exclusively from organic-rich sapropel layers and were closely affiliated with *P. aplysiae* (based on their 16S rRNA gene sequences). They possessed almost identical ERIC and substrate utilization patterns, even among strains from different sampling sites or from layers differing up to 100,000 years in age. Strains of cluster II originated from sapropels as well as from the surface and carbon-lean intermediate layers. They were related to *P. frigidiphilum*, but differed significantly in their fingerprint patterns and substrate spectra, even when these strains were obtained from the same sampling site and layer. Temperature range for growth (4 to 33 °C), salinity tolerance (5 to 100 ‰), pH requirements (5.5 - 9.3) and the composition of polar membrane lipids were similar for both clusters. All strains grew by fermentation (glucose, organic acids) and all but five by anaerobic respiration (nitrate, dimethyl sulfoxide, anthraquinone disulfonate or humic acids). These results indicate that the genus *Photobacterium* forms subsurface populations well adapted to life in the deep biosphere.

5.2 Introduction

In the recent years the marine deep subsurface received increasing attention. Geochemical analyses and modeling (D'Hondt et al., 2004) but also radiotracer-based activity measurements (Parkes et al., 1994, 2005), direct microscopic or viable counts (D'Hondt et al., 2004; Schippers et al., 2005) revealed the presence of active microbial communities in up to 15 Ma old marine sediments (Wellsbury et al., 2002). It was also shown that cell densities and metabolic activities in deeply buried sediments correlate with the availability of organic carbon (Coolen et al., 2002; Parkes et al., 2005) and/or electron acceptors (Cowen et al., 2003; D'Hondt et al., 2004; DeLong, 2004). Extensive molecular analyses were performed and unraveled an unexpectedly large microbial diversity (Kormas et al., 2003; Newberry et al., 2004), including some phylogenetic lineages appearing to be typical for deep subsurface habitats (Coolen et al., 2002; Webster et al., 2004). However, due to the low fraction of sediment microbes that have been brought into pure culture (D'Hondt et al., 2004; Toffin et al., 2004), there is a lack in knowledge of physiological adaptations of indigenous deep biosphere bacteria.

One aspect lowering the cultivation success might be the application of high substrate concentrations in standard microbiological media. Apparently, the use of media containing submillimolar substrate concentrations increased the cultivation efficiency and resulted in the subsequent isolation of a large culture collection from ancient Mediterranean sapropels (Süß et al., 2004). These dark, periodically (approximately every 20,000 years) occurring sediment layers differ from other subsurface environments in their unusually high organic carbon contents (up to 30 % of the dry weight; Rohling, 1994). It is assumed they were deposited during periods of intense precipitation with a high riverine influx of freshwater leading to increased productivity but also to haline stratification (Lourens et al., 1992). The latter prevented mixing of oxygen-rich surface and deep waters, eventually leading to anoxic conditions and enhanced preservation of organic material (Rohling, 1994). Sapropels are interspersed in-between carbonate-rich and extremely organic carbon poor hemipelagic sediments that were deposited under highly oligotrophic conditions like they prevail today. Although

the organic material within the sapropels consists mainly of highly recalcitrant kerogen (Killops & Killops, 2004), sapropels were shown to be subsurface ‘hot-spots’ with elevated microbial numbers and increased potential microbial activities (Cragg et al., 1998; Coolen & Overmann, 2000; Coolen et al., 2002).

The culture collection from sapropels of the Eastern Mediterranean included 98 strains covering 19 different phylotypes. Phylogenetic analysis revealed that about a third of the strains affiliated with the soil bacterium *Rhizobium radiobacter* (Süß et al., 2004). The occurrence of this phylotype as a member of the deep biosphere was recently confirmed by molecular methods (Süß et al., 2006). About another 30 % of the strains belonged to the genus *Photobacterium* (Süß et al., 2004). This genus was one of the first described bacterial taxa (Beijerinck, 1889) and was originally considered to be generally associated with marine animals (Salle, 1961). Although photobacteria were found to be widespread in marine sediments (*e.g. P. profundum* and *P. frigidiphilum*; Nogi et al., 1998; Seo et al., 2005a) their prevalence in the culture collection obtained from the up to 120,000 years old sapropels is unexpected and extends their ‘typical’ habitat range. Since *Photobacterium* sp. are among the dominant cultured subsurface bacterial groups, analysis of their physiology might help to deepen the knowledge of metabolic adaptations of sediment microbial communities.

Several recent studies have revealed a remarkable phylogenetic (Pukall et al., 1999; Acinas et al., 2004; Thompson et al., 2005b) or physiological (Moore et al., 1998; Bagwell & Lovell, 2000; Jaspers & Overmann, 2004) heterogeneity at the subspecies level within single bacterial taxa. The ecological significance of this phenomenon still remains elusive (Fuhrman & Campbell, 1998) but it was assumed that this microdiversity is amongst others a prerequisite for longevity of bacterial populations in changing environments (Arber et al., 1994; Cowan, 2000; Schloter et al., 2000). In the present study 22 *Photobacterium* strains originating from three different sampling sites in the Eastern Mediterranean Sea were examined with respect to their phylogenetic and metabolic diversity. The extent of the culture collection offered the opportunity to link variations in 16S rRNA genes and Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR fingerprinting

patterns to physiological differences and correlate these to spatial separation or to age and TOC-content of the sediment layers.

5.3 Material and Methods

5.3.1 Sample origin and isolation of the *Photobacterium* strains

All strains analyzed in this study were obtained from eastern Mediterranean sapropels and from hemipelagic carbon-lean intermediate layers sampled during R/V Meteor cruises M 40/4 and M 51/3 (Coolen & Overmann, 2000; Süß et al., 2004). Samples were taken from three different sites: Site 67 (34° 48.83' N, 27° 17.77' E, sampled in January 1998) and site 567 (34° 48.79' N, 27° 17.13' E, 200 m apart from each other sampled in November 2001) were located approximately 200 m apart from each other and situated about 100 km east of the island of Crete (Greece). Site 575 (34° 31.39' N, 31° 46.40' E) was sampled in November 2001 and located about 65 km west of Cyprus. Each core was cut longitudinally which left behind a potentially contaminated surface. After covering with cling film, rapidly freezing this surface with dry ice and lifting it off, 5 cm^3 subsamples were retrieved aseptically from the undisturbed sediment underneath using cut-off sterile plastic syringes (Coolen et al., 2002).

Most strains were isolated from highest positive dilutions of oxic and anoxic MPN series that were prepared onboard ship in a polyethylene chamber (AtmosBag, 280 l, Aldrich, Milwaukee, Wisconsin, USA) under a nitrogen atmosphere. Anoxic MPN plates were incubated in gas-tight plastic bags equipped with a gas generating and catalyst system (Anaerocult C mini, Merck, Darmstadt, Germany) (Süß et al., 2004). MPN series were supplemented with different carbon sources and electron acceptors (Tab. 17). Strains 67TD and 67FSB were obtained from anoxic enrichments directly inoculated with sapropel material. For isolation under anoxic conditions cultures were diluted in agar-solidified media in tubes (deep agar dilution series) under a N_2 atmosphere, whereas aerobes were obtained by repeated streaking on agar plates (Süß et al., 2004).

5.3.2 Extraction of nucleic acids

Genomic DNA of the strains was extracted following a standard protocol with cell lysis by lysozyme, SDS and “freeze and thaw” cycles with subsequent purification as described by Süß et al. (2006). Purified nucleic acids were resuspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and stored at -20 °C.

5.3.3 Phylogenetic analysis

The 16S rRNA genes of the strains were amplified and partial sequences determined as described elsewhere (Süß et al., 2004). Sequences (892 to 1300 bp long) were compared to those available in the GenBank database using the BLASTN tool. Six partial sequences from *Photobacterium* sp. (strains J2, J4, J158, S1D, Z0F, Z1E) isolated from the same sites but lost prior to this study were included in the phylogenetic analysis. Phylogenetic trees were constructed using the ARB software package (Ludwig et al., 2004). The maximum likelihood method was used for the construction of backbone-trees considering sequences of validly described species with sequence lengths of at least 1300 bp. Sequences of recently described species (Seo et al., 2005a,b; Thompson et al., 2005a; Yoon et al., 2005) not yet available in the ARB database release were retrieved from GenBank. Sequences < 1300 bp were added afterwards by parsimony interactive using a newly constructed specific *Photobacterium* filter. To verify the stability of the Mediterranean *Photobacterium* clusters, neighbour-joining and parsimony trees were calculated as described for the maximum likelihood method.

5.3.4 ERIC-PCR

To investigate subspecies diversity of closely related strains genomic fingerprinting using the primers ERIC1R and ERIC2 (Versalovic et al., 1991) was performed. The PCR reaction mix contained 1 U Red Taq DNA polymerase (Sigma, Munich, Germany) and the appropriate 10 × buffer, dNTPs (200 µM each), $MgCl_2$ (2.1 mM), BSA (0.2 ng · μl^{-1}), the primers ERIC1R and ERIC2 (5 pM each) and 4 ng μl^{-1} template DNA in a final volume of 50 µl. PCR was performed in a Perkin Elmer thermocycler (Perkin Elmer Gene Amp PCR System 9600, Welles-

ley, MA). The following protocol was used according to Versalovic et al. (1991): 4 min denaturation at 96 °C, followed by 35 cycles with denaturation for 30 sec at 94 °C, annealing for 1 min at 52 °C, and elongation for 8 min at 72 °C. Post elongation was performed for 10 min at 72 °C. Fragments were separated on 1 % (w/v) agarose gels (90 V for 3 h). Gels were stained for 20 min with ethidium bromide and documented by means of a digital imaging system and the respective software (BioDoc Analyze Biometra, Göttingen, Germany). The resulting band patterns were analyzed using the software package GelCompar II Version 2.5 (Applied Maths, St-Martens-Latem, Belgium). The computer-generated densitometric curves were compared using the Pearson coefficient. Dendrograms were generated by the UPGMA-method (unweighted-pair group method with arithmetic averages).

5.3.5 Growth media

Artificial seawater reflecting the average element composition of the Mediterranean Sea was used as a basal medium. This medium contained (in $g \cdot l^{-1}$): NaCl (24.3), $MgCl_2 \cdot 6\,H_2O$ (10), $CaCl_2 \cdot H_2O$ (1.5), KCl (0.66), Na_2SO_4 (4), KBr (0.1), H_3BO_3 (0.025), $SrCl_2 \cdot 6\,H_2O$ (0.04), NH_4Cl (0.021), KH_2PO_4 (0.0054), NaF (0.003). The medium was supplemented with $1\ ml \cdot l^{-1}$ trace element solution SL10 and $0.2\ ml \cdot l^{-1}$ of a selenite and tungstate solution (Widdel & Bak, 1992). The oxic medium was buffered with HEPES ($2.4\ g \cdot l^{-1}$). The pH of the medium was adjusted to 7.2 - 7.4 with NaOH prior to autoclaving. After autoclaving, the medium was cooled under air and supplemented with vitamins (Balch et al., 1979) and sodium bicarbonate (final concentration $0.2\ g \cdot l^{-1}$). For routine growth, temperature and pH tests a dilute yeast extract-peptone medium (Süß et al., 2004) was used containing yeast extract ($0.03\ g \cdot l^{-1}$), peptone ($0.06\ g \cdot l^{-1}$), sodium lactate ($5\ mmol \cdot l^{-1}$), glucose ($1\ mmol \cdot l^{-1}$), sodium thiosulfate ($1\ mmol \cdot l^{-1}$), vitamins and sodium bicarbonate ($0.2\ g \cdot l^{-1}$).

For anoxic incubations a slightly different medium was used. It contained resazurin ($0.25\ mg \cdot l^{-1}$) as a redox indicator. After autoclaving the anoxic medium was cooled under an atmosphere of N_2/CO_2 (80/20, v/v). Instead of HEPES, $30\ ml \cdot l^{-1}$ of a 1 M sodium hydrogen carbonate solution was added as a buffer

from a sterile stock. After addition of vitamins the medium was carefully reduced by addition of sterile dithionite until the resazurin turned colorless. The pH of the reduced medium was set to 7.2 - 7.4 with sterile HCl or Na_2CO_3 if necessary.

5.3.6 Substrate utilization under oxic conditions

For tests on substrate utilization, 56 different carbon sources were chosen for growth tests under oxic conditions. Assays were set up in polystyrene microtiter plates (Costar 3795, Corning, New York, NY). Each well was filled with 180 µl oxic basal medium. The following carbon sources were provided (final concentrations are given in brackets). (I) complex substrates: peptone (0.05 % w/v), casamino acids (0.05 % w/v), yeast extract (0.005 % w/v), (II) polysaccharides: cellulose (0.05 % w/v), starch (0.1 % w/v), chitin (0.05 % w/v), xylan (0.05 % w/v) and laminarin (0.05 % w/v), (III) mono- and disaccharides, sugar derivates: sucrose, cellobiose, maltose, trehalose, arabinose, xylose, fructose, glucose, mannose, rhammnose, mannitol, gluconate and glucosamine (each at 5 mM), (IV) organic acids: lactate and succinate (each at 10 mM), formate, acetate, malonate, fumarate, malate, 2-oxoglutarate, glycolate and pyruvate (each at 5 mM), butyrate (2.5 mM), tartrate (2 mM), citrate (2 mM), propionate (1 mM), capronate, caprylate, crotonate and valerate (each at 0.5 mM), (V) alcohols: ethanol, *n*-propanol, *n*-butanol, ethylene glycol, glycerol (each at 5 mM), methanol (2 mM) and Tween80 (0.001 % w/v), (VI) L-amino acids: alanine, arginine, cysteine, glutamine, isoleucine and phenylalanine (each at 2 mM), (VII) miscellaneous compounds: betaine, benzoate, salicylate and nicotinate (each at 2 mM). Cells were harvested from exponential phase liquid cultures and washed three times. Three replicates and two substrate free controls were inoculated for each strain as well as two inoculum free controls for each substrate. The plates were incubated at 20 °C for six weeks. Growth was determined by fluorometry (Martens-Habbena & Sass, 2006) as well as by phase contrast microscopy of selected wells.

5.3.7 Use of electron acceptors

The tests were performed in completely-filled, screw cap glass tubes with anoxic artificial seawater as basal medium. Acetate (10 mM) was used as electron donor and carbon source. NO_3^- (10 mM), $Fe(OH)_3$ (40 mM), manganese oxides (20 mM), thiosulfate (10 mM), elemental sulfur (20 mM), dimethyl sulfoxide (DSMO, 10 mM), 9,10-anthra-quinone-2,6-disulfonate (AQDS, 4 mM) and an iron-free humic acid suspension (1 mg ml^{-1}, Benz et al., 1998) were chosen to test anaerobic growth with alternative electron acceptors. For each strain and electron acceptor combination three replicates were inoculated as well as an inoculum-free control and a control without electron acceptor. The assays were incubated for six weeks at 20 °C in the dark. Growth was monitored by phase contrast or epifluorescence microscopy after staining with DAPI if necessary. The conversion of nitrate to nitrite or ammonium was determined photometrically according to Grasshoff et al. (1999). Reduction of Fe(III) to Fe(II) was generally indicated by the disappearance of reddish ferric hydroxide and the formation of black precipitates. The utilization of Mn(IV) led to decolorisation and finally disappearance of the brown manganese oxides and the precipitation of whitish manganese carbonates. The production of sulfide due to the reduction of thiosulfate, sulfite or elemental sulfur was measured photometrically after addition of an acidic cupric solution at 436 nm in accordance to Widdel (1980). Reduction of AQDS to the reduced antrahydroquinone was measured photometrically at 450 nm (Lovley et al., 1996).

5.3.8 Fermentative growth

Fermentative growth was tested in completely-filled screw cap glass tubes filled with anoxic artificial seawater supplied either with glucose (10 mM), a mixture of L-amino acids (alanine, threonine, lysin-monohydrochloride, arginine, asparagine, aspartate, leucine, isoleucine, glumatate, glutamine and methionine, each 5 mM), organic acids (malate, fumarate, succinate and lactate, each 15 mM) or a mixture of alcohols (methanol, ethanol, *n*-propanol, and *n*-butanol, each 10 mM) as substrates. For each substrate three replicates and a substrate free control were

incubated at 20 °C for six weeks. Growth was monitored by phase contrast microscopy. Assays proven positive for glucose consumption were analyzed by reversed phase HPLC using a Waters HPLC system (Waters, Milford, MA) equipped with a Syneri 4µ Hydro-RP column (Phenomex, Aschaffenburg, Germany) and using phosphate buffer (50 mM KH_2PO_4, pH 2.9) as eluent. As standards 12 different carboxylic acids (oxalate, gluconate, formate, pyruvate, malate, 2-oxoglutarate, lactate, acetate, citrate, fumarate, succinate and propionate) were used. Data analysis was done using the Millenium[32] 3.05.01. Software (Waters, Eschborn, Germany).

5.3.9 Similarity analysis of physiological data

The results of all physiological tests were used for the construction of a matrix with a binary code for the presence or absence of each phenotypic trait. Similarity check was performed using the MVSP 3.1 Software (Kovach Computing Services, Pentreath, UK) and the Dice coefficient for calculation of the distance matrix.

5.3.10 Determination of intact phospholipids and phospholipid fatty acids

Cells were grown in oxic media amended with lactate (10 mM), harvested at the end of the exponential growth phase by centrifugation, washed with phosphate buffer (130 mM NaCl, 5 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.4), freeze-dried and stored at -20 °C. Aliquots (30 - 100 mg) of the freeze-dried cells were extracted ultrasonically up to ten times for 10 minutes each using a solvent mixture of methanol/dichloromethane/ammonium acetate buffer pH 7.6 (2:1:0.8 by volume) in centrifuge tubes (modified after Vancanneyt et al., 1996) After centrifugation at 2200 × g at 15 °C for 10 min the supernatants were removed and collected in a separatory funnel. Dichloromethane and ammonium acetate buffer (pH 7.6) were added to the combined extracts to achieve a final ratio of methanol/dichloromethane/ammonium acetate buffer of 1:1:0.9 (by volume). After phase separation, the organic phase was removed and the aqueous phase was re-

extracted with dichloromethane five times. Combined extracts were dried over anhydrous sodium sulfate, evaporated to dryness and stored at -20 °C.

Lipid extracts were dissolved in 1 ml dichloromethane/methanol 9:1 (by volume) and then chromatographically separated according to Zink and Mangelsdorf (2004). Two glass columns in sequence filled with pure silica (1 g silica 60, 63 - 200 µm, dried at 110 °C for 16 h) and Florisil (1 g magnesium silica gel 150 - 250 µm, Merck, Darmstadt, Germany) were used to gain four fractions: (1) neutral lipids (eluted with 20 ml dichloromethane); (2) free fatty acids (50 ml methyl formate with 0.025 % v/v pure acetic acid); (3) glycolipids (20 ml acetone), and (4) phospholipids. To obtain the phospholipid fraction, the Florisil column was removed and only the silica column was eluted with 25 ml methanol. All fractions were evaporated to dryness and stored at -20 °C.

Aliquots of the phospholipid fractions were transesterified with trimethylsulfoniumhydroxide as described by Müller et al. (1998). The methyl esters obtained were quantified by GC-FID (Hewlett Packard HP 5890 Series II gas chromatograph, Hewlett Packard, Waldbronn, Germany) equipped with a DB-5HT capillary column (30 m × 0.25 mm, 0.1 µm film thickness, J&W Folsom, CA, USA) and identified by GC-MS using a Finnigan MAT SSQ 710B mass spectrometer (Finnigan-Thermoquest, San Jose, CA, USA). The carrier gas was helium (constant pressure of 12 psi). The oven temperature was raised from 60 °C (isothermal for 2 min) to 360 °C at a rate of 3 °C min^{-1} and held for 5 min. Mass spectra were collected in full scan mode (*m/z* 50 - 650, ionization energy 70 eV). Positions of double bonds were tentatively assigned by comparison with retention times of standards (Bacterial Acid Methyl Esters CP Mix; Supelco, Bellefonte, PA, USA).

5.4 Results

5.4.1 Phylogenetic affiliation of the *Photobacterium* strains

Based on short 16S rRNA gene fragments analyzed previously the isolates were originally found to be closely related to *Photobacterium profundum* (Süß et al., 2004). However, several novel *Photobacterium* species have been described since then (*P. aplysiae*, Seo et al., 2005b; *P. frigidiphilum*, Seo et al., 2005a; *P. lipolyticum*, Yoon et al., 2005 and *P. rosenbergii*, Thompson et al., 2005a), while the former *Hyphomicrobium indicum* was transferred to this genus (Xie & Yokota, 2004). These sequences, as well as almost complete 16S rRNA gene sequences (most of them 1100 to 1300 bp long) of the isolates from the Mediterranean Sea sediments were now included into the phylogenetic analysis.

It turned out that the isolates shared between 96 and 100 % sequence similarity of the 16S rRNA genes with each other. Their sequences affiliated with a phylogenetic branch containing *P. aplysiae*, *P. frigidiphilum*, *P. indicum*, *P. lipolyticum* and *P. profundum* (Fig. 9). The strains were most closely related to either *P. frigidiphilum* or *P. aplysiae* (sequence similarities 97.1 to 99.5 % and 96.8 to 99.5 %, respectively), suggesting that the strains belong to two separate phylogenetic clusters (Mediterranean cluster I and II; Fig. 9). Although phylogenetic trees obtained by different methods showed some variation in the branching patterns (what is supported by the low bootstrap values on the maximum likelihood tree shown in Fig. 9), consistently two separate clusters were obtained. Cluster I comprised strains obtained from all three sampling sites. Most of them were isolated from sapropels (strains S4, S10, S12, S14, S1E, J16, J34, 67FSB and 67TD), while only two strains originated from hemipelagic surface (strain Z0F) and intermediate (strain Z1E) layers. In contrast, strains of cluster II were obtained exclusively from a single site (567), but appeared to be less confined to sapropels or hemipelagic sediments.

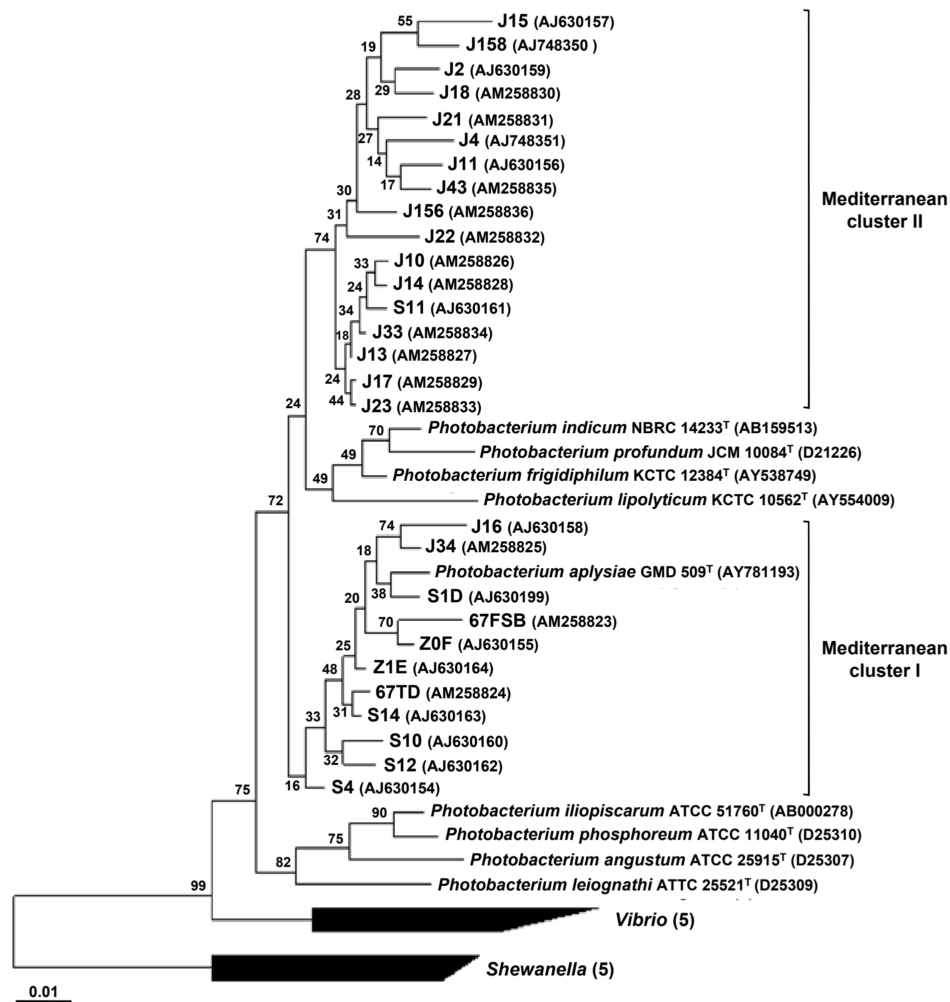

Fig. 9: Maximum likelihood tree showing the phylogenetic positions of the two *Photobacterium* clusters and closely related taxa. Sequences of *Shewanella* (5) and *Vibrio* (5) were taken as outgroup and ingroup, respectively. 16S rRNA gene sequences of Mediterranean isolates were added to a back-bone tree consisting of validly described species by maximum parsimony using the ARB software and a specific *Photobacterium* filter (Ludwig et al., 2004). Numbers at nodes are percentages bootstrap values, based on 1000 iterations.

5.4.2 Phenotypic characteristics

Microscopic inspection revealed that cells were rod-shaped, as considered to be typical for the genus *Photobacterium.* However, cells were often enlarged and irregularly shaped due to the accumulation of huge amounts of endogenous storage granula (Fig. 10). Sudan-black staining (0.05 % w/v in ethanol abs) revealed that these consisted of poly-3-hydroxyalkanoate. The cells were motile by monopolar flagella. Luminescence was not observed.

According to their growth behavior on dilute yeast extract-peptone-glucose agar plates the strains could be separated into two groups that reflected the two phylogenetic clusters. Cluster I strains were characterized by large cream-colored colonies that were formed normally within two days of incubation at 20 °C. The remaining strains grew distinctly slower and needed several days to weeks of incubation at 20 °C to form small whitish colonies (diameter generally less than 0.2 mm).

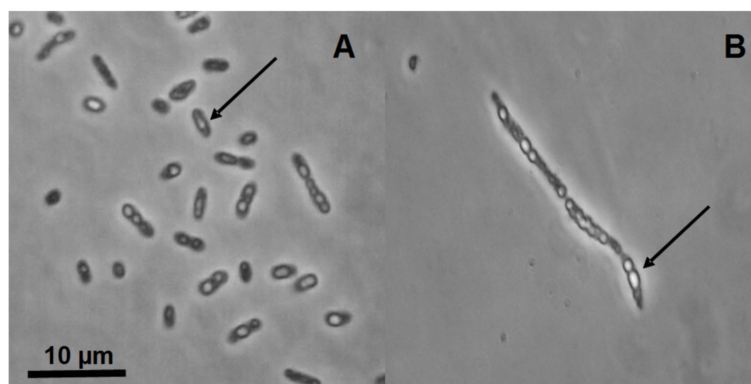

Fig. 10: Phase-contrast photomicrograph of *Photobacterium* strain J17 after four days (left) and two weeks (right) of incubation in oxic liquid medium with glucose as substrate. Arrows indicate endogenous storage granules consisting mainly of poly-3-hydroxyalkanoate.

5.4.3 Molecular characterization

For all strains tested, genomic fingerprinting based on ERIC-PCR yielded a clear and unique pattern of amplified DNA fragments of different lengths that could be used for cluster analysis (Fig. 11). Three groups of patterns were recognized. Strains of cluster I had very uniform ERIC band patterns clearly distinct from the

other strains. Within this cluster the strains grouped corresponding to the sampling site from which they were isolated.

Strains belonging to cluster II yielded two separate subclusters based on ERIC band patterns (Mediterranean cluster II subgroup A and B; Fig. 11). Subgroup A comprised most of the strains (five of seven) originating from sapropel 1 and two strains (J43 and J156) isolated from the intermediate layer beneath (Z1). The second subgroup (B) exhibited the highest variability in band patterns and comprised strains originating from all four depths analyzed.

DGGE analysis revealed that all of the *Photobacterium* strains possessed up to five different *rrn* gene copies (differing in one to three bases in approximately 500 bp). Based on the presence or absence of certain bands in the DGGE gel three subgroups were identified that confirmed the clusters defined on basis of the ERIC-PCR results (data not shown).

5.4.4 Aerobic substrate utilization

Under oxic conditions the strains grew on a range of different substrates. The majority of strains utilized the complex substrates yeast extract, casamino acids and peptone as well as poly- (starch, chitin) and monosccharides (glucose, fructose, mannose), monocarboxylic acids (pyruvate, lactate) and tricarboxylic acid cycle intermediates (citrate, 2-oxoglutarate, succinate) (Tab. 18). Only a very few strains (< 4) grew with *n*-propanol, ethylene glycol, aromatic compounds, certain carboxylic and amino acids (glycolate, tartrate, crotonate, isoleucine, phenylalanine), or with C_1 compounds (formate, methanol).

With respect to their metabolic capacities significant differences between cluster I and cluster II strains were observed. Strains of cluster I exhibited relatively uniform substrate utilization patterns (averaged pairwise similarities: 0.87 ± 0.05, Fig. 12) growing with 27 to 36 of the provided carbon sources. All of them grew on the amino acids alanine, arginine, asparagine, glutamine, proline, tryptophane, as well as on fumarate, malate, acetate, capronate and glucosamine, wile these substrates were utilized only by a very few strains belonging to cluster II.

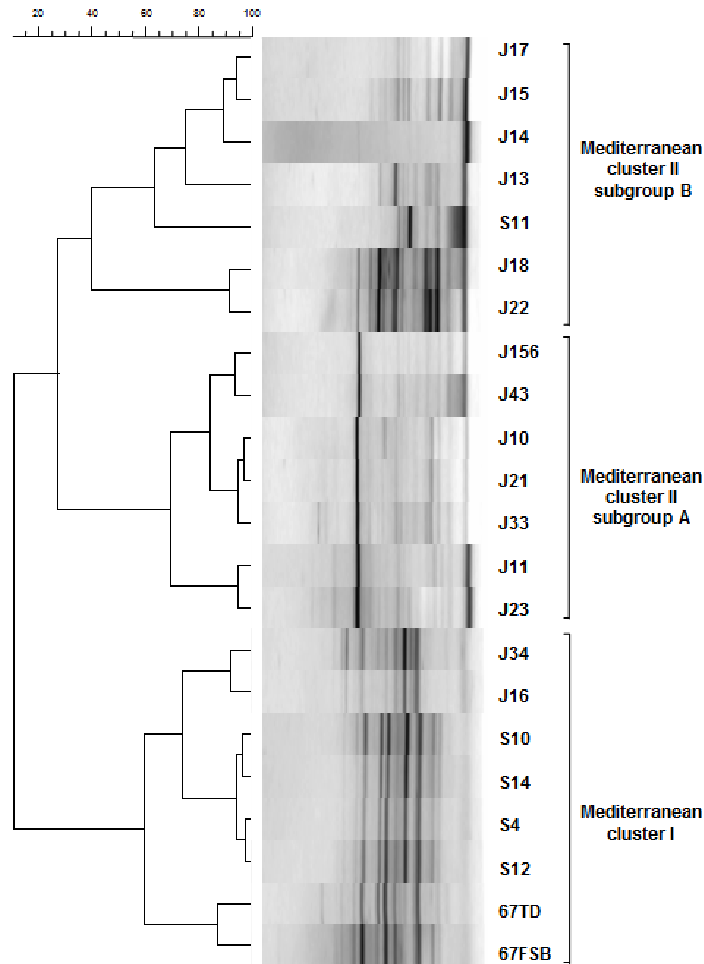

Fig. 11: Cluster analysis of ERIC-PCR fingerprinting band patterns of the *Photobacterium* strains. Strains of Mediterranean cluster II formed two subgroups (A and B). The dendrogram was calculated using Pearson correlation and UPGMA and is based on computer-generated densitometric curves obtained by analysis of negative images of ethidium bromide stained agarose gels.

Mediterranean cluster II strains showed irregular results (Fig. 12). Each strain exhibited its unique set of substrates that supported growth. When compared to the majority of the strains, a few isolates (J10, J33 and J15) appeared to be rather restricted in their metabolic capacities. However, despite aerobic growth on only 10 to 13 substrates, including yeast extract, glucose and asparagine, these three strains nonetheless readily fermented carbohydrates, amino acids and carboxylic acids (Tab. 17).

Tab. 17: Origin, isolation conditions, phenotypic traits and anaerobic growth capacities of the examined *Photobacterium* strains.

Strain	Origin		Isolation		Growth at			Reduction of			Fermentation of			
	Site	Layer		Substrate	Temperature (°C)	pH	Salinity (‰)	NO_3^-	DMSO	AQDS /HA	Glc	AS	TCA	ALC
Mediterranean cluster I														
67FSB	67	S1	anox	FS	4-33	5.5-9.3	10-75	+	+	+	+	+	+	+
67TD	67	S1	anox	Ac/thios	4-33	5.8-9.2	10-75	+			+		+	
S4	567	S1	ox	MKS	4-33	5.8-9.2	10-50	+	+		+	+	+	
S12		S1	ox	MKS	4-35	5.5-9.3	10-75	+	+		+	+	+	
S10		S5	ox	MKS	4-33	5.8-9.2	10-75	+		+	+	+	+	
S14		S5	ox	MKS	4-35	5.5-9.3	10-75	+	+		+		+	
J16	575	S3	anox	AS	4-30	5.5-9.3	10-75	+			+	+	+	
J34	575	S3	anox	AS	6-30	5.8-9.2	10-50	+	+	+[1]	+	+	+	
Mediterranean cluster II														
J15	567	Z0	anox	MKS	n.d.	n.d.	n.d.	+	+		+	+		
J17		Z0	anox	MKS	4-33	5.5-9.3	5-50				+	+	+	
J10		S1	anox	AS	4-35	5.5-9.3	10-75				+	+	+	
J11		S1	anox	AS	4-35	5.5-9.3	5-75	+	+		+	+	+	
J14		S1	anox	MKS	4-30	n.d.	n.d.	+	+		+	+	+	
J21		S1	anox	AS	4-33	n.d.	10-75	+			+	+	+	
J22		S1	anox	AS	4-33	5.5-9.3	10-75	+			+	+	+	
J23		S1	anox	MKS	4-35	5.5-9.3	10-75	+			+	+	+	
J33		S1	anox	AS	4-33	5.5-9.3	5-50				+	+	+	
S11		Z1	ox	MKS	4-33	5.5-9.3	10-75	+	+		+	+	+	
J43		Z1	anox	AS	4-33	5.8-9.2	n.d.				+	+		+
J156		Z1	anox	AS	4-33	5.8-9.2	n.d.				+	+		
J13		S5	anox	MKS	4-33	5.5-9.3	5-75		+		+			
J18		S5	anox	MKS	4-30	5.8-9.2	5-100				+			

Approximate age of sapropels according to Lourens et al. (1992): S1, 8,000 a; S3, 81,000 a; S5, 124,000 a. n.d.: not determined, Z0: surface layers, Z1: intermediate layer 1, FS: fatty acids, Ac/thios: acetate and thiosulfate, MKS: monomer mix (Süß et al., 2004), AS: amino acids, Glc: glucose, TCA: dicarboxylic acids and lactate, ALC: alcohols, HA: humic acids, [1] Use of humic acids

5.4.5 Anaerobic metabolism

All strains were able to grow in the absence of oxygen by fermentation or by anaerobic respiration (Tab. 17). All isolates of cluster I reduced nitrate to nitrite, while five strains grew with DMSO and two with AQDS as electron acceptor. One of the isolates grew in the assay amended with humic acids. In contrast, only eight of the fourteen strains belonging to cluster II grew by anaerobic respiration. Seven of these strains used nitrate and five reduced DMSO. AQDS reduction or growth on humic acids was not observed for this cluster. Interestingly, in cluster II nitrate reduction was found for most isolates from surface and the youngest sapropel S1 (six out of nine strains) but was less common in isolates originating

from the deeper layers (one of five strains). None of the strains reduced manganese oxides, ferric hydroxide, sulfite, thiosulfate or elemental sulfur.

All strains grew by fermentation of glucose. The mixture of dicarboxylic acids and lactate was fermented by all strains of cluster I and by nine out of fourteen strains of cluster II. Fermentative growth on amino acids was found for the majority of strains in both clusters. Two strains (67FSB and J43) grew even in the assay containing alcohols.

The fermentation assays with glucose as substrate were analyzed by HPLC. In the supernatant four to six different acids were identified, with formate, 2-oxoglutarate, lactate, acetate, and citrate, being detected in almost all cultures and suggesting mixed acid fermentation (data not shown). With concentrations often exceeding 13 $mmol \cdot l^{-1}$, formate was found to be the major fermentation product, except for four strains of cluster II. These strains (J13, J18, J43, and J156), that were isolated from the deeper layers Z1 and S5, were remarkable as they also failed to reduce nitrate and to grow fermentatively with amino acids or carboxylic acids.

5.4.6 Temperature range, salinity tolerance and pH requirements

With respect to their growth temperatures no clear differences between the two clusters were observed. With exception of isolate J34 (cluster I, T_{min} = 6 ºC), all strains grew at 4 °C. Most of them revealed an upper temperature limit for growth at 33 °C (Tab. 17), while four strains did not grow above 30 °C and five still grew at 35 °C. The strains were also quite uniform with respect to their salt and pH requirements. None of them grew at pH below 5.5, but they were tolerating rather alkaline conditions with pH values of up to 9.3. All strains required Na^+ and salinities of at least 5 ‰ for growth. The upper salinity limit for growth was between 50 to 75 ‰. Strain J18 was an exception, growing at salinities of 100 ‰.

Tab. 18: Selected phenotypic traits of Mediterranean *Photobacterium* strains compared to those of closely related, validly described *Photobacterium* species.

	***P. profundum* (1)**	***P. frigidiphilum* (2)**	***P. aplysiae* (3)**	***P. indicum* (4, 5)**	***P. lipolyticum* (6)**	**Mediterranean Cluster I**	**Mediterranean Cluster II**
Origin	Pacific deep-sea sediment	Pacific deep-sea sediment	Eggs of sea hare	Sea mud	Korean Intertidal sediments	Mediterranean sapropels	Mediterranean sediments
Water depth	5110 m	1450 m	12 m	400 m	surface	2150-2330 m	
Growth at 4°C	+	-	-	+	+	+	+
Growth at 30°C	-	-	+	-	+	+	+
Major fatty acids	C16:1, C16:0, *i*-C16:0, C18:1, C20:5ω3	C16:1, C16:0, C18:1, C20:5ω3	C16:1, C16:0, C18:1	C16:1, C16:0, C18:1	C16:1, and/or *i*-C15:0 2-OH, C16:0, C18:1	C16:1, C16:0, C18:1	C16:1, C16:0, C18:1
Presence of C20:5ω3[1]	+	+	n.d.	-	-	(+)	(+)
NO_3^- reduction	+	+	+	+	+	+	v
Fermentation of glucose	+	+	+	+	+	+	+
Utilization of							
N-acetyl-glucoseamine	-	+	+	n.d.	-	+	v
Cellobiose	-	-	+	n.d.	-	+	v
Fructose	-	+	+	n.d.	+	+	v
Maltose	+	+	+	+	+	+	+
Mannose	+	+	+	n.d.	-	+	+
Sucrose	-	+	+	+	+	+	+
Trehalose	+	+	+	n.d.	-	+	v
Arabinose	-	-	n.d.	-	-	-	4/15*
Glucose	+	+	n.d.	+	+	+	+
Rhamnose	-	n.d.	n.d.	n.d.	n.d.	-	v
Mannitol	+	+	+	n.d.	n.d.	+	v
Lactate	n.d.	+	+	+	-	+	+
Citrate	n.d.	n.d.	n.d.	+	-	+	+
Succinate	n.d.	+	+	+	+	+	+
Alanine	n.d.	+	+	-	n.d.	+	v
Tween 80	+	+	+	n.d.	+	v	4/15*
Glycerol	+	+	+	+	n.d.	+	v

(1) Nogi et al., (1998), (2) Seo et al., (2005a), (3) Seo et al., (2005b), (4) Johnson & Weisrock (1969), (5) Xie et al., (2004); (6) Yoon et al., (2005). n.d.: no data available, + growth of more than 60 % of the strains, v: growth of 40-60 % of the strains tested, * number of positive versus total number of strains. [1] C20:5ω3: eicosapentaenoic acid, typical for psychrophilic and piezophilic bacteria, (+) traces in the phospholipid fatty acid fraction.

5.4.7 Phospholipid content

Eight strains (four of each cluster) were analyzed for their phospholipids. Generally, all strains yielded very similar results with phosphatidylglycerol (12 – 20 %) and phosphatidylethanolamine (70 – 85 %) as the dominant phospholipid classes. Analysis of the polar lipid fatty acids revealed the dominance of *n*-16:0 (up to 32 %), *n*-16:1 (up to 48 %) and *n*-18:1 (up to 16.6 %) fatty acids in both clusters (Tab. 18). While all strains of cluster II contained *n*-20:5ω3 (0.3 - 2 %), traces of this fatty acid type were found only in two of the four strains of cluster I. The latter strains, in turn, contained *n*-18:2 and saturated short-chain fatty acids (*n*-9:0 to *n*-13:0).

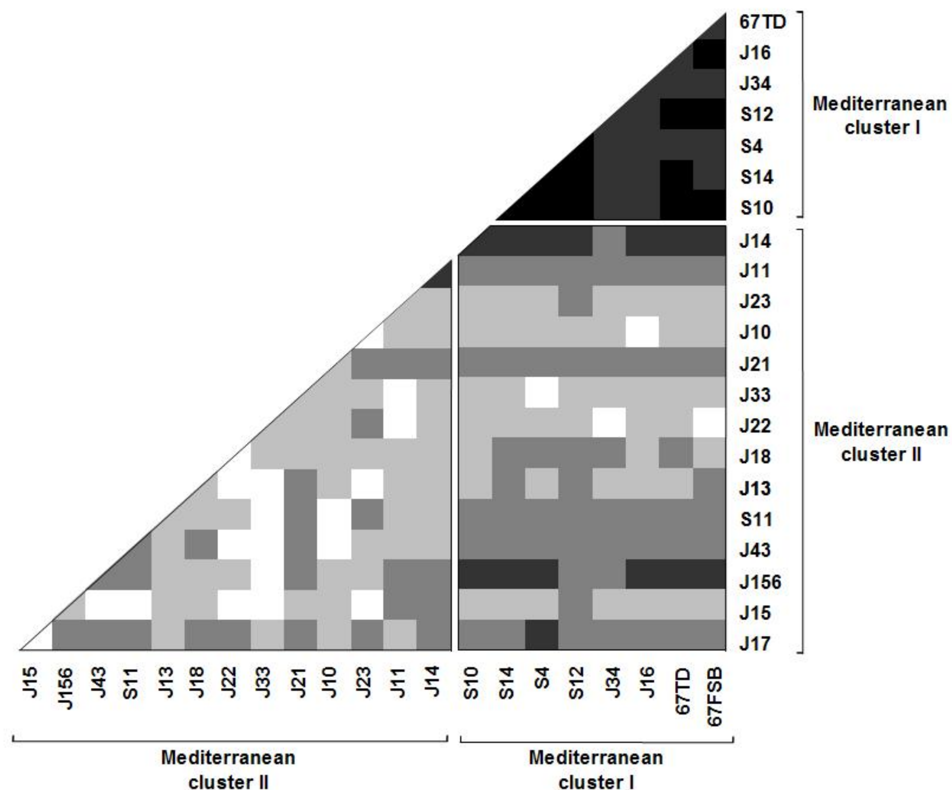

Fig. 12: Triangular similarity matrix based on aerobic and anaerobic growth characteristics. The distance matrix was calculated using the Dice coefficient (DC). The following color code was used: ■ DC > 0.9 ■ DC 0.75- 0.9 ■ DC 0.6-0.75 ■ DC 0.45-0.6 □ DC < 0.45

5.5 Discussion

In the present study we have analyzed a large number *Photobacterium* strains isolated from subsurface sediments. The strains appear well adapted to their environment and form two distinct clusters that differ with respect of genomic diversity, their physiological capacities, lipid composition and their habitat. Strains of cluster I were isolated almost exclusively from organic-rich sapropels whereas for the presence of the second cluster no correlation to certain sediment layers was found. Therefore, it can be concluded that the two clusters occupy different ecological niches.

5.5.1 *Photobacterium* sp. as indigenous subsurface microbes

Although *Photobacterium* species were so far known from pelagic environments and surface sediments, this genus apparently forms subsurface populations that thrive in this inhospitable environment. It can be regarded as almost certain that the isolates are indigenous deep-biosphere representatives. Deep-well plates containing the MPN series from which the majority of strains were isolated, had four additional rows that were not inoculated and served as control. None of the controls was proven positive, indicating that contamination during set-up of MPN series can be ruled out (Süß et al., 2004). Furthermore, the isolates were repeatedly obtained during different sampling campaigns (1998 and 2001) and applying various cultivation conditions (Tab. 17). The high viable counts (up to 3.3 % of the total count, Süß et al., 2004), also indicate that *Photobacterium* sp. are numerically important and therefore most likely metabolically active members of the sediment communities. If they were inactive, it could be expected that their numbers strongly decreased with depth: This was not the case. In addition, dormant stages such as spores or cysts are not described for this genus (Baumann & Baumann, 1984).

If the *Photobacterium* strains represent indigenous subsurface microorganisms they must be able to grow under *in situ* conditions. In fact, the isolates grew well under anoxic conditions and exhibited anaerobic pathways so far unknown for *Photobacterium* spp. (Baumann & Baumann, 1984) like fermentation of

dicarboxylic or amino acids. The latter capacity was, however, recently inferred from genome sequencing data of the sediment-dwelling *P. profundum* (Vezzi et al., 2005).

The Eastern Mediterranean Sea is characterized by low primary production and sedimentation rates. Consequently organic matter contents and microbial activities in the surface layers of the sediments are low (Tab. 19). Under these conditions oxygen and nitrate may penetrate as deep as the top sapropel S1 (van Santvoort et al., 1996; Slomp et al., 2002) and support growth of the *Photobacterium* strains. Profiles of Mn^{2+} and Fe^{2+} indicate that metal reduction is the dominant terminal oxidation process in this layer (van Santvoort et al., 1996). Although a clear drop in redox potential was found across sapropel 1, in the layers underneath still oxidized conditions with positive redox potentials prevail (van Santvoort et al., 1996). This is in line with the low sulfate reduction rates observed in the younger sapropels S1 to S7 (Tab. 19) and might explain the recovery of only very few sulfate reducers from sapropels (Süß et al., 2004) and the presence of only negligible sulfate gradients even as deep as 100 m below surface (Cragg et al., 1998). Oxidized conditions, in turn, might favor growth of facultative anaerobes such as *Photobacterium* sp. rather than of strict anaerobes and might explain their predominance in the culture collection obtained from this habitat (Süß et al., 2004), but also other deep-sea sediments (D'Hondt et al., 2004).

The finding that our isolates degrade a broad spectrum of monomeric and polymeric substrates classifies them as generalists and this nutritional versatility might be a useful adaptation to their habitat. Although sapropels are characterized by the presence of highly recalcitrant polymeric material (Killops & Killops, 2004), easily degradable substrates like carbohydrates or peptides are still present at very low concentrations or adsorbed to the kerogen matrix of the sapropels (Coolen & Overmann, 2000). However, the degradation capacities of our isolates might be even underestimated as all growth tests were performed under atmospheric pressure. Gene expression analyses of the deep-sea strain *P. profundum* SS9 indicated that certain metabolic pathways, such as fermentation of amino acids or the degradation of biopolymers, might be expressed only at elevated hydrostatic pressure (Vezzi et al., 2005).

Tab. 19: Selected physico- and biogeochemical data of Eastern Mediterranean sapropels, surface and intermediate layers obtained from literature (ten Haven et al., 1987; Passier et al., 1996; van Santvoort et al., 1996; Kroon et al., 1998; Slomp et al., 2002). n.a.: data not available.

Sediment	Average age	Porewater	TOC	pH	CaCO3	Porosity	Sulfate reduction
	(10^3 a)	(mmol l^{-1})	(%)		(%)		(µmol cm^{-2} a^{-1})
Surface		31.1 - 31.4	0.06 - 0.29	7.6 - 7.7	48-59	0.61-0.65	
S1	8	32.0	2.7 - 4.5	7.6	36-54	0.78-0.84	0.19 – 5.8
Z1		31.1-31.9	0.1 - 0.27	7.6 - 7.8	42-54	0.56-0.69	
S5	124	27.5-27.8	8.7 - 19.8	7.6 - 7.8	26-30	0.84	0.8 – 2.7
Z5		26.8	0.1 - 0.2	7.7	44-47	0.6	
S6	172	32.2-32.8	2.1 - 5.1	7.1 - 7.3	47-55	0.68-0.73	1.1 – 2.0
Z6		32.2-32.3	0 - 0.02	7.4-7.6	47-61	0.50-0.51	
S7	195	32.5-32.6	3 - 9.8	7.3 - 7.4	42-50	0.75-0.77	2.3 – 2.5
Z7		n.a.	0 - 0.01	7.4 - 7.5	33-45	0.5	

5.5.2 Microdiversity in subsurface environments

The *Photobacterium* clusters investigated in this study differed with respect to their microdiversity. Mediterranean cluster I strains, that were predominantly isolated from sapropel layers, apparently maintained a highly stable genome organization over thousands of years while Mediterranean cluster II strains exhibited a higher level of microdiversity. However, this degree of genomic heterogeneity was much lower than that generally found in pelagic bacteria (Sass et al., 2001; Jaspers & Overmann, 2004) or those inhabiting surface sediments (Sass et al., 1998c; Bagwell & Lovell, 2000). At first sight this might correlate with the highly constant environmental conditions in subsurface environments. Seasonal changes of physicochemical conditions and temperature (Thompson et al., 2004), varying light conditions (Moore et al., 1998) or the increasing pressure from the sea surface to the deep-sea (López-López et al., 2005) were shown to stimulate the development of specifically adapted subpopulations within single species; and even protozoan grazing or virus infection (Feil, 2004) were supposed to increase microdiversity. These stimuli in combination with short generation times may stimulate genotypic and phenotypic diversification in bacteria from pelagic environments and surface sediments but cannot sufficiently explain existence of microdiversity in subsurface bacteria like our *Photobacterium* strains. In fact, genome stability can be interpreted as an indication for metabolic activity. Starving or resting cells were shown to enhance the generation of diversity (Arber et al., 1994) and ecologically successful populations do not necessarily need to develop a high genetic diversity (Hahn et al., 2005). This could explain the low level of diversity in Mediterranean cluster I. On the other hand, increased variability within a population, *e.g.* in Mediterranean cluster II, can be considered as complementary strategy for long-term survival. The development of multiple ecotypes allows an immediate response to a wide range of environmental conditions (Moore et al., 1998; Gray et al., 1999; Klepac-Ceraj et al., 2004).

While the concept of periodic selection predicts that two bacterial populations cannot occupy the same ecological niche and that one type would outcompete the other (Palys et al., 1997), Thompson and coworkers (Thompson et al., 2005b) reported on the consistent co-occurrence of a large diversity of *Vibrio*

genomovars within a natural community. They concluded that most of the genotypic diversity they detected must be "ecologically neutral" and explained the co-occurrence with the erratic and unpredictable appearance of resources and grazing that could eliminate dominant subpopulations. These factors can be ruled out for subsurface bacteria. On the other hand it might be that the low *in situ* growth rates do not allow one subpopulation overgrowing another and therefore enable co-existence. The highly complex and diverse structure of the sapropelic kerogen (*e.g.* polyphenylic and aliphatic residues, Killops & Killops, 2004) most likely requires a specialized bacterial community for degradation. Since *Photobacterium* cluster I exhibited low diversity and was detected mostly in the sapropels it can be expected that it is involved in this process. Strains of cluster II in contrast were found in all layers and showed a higher diversity in terms of substrate utilization and genomic level. This might indicate a role as opportunistic bacterium which quickly reacts to changing conditions, *e.g.* substrate availability. This indicates that the two *Photobacterium* clusters occupy distinct ecological niches.

5.6 Acknowledgments

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6 Intact polar membrane lipids in deep tidal flat sediments as indicators of active microbial communities and advective pore water transport

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6.1 Abstract

Amount and distribution pattern of intact polar lipids (IPLs) as biomarkers for 'viable' microorganisms were determined in a 5 m sediment core from a tidal flat margin of the southern North Sea coast. The upper 130 cm of the core were mostly sand whereas the layers beneath were dominated by mixed/mud and mud sediments. IPLs were analyzed using HPLC-high resolution tandem mass spectrometry. Exceptionally high contents of phosphate-free IPLs were detected in the upper 130 cm: betaine lipids (BLs), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG) and sulfoquinovosyldiacylglycerol (SQDG). Fatty acid patterns of these phosphate-free IPLs indicated eukaryotic cells as a major source of total IPLs. The presence of eukaryotes, *e.g.* diatoms, at several tens of centimeters depth may be explained by intense advective pore water flow and subsurface water run-off during ebb tide. BLs and SQDGs with odd-carbon-numbered fatty acid side chains suggest also a bacterial contribution to the phosphate-free IPL fraction. Relative contents of most bacterial phospholipids remained largely constant throughout the core. An exception was diphosphatidylglycerol which strongly increased in abundance beneath 130 cm depth and there represented about 50 % of all IPLs. Acyl-ether and diether phospholipids were detected throughout the core, with highest amounts in the middle section suggesting strictly anaerobic bacteria as source organisms. The only archaeal IPLs detected were phospholipid-based archaeols and hydroxy-archaeols as they are found in *Methanosarcinales*. The contents of archaeal IPLs were an order of magnitude lower than those of bacterial origin but the strong correlation of the abundances of both in the deeper sediments suggests a syntrophic association. The $\delta^{13}C$ values of total organic carbon (TOC) showed a predominance of marine organic matter in the upper part of the core and a significant shift to lighter terrigenous organic matter beneath 60 cm depth. A similar shift was found in the IPL-derived fatty acids. In the upper 130 cm, the typical bacterial *anteiso* $C_{15:0}$ fatty acid was isotopically even heavier than bulk TOC, indicating the supply and preferential assimilation of marine organic matter even at one meter depth.

6.2 Introduction

The Wadden Sea of the southern North Sea coast is one the world's largest continuous tidal flat systems characterized by high productivity due to nutrient supply from land and sea (Poremba et al., 1999; van Beusekom & de Jonge, 2002). Despite tight coupling within the pelagic microbial food web a significant part of the primary organic matter is deposited in the sediments. This material, together with organic matter produced by benthic primary production, is an important carbon source for heterotrophic sediment microbial communities (MacIntyre et al., 1996; Stal, 2003). In general, intense remineralization causes a depletion of oxygen within the top few millimeters of sediment and leads to steep geochemical gradients (Böttcher et al., 2000; Llobet-Brossa et al., 2002). Otherwise, bottom currents across small mounds and ripples of permeable sediments can cause rapid exchange of pore and overlying water and hence lead to enhanced oxygen and organic matter uptake. At the same time, inorganic degradation products like ammonium or hydrogen sulfide may be flushed out of the sediment (Huettel & Rusch, 2000; Rusch et al., 2001; Billerbeck et al., 2006b). At tidal flat margins tide-driven hydraulic pressure gradients enhance deep pore water flow which can supply even deeper permeable sediments down to several meters depth with electron acceptors and donors (Billerbeck et al., 2006b; Beck et al., 2008b; Røy et al., 2008). Consequently, despite their relatively low organic matter content, sandy tidal flat sediments exhibit mineralization rates similar to organic-matter-rich mud and harbor diverse microbial populations (Huettel & Rusch, 2000; Ishii et al., 2004; Billerbeck et al., 2006a; Gittel et al., 2008).

The tidal flats in the backbarrier area of Spiekeroog Island, NW Germany, comprise sediments dominated by varying lithologies. Muddy sediments are generally found closer to the mainland and sandy sediments seawards. A handful of representative sites has been extensively studied using geochemical, microbiological and molecular ecological tools (Köpke et al., 2005; Wilms et al., 2006a; Fichtel et al., 2008; Gittel et al., 2008). One of these sites, Janssand (Fig. 13), located at the tidal flat margin, is dominated by sand and characterized

by an unusual distribution of microbial cell numbers which, in contrast to open ocean marine sediments, showed hardly a decrease with depth (Gittel et al., 2008). This cell number distribution is an indication for highly active communities even in the deeper layers.

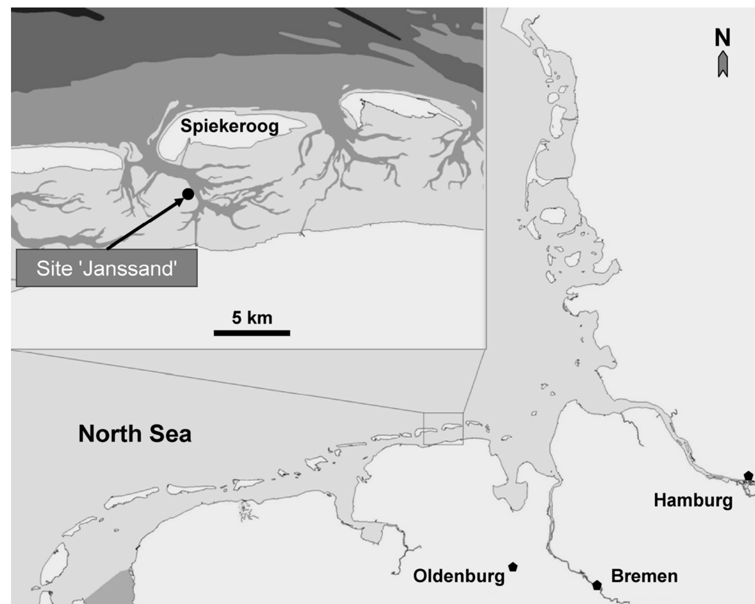


Fig. 13: Location of the sampling site 'Janssand' in the backbarrier tidal flats of Spiekeroog Island in the Northwest German Wadden Sea (southern North Sea).

In the present study, an approach based on the analysis of intact polar lipids (IPLs) to assess the structure of microbial communities is applied to surface and subsurface sediments from site Janssand. IPLs are biomarkers suitable to trace vegetative bacteria as they are rapidly degraded after cell lysis and are present in all organisms (White et al., 1979; Harvey et al., 1986). The analysis of these biomarkers is an unbiased analytical approach to assess the 'bulk' biomass and can help to complement information obtained using microbiological and molecular biological methods (Rütters et al., 2002b; Sturt et al., 2004; Biddle et al., 2006; Freese et al., 2009). The recent development of high performance liquid chromatography coupled to mass spectrometry allows direct analysis of IPLs in complex samples from a variety of aquatic environments, surface and deep subsurface sediments (Rütters et al., 2002a; Zink et al., 2003; Biddle et al., 2006; Ertefai et al., 2008; Lipp et al., 2008; Freese et al., 2009; Van Mooy et al., 2009).

Investigations of IPLs in tidal flat sediments were so far restricted to the upper layers (Rütters et al., 2002a,b; Freese et al., 2009) and only recently, IPL-derived fatty acids were analyzed down to several meters depth (Freese et al., 2008a). Unfortunately, during hydrolysis valuable information about the compositions of the IPLs, *i.e.* head group-side chain combinations, gets lost and therefore a more precise assignment to possible source organisms is prevented (Rütters et al., 2002a).

Here, we report about the detailed and sediment layer-specific analysis of the distribution of IPLs in a 5 m long sediment core recovered close to the tidal flat margin of site Janssand. We will discuss the different potential sources of IPLs (eukaryotic versus prokaryotic) and relate them to the geochemical and lithological profiles. To elucidate possible carbon sources used by the different source microorganisms, we analyzed the stable carbon isotopic composition of the total organic carbon and hydrolyzed polar lipids using GC-IRMS. This work is part of an interdisciplinary study with complementary microbiological and molecular ecological investigations published by Fichtel et al. (2008) and Gittel et al. (2008).

6.3 Methods

6.3.1 Study site and sampling

A five-meter-long sediment core was taken in April 2005 at the north-eastern margin of the Janssand tidal flat (53°44.177′ N, 007°41.970′ E) located in the backbarrier area of the island of Spiekeroog, southern North Sea, Germany (Fig. 13). The surface water biogeochemistry and hydrography of the site have been described by Billerbeck et al. (2006b).

An aluminum tube of 6 m length and 8 cm in diameter was driven into the sediment by vibro-coring as described by Wilms et al. (2006a). The sedimentary structures and lithology of the cores were determined by visual inspection. Samples for IPL analysis were taken from the innermost part of the core to avoid contamination. The core was sampled at about 10 cm increments allowing the

separation of specific layers dominated by different sediment types. Sediment samples were freeze-dried and stored at -20 °C until processing.

6.3.2 Bulk sediment parameters

Total organic carbon (TOC) contents of the sediment samples were determined as the difference between total carbon determined by combustion in a CS-444 instrument (Leco Instruments GmbH, Mönchengladbach, Germany) and inorganic carbon measured with a CM5012 CO_2 coulometer coupled to a CM5130 acidification module (UIC Inc., Joliet, IL). The isotopic composition of bulk organic matter ($\delta^{13}C$ value) was analyzed with an elemental analyzer (Carlo Erba EA 1108, Mt. Laurel, USA), coupled to an isotope-ratio monitoring mass spectrometer (Finnigan MAT 252, Bremen, Germany) via a Conflo II split interface, after removal of carbonate by acid treatment. Pore water sulfate, methane concentrations, and total cell counts (obtained after acridine orange staining) were published previously by Gittel et al. (2008).

6.3.3 Extraction of sediment samples and subsequent fractionation

The freeze-dried sediment samples (ca. 50 g) were ultrasonically extracted up to ten times for 10 minutes using a solvent mixture of methanol/dichloromethane/ammonium acetate buffer (50 mM, pH 7.6), 2:1:0.8 by volume, in centrifuge tubes (Bligh & Dyer, 1959, modified after Vancanneyt et al., 1996). After centrifugation at 2200 × g for 10 minutes at 15 °C the supernatants were removed and collected in a separatory funnel. Dichloromethane and ammonium acetate buffer (50 mM, pH 7.6) were added to the combined extracts to achieve a final ratio of methanol/dichloromethane/ammonium acetate buffer of 1:1:0.9 (by volume).

After phase separation, the organic phase was removed and the aqueous phase was reextracted five times with dichloromethane. Combined extracts were dried over anhydrous sodium sulfate, evaporated to dryness and stored at -20 °C. Lipid extracts were dissolved in 1 ml dichloromethane/methanol 9:1 (by volume) and then chromatographically separated according to Zink & Mangelsdorf (2004).

Two glass columns in sequence filled with pure silica (1 g silica 60, 63 - 200 µm, Merck, dried at 110 °C for 16 h) and Florisil (1 g magnesium silica gel 150 - 250 µm, Merck) were used to obtain four fractions: (1) neutral lipids (eluted with 20 ml dichloromethane); (2) free fatty acids (50 ml methyl formate with 0.025 % pure acetic acid); (3) glycolipids (20 ml acetone), and (4) polar lipids. To obtain the polar lipid fraction, the Florisil column was removed and only the silica column was eluted with 25 ml methanol. All fractions were evaporated to dryness and stored at -20 °C.

6.3.4 Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)

Aliquots of the polar lipid fractions were transesterified with trimethylsulfonium-hydroxide as described by Müller et al. (1993, 1998). The methyl esters obtained were quantified by GC (Hewlett Packard HP 5890 Series II gas chromatograph, Hewlett Packard, Waldbronn, Germany; equipped with a DB-5HT capillary column 30 m × 0.25 mm, 0.1 µm film thickness, J&W, Folsom, CA, USA) coupled to a flame ionization detector (FID) and identified by GC-MS using a Finnigan MAT SSQ 710B mass spectrometer (Finnigan-Thermoquest, San Jose, CA, USA). The carrier gas was helium (constant pressure of 12 psi). The oven temperature was raised from 60 °C (isothermal for 2 min) to 360 °C at rate of 3 °C min^{-1} and held for 5 min. Mass spectra were collected in full scan mode (*m/z* 50 - 650, ionization energy 70 eV). Positions of double bonds were tentatively assigned by comparison with retention times of standards (Bacterial Acid Methyl Esters CP Mix; Supelco, Bellefonte, PA, USA).

6.3.5 Gas chromatography combustion isotope ratio mass spectrometry (GC-IRMS)

For analysis of the isotopic composition of the polar lipid fraction aliquots were acid hydrolyzed to obtain polar lipid fatty acid methyl esters and ether core lipids. Aliquots of polar lipid fractions were refluxed in 3 ml methanol/dichloromethane/HCl (2 N) 10:1:1 (v/v/v) at 80 °C for 2 h under nitrogen.

After cooling the mixture was diluted with 1 ml H_2O and extracted three times with 3 ml *n*-hexane/dichloromethane 4:1 (v/v). The free hydroxy groups of the neutral lipids were transformed to the trimethylsilyl (TMS) ether derivatives using *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) at 70 °C for 2 h.

Compound-specific stable carbon isotope ratios of IPL derivatives were measured (minimum of three replications) on an HP 6890 GC equipped with a Gerstel CIS 4 injector and a J&W HP-5 capillary column (L 30 m; ID 0.25 mm; 1µm film thickness). The column temperature was raised from 60 °C (2 min) at 3 °C min^{-1} to 300 °C (36 min). The GC was coupled via a GC combustion interface III (set at 940 °C) to a DELTA V Advantage isotope-ratio-monitoring mass spectrometer (ThermoFinnigan, Bremen, Germany). Carbon isotope ratios are reported in the δ notation as per mil (‰) deviation from the Vienna Pee Dee Belemnite standard (V-PDB). Internal standards ($C_{23:0}$ fatty acid, squalane) within each run were used to monitor reproducibility and precision during the measurements. Reported $\delta^{13}C$ values have an analytical error of less than ± 1.0 ‰ and were corrected for addition of trimethylsilyl groups and the extra carbon atom in the methyl esters.

6.3.6 High performance liquid chromatography-mass spectrometry (HPLC-MS) and HPLC-MS/MS

Aliquots of the glycolipids and polar lipid fraction were analyzed using an HPLC instrument (Waters 2695 Separations Module, Waters, Manchester, UK) coupled to a hybrid-quadrupole/time-of-flight mass spectrometer (Micromass Q-TOF micro, Waters, Manchester, UK) equipped with an electrospray source. HPLC separation was achieved on a diol phase (Lichrospher 100 Diol 5µ, Merck, Germany) using a 2 × 125 mm column. A flow rate of 0.40 ml min^{-1} was employed with the following linear solvent gradient (modified after Rütters et al., 2002a): 1 min 100 % A, increasing over 20 min to 35 % A and 65 % B, followed by 40 min reconditioning with Eluent A. Eluent A was a 78:20:1.2:0.04 (v/v) mixture of *n*-hexane/*i*-propanol/formic acid/ammonia (25 % solution in water), Eluent B was 88:10:1.2:0.04 (v/v) *i*-propanol/water/formic acid/ammonia (25 % solution in water).

Quantification was done after external calibration of MS signals with polar lipid standards representing major IPL types (purchased from Avanti Polar Lipids, Alabaster, USA; Matreya, Pleasant Gap, USA; Sigma Aldrich, München, Germany, and Lipid Products, Redhill, UK). The mass spectrometer was operated in negative ion mode with the capillary voltage set to 3000 V and a sample cone voltage of 35 V. The source temperature was 110 °C with the desolvation temperature being 220 °C. The desolvation gas used was nitrogen. During full scan mode (*m/z* 500 - 2000) the voltage in the collision cell was set to 7 V. To identify structures of the glycolipids and polar lipids MS/MS experiments were carried out by running a profile in the collision cell from 30 – 40 V (in 5 V steps) with argon as collision gas. All MS and MS/MS measurements were performed at high resolution using the lock spray channel of the Q-TOF and a 10 mmol l^{-1} sodium formate solution (in acetonitrile) as calibration standard. Due to the lack of commercially available standards for quantification of betaine lipids or unknown polar lipids the response factor of the phosphatidylglycerol standard was used, because its response factor is an average of the other intact polar lipids used in the HPLC-ESI-MS application. Detection limit was 7.5 ng for each injected IPL species per injection for quantification with the MS instrument.

6.3.7 Statistical analysis

To assess the strength of the relationship between the different classes of IPLs the Spearman's rank correlation coefficient was calculated. This coefficient was used because IPL concentration data were non-parametric. Potential correlations between dissolved organic carbon (DOC) and IPLs were estimated by calculating Pearson's Correlation after log-transformation of the IPL data. Single classification analysis of variance (ANOVA) was used to determine the dependence of IPL classes with respect to lithology, TOC and depth on log-transformed IPL data.

6.4 Results

6.4.1 Geochemical characterization of the sediments

The Janssand core from the backbarrier intertidal flat of the Spiekeroog Island (Fig. 13) mainly consists of fine- to medium-sized sand down to 5 m depth (Fig. 14A) and contains interspersed mud-rich layers which increase in thickness with depth. At about 1 m depth a thin shell layer was found. An age of approximately 1000 to 2000 years was estimated for the sediments at the bottom of the core based on an average sedimentation rate of 22 to 30 cm per 100 years as reported by Kunz (1993).

The total organic carbon (TOC) content (Fig. 14D) strongly depended on the lithology ($p < 0.001$, calculated using ANOVA). Sand-dominated layers had TOC contents below 0.5 % whereas in the black mud intercalations the values ranged from 1.2 % to 2.2 %. Mixed mud/sand sediments were characterized by intermediate TOC contents. Pore water DOC contents increased slightly with depth from 50 ppm close to the surface to 130 ppm at 490 cm depth (Fig. 14C). DOC values did not exhibit any significant correlation with lithology.

Pore water sulfate concentrations decreased strongly with depth (Fig. 14B). In sediments layers beneath 200 cm sulfate concentrations were below the detection limit of 0.1 mmol l^{-1}. Methane was detected in all analyzed sediments layers and penetrated far into the sulfate zone resulting in an extended sulfate-methane transition zone.

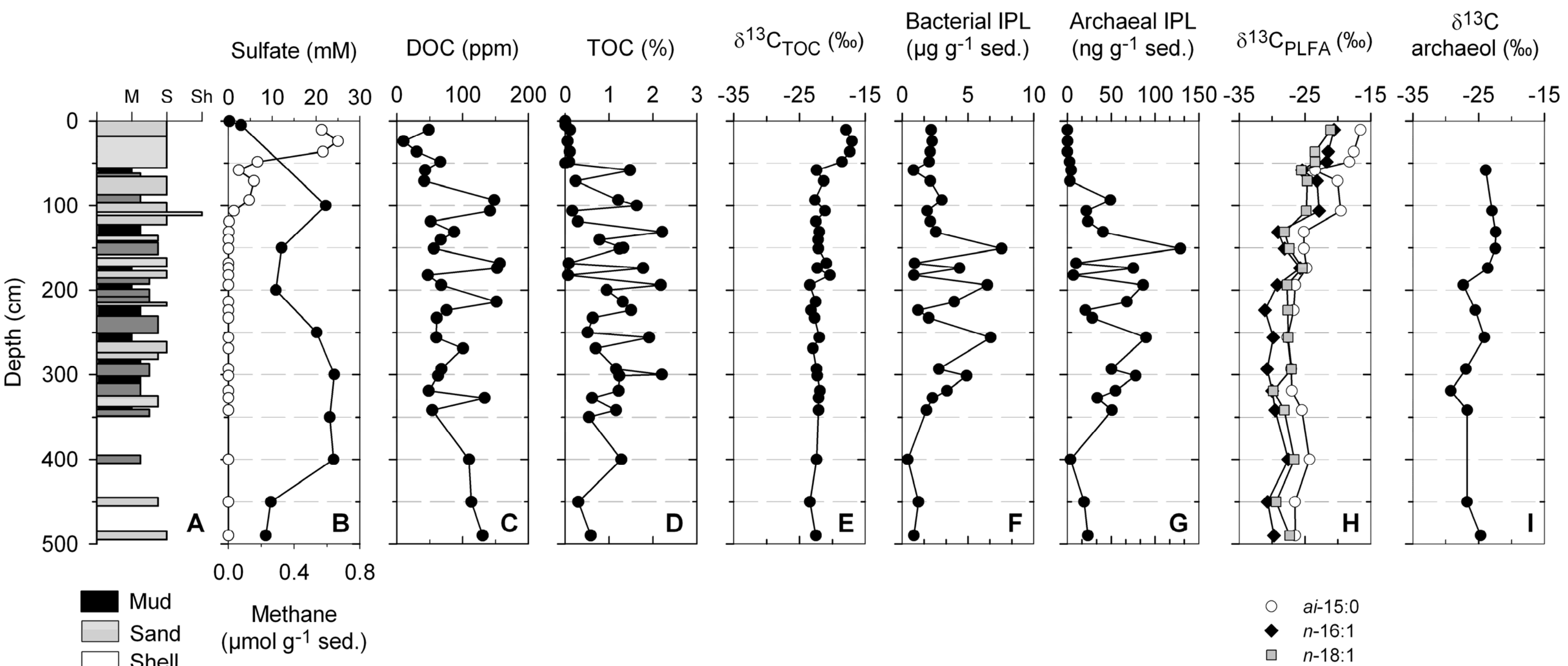


Fig. 14: Depth profiles of biogeochemical parameters in a 5 m long core from the tidal flat Janssand. **A:** Simplified lithological profile with main sediment types (M: mud, S: sand, Sh: shells); **B:** Pore water concentrations of sulfate (open circles) and methane (filled circles); **C:** Dissolved organic carbon (DOC); **D:** Total organic carbon content (TOC); **E:** $\delta^{13}C$ values of TOC; **F:** Contents of bacterial IPLs (including eukaryotic IPLs in the tide-influenced upper part of the core); **G:** Contents of archaeal IPLs; **H:** $\delta^{13}C$ values of selected IPL-derived branched *anteiso* 15:0 (*ai*-15:0) fatty acid (open circles), unbranched 16:1 (*n*-16:1) fatty acids (filled diamonds), and unbranched 18:1 (*n*-18:1) fatty acids (grey squares). Note that the values of 16:1 and 18:1 fatty acids are sums of $\delta^{13}C$ values of isomers with different double bond positions; **I:** $\delta^{13}C$ values of archaeol-containing IPLs.

6.4.2 Depth profiles of non-archaeal IPLs

IPLs in the upper sediment layers were from both, bacterial and eukaryotic sources and are therefore referred to as non-archaeal IPLs below. The variance of the profile of these biomarkers along the core was not obviously connected to either depth, lithology or TOC content (Fig. 14F). However, the contents were highest in mixed and black mud-dominated sediment layers at about 150, 190 and 250 cm depth. Phospholipids contributed about 60 % to the total IPL fraction in the upper 60 cm of the core with increasing abundance in the deeper layers to a maximum of 95 % at 450 cm (Fig. 15). Accordingly, the highest abundance of IPLs without phosphate-containing head groups (see Fig. 16 for structures), *i.e.* betaine lipids (BL), digalactosyldiacylglycerol (DGDG), monogalactosyldiacyl-glycerol (MGDG) and sulfoquinovosyldiacylglycerol (SQDG), was found in the uppermost 60 cm of the core and decreased to less than 20 % in sediments beneath 100 cm depth. In the following, only significant depth or lithology-related trends for the different IPL classes are described (using analysis of variance; ANOVA).

The three main IPL classes diphosphatidylglycerol (DPG), phosphatidyl-glycerol (PG), and phosphatidylethanolamine (PE) were detected in all sediment samples (Fig. 15B). They accounted for approximately 50 % of all classes of detected IPLs in the upper 60 cm and for up to 90 % in layers below 100 cm depth.

Phosphatidylglycerol (PG) was the dominant IPL class in the sands in the upper 50 cm where it accounted for 30 % of all detected IPLs (Fig. 15B). Its diacylglycerol (DAG) derivative was the major IPL in the uppermost core layers, but its content decreased strongly within the upper 60 cm (Fig. 17E).

DPG had significantly higher concentrations in the mixed mud/sand and mud layers than in the sands ($p < 0.005$). The low DPG content in the upper sand layers was not caused by tidal flushing because statistical analysis revealed that there was no significant difference between the upper and deeper sand layers of the core in this respect.

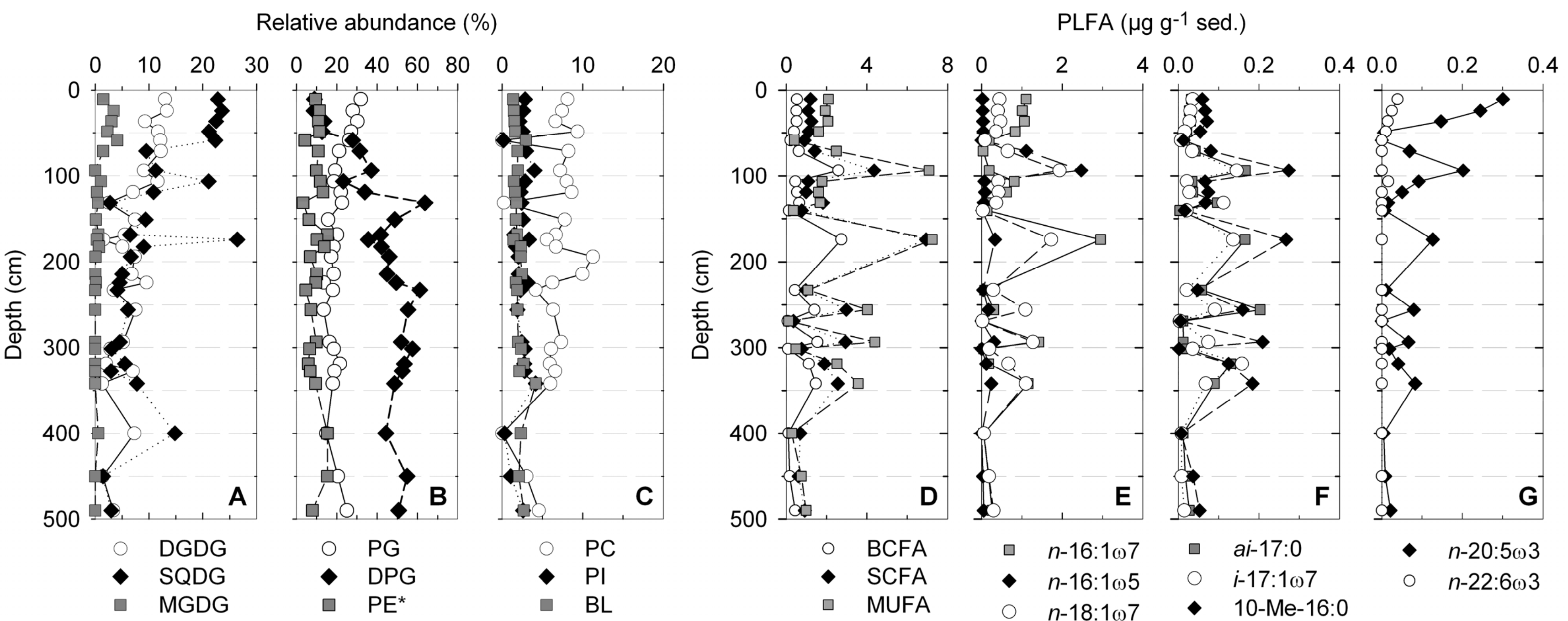


Fig. 15: Left: Depth profiles of relative abundance of non-archaeal IPLs based on head groups. **A:** Digalactosyldiacylglycerol (DGDG), sulfo-quinovosyldiacylglycerol (SQDG), and monogalactosyldiacylglycerol (MGDG); **B:** phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and phosphatidylethanolamine (PE); **C:** phosphatidylcholine (PC), phosphatidylinositol (PI), and betaine lipids (BL; including DGTS and DGTA); phospholipids include diacyl, acyl-ether and diether glycerol core lipids if present (for abbreviations of IPL classes see text and Fig. 16). * = including PMME and PDME. **Right:** Depth profiles of IPL-derived fatty acids. **D:** branched-chain fatty acids (BCFA), straight-chain fatty acids (SCFA), and mono-unsaturated fatty acids (MUFA); **E:** selected unbranched 16:1 and 18:1 fatty acids (including *cis* and *trans* isomers if present); **F:** selected branched bacterial PLFA; **G:** polyunsaturated fatty acids. Note different x-scales in D, E and F/G.

DPG was a minor IPL in the upper 50 cm of the core with a proportion of less than 10 % but became the dominant IPL at greater depth with relative abundances between 25 % and 65 %; it was the main IPL below 100 cm depth. DPG was only detected with diacyl fatty acid side chains and contained mainly monounsaturated and saturated C_{16}, C_{17} and C_{18} fatty acid side chains (Fig. 17H).

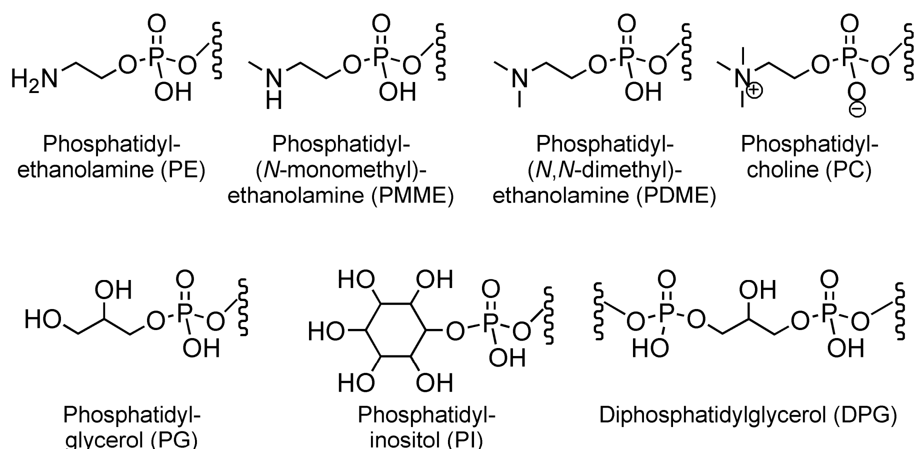
PE, including its methyl derivatives phosphatidyl-(*N*-monomethyl)-ethanolamine (PMME) and phosphatidyl-(*N*,*N*-dimethyl)-ethanolamine (PDME), accounted for approximately 10 to 15 % of the total IPLs throughout the entire core. The depth distribution of these compounds was distinctive with regard to the very low contents in the mud-dominated layers (Fig. 17F). The PE side chains were dominated by a mixture of saturated and monounsaturated fatty acids with odd and even carbon numbers like $C_{16:0}/C_{16:1}$, $C_{17:0}/C_{16:1}$ and $C_{17:1}/C_{17:1}$.

Phosphatidylcholine (PC) generally contributed between 4 and 12 % to the total IPL pool (Fig. 15C). PC DAG was found at highest concentrations in the mixed and mud layers ($p < 0.025$). PC contents in the sand layers were significantly depth-dependent with higher concentrations above 150 cm depth ($p < 0.01$). The detected PC DAG species mainly contained fatty acid side chains with 16 and 18 carbon atoms (Fig. 17I).

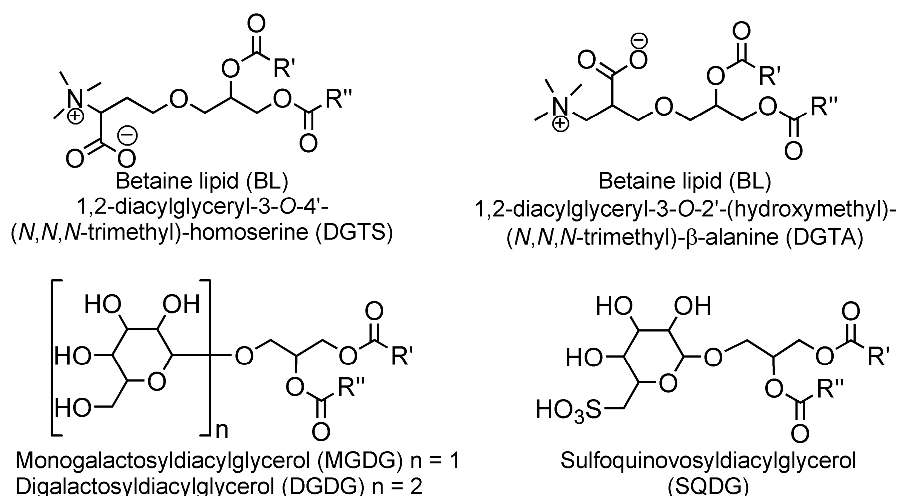
With the exception of two samples from 60 and 400 cm depth, phosphatidylinositol (PI) was detected throughout the entire sediment core as a minor constituent with less than 4 % of total IPLs (Fig. 15C).

SQDG and DGDG were the dominant phosphate-free IPLs at all sediment depths (Fig. 15A). SQDG represented about 23 % of all detected IPLs in the upper 60 cm of the sand-dominated sediments but decreased to values lower than 10 % in the deeper parts of the core. SQDG comprised an assemblage of several different species with a variety of side chains from 14 to 19 carbon atoms including saturated and monounsaturated fatty acids, *e.g.* $C_{16:0}/C_{16:1}$ and $C_{15:0}/C_{15:0}$ (Fig. 17A).

Phospholipid head groups



Non-phosphorus head groups



Core lipids

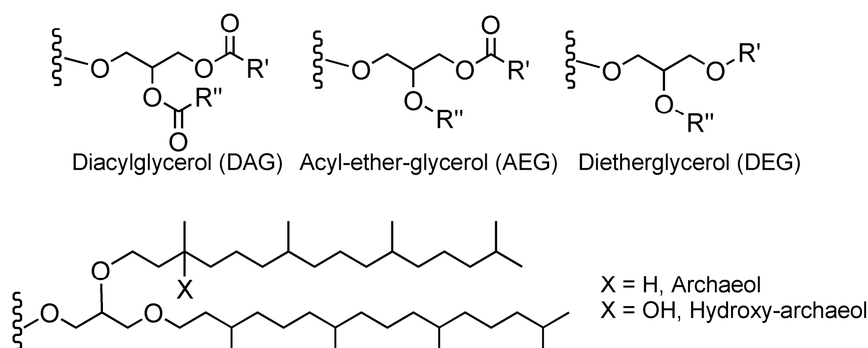


Fig. 16: Structures and head groups of IPLs identified in this study. Note that the structure of the hexose head group of glycolipids was not determined. The two different betaine lipid species DGTS and DGTA were not distinguishable with the mass spectrometry method applied.

DGDG accounted for about 13 % of total IPLs in the upper 70 cm of the core but decreased to abundances of less than 10 % in the deeper parts (Fig. 15A). The detected DGDG species almost exclusively contained saturated or monounsaturated C_{16} and C_{18} fatty acids (Fig. 17C). Small amounts of DGDG with the side chain combination $C_{16:1}/C_{20:5}$ were even detected in the deeper sediment layers, suggesting a bacterial source.

MGDG was a minor IPL class with less than 5 % even in the upper sediments (Fig. 15A). The abundance of MGDG species with polyunsaturated side chains, *e.g.* $C_{16:2}/C_{20:5}$, strongly decreased with depth, and these compounds were not detected beneath a depth of 200 cm (Fig. 17B). The elevated contents of MGDG in the upper part of the core were significantly correlated with depth ($p < 0.001$) but showed no clear correlation with lithology.

The detected betaine lipids (BL) comprised two different structural groups of lipids, namely 1,2-diacylglyceryl-3-*O*-4'-(*N*,*N*,*N*-trimethyl)-homoserine (DGTS) and 1,2-diacylglyceryl-3-*O*-2'-(hydroxymethyl)-(*N,N,N*-trimethyl)-β-alanine (DGTA). They were detected throughout the entire sediment column with abundances less than 4 % (Fig. 15C). The major BL species mainly contained saturated and monounsaturated C_{16} and C_{18} fatty acids. The detection of a BL species with a C_{17} fatty acid side chain strongly suggests a bacterial origin in the deeper sediments layers (Fig. 17D).

Spearman correlation analysis showed that the abundances of most IPLs significantly correlated with each other (Tab. 20). Furthermore, the total content of bacterial IPLs strongly correlated with almost all IPL classes, including those of archaeal sources. A particularly close correlation was detected for DPG and archaeal lipids ($r = 0.938$, $p = 0.002$, $n = 27$). Close correlations were also found for PE and PC ($r = 0.928$, $p = 0.002$, $n = 27$) as well as for BL and PI ($r = 0.919$, $p = 0.002$, $n = 27$). MGDG showed, in contrast to all other IPLs, no or only insignificant correlations with other polar lipids. In particular, it negatively correlated with DPG ($r = -0.492$, $p = 0.01$, $n = 27$) whereas a slight positive correlation with SQDG ($r = 0.577$, $p = 0.002$, $n = 27$) was found.

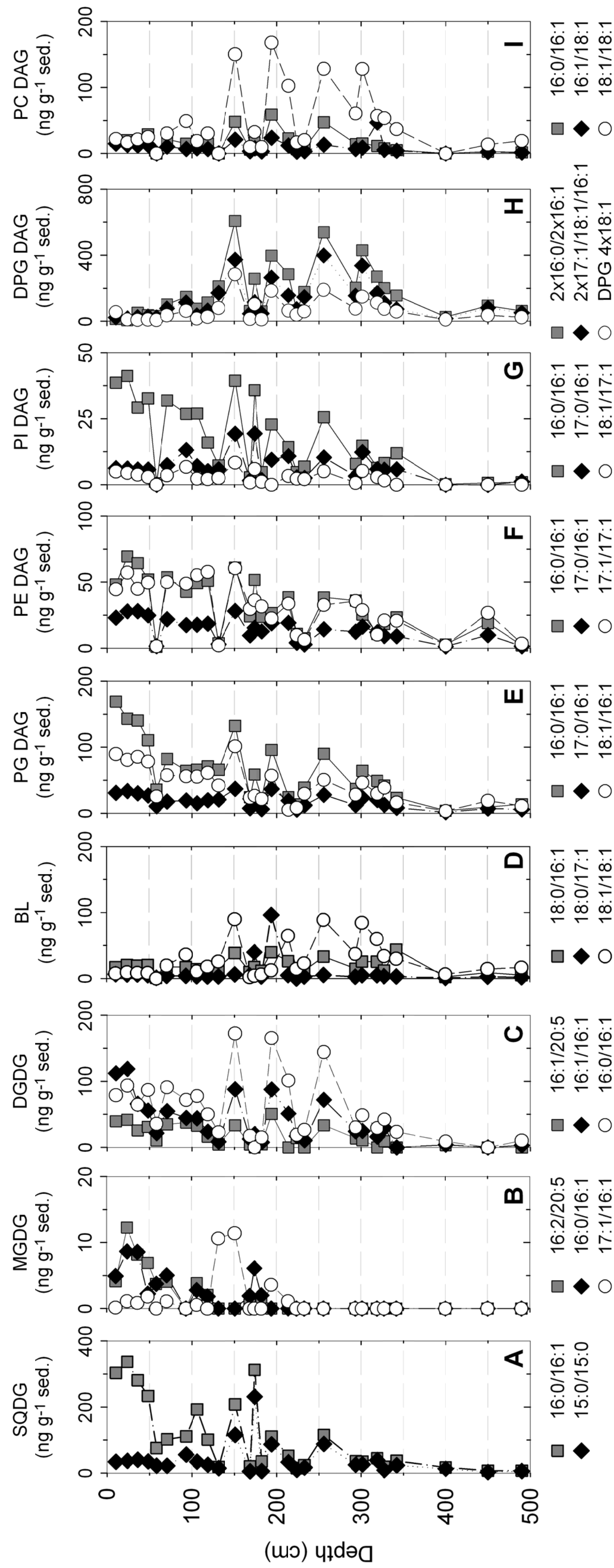


Fig. 17: Depth profiles of selected phosphate-free IPLs and phospholipids with diacylglycerol core lipids (DAG; for abbreviations cf. Fig. **16**).

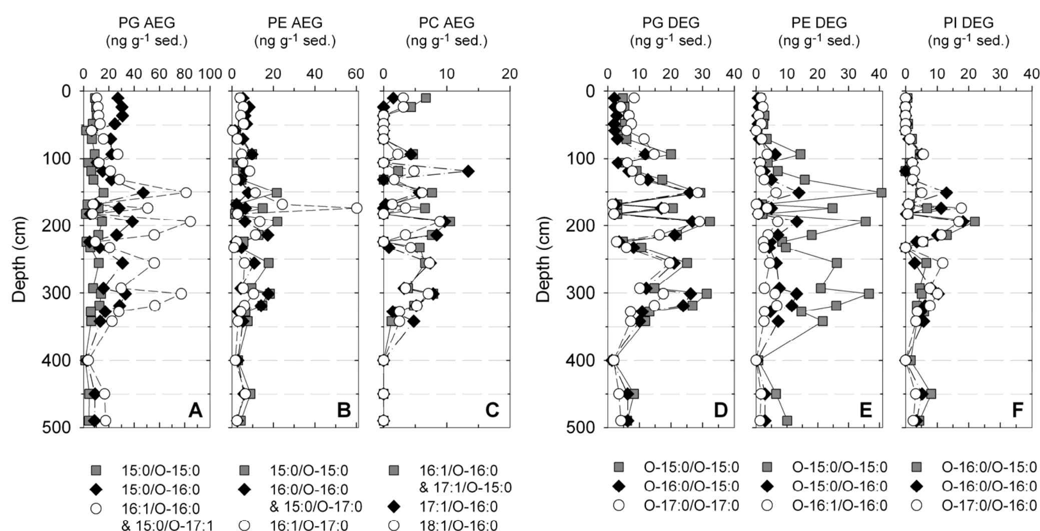


Fig. 18: Depth profiles of selected phospholipids with mixed acyl-ether glycerol (AEG) and diether glycerol core lipids (DEG; for abbreviations cf. Fig. 16).

6.4.3 Phospholipids with diether (DEG) and acyl-ether (AEG) glycerol cores

All analyzed sediment samples were dominated by diacylglycerol (DAG) core lipids, which formed the major group of polar lipids throughout the core but showed a decrease from more than 90 % of the detected IPLs in the upper layers to 78 % at the bottom of the core. Acyl-ether glycerols (AEGs), in turn, increased from 7 % of the core lipids in the upper 100 cm to 15 % at greater depth. IPLs with diether glycerol lipids (DEGs) increased from 2 % at the top to 9 % at the bottom of the core (Fig. 18).

A major fraction of the AEG lipids were linked to a PG head group (Fig. 18A). Most of the AEG IPLs comprised saturated ether- and ester-linked side chains with 15 to 18 carbon atoms. Noteworthy, no PCs with DEG side chains were detected but PG, PE, and PI with glycerol diether core lipids were found (Fig. 18D-F). The contents of DEGs were significantly elevated in the mixed mud/sand and mud layers ($p < 0.025$). Additional analysis showed that this was mainly determined by the high contents of PG DEGs and PE DEGs ($p < 0.025$).

Tab. 20: Spearman rank correlation coefficients calculated for the different concentrations of IPL classes (including the different core lipids). Number of samples: $n = 27$. Statistical significance: correlation is significant with $p < 0.002$ (dark grey), $p < 0.01$ (light grey) or $p < 0.02$ (open); no significant correlation (italics, no filling; $p > 0.05$). For abbreviations for IPL classes see text and Fig. 16.

	Bacterial IPLs[a]	**Archaeal IPLs**	**MGDG**	**BL**	**PG**	**DPG**	**PE**[b]	**PC**	**DGDG**	**SQDG**
PI	0.917	0.729	*-0.155*	0.919	0.820	0.739	0.799	0.799	0.557	0.581
SQDG	0.579	*0.069*	0.577	0.466	0.705	*0.083*	0.720	0.720	0.734	
DGDG	0.641	*0.136*	*0.332*	0.497	0.692	*0.219*	0.696	0.696		
PC	0.881	0.611	*-0.071*	0.811	0.820	0.634	0.928			
PE	0.839	0.508	*0.064*	0.726	0.785	0.542				
DPG	0.771	0.938	-0.492	0.847	0.586					
PG	0.917	0.473	*0.124*	0.814						
BL	0.899	0.799	*-0.247*							
MGDG	*-0.085*	-0.549								
Archaeal IPLs	0.685									

[a] Including IPLs from eukaryotic sources in the tide-influenced upper part of the core.
[b] Including its methyl derivatives PMME and PDME.

6.4.4 Depth profiles of archaeal IPLs

Archaeal phospholipid contents were about one order of magnitude lower than those of bacterial IPLs and did not exceed a relative abundance of 3 % of total IPLs (Fig. 14G). The detected archaeal IPLs were linked to PG and PI head groups with archaeol-containing phospholipids being more abundant than the corresponding hydroxy-archaeol core lipids.

An initial statistical analysis of the contents of archaeal phospholipids revealed a significant correlation with lithology ($p < 0.025$) with lower amounts occurring in the sand layers. A more detailed depth-related analysis of the data revealed, however, that the correlation was skewed by the extremely low concentrations of archaeal IPLs in the upper 150 cm of the sand body ($p < 0.05$), *i.e.* the part of the section that is influenced by the tide-driven flow of pore water. In contrast, the deeper sand layers not influenced by the tide had concentrations similar to those found in the mixed mud/sand and mud layers (Fig. 14G). Furthermore, archaeal IPLs correlated with DOC ($r = 0.406$, $p < 0.05$, $n = 27$, using Pearson's Correlation).

6.4.5 Polar lipid-derived fatty acids (PLFAs)

The chain length of polar lipid-derived fatty acids (PLFAs) ranged between 14 and 30 carbon atoms. PLFAs between C_{23} and C_{30} were minor, however, and only contributed less than 1 µg g^{-1} sediment. They were excluded from further analysis because they might also be derived from other complex polar lipids eluting in the polar lipid fraction during sample preparation (Aries et al., 2001).

Straight-chain and mono-unsaturated fatty acids (MUFAs in Fig. 15D) with 16 and 18 carbon atoms dominated throughout the whole sediment column and contributed up to 50 % of all detected PLFAs. The *n*-16:1 and *n*-18:1 PLFAs were the most abundant MUFAs at nearly all sediment depths (Fig. 15E). The second-most abundant PLFAs were straight-chain saturated fatty acids with carbon atoms between 14 and 20 (SCFAs in Fig. 15D). The C_{16} and C_{18} fatty acids were most abundant, but also the *n*-14:0 and *n*-17:0 fatty acids were found. Branched-chain fatty acids were detected in the range from C_{13} to C_{18} throughout

the entire core (BCFA in Fig. 15D) with the *iso*- and *anteiso*-branched C_{15} to C_{17} PLFAs as the most abundant compounds in this series.

Selected biomarker PLFAs assumed to be specific for sulfate-reducing bacteria (SRBs) were detected throughout the entire core even in the sulfate-free layers (Fig. 15F). The SRB-marker-PLFAs were an order of magnitude lower in concentration than the major MUFAs shown in Fig. 15D. At almost all depths, the most abundant SRB PLFA was 10-Me-16:0, claimed to be indicative for *Desulfo-bacteraceae* (Dowling et al., 1986). Further abundant SRB-related PLFAs were *anteiso* (*ai*)-17:0 and *iso* (*i*)-17:1, the latter being considered as marker for *Desul-fovibrionaceae* (Kohring et al., 1994).

The polyunsaturated eicosapentaenoic acid (EPA; *n*-20:5ω3) was detected throughout the entire core (Fig. 15G). Contents were highest near the surface (ca. 300 ng g^{-1} sediment) and decreased strongly within the uppermost 50 cm, although in deeper sediments, significant contents (up to 100 ng g^{-1} sediment) were found in distinctive layers between 250 and 350 cm depth. In contrast, the polyunsaturated polar lipid-derived docosahexaenoic acid (*n*-22:6ω3) was only detected in the upper 50 cm and only in minor amounts at a depth of 100 cm (Fig. 15G), but was completely absent below.

6.4.6 Stable carbon isotopic composition

The bulk isotopic values of total organic carbon (TOC) in the upper sand-dominated 50 cm were in the range of -17 ‰ and to -18 ‰ whereas in deeper sediments below 50 cm values between -20 ‰ and -23 ‰ were found (Fig. 14E).

The ^{13}C values of the IPL-derived *ai*-15:0 fatty acid and of the unbranched 16:1 and 18:1 fatty acids showed a distinctive pattern with depth (Fig. 14H). All three PLFAs were isotopically heaviest in the upper 100 cm of the mainly sandy sediments ($\delta^{13}C$ between -16 and -24 ‰). In the intercalated mud layer at ap-proximately 50 cm sediment depth the isotope composition is about 5 ‰ lighter (Fig. 14H). At depths below 130 cm the PLFAs were enriched in the lighter isotope with $\delta^{13}C$ values between -25 ‰ and -31 ‰. The corresponding isotopic value of IPL-derived archaeol ranged between -22 and -25 ‰ in the upper 200 cm

(Fig. 14I). In general, archaeol exhibited $\delta^{13}C$ values lighter than -25 ‰ in the sediments beneath 200 cm.

6.5 Discussion

In the present study, high contents of intact polar lipids (IPLs) were found in a 5 m long sediment core from a tidal flat. While IPL contents generally decreased only little with depth, highest values were found in TOC-enriched muddy and mixed mud/sand layers between 1 and 3.5 m depth. This is unexpected as intact polar lipids are an indicator for living microbial cells (Rütters et al., 2002b; Zink et al., 2003; Sturt et al., 2004) which in marine sediments normally decrease in number with depth (Parkes et al., 2000). The investigated sediments were also remarkable with respect to depth distribution and composition of their lipids. The core was characterized by high contents of betaine lipids (BL), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG) and in particular sulfoquinovosyldiacylglycerol (SQDG). These phosphate-free lipids are typically found in phototrophic microeukaryotes and macrophytes (Berge et al., 1995; Kato et al., 1996; Sanina et al., 2004) and in pelagic ecosystems (Ertefai et al., 2008; Van Mooy et al., 2009) but only very rarely in sediments. However, these lipids were not restricted to a thin layer at the sediment surface but were among the dominant lipids down to a depth of about 1.3 m above a thick mud layer. But even in the layers beneath this mud layer significant amounts of SQDG, BL and DGDG occurred. The potential origin of these unusual lipids will be discussed below.

6.5.1 Potential origin of phosphate-free IPLs (MGDG, BL, DGDG, SQDG)

SQDG, DGDG, MGDG and BL detected in sediments have generally been attributed to the presence of eukaryotic phototrophs, *e.g.* green algae like *Enteromorpha* spp. (Mazzella et al., 2007). The presence of benthic phototrophs, which are common in intertidal areas (Billerbeck et al., 2007) can explain the presence of SQDG and the other phosphate-free lipids at the sediment surface but not in the deeper layers, as the layers around 100 cm depth have a putative age of some tens of years to a few hundred years (Kunz, 1993). Nonetheless, many SQDG and

MGDG species in these shallow subsurface layers show typical eukaryotic traits, as for example the side chain combination $C_{16:2}/C_{20:5}$ (Fig. 17B).

Downward transport of intact diatom cells into the sediment caused by bottom currents has been shown to occur in coarse sand sediment but is generally restricted to the uppermost 10 cm (Huettel & Rusch, 2000; Rusch et al., 2001). But since the analyzed core was taken close to the margin of the tidal flat and at the low-water line, the sediments are characterized by intense subsurface pore water discharge (Beck et al., 2009). During ebb tide a significant part of the water runoff flows through the upper meter of sediment, which is reflected by the sulfate profile (Beck et al., 2008a; Røy et al., 2008). It is most likely that this ‘tidal pumping’ transports intact cells from the zone of benthic photosynthesis into the deeper layers. Such transport of colloids and small suspended particles by pore water has been described, for example, for permeable sand aquifers by McCarthy & Zachara (1989). The presence of diatom cells in the sediment layers down to 1 m depth was corroborated by the detection of 16S rRNA genes of diatom chloroplasts using a PCR-DGGE approach (Wilms et al., 2006b).

Even though many of the phosphate-free IPLs in the uppermost 130 cm of the sediment show typical eukaryotic side chain combinations, there also appears to be a significant bacterial contribution to this lipid pool, for example SQDG, DGDG and BL species with odd-numbered carbon side chains that are rarely found in eukaryotes (Sanina et al., 2004). In contrast to MGDGs with PUFA side chains these ‘bacterial’ lipids were detected throughout the core. Potential bacterial sources of such SQDGs are *Alphaproteobacteria* of the genera *Brevundimonas*, *Maricaulis*, and *Rhizobium* (Cedergren & Hollingsworth, 1994; Abraham et al., 1997) or the Firmicute *Marinococcus* (Sprott et al., 2006), whereas DGDGs have been found in *Bacillus* spp. (Clejan et al., 1986). BLs were detected in some *Alphaproteobacteria,* namely *Rhodobacter sphaeroides* and *Rhizobium meliloti* (Benning et al., 1995; Geiger et al., 1999). Most of these bacterial genera have either been isolated from tidal or deep subsurface sediments or were detected using molecular methods (Köpke et al., 2005; Süß et al., 2006).

6.5.2 Bacterial phospholipids

Whereas the relative contribution of phosphate-free IPLs (MGDG, DGDG, SQDG) decreased with depth, the abundances of most phospholipid classes (PE, PG, PI, PC) remained more or less constant (Fig. 15). This reflects that the cell numbers in the sediment core have been found to be relatively constant with depth (Gittel et al., 2008). One exception was DPG that, beneath the mud layer at about 130 cm depth, strongly increased and represented even about 50 % of all IPLs at greater depth. DPGs are common bacterial phospholipids and were found in most cultured phyla like the *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (Clejan et al., 1986; Nunes et al., 1992; Mukamolova et al., 1995; Choma & Komaniecka, 2002; Freese et al., 2009). In some *Gamma*- and *Deltaproteobacteria* isolates from the Wadden Sea, DPG was only one of the minor phospholipid classes (Rütters et al., 2002a; Freese et al., 2009). However, most investigations on pure cultures were performed under optimal growth conditions to achieve high biomass yields and it is questionable whether these reflect *in situ* conditions. In fact, it was shown that several bacterial species increase their DPG contents when exposed to physical or chemical stress or under starvation (Oliver & Stringer, 1984; Wanner & Egli, 1990; Mukamolova et al., 1995). For example, after 100 days of starvation IPLs of *Micrococcus luteus* NCIMB 13267 consisted almost entirely of DPG, whereas in freshly grown cultures DPG represented only about 38 % of the IPL fraction (Mukamolova et al., 1995). Considering the larger age (some hundred years) of the deeper layers (> 150 cm), the more recalcitrant organic matter and that these are apparently cut off from nutrient supply by tidal pumping as seen in the sulfate profile, it can be expected that the microbial communities are nutrient-limited and hence contain higher amounts of DPG.

In pure bacterial cultures the increase in DPG under stress is at the expense of other phospholipids, in most cases PE (Oliver & Stringer, 1984; Mukamolova et al., 1995). Hence, in the upper sand-dominated section of the core, where most phospholipids (PE, PG, PI, and PC) remained more or less constant, the moderate increase in DPG content may reflect only an increase in bacterial cell numbers. In contrast, the very strong increase in DPG content and the almost complete absence of PE in the mud layer at 130 cm depth strongly suggest nutri-

ent limitation. The nutrient supply in fine-grained sediments is limited due to much lower diffusion rates compared to sand and results in generally much lower activity of the microbial communities (Musslewhite et al., 2003). Similar findings were reported for mud-rich sediments (Rütters et al., 2002b; Freese et al., 2009): From the surface to 20 cm depth a general decrease of phospholipid contents was observed whereas DPG contents showed only a moderate decrease and no quantifiable amounts of PE or PI were found at this depth.

Like the phosphate-free lipids, PI and PC are rarely detected in sediment cores beneath the surface layer (Freese et al., 2009). Both lipids are not very common in bacteria (Sohlenkamp et al., 2003) although they have been found in some *Alphaproteobacteria*, *e.g. Paracoccus* and *Rhizobium* spp. (Thiele et al., 1980; de Rudder et al., 1997) or the sulfate reducer *Desulforhabdus amnigenus* (Rütters et al., 2001). Some of these genera are also potential sources of phosphate-free IPLs, *e.g. Rhizobium* spp. for BL and SQDG (Geiger et al., 1999). Hence, the high correlation between the presence of PI and that of BL or SQDG in Janssand sediments may be indicative for the presence of these *Alphaproteobacteria*.

6.5.3 Presence of diether and acyl-ether glycerol phospholipids

The occurrence of mixed acyl-ether (AEGs) and diether (DEG) phospholipids throughout the entire sediment core suggests strictly anaerobic bacteria as source organisms. In a number of studies either intact dialkyl and monoalkyl ether lipids or their core lipids (without head groups) were found in sediments harbouring anaerobic methanotrophic communities (Orphan et al., 2001; Pancost et al., 2001; Aloisi et al., 2002; Blumenberg et al., 2004; Elvert et al., 2005), in sediments from hydrothermal fields (Bradley et al., 2009), or in water samples from the anoxic hypolimnion of a meromictic lake (Ertefai et al., 2008). In previous studies on Wadden Sea sediments (0 - 50 cm) only mixed acyl-ether PGs and PCs were detected, but neither PE AEGs nor diether IPLs (Rütters et al., 2002b; Freese et al., 2009).

In most of these studies ether lipids were explained by the presence of sulfate-reducing bacteria. In fact, AEGs have been found in the mesophilic sulfate-

reducing bacteria *Desulfosarcina variabilis* and *Desulforhabdus amnigenus* (Rütters et al., 2001), and strikingly the AEG patterns in these two species are very similar to those found at site the Janssand site (PG AEG, PE AEG, PC AEG). But since both, AEG and DEG showed maximum abundance in the sulfate-free layers, SRB as source organisms may appear improbable at first sight. However, SRBs were detected throughout the entire Janssand core using CARD-FISH (Gittel et al., 2008), and the relative contribution to the total cell counts did not differ significantly between the sulfate-containing and sulfate-free layers. Since *Desulfosarcina*-related SRB did not exceed 0.7 % of the total cell counts (Gittel et al., 2008) other SRB may be the major source for the AEGs. Candidates may be relatives of the second known AEG-containing SRB species, *Desulforhabdus amnigenus*, like for example *Syntrophobacter* spp. for which no intact lipid data are available. We can only speculate about their presence at site Janssand as Gittel et al. (2008) did not use probes specific for the *Syntrophobacteraceae*. However, the *Syntrophobacteraceae* comprise organisms that can grow as sulfate reducers or in syntrophic relationship with methanogens.

Whereas AEGs were found in mesophilic SRBs, DEG lipids are mostly typical of deeply branching thermophilic bacteria like the *Aquificales* (De Rosa et al., 1988; Huber et al., 1992; Huber et al., 1996; Jahnke et al., 2001) and some *Thermodesulfobacterium* spp. (Sturt et al., 2004). On one hand, these organisms are unlikely to be present in quantities large enough to be the source organisms of theses biomarkers at site Janssand. On the other hand ether lipids have been found in mesophilic myxobacteria: *Stigmatella* spp. contain DEGs (Caillon et al., 1983), whereas AEGs were found in *Myxococcus* spp. (Kleinig, 1972; Ring et al., 2006). Interestingly, *Stigmatella aurantiaca* contains the same classes of diether (PG, PE, and PI) and mixed ether-ester lipid cores as detected in the Janssand sediments (Fig. 18D-F). Although, myxobacteria were originally described as strictly aerobic and terrestrial organisms, an increasing number of reports suggest that they are also common in anoxic and marine environments. Some members of the *Myxococcales* were shown to dissimilatorily reduce iron (He & Sanford, 2003; Treude et al., 2003) or nitrate (Coates et al., 2002). Coates et al. (2002) described a strictly anaerobic *Stigmatella*-related bacterium that degraded humic substances.

In addition, myxobacterial sequences were detected in a wide range of anoxic marine sediments (Ravenschlag et al., 1999; Bowman & McCuaig, 2003; Knittel et al., 2003; Bissett et al., 2006) including deeper tidal flat sediments from site Janssand (Wilms et al., 2006a).

In addition, mixed acyl-ether phospholipids with monounsaturation in the ether-linked side chains were detected in the Janssand core. These were most likely plasmalogens with alkenyl-acyl side chains. Plasmalogens have been considered being specific for anaerobic bacteria affiliated with the *Deltaproteobacteria* and the *Firmicutes* like the sulfate-reducing *Desulfovibrio*, *Desulfosporosinus* and *Desulfotomaculum* spp., the fermenting *Clostridium* spp. and the homoacetogenic *Selenomonas* spp. (Lechevalier & Lechevalier, 1988; Paltauf, 1994; Rütters, 2001; Kaksonen et al., 2006). However, relatively recently plasmalogens were also detected in facultatively anaerobic *Actinobacteria* (Männistö & Häggblom, 2006), for example in *Granulicoccus phenolivorans.* In this organism, plasmalogens appear to be the major phospholipids as their dimethyl acetals hydrolysis products represent about 40 % of the PLFA pattern (Maszenan et al., 2007). Since *Desulfovibrio* spp. as potential source organisms were found only at the sediment surface (Gittel et al., 2008), plasmalogens may originate mainly from members of the Gram-positive phyla *Firmicutes* and *Actinobacteria*. Actinobacterial sequences were detected at site Janssand at most depths, whereas no *Firmicutes* were found (Wilms et al., 2006a). Gittel et al. (2008), in contrast, detected *Desulfotomaculum* spp. (up to 0.3 % of the total cell count) at a few scattered depths, and in a culture-based study Köpke et al. (2005) found predominantly *Firmicutes* (*e.g. Clostridium* and *Desulfosporosinus* spp.) in the deeper sediment layers of tidal flat sediments.

6.5.4 Dominance of bacterial over archaeal abundance

Whereas bacterial phospholipids were detected throughout the core, archaeal phospholipids appeared to be confined to the deeper layers (> 70 cm, Fig. 14G). This may indicate that *Archaea* at site Janssand are mainly strict anaerobes that prefer stable anoxic conditions, even though mesophilic aerobic *Archaea* have recently been isolated from other marine habitats (Könneke et al., 2005). As

tetraether lipids were not found even in the surface layers these *Archaea* appear to contribute only very little to the microbial community. In contrast, beneath 70 cm depth the numbers of *Archaea* as inferred from IPL contents follow very closely those of *Bacteria* ($R^2 = 0.93$, Fig. 19B). This correlation strongly suggests that *Bacteria* and *Archaea* exist in syntrophic association, *i.e.* methanogenic *Archaea* scavenge hydrogen produced by fermenting *Bacteria*. The assumption that methanogens are the major *Archaea* in the sulfate-free deeper layers of the core is supported by the presence of PG and PI archaeols and hydroxy-archaeols as major archaeal lipids, since these are also the main IPLs in the *Methanosarcinales* (Koga & Nakano, 2008).

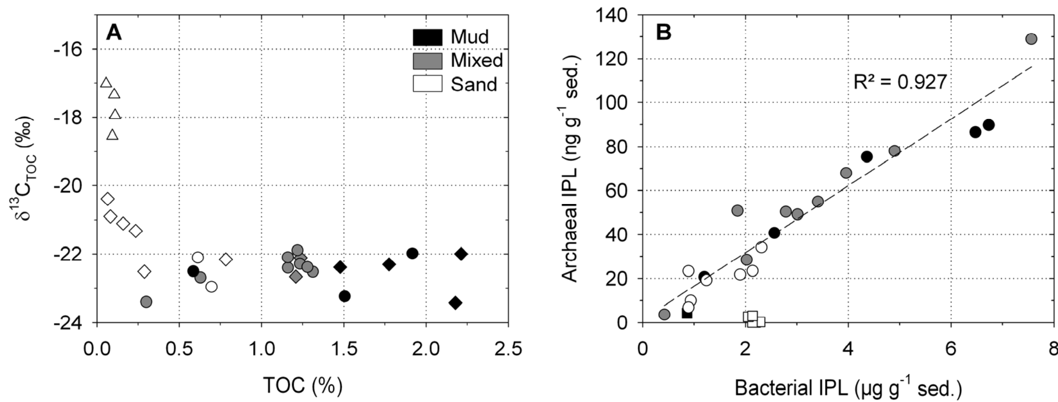


Fig. 19: A: Cross-plot of $\delta^{13}C$ values of TOC ($\delta^{13}C_{TOC}$) versus total organic carbon content (TOC). Sediments down to 50 cm depth are shown as triangles, from 50 to 200 cm as diamonds, and from depths > 200 cm as circles. Sediment types are represented as fillings of the symbols: mud = filled, mixed mud/sand sediments = shaded, sand = open. **B:** Cross-Plot of archaeal IPL contents vs. bacterial IPL contents. Shallow subsurface samples to a depth of 71 cm are shown as squares, circles represent samples from sediment depths below 71 cm. R^2 is shown for a linear regression of IPL contents excluding samples of depths shallower than 71 cm. Filling of the symbols as in Fig. 19A.

The predominance of *Bacteri*a over *Archaea* in the Janssand core is in agreement with quantitative molecular ecological studies on other tidal flats by Wilms et al. (2007) and Ishii et al. (2004). Like in our study, Wilms et al. (2007) found numbers of *Archaea* being an order of magnitude lower than those of *Bacteria*. This is in contrast to the deep biosphere, where *Archaea* may represent the majority of the microbial cells (Biddle et al., 2006; Valentine, 2007; Lipp et al., 2008). In deep sub-seafloor samples monoglycosyl- or diglycosyl-glycerol dibiphytanyl glycerol tetraethers and archaeols were found to be the dominant

archaeal IPLs. These lipids were not detected at site Janssand. Conversely, the archaeol containing phospholipids like they were detected at site Janssand occur in the deep biosphere only in very low abundances (Biddle et al., 2006; Lipp et al., 2008).

6.5.5 ^{13}C isotopic composition indicating possible carbon sources

Within the upper 60 cm of the core carbon isotope composition of the bulk organic matter was heaviest indicating a predominance of marine organic matter (Fig. 14E; Fry & Sherr, 1984). On the one hand, this might be explained by rapid sedimentation and sediment resuspension that supply fresh organic material at prograding flat margins (Beck et al., 2009). However, our IPL data also indicate that these values can be explained by a transport of cells of benthic phototrophs or by a supply of fresh organic matter (*e.g.* exudates) into the deeper permeable sediment layers by intensive advective pore water transport and tidal pumping.

A significant shift to isotopically lighter organic matter can be found at 60 cm depth indicating a higher portion of terrigenous material and a stronger degradation of marine organic matter within the deeper buried sediments. The $\delta^{13}C$ values are in a range as observed for other near-surface intertidal sediments which are characterized by higher mud contents (Rütters et al., 2002b). For the sand layers in the intermediate section (50 - 200 cm) of the core (open diamonds in Fig. 19A) there appears to be a negative correlation between the TOC content and the isotopic signal: the higher the TOC content the lower the contribution of marine organic matter. This correlation is even more pronounced if mud or mixed mud/sand and sand layers are compared to each other. A closer examination of the data showed that the two sand beds (119 and 140 cm) in the 50 to 200 cm section (open diamonds in Fig. 19A) that showed the most negative $\delta^{13}C$ values were adjacent to two thicker mud layers. This indicates that some of the supposedly terrigenous organic matter is diffusing from the mud layers into the sand layers (McMahon & Chapelle, 1991). The low TOC values indicate that fresh marine organic material is rapidly degraded in the upper sand-dominated sediments (Fig. 14D). Nonetheless, even in the deepest layers a considerable contribution of marine organic matter was indicated as $\delta^{13}C$ values did not decrease to values

below -24 ‰. Such values have been reported for other cores taken at other, mixed mud/sand locations in the back-barrier tidal flats of Spiekeroog Island (Volkman et al., 2000; Freese et al., 2008a). Therefore, intensive advective pore water flow at the tidal flat margin of site Janssand not only seems to transport particulate organic matter into the upper 130 cm of permeable sediments as indicated by the high contents of phosphate-free IPLs of eukaryotic origin. The $\delta^{13}C$ values of bulk TOC may also indicate that the transport of marine organic matter by 'tidal pumping' and deep advective pore water transport has some influence on the organic matter composition in permeable sediments even at several meters depth.

The carbon isotopic values of the IPL-derived fatty acids (PLFA in Fig. 14H) followed generally the shift of the $\delta^{13}C$ values of bulk TOC. However, in most cases the isotopic signatures of the fatty acids were more negative than that of bulk TOC, particularly in the deeper layers (> 200 cm). Exceptions were the sand layers between 50 and 130 cm. At first sight the depth trend of the isotope signature of PLFAs may indicate a strong contribution of eukaryotic cells like marine phytoplankton ($\delta^{13}C$ values between -22 to -19 ‰; Volkman et al., 2000). However, the typical bacterial *ai*-15:0 fatty acid was isotopically heavier than the two even-carbon-numbered fatty acids that occur in both, bacteria and eukaryotic cells. Hence, it appears more likely that the isotope signature reflects the utilization of marine organic matter (including buried cells) than a direct contribution from marine phototrophs.

It could be argued that the depletion in $\delta^{13}C$ at about 50 cm depth is indicative for the presence of microbial consortia that are involved in the anaerobic oxidation of methane (AOM). The lipids found in the sediment (PG- and PI-archaeols and the corresponding hydroxy-archaeols) indicate the presence of ANME consortia and/or methanogens, as the methane-oxidizing ANME-2 and ANME-3 *Archaea* and the closely related *Methanosarcinales* contain very similar lipids (Elvert et al., 2003, 2005; Blumenberg et al., 2004; Koga & Nakano, 2008; Rossel et al., 2008). However, the isotopic shift to lighter values at approximately 50 cm depth was observed in bacterial PLFAs but not in archaeol (Fig. 14H and Fig. 14I). If ANME consortia were among the dominating *Archaea*, the isotopic

shift in the archaeal lipids should have been at least as pronounced as in the bacterial PLFAs. The high isotopic values in the upper sediment layers rather indicate a microbial community driven by photosynthetically produced organic matter and a minor role of the anaerobic oxidation of methane. This is corroborated by results of Ishii et al. (2004), who detected only very few ANME-2 and no ANME-1 consortia in Wadden Sea sediments of site Janssand.

6.6 Conclusions

The analysis of intact polar lipids (IPLs) applied to surface and subsurface sediments of an intertidal flat margin has been proven to be a powerful tool for the analysis of the *in situ* microbial communities. The analysis of IPLs is unbiased as these molecules are unstable outside intact cells (White et al., 1979; Harvey et al., 1986) and the combined information on head group and side chains allows a much better phylogenetic delineation than the use of polar lipid fatty acids alone (Rütters et al., 2002b; Freese et al., 2009). Although IPLs specific for different phylogenetic groups were identified, the amount of information encoded in a single IPL molecule, and hence the phylogenetic resolution, is limited. Nevertheless, IPL analysis can ideally complement molecular biological investigations like the analysis of 16S rRNA genes. In a previous study, the 16S rRNA genes of diatom chloroplasts were detected in the deeper layers of the tidal flat Janssand (Wilms et al., 2006a). Originally, it was assumed that this signal was derived from ‘fossil’ DNA, as it was shown that nucleic acids of phototrophic microbes may survive for tens of thousands of years outside the cell if adsorbed by the sediment matrix (Coolen & Overmann, 2007). The results presented here, *i.e.* the detection of diatom-typical IPLs (Berge et al., 1995; Kato et al., 1996) in the deeper layers of site Janssand, in turn suggest intact cells as a more likely source. The presence of intact eukaryotic lipids showed that the subsurface water discharge not only supplies solutes (Beck et al., 2009) but apparently also fresh particulate organic material, *i.e.* cells. The isotopic analysis of the IPL-derived fatty acids revealed that the *in situ* bacterial communities assimilate this fresh material. These results could hardly been obtained by a standard molecular biological study and show

that the analysis of intact polar lipids is a valuable complementary tool for the analysis of microbial communities.

Whereas the analysis of IPLs in environmental samples is becoming more accessible, the interpretation of the results is still hampered by the relatively small number of microorganisms analyzed for their intact lipids. In addition, the majority of microbial phyla has not been brought into pure culture (Rappé & Giovannoni, 2003) and hence the lipid composition of many microorganisms remains unknown. These yet-to-be-cultured microorganisms may be the source of a number of environmental lipid classes, *e.g.* diether alkyl phospholipids, for which no cultured counterpart is known so far. Therefore, the analysis of a larger numbers of bacterial, archaeal and microeukaryotic species should be pursued, and this work may also include microbial enrichments if no pure cultures are available. The high amounts of ether lipids in the mud layers in the middle section of the core are remarkable, because they are not always accompanied by high cell counts (*e.g.* at 200 cm depth, Fig. 18). Although it was shown that phospholipids are being degraded within days after release from cells (White et al., 1979; Harvey et al., 1986), it is not known whether the degradation rate is the same for ester and ether lipids, phosphate-free polar lipids and phospholipids or whether lithology has an influence. This knowledge, however, is necessary for an accurate interpretation of the environmental IPL data and should be gained in the future.

6.7 Acknowledgements

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7 Gesamtbetrachtung und Ausblick

Die verwendete Kombination von mikrobiologischen und geochemischen Methoden hat aufschlussreiche Ergebnisse über die Anpassungsmechanismen von Mikroorganismen und die Prozesse am Standort Janssand im Rückseitenwatt der Insel Spiekeroog geliefert. Die gegenwärtige Studie zeigt daher, dass sich biogeochemische ideal mit mikrobiologischen Studien ergänzen.

7.1 Kombination von mikrobiologischen und geochemischen Studien an bakteriellen Reinkulturen

Ein wichtiger Aspekt dieser Arbeit war es, neue Erkenntnisse über die Zusammensetzung der Membranlipide und die physiologische Anpassung von sulfatreduzierenden Bakterien aus Wattsedimenten zu gewinnen. Zuerst wurden acht Stämme von *Desulfovibrio acrylicus* untersucht, die von drei unterschiedlichen Standorten des Wattenmeeres stammen. Unerwartet war, dass diese Stämme große Mengen phosphatfreier Ornithinlipide aufwiesen. Derartige Lipide waren zuvor nur bei einem anderen Vertreter der *Deltaproteobacteria* nachgewiesen worden (Kapitel 2). Weiterführende Untersuchungen zeigten, dass der relative Anteil der Ornithinlipide mit der Wachstumstemperatur zunimmt und dass sie offenbar eine wichtige Rolle bei der Regulierung membrangebundener Proteine spielen (Kapitel 3). Die Analyse von Membranfettsäuren wurde außerdem für die chemotaxonomische Charakterisierung eines weiteren Sulfatreduzierers genutzt, der ebenfalls aus dem Watt isoliert worden war. Die Kombination aus chemotaxonomischen, phylogenetischen und physiologischen Untersuchungen und der Vergleich mit verwandten, bereits beschriebenen Arten haben dazu geführt, dass eine neue Gattung vorgeschlagen wird (Kapitel 4). In einem weiteren Kapitel wurde die Zusammensetzung der polaren Membranlipide von *Photobacterium* spp. analysiert, die aus der tiefen Biosphäre isoliert worden waren. Die Ergebnisse der Lipidanalyse bestätigten die auf molekularbiologischen Untersuchungen basierende Unterteilung der Isolate in zwei getrennte Populationen (Kapitel 5).

7.2 Anpassungsstrategien von *Desulfovibrio acrylicus* bei veränderten Wachstumsbedingungen

Desulfovibrio acrylicus zeichnet sich durch Membranfettsäuren und Phospholipidmuster aus, wie sie für sulfatreduzierende Bakterien innerhalb der *Deltaproteobacteria* typisch sind (Rütters, 2001). Neben Phosphatidylethanolamin (PE), Phosphatidylglycerol (PG) und Diphosphatidylglycerol (DPG) wurden unerwartet hohe Anteile phosphatfreier Ornithinlipide (OL) identifiziert, die bis zu 60 % der Membranlipide ausmachten. Ornithinlipide waren bislang nur in wenigen Vertretern der *Proteobacteria*, *Actinobacteria*, *Firmicutes* und *Bacteroidetes* gefunden worden. Der einzige beschriebene Sulfatreduzierer mit OL war bislang *Desulfovibrio gigas* (Makula & Finnerty, 1975).

Die *Desulfovibrio acrylicus*-Stämme wurden hinsichtlich ihrer temperaturabhängigen Veränderungen in Phänotyp, Wachstum, Aktivität und Zusammensetzung der intakten polaren Membranlipide untersucht. Die Stämme wuchsen in einem Temperaturbereich von 4 bis 37 °C, ein Stamm sogar bis 40 °C. Obwohl alle acht Stämme zu einem einzigen Phylotyp gehören, wurden fünf Stämme als mesophil charakterisiert. Ihre höchsten Wachstumserträge wurden hauptsächlich im mittleren Temperaturbereich erzielt, während die Wachstumserträge zu den Grenzen hin stark abnahmen. Im Gegensatz dazu wuchsen die drei als psychrotroph eingeordneten Stämme mit hohen Erträgen über den gesamten unteren Temperaturbereich von der Minimum- bis zur optimalen Wachstumstemperatur. Die Unterschiede in der Temperaturanpassung innerhalb eines Phylotyps sind bemerkenswert. Sie zeigen, dass innerhalb einer Art signifikante physiologische Unterschiede auftreten können und verdeutlichen, dass rein molekularbiologische Studien derartige Anpassungen allein aus der Analyse der 16S rRNA Gene nicht eindeutig nachweisen können.

7.3 Die mögliche Rolle der Ornithinlipide

Die Anpassung der Membranfluidität einer Zelle dient der Aufrechterhaltung lebenswichtiger Prozesse wie dem Elektronentransport in der Atmungskette, die Aufnahme von Substraten oder Chemotaxis. In früheren Studien zur Temperatur-

anpassung wurden häufig nur Membranfettsäuren oder polare Lipidgruppen untersucht (Donato et al., 2000; Freese et al., 2008b). Die in der vorliegenden Studie durchgeführte molekulare Analyse der intakten polaren Lipide (IPL) ergänzt diese Untersuchungen und ermöglichte außerdem den Vergleich des Temperatureffekts auf Lipidkopfgruppen und Seitenketten.

Bei steigender Wachstumstemperatur wurden in den untersuchten Stämmen hauptsächlich PE gegen OL ausgetauscht. Die molekularen Untersuchungen der IPL haben jedoch gezeigt, dass die Seitenketten der OL ähnlichen Veränderungen unterliegen wie die der Phospholipide. Offenbar ist der Hauptmechanismus zur Anpassung der Membranviskosität die Veränderung der Seitenketten der IPL. Daraus wurde der Schluss gezogen, dass der bei steigender Temperatur zunehmende Gehalt von OL nicht vorrangig der Anpassung der Membranfluidität dient, sondern der Regulierung membrangebundener Proteine, so wie es in einer früheren Studie von Aygun-Sunar et al. (2006) gezeigt wurde. Außerdem unterschieden sich die acht Isolate von *Desulfovibrio acrylicus* hinsichtlich ihrer physiologischen Anpassung an wechselnde Wachstumstemperaturen, aber nicht in den beobachteten Anpassungsmechanismen. Das verdeutlicht, dass psychrotrophe und mesophile Bakterien die gleichen Mechanismen nutzen, um die Membranfluidität aufrecht zu erhalten. Ungeklärt ist, inwiefern diese Anpassung durch die *de-novo*-Synthese neuer IPL erfolgt oder durch Enzyme, die die bereits vorhandenen Seitenketten in der Cytoplasmamembran verändern. Die Klärung dieser Frage sollte Gegenstand zukünftiger Untersuchungen sein.

7.4 Phosphatfreie Membranlipide in Biomarkerstudien des Standorts Janssand

Die Analyse von IPL als Biomarker für lebende Zellen hat aufschlussreiche Ergebnisse über die Zusammensetzung der mikrobiellen Gemeinschaft in einem 5 m langen Sedimentkern aus dem Wattenmeer geliefert (Kapitel 6). Intakte polare Lipide wurden als Biomarker für intakte Zellen postuliert (Rütters et al., 2002a), da sie innerhalb von Tagen nach Zelllyse abgebaut werden (White et al., 1979; Harvey et al., 1986). Im Rahmen der vorliegenden Studie hat es sich als

besonders vorteilhaft erwiesen, dass diese Biomarker einen großen analytischen Rahmen ermöglichen, der die uneingeschränkte Analyse von Organismen eukaryotischen und prokaryotischen Ursprungs einschließt. Dadurch war es einerseits möglich, eine abundante mikrobielle Gemeinschaft selbst in den tieferen Sedimenten des Janssands nachzuweisen. Andererseits wurden so auch Erkenntnisse darüber gewonnen, bis in welche Tiefe der Transport benthischer Algen in die permeablen Sandschichten durch den Porenwasserfluss erfolgt, wo sie den Mikroorganismen als Substrat dienen.

Hohe Gehalte von phosphatfreien Betainlipiden (BL), Glycolipiden und Sulfochinovosyldiacylglycerol (SQDG) wurden in den oberen sanddominierten 130 cm des Sedimentkerns gefunden. Mehrfach ungesättigte Fettsäureseitenketten dieser phosphatfreien IPL deuten auf den Eintrag eukaryotischer Algen mit intakten Cytoplasmamembranen hin. Dies ist ein Hinweis darauf, dass der advektive Porenwasserfluss am Platenrand des Janssands nicht nur gelöstes organisches Material (Beck et al., 2009), sondern auch frisches partikuläres organisches Material in die tieferen Sedimentschichten transportiert. Die Analyse der Kohlenstoffisotopenverhältnisse der hydrolisierten IPL-Fettsäuren hat außerdem gezeigt, dass dieses marine organische Material auch in mehr als einem Meter Tiefe von den autochthonen Mikroorganismen bevorzugt als Kohlenstoffquelle verwendet wird. Ornithinlipide, die auf eine hohe Abundanz von *Desulfovibrio acrylicus* hinweisen könnten, wurden im Sedimentkern nicht nachgewiesen. Unerwartet war jedoch der Nachweis phosphatfreier SQDG und BL bakteriellen Ursprungs in bis zu fünf Metern Tiefe. Bisher waren keine anaeroben Bakterien bekannt, die diese IPL synthetisieren. Erst vor kurzem wurden auch anaerob wachsende Bakterien isoliert, die SQDG (H. Sass und H. Rütters, persönliche Mitteilung) und Betainlipide (K.-U. Hinrichs, persönliche Mitteilung) enthalten.

7.5 Phospholipide von noch unkultivierten Mikroorganismen

Acyletherglycerole (AEG) und Dietherglycerole (DEG) wurden im gesamten Sedimentkern des Janssands detektiert, wobei die Gehalte im mittleren Teil des Kerns am höchsten waren. Das deutet auf obligat anaerobe Bakterien als Quellor-

ganismen hin. Bisher gibt es nur wenige Isolate von mesophilen Bakterien, die ethergebundene Seitenketten haben. Membranlipide mit AEG-Gerüsten wurden bisher nur in mesophilen sulfatreduzierenden Bakterien gefunden (Rütters et al., 2001). Außerdem wurden Lipide mit DEG-Gerüsten meist nur in thermophilen Bakterien beschrieben (Langworthy et al., 1983; Sturt et al., 2004). Vertreter der mesophilen *Myxobacteria* scheinen jedoch eine naheliegende Quelle für DEG- und AEG-Lipide zu sein. Die in dieser Ordnung beschriebenen Etherlipide (Kleinig, 1972; Caillon et al., 1983; Ring et al., 2006) gleichen den IPL, die im Janssand detektiert wurden. Gensequenzen dieser Ordnung wurden außerdem in einer Reihe von anoxischen marinen Sedimenten und in tieferen Sedimenten des Janssands gefunden (Knittel et al., 2003; Bissett et al., 2006; Wilms et al., 2006a). Viele der Vertreter dieser Ordnung, auf die es molekularbiologische Hinweise gibt, liegen jedoch nicht in Reinkultur vor. Außerdem existieren auch von den bereits vorhandenen Reinkulturen kaum IPL-Daten. Die Analyse ihrer IPL und die Kultivierung weitere Stämme sind daher wichtige Aufgaben für zukünftige Studien.

7.6 Bakterien dominieren den untersuchten Standort

In den Sedimenten des Janssands wurden ausschließlich Archaeenlipide detektiert, die typisch für methanogene Vertreter der *Methanosarcinales* aus dem Subphylum der *Euryarchaeota* sind. Archaeen, die Tetraether synthetisieren wie z.B. die *Crenarchaeota*, scheinen somit nur einen sehr geringen Anteil an der mikrobiellen Gemeinschaft auszumachen. Der Gehalt der Archaeenlipide war im gesamten Kern um mindestens eine Größenordnung niedriger als der der Bakterienlipide. Die Gehalte der Bakterien- und Archaeenlipide korrelierten in den tieferen Sedimentschichten jedoch sehr stark miteinander. Das deutet darauf hin, dass Archaeen und Bakterien syntroph vorkommen, z.B. wasserstoffverbrauchende Archaeen als syntrophe Partner von Gärern, die Wasserstoff produzieren. Das Fehlen einer ausgeprägten Sulfat-Methan-Übergangszone und die Isotopensignatur der hydrolisierten IPL ließen keinen eindeutigen Rückschluss auf eine hohe

Abundanz von Mikroorganismen zu, die an der anaeroben Oxidation von Methan (AOM) beteiligt sind und ^{13}C-abgereichertes Methan in ihre Biomasse einbauen.

7.7 Ausblick

Die Fortschritte in der Analytik, z.B. die Verwendung von HPLC-ESI-MS, ermöglichen mittlerweile die Analyse intakter polarer Lipide aus Umweltproben, die dadurch zunehmend Anwendung als Biomarker in der Biogeochemie finden.
Die Interpretation dieser Daten ist jedoch mit einigen Unsicherheiten behaftet, da die Datenbasis zur Zusammensetzung der intakten polaren Membranlipide von Mikroorganismen noch immer sehr unvollständig ist. Dies betrifft zum einen kultivierte Arten von Mikroorganismen, deren intakte polare Lipide bisher nur sehr selten analysiert wurden. Während dies aber nachgeholt werden kann, stellen bisher unkultivierte (‚*yet-to-be-cultured*') Mikroorganismen ein größeres Problem für die Interpretation von IPL-Mustern in Umweltproben dar, insbesondere in tieferen Sedimentschichten, die von Bakterien- und Archaeen-Phyla ohne jegliche kultivierten Vertreter wie z.B. von *Chloroflexi* oder SAGMEG-Archaeen (*South African Goldmine Euryarchaeotal Group*) dominiert werden. In diesen Schichten stellen diese Organismen aber vermutlich die Hauptquelle von Membranlipiden dar, die entsprechend noch nicht eindeutig ihren Ursprungsorganismen zugeordnet werden konnten. Ein Beispiel für derartige Lipide sind die bakteriellen IPL mit Dietherglycerolgerüsten, für die es bisher keine plausiblen mesophilen Ursprungsorganismen gibt. Deshalb sollte die Charakterisierung der Reinkulturen von Archaeen und Bakterien intensiviert, aber auch auf Mikroeukaryoten erweitert werden. Die Untersuchungen sollten auch Anreicherungskulturen einschließen, falls keine Reinkulturen vorliegen oder erhalten werden können.

Der Nachweis hoher Anteile phosphatfreier bakterieller Membranlipide in den Reinkulturen und den Sedimentproben war überraschend. So kann z.B. über die Verbreitung von Ornithinlipiden innerhalb der *Deltaproteobacteria* im Augenblick nur spekuliert werden. Der Datensatz über die IPL von bakteriellen Reinkulturen ist noch sehr lückenhaft, und Ornithinlipide wurden vermutlich auch deshalb nicht gefunden, weil häufig nur die Fettsäuren bakterieller Reinkulturen

analysiert wurden. Ein Hinweis für die weitere Verbreitung von Ornithinlipiden innerhalb der Gattung *Desulfovibrio* könnte der erhöhte Anteil von 3-Hydroxyfettsäuren in der Phospholipidfraktion sein, die in verschiedenen Stämmen von *Desulfovibrio* nachgewiesen wurden (Edlund et al., 1985). Interessanterweise wurde vor kurzem auch ein Vertreter von *Desulfovibrio* aus der tiefen Biosphäre kultiviert, in dem Ornithinlipide nachgewiesen worden waren (Bert Engelen, persönliche Mitteilung).

Die Strukturen einer Vielzahl von IPL wurden außerdem noch nicht aufgeklärt. Frühere Untersuchungen von IPL-Gruppen in bakteriellen Reinkulturen mittels Dünnschichtchromatographie erschwerten häufig eine strukturelle Aufklärung, sodass z.B. nicht näher charakterisierte Aminolipide detektiert wurden (z.B. Labrenz et al., 1998, 2000). Eine erneute Untersuchung dieser Reinkulturen mittels der neu etablierten hochauflösenden HPLC-ESI-MS/MS-Applikation könnte Aufschluss darüber geben, ob es sich hierbei ebenfalls um Ornithinlipide oder um andere phosphatfreie Lipide (z.B. Lysinlipide oder Glutaminlipide) handelt.

Die Rolle der phosphatfreien Lipide ist nicht abschließend geklärt. In einigen Studien wurde gezeigt, dass einige Mikroorganismen in der Lage sind, unter Phosphatlimitierung Phospholipide gegen phosphatfreie Membranlipide auszutauschen (Minnikin & Abdolrahimzadeh, 1974; Benning et al., 1995; Weissenmayer et al., 2002). Es wurde postuliert, dass diese Organismen dadurch einen Wachstumsvorteil gegenüber Bakterien haben, die ausschließlich Phospholipide synthetisieren können (Van Mooy et al., 2006, 2009). Dieser Vorteil spielt vermutlich keine entscheidende Rolle in Wattsedimenten, die nicht phosphatlimitiert sind (Beck et al., 2008a). Die Ergebnisse der vorliegenden Studie deuten vielmehr darauf hin, dass die Funktion der Membranlipide sehr viel komplexer ist. Weitere Untersuchungen sind nötig, um zu klären, ob beispielsweise die hohen Gehalte von Diphosphatidylglycerol und phosphatfreien IPL im untersuchten Sedimentkern darauf zurückzuführen sind, dass die Mikroorganismen die Zusammensetzung ihrer Zellmembranen unter erhöhtem Nährstoffstress angepasst haben. Die meisten Studien an Membranlipiden von Reinkulturen wurden bisher mit nährstoffreichen Wachstumsmedien durchgeführt. Langzeitexperimente mit Reinkul-

turen unter Nährstoffmangel könnten weiteren Aufschluss über diese Anpassungsmechanismen in den Zellmembranen geben.

Bemerkenswert war auch der hohe Gehalt an intakten bakteriellen Etherlipiden im mittleren Teil des untersuchten Sedimentkerns, der nicht immer mit hohen Zellzahlen einherging. Die bisher am ICBM durchgeführten Vorversuche mit künstlich zugesetzten Einzelstandards lassen vermuten, dass sowohl ether- als auch esterbasierte Phospholipide in Wattsedimenten in weniger als hundert Tagen unter anoxischen Bedingungen abgebaut werden (Burchart et al., 2007). Bei diesen Versuchen wurden den Sedimentproben jedoch keine intakten Zellen zugesetzt. Außerdem gibt es bisher nur sehr wenige Studien über den Abbau von IPL in Umweltproben, die zudem auf Untersuchungen zum Abbau bakterieller Phospholipide beschränkt waren (White et al., 1979; Harvey et al., 1986). Ungeklärt ist daher die Frage, wie schnell abgestorbene Zellen tatsächlich lysieren oder ob phosphatfreie Lipide und Archaeenlipide ähnlich schnell abgebaut werden wie bakterielle Phospholipide. Derzeit laufen daher Planungen, Versuche mit Sedimenten durchzuführen, denen intaktes Zellmaterial zugesetzt wird. Diese Versuche sollen die tatsächlichen *in-situ*-Bedingungen realistischer widerspiegeln und auch zeigen, ob sich z.B. der Abbau von Archaeenzellen von denen der Bakterien unterscheidet.

Die vorliegende Studie hat auch gezeigt, dass der advektive Porenwasserfluss am Platenrand organisches Material marinen Ursprungs in die tieferen permeablen Sandschichten transportiert, welches bevorzugt von den autochthonen Mikroorganismen verwertet wird. Eine weitere analytische Herausforderung stellt die genaue Analyse der Zusammensetzung des organischen Materials dar, das in gelöster oder partikulärer Form eingetragen wird. Für das grundlegende Verständnis der mikrobiellen und geochemischen Stoffkreisläufe im Wattenmeer sollten daher zukünftige Untersuchungen auch klären, welche Substrate in die tieferen Sedimentschichten transportiert und welche Abbauprodukte anschließend wieder zurück in die Wassersäule gespült werden.

Literatur

Abboud, R., Popa, R., Souza-Egipsy, V., Giometti, C.S., Tollaksen, S., Mosher, J.J., Findlay, R.H., Nealson, K.H., 2005. Low-temperature growth of *Shewanella oneidensis* MR-1. Applied and Environmental Microbiology, 71, 811-816.

Abraham, W.R., Meyer, H., Lindholst, S., Vancanneyt, M., Smit, J., 1997. Phospho- and sulfolipids as biomarkers of *Caulobacter sensu lato*, *Brevundimonas* and *Hyphomonas*. Systematic and Applied Microbiology, 20, 522-539.

Acinas, S.G., Klepac-Ceraj, V., Hunt, D.E., Pharino, C., Ceraj, I., Distel, D.L., Polz, M.F., 2004. Fine-scale phylogenetic architecture of a complex bacterial community. Nature, 430, 551-554.

Aloisi, G., Bouloubassi, I., Heijs, S.K., Pancost, R.D., Pierre, C., Sinninghe Damsté, J.S., Gottschal, J.C., Forney, L.J., Rouchy, J.M., 2002. CH_4-consuming microorganisms and the formation of carbonate crusts at cold seeps. Earth and Planetary Science Letters, 203, 195-203.

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., Stahl, D.A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology, 56, 1919-1925.

Amann, R.I., Ludwig, W., Schleifer, K.-H., 1995. Phylogenetic identification and *in-situ* detection of individual microbial cells without cultivation. Microbiological Reviews, 59, 143-169.

Arber, W., Naas, T., Blot, M., 1994. Generation of genetic diversity by DNA rearrangements in resting bacteria. FEMS Microbiology Ecology, 15, 5-13.

Aries, E., Doumenq, P., Artaud, J., Molinet, J., Bertrand, J.C., 2001. Occurrence of fatty acids linked to non-phospholipid compounds in the polar fraction of a marine sedimentary extract from Carteau cove, France. Organic Geochemistry, 32, 193-197.

Asselineau, J., Pichinoty, F., Promé, D., Promé, J.-C., 1988. Composition des lipids complexes de *Flavobacterium meningosepticum*. Annales de l'Institut Pasteur-Microbiologie, 139, 159-170.

Asselineau, J., 1991. Bacterial lipids containing amino acids or peptides linked by amide bonds. Fortschritte der Chemie Organischer Naturstoffe, 56, 1-85.

Austin, B., 1988. Marine microbiology. 1. Aufl., Cambridge University Press, Cambridge.

Aygun-Sunar, S., Mandaci, S., Koch, H.-G., Murray, I.V.J., Goldfine, H., Daldal, F., 2006. Ornithine lipid is required for optimal steady-state amounts of *c*-type cytochromes in *Rhodobacter capsulatus*. Molecular Microbiology, 61, 418-435.

Badziong, W., Thauer, R.K., 1978. Growth yields and growth rates of *Desulfovibrio vulgaris* (Marburg) growing on hydrogen plus sulfate and hydrogen plus thiosulfate as the sole energy source. Archives of Microbiology, 117, 209-214.

Bagwell, C.E., Lovell, C.R., 2000. Microdiversity of culturable diazotrophs from the rhizoplanes of the salt marsh grasses *Spartina alterniflora* and *Juncus roemerianus*. Microbial Ecology, 39, 128-136.

Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R., Wolfe, R.S., 1979. Methanogens: reevaluation of a unique biological group. Microbiology and Molecular Biology Reviews, 43, 260-296.

Batrakov, S.G., 1982. Ornithino lipids - a novel type of microbial lipids. Izvestiya Akademii Nauk SSSR Seriya Biologicheskaya, 4, 591-604.

Batzke, A., Engelen, B., Sass, H., Cypionka, H., 2007. Phylogenetic and physiological diversity of cultured deep biosphere bacteria from equatorial pacific ocean and Peru Margin sediments. Geomicrobiology Journal, 24, 261-273.

Baumann, P., Baumann, L., 1984. Genus II *Photobacterium* Beijerinck 1889, 401 AL. In: N.R. Krieg, J.G. Holt (Hrsg.), Bergey's manual of systematic bacteriology, S. 539-545. Williams and Wilkins, Baltimore.

Beck, M., Dellwig, O., Kolditz, K., Freund, H., Liebezeit, G., Schnetger, B., Brumsack, H.-J., 2007. In situ pore water sampling in deep intertidal flat sediments. Limnology and Oceanography-Methods, 5, 136-144.

Beck, M., Dellwig, O., Holstein, J.M., Grunwald, M., Liebezeit, G., Schnetger, B., Brumsack, H.-J., 2008a. Sulphate, dissolved organic carbon, nutrients and terminal metabolic products in deep pore waters of an intertidal flat. Biogeochemistry, 89, 221-238.

Beck, M., Dellwig, O., Liebezeit, G., Schnetger, B., Brumsack, H.-J., 2008b. Spatial and seasonal variations of sulphate, dissolved organic carbon, and nutrients in deep pore waters of intertidal flat sediments. Estuarine, Coastal and Shelf Science, 79, 307-316.

Beck, M., Köster, J., Engelen, B., Holstein, J.M., Gittel, A., Könneke, M., Riedel, T., Wirtz, K., Cypionka, H., Rullkötter, J., Brumsack, H.-J., 2009. Deep pore water profiles reflect enhanced microbial activity towards tidal flat margins. Ocean Dynamics, 59, 371-384.

de Beer, D., Wenzhöfer, F., Ferdelman, T., Boehme, S.E., Huettel, M., van Beusekom, J.E.E., Böttcher, M.E., Musat, N., Dubilier, N., 2005. Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. Limnology and Oceanography, 50, 113-127.

Beijerinck, M., 1889. Le *Photobacterium luminosum*. Bactérie lumineuse de la Mer Nord. Archives Néerlandaises des Sciences Exactes et Naturelles, 23, 401-427.

Benning, C., Huang, Z.H., Gage, D.A., 1995. Accumulation of a novel glycolipid and a betaine lipid in cells of *Rhodobacter sphaeroides* grown under phosphate limitation. Archives of Biochemistry and Biophysics, 317, 103-111.

Benning, C., 1998. Biosynthesis and function of the sulfolipid sulfoquinovosyl diacylglycerol. Annual Review of Plant Physiology and Plant Molecular Biology, 49, 53-75.

Benz, M., Schink, B., Brune, A., 1998. Humic acid reduction by *Propionibacterium freudenreichii* and other fermenting bacteria. Applied and Environmental Microbiology, 64, 4507-4512.

Bergamaschi, B.A., Tsamakis, E., Keil, R.G., Eglinton, T.I., Montluçon, D.B., Hedges, J.I., 1997. The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru Margin sediments. Geochimica et Cosmochimica Acta, 61, 1247-1260.

Berge, J.P., Gouygou, J.P., Dubacq, J.P., Durand, P., 1995. Reassessment of lipid composition of the diatom, *Skeletonema costatum*. Phytochemistry, 39, 1017-1021.

van Beusekom, J.E.E., de Jonge, V.N., 2002. Long-term changes in Wadden Sea nutrient cycles: importance of organic matter import from the North Sea. Hydrobiologia, 475/476, 185-194.

Bhakoo, M., Herbert, R.A., 1980. Fatty acid and phospholipid composition of five psychrotrophic *Pseudomonas* spp. grown at different temperatures. Archives of Microbiology, 126, 51-55.

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H., Hinrichs, K.-U., 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. Proceedings of the National Academy of Sciences of the United States of America, 103, 3846-3851.

Billerbeck, M., Werner, U., Bosselmann, K., Walpersdorf, E., Huettel, M., 2006a. Nutrient release from an exposed intertidal sand flat. Marine Ecology-Progress Series, 316, 35-51.

Billerbeck, M., Werner, U., Polerecky, L., Walpersdorf, E., de Beer, D., Huettel, M., 2006b. Surficial and deep pore water circulation governs spatial and temporal scales of nutrient recycling in intertidal sand flat sediment. Marine Ecology-Progress Series, 326, 61-76.

Billerbeck, M., Røy, H., Bosselmann, K., Huettel, M., 2007. Benthic photosynthesis in submerged Wadden Sea intertidal flats. Estuarine, Coastal and Shelf Science, 71, 704-716.

Bissett, A., Bowman, J., Burke, C., 2006. Bacterial diversity in organically-enriched fish farm sediments. FEMS Microbiology Ecology, 55, 48-56.

Black, G.E., Snyder, A.P., Heroux, K.S., 1997. Chemotaxonomic differentiation between the *Bacillus cereus* group and *Bacillus subtilis* by phospholipid extracts analyzed with electrospray ionization tandem mass spectrometry. Journal of Microbiological Methods, 28, 187-199.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911-917.

Blumenberg, M., Seifert, R., Reitner, J., Pape, T., Michaelis, W., 2004. Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. Proceedings of the National Academy of Sciences of the United States of America, 101, 11111-11116.

Boon, J.J., de Leeuw, J.W., van der Hoek, G.J., Vosjan, J.H., 1977. Significance and taxonomic value of *iso* and *anteiso* monoenoic fatty acids and branched β-hydroxy acids in *Desulfovibrio desulfuricans*. Journal of Bacteriology, 129, 1183-1191.

Böttcher, M.E., Oelschläger, B., Höpner, T., Brumsack, H.-J., Rullkötter, J., 1998. Sulfate reduction related to the early diagenetic degradation of organic matter and "black spot" formation in tidal sandflats of the German Wadden Sea (southern North Sea): stable isotope (^{13}C, ^{34}S, ^{18}O) and other geochemical results. Organic Geochemistry, 29, 1517-1530.

Böttcher, M.E., Hespenheide, B., Llobet-Brossa, E., Beardsley, C., Larsen, O., Schramm, A., Wieland, A., Böttcher, G., Berninger, U.G., Amann, R., 2000. The biogeochemistry, stable isotope geochemistry, and microbial community structure of a temperate intertidal mudflat: an integrated study. Continental Shelf Research, 20, 1749-1769.

Boudreau, B.P., Huettel, M., Forster, S., Jahnke, R.A., McLachlan, A., Middelburg, J.J., Nielsen, P., Sansone, F., Taghon, G., van Raaphorst, W., Webster, I., Weslawski, J.M., Wiberg, P., Sundby, B., 2001. Permeable marine sediments: overturning an old paradigm. EOS, Transactions American Geophysical Union, 82, 133-136.

Bowman, J.P., McCuaig, R.D., 2003. Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. Applied and Environmental Microbiology, 69, 2463-2483.

Boyle, A.W., Phelps, C.D., Young, L.Y., 1999. Isolation from estuarine sediments of a *Desulfovibrio* strain which can grow on lactate coupled to the reductive dehalogenation of 2,4,6-tribromophenol. Applied and Environmental Microbiology, 65, 1133-1140.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Bradley, A.S., Hayes, J.M., Summons, R.E., 2009. Extraordinary ^{13}C enrichment of diether lipids at the Lost City Hydrothermal Field indicates a carbon-limited ecosystem. Geochimica et Cosmochimica Acta, 73, 102-118.

Burchart, T., Seidel, M., Köster, J., Rullkötter, J., 2007. Laboratory investigation of the rate of microbial degradation of intact polar lipids in Wadden Sea sediments. 23rd International Meeting on Organic Geochemistry (IMOG), 177-178.

Caillon, E., Lubochinsky, B., Rigomier, D., 1983. Occurrence of dialkyl ether phospholipids in *Stigmatella aurantiaca* DW4. Journal of Bacteriology, 153, 1348-1351.

Cedergren, R.A., Hollingsworth, R.I., 1994. Occurrence of sulfoquinovosyl diacylglycerol in some members of the family *Rhizobiaceae*. Journal of Lipid Research, 35, 1452-1461.

Choma, A., Komaniecka, I., 2002. Analysis of phospholipids and ornithine-containing lipids from *Mesorhizobium* spp. Systematic and Applied Microbiology, 25, 326-331.

Christie, W.W., 2003. Lipid analysis - isolation, separation, identification and structural analysis of lipids. 3. Aufl., The Oily Press, Bridgwater, UK.

Clejan, S., Krulwich, T.A., Mondrus, K.R., Seto-Young, D., 1986. Membrane lipid composition of obligately and facultatively alkalophilic strains of *Bacillus* spp. Journal of Bacteriology, 168, 334-340.

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnology and Oceanography, 14, 454-458.

Coates, J.D., Cole, K.A., Chakraborty, R., O'Connor, S.M., Achenbach, L.A., 2002. Diversity and ubiquity of bacteria capable of utilizing humic sub-

stances as electron donors for anaerobic respiration. Applied and Environmental Microbiology, 68, 2445-2452.

Cole, M.J., Enke, C.G., 1994. Microbial characterization by phospholipid profiling - practice and problems in microbial identification, Mass spectrometry for the characterization of microorganisms, 541, ACS Symposium Series, S. 36-61. American Chemical Society, Washington.

Coolen, M.J.L., Overmann, J., 2000. Functional exoenzymes as indicators of metabolically active bacteria in 124,000-year-old sapropel layers of the eastern Mediterranean Sea. Applied and Environmental Microbiology, 66, 2589-2598.

Coolen, M.J.L., Cypionka, H., Sass, A.M., Sass, H., Overmann, J., 2002. Ongoing modification of Mediterranean Pleistocene sapropels mediated by prokaryotes. Science, 296, 2407-2410.

Coolen, M.J.L., Overmann, J., 2007. 217 000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment. Environmental Microbiology, 9, 238-249.

Cord-Ruwisch, R., 1985. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. Journal of Microbiological Methods, 4, 33-36.

Cowan, D.A., 2000. Microbial genomes – the untapped resource. Trends in Biotechnology, 18, 14-16.

Cowen, J.P., Giovannoni, S.J., Kenig, F., Johnson, H.P., Butterfield, D., Rappé, M.S., Hutnak, M., Lam, P., 2003. Fluids from aging ocean crust that support microbial life. Science, 299, 120-123.

Cragg, B.A., Law, K.M., Cramp, A., Parkes, R.J., 1998. The response of bacterial populations to sapropels in deep sediments of the Eastern Mediterranean (site 969). In: A.H.F. Robertson, K.-C. Emeis, C. Richter, A. Camerlenghi (Hrsg.), Proceedings of the Ocean Drilling Program, Scientific results, 160, S. 303-307, College Station, TX.

Cronan, J.E., Jr., Gelmann, E.P., 1975. Physical properties of membrane lipids: biological relevance and regulation. Bacteriological Reviews, 39, 232-256.

Cypionka, H., 1994. Sulfate transport. Methods in Enzymology, 243, 3-14.

D'Andrea, A.F., Aller, R.C., Lopez, G.R., 2002. Organic matter flux and reactivity on a South Carolina sandflat: the impacts of porewater advection and macrobiological structures. Limnology and Oceanography, 47, 1056-1070.

D'Hondt, S., Jørgensen, B.B., Miller, D.J., Batzke, A., Blake, R., Cragg, B.A., Cypionka, H., Dickens, G.R., Ferdelman, T., Hinrichs, K.-U., Holm, N.G., Mitterer, R., Spivack, A., Wang, G.Z., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S.D., Sass, H., Skilbeck, C.G., Aiello, I.W., Guèrin, G., House, C.H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R.J., Schippers, A., Smith, D.C., Teske, A., Wiegel, J., Padilla, C.N., Acosta, J.L.S., 2004. Distributions of microbial activities in deep subseafloor sediments. Science, 306, 2216-2221.

De Rosa, M., Gambacorta, A., 1988. The lipids of archaebacteria. Progress in Lipid Research, 27, 153-175.

De Rosa, M., Gambacorta, A., Huber, R., Lanzotti, V., Nicolaus, B., Stetter, K.O., Trincone, A., 1988. A new 15,16-dimethyl-30-glyceryloxytriacontanoic acid from lipids of *Thermotoga maritima*. Journal of the Chemical Society, Chemical Communications, 19, 1300-1301.

Dell'Anno, A., Fabiano, M., Duineveld, G.C.A., Kok, A., Danovaro, R., 1998. Nucleic acid (DNA, RNA) quantification and RNA/DNA ratio determination in marine sediments: Comparison of spectrophotometric, fluorometric, and high-performance liquid chromatography methods and estimation of detrital DNA. Applied and Environmental Microbiology, 64, 3238-3245.

DeLong, E.F., Yayanos, A.A., 1986. Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes. Applied and Environmental Microbiology, 51, 730-737.

DeLong, E.F., Wickham, G.S., Pace, N.R., 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science, 243, 1360-1363.

DeLong, E.F., 2004. Microbial life breathes deep. Science, 306, 2198-2200.

Dembitsky, V.M., 1993. Lipids of bryophytes. Progress in Lipid Research, 32, 281-356.

Denich, T.J., Beaudette, L.A., Lee, H., Trevors, J.T., 2003. Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. Journal of Microbiological Methods, 52, 149-182.

Dinh, H.T., 2003. Microbiological study of the anaerobic corrosion of iron. Dissertation, Universität Bremen.

Dinh, H.T., Kuever, J., Mußmann, M., Hassel, A.W., Stratmann, M., Widdel, F., 2004. Iron corrosion by novel anaerobic microorganisms. Nature, 427, 829-832.

Donato, M.M., Jurado, A.S., Antunes-Madeira, M.C., Madeira, V.M.C., 2000. Membrane lipid composition of *Bacillus stearothermophilus* as affected by lipophilic environmental pollutants: an approach to membrane toxicity assessment. Archives of Environmental Contamination and Toxicology, 39, 145-153.

Dowhan, W., 1997. Molecular basis for membrane phospholipid diversity: Why are there so many lipids? Annual Review of Biochemistry, 66, 199-232.

Dowling, N.J.E., Widdel, F., White, D.C., 1986. Phospholipid ester-linked fatty-acid biomarkers of acetate-oxidizing sulfate-reducers and other sulfide-forming bacteria. Journal of General Microbiology, 132, 1815-1825.

Dunkelblum, E., Tan, S.H., Silk, P.J., 1985. Double bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry - application to analysis of fatty acids in pheromone glands of 4 *Lepidoptera*. Journal of Chemical Ecology, 11, 265-277.

Edlund, A., Nichols, P.D., Roffey, R., White, D.C., 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. Journal of Lipid Research, 26, 982-988.

Elvert, M., Boetius, A., Knittel, K., Jørgensen, B.B., 2003. Characterization of specific membrane fatty acids as chemotaxonomic markers for sulfate-reducing bacteria involved in anaerobic oxidation of methane. Geomicrobiology Journal, 20, 403-419.

Elvert, M., Hopmans, E.C., Treude, T., Boetius, A., Suess, E., 2005. Spatial variations of methanotrophic consortia at cold methane seeps: implications from a high-resolution molecular and isotopic approach. Geobiology, 3, 195-209.

Engelen, B., Cypionka, H., 2009. The subsurface of tidal-flat sediments as a model for the deep biosphere. Ocean Dynamics, 59, 385-392.

Ertefai, T.F., Fisher, M.C., Fredricks, H.F., Lipp, J.S., Pearson, A., Birgel, D., Udert, K.M., Cavanaugh, C.M., Gschwend, P.M., Hinrichs, K.-U., 2008. Vertical distribution of microbial lipids and functional genes in chemically distinct layers of a highly polluted meromictic lake. Organic Geochemistry, 39, 1572-1588.

Evans, R.I., McClure, P.J., Gould, G.W., Russell, N.J., 1998. The effect of temperature on the phospholipid and fatty acyl compositions of non-proteolytic *Clostridium botulinum*. International Journal of Food Microbiology, 40, 159-167.

Fang, J.S., Barcelona, M.J., 1998. Structural determination and quantitative analysis of bacterial phospholipids using liquid chromatography electrospray ionization mass spectrometry. Journal of Microbiological Methods, 33, 23-35.

Fang, J.S., Barcelona, M.J., Alvarez, P.J.J., 2000a. A direct comparison between fatty acid analysis and intact phospholipid profiling for microbial identification. Organic Geochemistry, 31, 881-887.

Fang, J.S., Barcelona, M.J., Nogi, Y., Kato, C., 2000b. Biochemical implications and geochemical significance of novel phospholipids of the extremely barophilic bacteria from the Marianas Trench at 11,000 m. Deep Sea Research Part I: Oceanographic Research Papers, 47, 1173-1182.

Feil, E.J., 2004. Small change: keeping pace with microevolution. Nature Reviews Microbiology, 2, 483-495.

Fichtel, J., Köster, J., Scholz-Böttcher, B., Sass, H., Rullkötter, J., 2007. A highly sensitive HPLC method for determination of nanomolar concentrations of dipicolinic acid, a characteristic constituent of bacterial endospores. Journal of Microbiological Methods, 70, 319-327.

Fichtel, J., Köster, J., Rullkötter, J., Sass, H., 2008. High variations in endospore numbers within tidal flat sediments revealed by quantification of dipicolinic acid. Geomicrobiology Journal, 25, 371-380.

Franke, U., Polerecky, L., Precht, E., Huettel, M., 2006. Wave tank study of particulate organic matter degradation in permeable sediments. Limnology and Oceanography, 51, 1084-1096.

Freese, E., Köster, J., Rullkötter, J., 2008a. Origin and composition of organic matter in tidal flat sediments from the German Wadden Sea. Organic Geochemistry, 39, 820-829.

Freese, E., Sass, H., Rütters, H., Schledjewski, R., Rullkötter, J., 2008b. Variable temperature-related changes in fatty acid composition of bacterial isolates from German Wadden sea sediments representing different bacterial phyla. Organic Geochemistry, 39, 1427-1438.

Freese, E., Rütters, H., Köster, J., Rullkötter, J., Sass, H., 2009. *Gammaproteobacteria* as a possible source of eicosapentaenoic acid in anoxic intertidal sediments. Microbial Ecology, 57, 444-454.

Fritsche, W., Laplace, F., 1990. Mikrobiologie. 2. Aufl., Gustav Fischer Verlag, Jena.

Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., Maynard, V., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et Cosmochimica Acta, 43, 1075-1090.

Frolova, G.M., Pavel, K.G., Shparteeva, A.A., Nedashkovskaya, O.I., Gorshkova, N.M., Ivanova, E.P., Mikhailov, V.V., 2005. Lipid composition of novel *Shewanella* species isolated from far eastern seas. Microbiology, 74, 664-669.

Frostegård, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., Simonet, P., 1999. Quantification of bias related to the extraction of DNA directly from soils. Applied and Environmental Microbiology, 65, 5409-5420.

Fry, B., Sherr, E.B., 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and fresh-water ecosystems. Contributions in Marine Science, 27, 13-47.

Fuhrman, J.A., Campbell, L., 1998. Microbial microdiversity. Nature, 393, 410-411.

Galbraith, L., Jonsson, M.H., Rudhe, L.C., Wilkinson, S.G., 1999. Lipids and fatty acids of *Burkholderia* and *Ralstonia* species. FEMS Microbiology Letters, 173, 359-364.

Galushko, A.S., Schink, B., 2000. Oxidation of acetate through reactions of the citric acid cycle by *Geobacter sulfurreducens* in pure culture and in syntrophic coculture. Archives of Microbiology, 174, 314-321.

Geiger, O., Rohrs, V., Weissenmayer, B., Finan, T.M., Thomas-Oates, J.E., 1999. The regulator gene *phoB* mediates phosphate stress-controlled synthesis of the membrane lipid diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine in *Rhizobium* (*Sinorhizobium*) *meliloti*. Molecular Microbiology, 32, 63-73.

Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R., 1994. Methods for general and molecular bacteriology. 1. Aufl., ASM, Washington.

Giovannoni, S.J., Stingl, U., 2007. The importance of culturing bacterioplankton in the 'omics' age. Nature Reviews Microbiology, 5, 820-826.

Gittel, A., Mußmann, M., Sass, H., Cypionka, H., Könneke, M., 2008. Identity and abundance of active sulfate-reducing bacteria in deep tidal flat sediments determined by directed cultivation and CARD-FISH analysis. Environmental Microbiology, 10, 2645-2658.

Gliozzi, A., Paoli, G., Derosa, M., Gambacorta, A., 1983. Effect of isoprenoid cyclization on the transition temperature of lipids in thermophilic archaebacteria. Biochimica et Biophysica Acta, 735, 234-242.

Gounot, A.M., 1991. Bacterial life at low temperature - physiological aspects and biotechnological implications. Journal of Applied Bacteriology, 71, 386-397.

Grasshoff, K., Kremling, K., Ehrhardt, M., 1999. Methods of seawater analysis. 3. Aufl., Wiley-VCH, Weinheim.

Gray, N.D., Howarth, R., Rowan, A., Pickup, R.W., Jones, J.G., Head, I.M., 1999. Natural communities of *Achromatium oxaliferum* comprise genetically, morphologically, and ecologically distinct subpopulations. Applied and Environmental Microbiology, 65, 5089-5099.

Green, C.T., Scow, K.M., 2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeology Journal, 8, 126-141.

Guckert, J.B., Hood, M.A., White, D.C., 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae* - increases in the *trans*/*cis* ratio and proportions of cyclopropyl fatty acids. Applied and Environmental Microbiology, 52, 794-801.

Guezennec, J., Fiala-Medioni, A., 1996. Bacterial abundance and diversity in the Barbados Trench determined by phospholipid analysis. FEMS Microbiology Ecology, 19, 83-93.

Haack, S.K., Garchow, H., Odelson, D.A., Forney, L.J., Klug, M.J., 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology, 60, 2483-2493.

Hahn, M.W., Pöckl, M., 2005. Ecotypes of planktonic *Actinobacteria* with identical 16S rRNA genes adapted to thermal niches in temperate, subtropical, and tropical freshwater habitats. Applied and Environmental Microbiology, 71, 766-773.

Hahn, M.W., Pöckl, M., Wu, Q.L., 2005. Low intraspecific diversity in a *Polynucleobacter* subcluster population numerically dominating bacterioplankton of a freshwater pond. Applied and Environmental Microbiology, 71, 4539-4547.

Hamamoto, T., Takata, N., Kudo, T., Horikoshi, K., 1994. Effect of temperature and growth phase on fatty acid composition of the psychrophilic *Vibrio* sp. strain No-5710. FEMS Microbiology Letters, 119, 77-81.

Han, X.L., Gross, R.W., 1995. Structural determination of picomole amounts of phospholipids via electrospray ionization tandem mass spectrometry. Journal of the American Society for Mass Spectrometry, 6, 1202-1210.

Haque, M.A., Russell, N.J., 2004. Strains of *Bacillus cereus* vary in the phenotypic adaptation of their membrane lipid composition in response to low water activity, reduced temperature and growth in rice starch. Microbiology, 150, 1397-1404.

Harder, W., Veldkamp, H., 1971. Competition of marine psychrophilic bacteria at low temperature. Antonie van Leeuwenhoek, 37, 51-63.

Harrison, S.J., Phizacklea, A.P., 1987. Vertical temperature gradients in muddy intertidal sediments in the Forth estuary, Scotland. Limnology and Oceanography, 32, 954-963.

Harvey, H.R., Fallon, R.D., Patton, J.S., 1986. The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. Geochimica et Cosmochimica Acta, 50, 795-804.

Hasegawa, Y., Kawada, N., Nosoh, Y., 1980. Change in chemical composition of membrane of *Bacillus caldotenax* after shifting the growth temperature. Archives of Microbiology, 126, 103-108.

ten Haven, H.L., de Lange, G.J., McDuff, R.E., 1987. Interstitial water studies of late Quarternary Eastern Mediterranean sediments with emphasis on early diagenetic reactions and evaporitic salt influences. Marine Geology, 75, 119-136.

Hazel, J.R., Williams, E.E., 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Progress in Lipid Research, 29, 167-227.

Hazel, J.R., 1995. Thermal adaptation in biological membranes - is homeoviscous adaptation the explanation? Annual Review of Physiology, 57, 19-42.

He, Q., Sanford, R.A., 2003. Characterization of Fe(III) reduction by chlororespiring *Anaeromxyobacter dehalogenans*. Applied and Environmental Microbiology, 69, 2712-2718.

Head, I.M., Saunders, J.R., Pickup, R.W., 1998. Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. Microbial Ecology, 35, 1-21.

Heipieper, H.J., Diefenbach, R., Keweloh, H., 1992. Conversion of *cis* unsaturated fatty acids to *trans*, a possible mechanism for the protection of phe-

nol-degrading *Pseudomonas putida* P8 from substrate toxicity. Applied and Environmental Microbiology, 58, 1847-1852.

Henderson, R.J., Millar, R.M., Sargent, J.R., 1995. Effect of growth temperature on the positional distribution of eicosapentaenoic acid and *trans* hexadecenoic acid in the phospholipids of a *Vibrio* species of bacterium. Lipids, 30, 181-185.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., Nelson, E.B., 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology, 15, 25-36.

Hinrichs, K.U., Summons, R.E., Orphan, V., Sylva, S.P., Hayes, J.M., 2000. Molecular and isotopic analysis of anaerobic methane-oxidizing communities in marine sediments. Organic Geochemistry, 31, 1685-1701.

Hölzl, G., Dörmann, P., 2007. Structure and function of glycoglycerolipids in plants and bacteria. Progress in Lipid Research, 46, 225-243.

Huber, R., Wilharm, T., Huber, D., Trincone, A., Burggraf, S., König, H., Rachel, R., Rockinger, I., Fricke, H., Stetter, K.O., 1992. *Aquifex pyrophilus* gen. nov. sp. nov represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacteria. Systematic and Applied Microbiology, 15, 340-351.

Huber, R., Rossnagel, P., Woese, C.R., Rachel, R., Langworthy, T.A., Stetter, K.O., 1996. Formation of ammonium from nitrate during chemolithoautotrophic growth of the extremely thermophilic bacterium *Ammonifex degensii* gen. nov. sp. nov. Systematic and Applied Microbiology, 19, 40-49.

Huettel, M., Gust, G., 1992. Impact of bioroughness on interfacial solute exchange in permeable sediments. Marine Ecology-Progress Series, 89, 253-267.

Huettel, M., Ziebis, W., Forster, S., Luther, G.W., 1998. Advective transport affecting metal and nutrient distributions and interfacial fluxes in permeable sediments. Geochimica et Cosmochimica Acta, 62, 613-631.

Huettel, M., Rusch, A., 2000. Transport and degradation of phytoplankton in permeable sediment. Limnology and Oceanography, 45, 534-549.

Inagaki, F., Okada, H., Tsapin, A.I., Nealson, K.H., 2005. The Paleome: a sedimentary genetic record of past microbial communities. Astrobiology, 5, 141-153.

Isaksen, M.F., Jørgensen, B.B., 1996. Adaptation of psychrophilic and psychrotrophic sulfate-reducing bacteria to permanently cold marine environments. Applied and Environmental Microbiology, 62, 408-414.

Ishii, K., Mußmann, M., MacGregor, B.J., Amann, R., 2004. An improved fluorescence in situ hybridization protocol for the identification of bacteria and archaea in marine sediments. FEMS Microbiology Ecology, 50, 203-212.

Ivanova, E.P., Sawabe, T., Gorshkova, N.M., Svetashev, V.I., Mikhailov, V.V., Nicolau, D.V., Christen, R., 2001. *Shewanella japonica* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 51, 1027-1033.

Jahnke, L.L., Eder, W., Huber, R., Hope, J.M., Hinrichs, K.-U., Hayes, J.M., Des Marais, D.J., Cady, S.L., Summons, R.E., 2001. Signature lipids and stable carbon isotope analyses of Octopus Spring hyperthermophilic communities compared with those of *Aquificales* representatives. Applied and Environmental Microbiology, 67, 5179-5189.

Jaspers, E., Overmann, J., 2004. Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies. Applied and Environmental Microbiology, 70, 4831-4839.

Johnson, R.M., Weisrock, W.P., 1969. *Hyphomicrobium indicum* sp. nov. (*Hyphomicrobiaceae* Douglas). International Journal of Systematic and Evolutionary Microbiology, 19, 295-307.

Jørgensen, B.B., 1977. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnology and Oceanography, 22, 814-832.

Jørgensen, B.B., 1982. Mineralization of organic matter in the sea bed - the role of sulfate reduction. Nature, 296, 643-645.

Kaksonen, A.H., Spring, S., Schumann, P., Kroppenstedt, R.M., Puhakka, J.A., 2006. *Desulfotomaculum thermosubterraneum* sp. nov., a thermophilic sulfate-reducer isolated from an underground mine located in a geother-

mally active area. International Journal of Systematic and Evolutionary Microbiology, 56, 2603-2608.

Kannenberg, E.L., Poralla, K., 1999. Hopanoid biosynthesis and function in bacteria. Naturwissenschaften, 86, 168-176.

Kato, M., Sakai, M., Adachi, K., Ikemoto, H., Sano, H., 1996. Distribution of betaine lipids in marine algae. Phytochemistry, 42, 1341-1345.

Kayganich, K.A., Murphy, C., 1992. Fast atom bombardement tandem mass spectrometric identification of diacyl, alkylacyl, and alk-1-enylacyl molecular polymorphonuclear leukocytes. Analytical Chemistry, 64, 2965-2971.

Kell, D.B., 1984. Diffusion of protein complexes in prokaryotic membranes: fast, free, random or directed? Trends in Biochemical Sciences, 9, 86-88.

Kepner, R.L., Pratt, J.R., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples - past and present. Microbiological Reviews, 58, 603-615.

Killops, S.D., Killops, V.J., 2004. An introduction to organic geochemistry. 2. Aufl., Blackwell, Oxford.

Kleinig, H., 1972. Membranes from *Myxococcus fulvus* (*Myxobacterales*) containing carotenoid glucosides. 1. Isolation and composition. Biochimica et Biophysica Acta, 274, 489-498.

Klepac-Ceraj, V., Bahr, M., Crump, B.C., Teske, A.P., Hobbie, J.E., Polz, M.F., 2004. High overall diversity and dominance of microdiverse relationships in salt marsh sulphate-reducing bacteria. Environmental Microbiology, 6, 686-698.

Knittel, K., Boetius, A., Lemke, A., Eilers, H., Lochte, K., Pfannkuche, O., Linke, P., Amann, R., 2003. Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia margin, Oregon). Geomicrobiology Journal, 20, 269-294.

Knoblauch, C., Jørgensen, B.B., 1999. Effect of temperature on sulphate reduction, growth rate and growth yield in five psychrophilic sulphate-reducing bacteria from Arctic sediments. Environmental Microbiology, 1, 457-467.

Knoche, H.W., Shively, J.M., 1972. The structure of an ornithine-containing lipid from *Thiobacillus thiooxidans*. Journal of Biological Chemistry, 247, 170-178.

Koga, Y., Nishihara, M., Morii, H., Akagawamatsushita, M., 1993. Ether polar lipids of methanogenic bacteria - structures, comparative aspects, and biosyntheses. Microbiological Reviews, 57, 164-182.

Koga, Y., Nakano, M., 2008. A dendrogram of archaea based on lipid component parts composition and its relationship to rRNA phylogeny. Systematic and Applied Microbiology, 31, 169-182.

Kohring, L.L., Ringelberg, D.B., Devereux, R., Stahl, D.A., Mittelman, M.W., White, D.C., 1994. Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria. FEMS Microbiology Letters, 119, 303-308.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature, 437, 543-546.

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., Sass, H., 2005. Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. Applied and Environmental Microbiology, 71, 7819-7830.

Kormas, K.A., Smith, D.C., Edgcomb, V., Teske, A., 2003. Molecular analysis of deep subsurface microbial communities in Nankai Trough sediments (ODP Leg 190, Site 1176). FEMS Microbiology Ecology, 45, 115-125.

Kroon, D., Alexander, I., Little, M., Lourens, L.J., Matthewson, A., Robertson, A.H.F., Sakamoto, T., 1998. Oxygen isotope and sapropel stratigraphy in the Eastern Mediterranean during the last 3.2 million years. In: A.H.F. Robertson, K.-C. Emeis, C. Richter, A. Camerlenghi (Hrsg.), Proceedings of the Ocean Drilling Program, Scientific Results Volume 160, S. 181-189.

Kunz, H., 1993. Klimaänderungen und ihre Folgen für Wasserhaushalt, Gewässernutzung und Gewässerschutz. In: H.J. Schellnhuber, H. Sterr (Hrsg.), Klimaänderung und Küste, S. 97-136. Springer Verlag, Berlin.

Laanbroek, H.J., Blok, J.P., Steenhuis, L., 1985. Variability in fermentation patterns of sugar utilizing bacteria isolated from anaerobic, intertidal sediments. Microbial Ecology, 11, 117-125.

Labrenz, M., Collins, M.D., Lawson, P.A., Tindall, B.J., Braker, G., Hirsch, P., 1998. *Antarctobacter heliothermus* gen. nov., sp. nov., a budding bacterium from hypersaline and heliothermal Ekho Lake. International Journal of Systematic Bacteriology, 48, 1363-1372.

Labrenz, M., Tindall, B.J., Lawson, P.A., Collins, M.D., Schumann, P., Hirsch, P., 2000. *Staleya guttiformis* gen. nov., sp. nov. and *Sulfitobacter brevis* sp. nov., α-3-*Proteobacteria* from hypersaline, heliothermal and meromictic antarctic Ekho Lake. International Journal of Systematic and Evolutionary Microbiology, 50, 303-313.

Lai, D., Springstead, J.R., Monbouquette, H.G., 2008. Effect of growth temperature on ether lipid biochemistry in *Archaeoglobus fulgidus*. Extremophiles, 12, 271-278.

Langworthy, T.A., Mayberry, W.R., Smith, P.F., 1976. Sulfonolipid and novel glucosamidyl glycolipids from extreme thermoacidophile *Bacillus acidocaldarius*. Biochimica et Biophysica Acta, 431, 550-569.

Langworthy, T.A., Holzer, G., Zeikus, J.G., Tornabene, T.G., 1983. *Iso*-branched and *anteiso*-branched glycerol diethers of the thermophilic anaerobe *Thermodesulfotobacterium commune*. Systematic and Applied Microbiology, 4, 1-17.

Leadbetter, J.R., 2003. Cultivation of recalcitrant microbes: cells are alive, well and revealing their secrets in the 21st century laboratory. Current Opinion in Microbiology, 6, 274-281.

Lechevalier, H., Lechevalier, M.P., 1988. Chemotaxonomic use of lipids - an overview. In: C. Ratledge, S.G. Wilkinson (Hrsg.), Microbial Lipids, 1, S. 869-902. Academic Press, London.

Lechevalier, M.P., 1977. Lipids in Bacterial taxonomy - taxonomist's view. CRC Critical Reviews in Microbiology, 5, 109-210.

Lien, T., Beeder, J., 1997. *Desulfobacter vibrioformis* sp. nov., a sulfate reducer from a water-oil separation system. International Journal of Systematic Bacteriology, 47, 1124-1128.

Lipp, J.S., Morono, Y., Inagaki, F., Hinrichs, K.-U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. Nature, 454, 991-994.

Llobet-Brossa, E., Rosselló-Móra, R., Amann, R., 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. Applied and Environmental Microbiology, 64, 2691-2696.

Llobet-Brossa, E., Rabus, R., Böttcher, M.E., Könneke, M., Finke, N., Schramm, A., Meyer, R.L., Grötzschel, S., Rosselló-Móra, R., Amann, R., 2002. Community structure and activity of sulfate-reducing bacteria in an intertidal surface sediment: a multi-method approach. Aquatic Microbial Ecology, 29, 211-226.

López-Lara, I.M., Sohlenkamp, C., Geiger, O., 2003. Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. Molecular Plant-Microbe Interactions, 16, 567-579.

López-López, A., Bartual, S.G., Stal, L., Onyshchenko, O., Rodríguez-Valera, F., 2005. Genetic analysis of housekeeping genes reveals a deep-sea ecotype of *Alteromonas macleodii* in the Mediterranean Sea. Environmental Microbiology, 7, 649-659.

Lourens, L.J., Hilgen, F.J., Gudjonsson, L., Zachariasse, W.J., 1992. Late Pliocene to early Pleistocene astronomically forced sea surface productivity and temperature variations in the Mediterranean. Marine Micropaleontology, 19, 49-78.

Lovley, D.R., Coates, J.D., Blunt-Harris, E.L., Phillips, E.J.P., Woodward, J.C., 1996. Humic substances as electron acceptors for microbial respiration. Nature, 382, 445-448.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettske, I., Gerber,

S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüßmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., Schleifer, K.-H., 2004. ARB: a software environment for sequence data. Nucleic Acids Research, 32, 1363-1371.

Luna, G.M., Manini, E., Danovaro, R., 2002. Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination and ecological significance. Applied and Environmental Microbiology, 68, 3509-3513.

van der Maarel, M.J.E.C., van Bergeijk, S., van Werkhoven, A.F., Laverman, A.M., Meijer, W.G., Stam, W.T., Hansen, T.A., 1996. Cleavage of dimethylsulfoniopropionate and reduction of acrylate by *Desulfovibrio acrylicus* sp. nov. Archives of Microbiology, 166, 109-115.

MacIntyre, H.L., Geider, R.J., Miller, D.C., 1996. Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. 1. Distribution, abundance and primary production. Estuaries, 19, 186-201.

Macnaughton, S.J., Jenkins, T.L., Wimpee, M.H., Cormier, M.R., White, D.C., 1997. Rapid extraction of lipid biomarkers from pure culture and environmental samples using pressurized accelerated hot solvent extraction. Journal of Microbiological Methods, 31, 19-27.

Madigan, M.T., Martinko, J.M., Parker, J., 2006. Brock - Mikrobiologie. 11. Aufl., Pearson Education Deutschland GmbH, München.

Makula, R.A., Finnerty, W.R., 1974. Phospholipid composition of *Desulfovibrio* species. Journal of Bacteriology, 120, 1279-1283.

Makula, R.A., Finnerty, W.R., 1975. Isolation and characterization of an ornithine-containing lipid from *Desulfovibrio gigas*. Journal of Bacteriology, 123, 523-529.

Mangelsdorf, K., Zink, K.G., Birrien, J.L., Toffin, L., 2005. A quantitative assessment of pressure dependent adaptive changes in the membrane lipids of piezosensitive deep sub-seafloor bacterium. Organic Geochemistry, 36, 1459-1479.

Männistö, M.K., Puhakka, J.A., 2001. Temperature- and growth-phase-regulated changes in lipid fatty acid structures of psychrotolerant groundwater Proteobacteria. Archives of Microbiology, 177, 41-46.

Männistö, M.K., Häggblom, M.M., 2006. Characterization of psychrotolerant heterotrophic bacteria from Finnish Lapland. Systematic and Applied Microbiology, 29, 229-243.

Martens-Habbena, W., Sass, H., 2006. Sensitive determination of microbial growth by nucleic acid staining in aqueous suspension. Applied and Environmental Microbiology, 72, 87-95.

Maszenan, A.M., Jiang, H.L., Tay, J.H., Schumann, P., Kroppenstedt, R.M., Tay, S.T.L., 2007. *Granulicoccus phenolivorans* gen. nov., sp. nov., a Gram-positive, phenol-degrading coccus isolated from phenol-degrading aerobic granules. International Journal of Systematic and Evolutionary Microbiology, 57, 730-737.

Mazzella, N., Molinet, J., Syakti, A.D., Bertrand, J.C., Doumenq, P., 2007. Assessment of the effects of hydrocarbon contamination on the sedimentary bacterial communities and determination of the polar lipid fraction purity: relevance of intact phospholipid analysis. Marine Chemistry, 103, 304-317.

McCarthy, J.F., Zachara, J.M., 1989. Subsurface transport of contaminants - mobile colloids in the subsurface environment may alter the transport of contaminants. Environmental Science and Technology, 23, 496-502.

McInerney, M.J., Bryant, M.P., 1981. Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of H_2 on acetate degradation. Applied and Environmental Microbiology, 41, 346-354.

McMahon, P.B., Chapelle, F.H., 1991. Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry. Nature, 349, 233-235.

de Mendoza, D., Cronan, J.E., 1983. Thermal regulation of membrane lipid fluidity in bacteria. Trends in Biochemical Sciences, 8, 49-52.

Meyer, B., Kuever, J., 2007. Phylogeny of the alpha and beta subunits of the dissimilatory adenosine-5'-phosphosulfate (APS) reductase from sulfate-reducing prokaryotes - origin and evolution of the dissimilatory sulfate-reduction pathway. Microbiology, 153, 2026-2044.

Minnikin, D.E., Abdolrahimzadeh, H., 1974. Replacement of phosphatidylethanolamine and acidic phospholipids by an ornithine-amide lipid and a minor phosphorus-free lipid in *Pseudomonas fluorescens* NCMB 129. FEBS Letters, 43, 257-260.

Minz, D., Fishbain, S., Green, S.J., Muyzer, G., Cohen, Y., Rittmann, B.E., Stahl, D.A., 1999. Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Applied and Environmental Microbiology, 65, 4659-4665.

Moore, L.R., Rocap, G., Chisholm, S.W., 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus ecotypes*. Nature, 393, 464-467.

Morita, R.Y., 1975. Psychrophilic bacteria. Bacteriological Reviews, 39, 144-167.

Mukamolova, G.V., Yanopolskaya, N.D., Votyakova, T.V., Popov, V.I., Kaprelyants, A.S., Kell, D.B., 1995. Biochemical changes accompanying the long-term starvation of *Micrococcus luteus* cells in spent growth medium. Archives of Microbiology, 163, 373-379.

Müller, K.D., Nalik, H.P., Schmid, E.N., Husmann, H., Schomburg, G., 1993. Fast identification of *Mycobacterium* species by GC analysis with trimethylsulfonium hydroxide (TMSH) for transesterification. Journal of High Resolution Chromatography, 16, 161-165.

Müller, K.D., Schmid, E.N., Kroppenstedt, R.M., 1998. Improved identification of *Mycobacteria* by using the microbial identification system in combination with additional trimethylsulfonium hydroxide pyrolysis. Journal of Clinical Microbiology, 36, 2477-2480.

Murray, R.G.E., Doetsch, R.N., Robinow, C.F., 1994. Determinative and cytological light microscopy. In: P. Gerhardt, R.G.E. Murray, W.A. Wood, N.R. Krieg (Hrsg.), Methods for General and Molecular Bacteriology. American Society of Microbiology, Washington, D.C., USA.

Musat, N., Werner, U., Knittel, K., Kolb, S., Dodenhof, T., van Beusekom, J.E.E., de Beer, D., Dubilier, N., Amann, R., 2006. Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea. Systematic and Applied Microbiology, 29, 333-348.

Musslewhite, C.L., McInerney, M.J., Dong, H.L., Onstott, T.C., Green-Blum, M., Swift, D., Macnaughton, S., White, D.C., Murray, C., Chien, Y.J., 2003. The factors controlling microbial distribution and activity in the shallow subsurface. Geomicrobiology Journal, 20, 245-261.

Mußmann, M., Ishii, K., Rabus, R., Amann, R., 2005. Diversity and vertical distribution of cultured and uncultured *Deltaproteobacteria* in an intertidal mud flat of the Wadden Sea. Environmental Microbiology, 7, 405-418.

Nedwell, D.B., Rutter, M., 1994. Influence of temperature on growth rate and competition between two psychrotolerant Antarctic bacteria: low temperature diminishes affinity for substrate uptake. Applied and Environmental Microbiology, 60, 1984-1992.

Nedwell, D.B., 1999. Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperatures. FEMS Microbiology Ecology, 30, 101-111.

Newberry, C.J., Webster, G., Cragg, B.A., Parkes, R.J., Weightman, A.J., Fry, J.C., 2004. Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. Environmental Microbiology, 6, 274-287.

Nichols, D., Bowman, J., Sanderson, K., Nichols, C.M., Lewis, T., McMeekin, T., Nichols, P.D., 1999. Developments with Antarctic microorganisms: culture collections, bioactivity screening, taxonomy, PUFA production and cold-adapted enzymes. Current Opinion in Biotechnology, 10, 240-246.

Nichols, D.S., Nichols, P.D., Russell, N.J., Davies, N.W., McMeekin, T.A., 1997. Polyunsaturated fatty acids in the psychrophilic bacterium *Shewanella gelidimarina* ACAM 456^T: molecular species analysis of major phospholipids and biosynthesis of eicosapentaenoic acid. Biochimica et Biophysica acta-Lipids and Lipid Metabolism, 1347, 164-176.

Nogi, Y., Masui, N., Kato, C., 1998. *Photobacterium profundum* sp. nov., a new, moderately barophilic bacterial species isolated from a deep-sea sediment. Extremophiles, 2, 1-7.

Nunes, O.C., Donato, M.M., Manaia, C.M., Dacosta, M.S., 1992. The polar lipid and fatty acid composition of *Rhodothermus* strains. Systematic and Applied Microbiology, 15, 59-62.

O'Leary, W.M., Wilkinson, S.G., 1988. Gram-positive bacteria. In: Ratledge, C., Wilkinson S. G. (Hrsg.), Microbial Lipids Vol. 1, Academic Press, London, 117-203.

Oliver, J.D., Stringer, W.F., 1984. Lipid composition of a psychrophilic marine *Vibrio* sp. during starvation-induced morphogenesis. Applied and Environmental Microbiology, 47, 461-466.

Orphan, V.J., Hinrichs, K.-U., Ussler, W., Paull, C.K., Taylor, L.T., Sylva, S.P., Hayes, J.M., DeLong, E.F., 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Applied and Environmental Microbiology, 67, 1922-1934.

Paltauf, F., 1994. Ether lipids in biomembranes. Chemistry and Physics of Lipids, 74, 101.

Palys, T., Nakamura, L.K., Cohan, F.M., 1997. Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. International Journal of Systematic Bacteriology, 47, 1145-1156.

Pancost, R.D., Bouloubassi, I., Aloisi, G., Sinninghe Damsté, J.S., 2001. Three series of non-isoprenoidal dialkyl glycerol diethers in cold-seep carbonate crusts. Organic Geochemistry, 32, 695-707.

Park, C.-E., Berger, L.R., 1967. Complex lipids of *Rhodomicrobium vannielii*. Journal of Bacteriology, 93, 221-229.

Parkes, R.J., Cragg, B.A., Bale, S.J., Getliff, J.M., Goodman, K., Rochelle, P.A., Fry, J.C., Weightman, A.J., Harvey, S.M., 1994. Deep bacterial biosphere in Pacific ocean sediments. Nature, 371, 410-413.

Parkes, R.J., Cragg, B.A., Wellsbury, P., 2000. Recent studies on bacterial populations and processes in subseafloor sediments: A review. Hydrogeology Journal, 8, 11-28.

Parkes, R.J., Webster, G., Cragg, B.A., Weightman, A.J., Newberry, C.J., Ferdelman, T.G., Kallmeyer, J., Jørgensen, B.B., Aiello, I.W., Fry, J.C., 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. Nature, 436, 390-394.

Passier, H.F., Middelburg, J.J., van Os, B.J.H., de Lange, G.J., 1996. Diagenetic pyritisation under Eastern Mediterranean sapropels caused by downward sulphide diffusion. Geochimica et Cosmochimica Acta, 60, 751-763.

Pernthaler, A., Pernthaler, J., Amann, R., 2002. Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. Applied and Environmental Microbiology, 68, 3094-3101.

Pluschke, G., Overath, P., 1981. Function of phospholipids in *Escherichia coli*. Influence of changes in polar head group composition on the lipid phase transition and characterization of a mutant containing only saturated phospholipid acyl chains. Journal of Biological Chemistry, 256, 3207-3212.

Poremba, K., Tillmann, U., Hesse, K.J., 1999. Tidal impact on planktonic primary and bacterial production in the German Wadden Sea. Helgoland Marine Research, 53, 19-27.

Precht, E., Huettel, M., 2004. Rapid wave-driven advective pore water exchange in a permeable coastal sediment. Journal of Sea Research, 51, 93-107.

Pukall, R., Päuker, O., Buntefuß, D., Ulrichs, G., Lebaron, P., Bernard, L., Guindulain, T., Vives-Rego, J., Stackebrandt, E., 1999. High sequence diversity of *Alteromonas macleodii*-related cloned and cellular 16S rDNAs from a Mediterranean seawater mesocosm experiment. FEMS Microbiology Ecology, 28, 335-344.

Quinn, P.J., 1981. The fluidity of cell membranes and its regulation. Progress in Biophysics and Molecular Biology, 38, 1-104.

Rabus, R., Brüchert, V., Amann, J., Könneke, M., 2002. Physiological response to temperature changes of the marine, sulfate-reducing bacterium *Desulfobacterium autotrophicum*. FEMS Microbiology Ecology, 42, 409-417.

Raetz, C.R.H., 1986. Molecular genetics of membrane phospholipid synthesis. Annual Review of Genetics, 20, 253-291.

Rajendran, N., Matsuda, O., Imamura, N., Urushigawa, Y., 1995. Microbial community structure analysis of euxinic sediments using phospholipid fatty acid biomarkers. Journal of Oceanography, 51, 21-38.

Rappé, M.S., Giovannoni, S.J., 2003. The uncultured microbial majority. Annual Review of Microbiology, 57, 369-394.

Ratledge, C., Wilkinson, S.G., 1988. Microbial Lipids. Academic Press, London.

Ravenschlag, K., Sahm, K., Pernthaler, J., Amann, R., 1999. High bacterial diversity in permanently cold marine sediments. Applied and Environmental Microbiology, 65, 3982-3989.

Ring, M.W., Schwar, G., Thiel, V., Dickschat, J.S., Kroppenstedt, R.M., Schulz, S., Bode, H.B., 2006. Novel iso-branched ether lipids as specific markers of developmental sporulation in the myxobacterium *Myxococcus xanthus*. Journal of Biological Chemistry, 281, 36691-36700.

Rohling, E.J., 1994. Review and new aspects concerning the formation of Eastern Mediterranean sapropels. Marine Geology, 122, 1-28.

Rossel, P.E., Lipp, J.S., Fredricks, H.F., Arnds, J., Boetius, A., Elvert, M., Hinrichs, K.-U., 2008. Intact polar lipids of anaerobic methanotrophic archaea and associated bacteria. Organic Geochemistry, 39, 992-999.

Rosselló-Móra, R., Amann, R., 2001. The species concept for prokaryotes. FEMS Microbiology Reviews, 25, 39-67.

Røy, H., Huettel, M., Jørgensen, B.B., 2002. The role of small-scale sediment topography for oxygen flux across the diffusive boundary layer. Limnology and Oceanography, 47, 837-847.

Røy, H., Lee, J.S., Jansen, S., de Beer, D., 2008. Tide-driven deep pore-water flow in intertidal sand flats. Limnology and Oceanography, 53, 1521-1530.

de Rudder, K.E.E., Thomas-Oates, J.E., Geiger, O., 1997. *Rhizobium meliloti* mutants deficient in phospholipid *N*-methyltransferase still contain phosphatidylcholine. Journal of Bacteriology, 179, 6921-6928.

Rusch, A., Huettel, M., 2000. Advective particle transport into permeable sediments - evidence from experiments in an intertidal sandflat. Limnology and Oceanography, 45, 525-533.

Rusch, A., Forster, S., Huettel, M., 2001. Bacteria, diatoms and detritus in an intertidal sandflat subject to advective transport across the water-sediment interface. Biogeochemistry, 55, 1-27.

Rusch, A., Huettel, M., Reimers, C.E., Taghon, G.L., Fuller, C.M., 2003. Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sands. FEMS Microbiology Ecology, 44, 89-100.

Rusch, A., Huettel, M., Wild, C., Reimers, C.E., 2006. Benthic oxygen consumption and organic matter turnover in organic-poor, permeable shelf sands. Aquatic Geochemistry, 12, 1-19.

Russell, N.J., 1984. Mechanisms of thermal adaptation in bacteria - blueprints for survival. Trends in Biochemical Sciences, 9, 108-112.

Russell, N.J., Nichols, D.S., 1999. Polyunsaturated fatty acids in marine bacteria - a dogma rewritten. Microbiology, 145, 767-779.

Rütters, H., 2001. Tracing viable bacteria in Wadden Sea sediments using phospholipid analysis. Dissertation, Carl von Ossietzky Universität Oldenburg.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2001. Monoalkylether phospholipids in the sulfate-reducing bacteria *Desulfosarcina variabilis* and *Desulforhabdus amnigenus*. Archives of Microbiology, 176, 435-442.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2002a. Phospholipid analysis as a tool to study complex microbial communities in marine sediments. Journal of Microbiological Methods, 48, 149-160.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2002b. Microbial communities in a Wadden Sea sediment core - clues from analyses of intact glyceride lipids, and released fatty acids. Organic Geochemistry, 33, 803-816.

Salle, A.M., 1961. Fundamental principles of bacteriology. 5. Aufl., McGraw-Hill, New York, Toronto, London.

Sanina, N.M., Goncharova, S.N., Kostetsky, E.Y., 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. Phytochemistry, 65, 721-730.

van Santvoort, P.J.M., de Lange, G.J., Thomson, J., Cussen, H., Wilson, T.R.S., Krom, M.D., Ströhle, K., 1996. Active post-depositional oxidation of the

most recent sapropel (S1) in sediments of the Eastern Mediterranean. Geochimica et Cosmochimica Acta, 60, 4007-4024.

Sass, A.M., Sass, H., Coolen, M.J.L., Cypionka, H., Overmann, J., 2001. Microbial communities in the chemocline of a hypersaline deep-sea basin (Urania Basin, Mediterranean Sea). Applied and Environmental Microbiology, 67, 5392-5402.

Sass, A.M., Rütters, H., Cypionka, H., Sass, H., 2002. *Desulfobulbus mediterraneus* sp. nov., a sulfate-reducing bacterium growing on mono- and disaccharides. Archives of Microbiology, 177, 468-474.

Sass, H., Cypionka, H., Babenzien, H.-D., 1996. Sulfate-reducing bacteria from the oxic sediment layers of the oligotrophic Lake Stechlin. Archiv für Hydrobiologie, Special Issues Advances in Limnology, 48, 241-246.

Sass, H., Berchtold, M., Branke, J., König, H., Cypionka, H., Babenzien, H.-D., 1998a. Psychrotolerant sulfate-reducing bacteria from an oxic freshwater sediment, description of *Desulfovibrio cuneatus* sp. nov. and *Desulfovibrio litoralis* sp. nov. Systematic and Applied Microbiology, 21, 212-219.

Sass, H., Wieringa, E., Cypionka, H., Babenzien, H.-D., Overmann, J., 1998b. Unexpected genetic and physiological diversity of sulfate-reducing bacteria in an oligotrophic lake sediment. Archives of Microbiology, 170, 243-251.

Sass, H., Wieringa, E., Cypionka, H., Babenzien, H.-D., Overmann, J., 1998c. High genetic and physiological diversity of sulfate-reducing bacteria isolated from an oligotrophic lake sediment. Archives of Microbiology, 170, 243-251.

Sass, H., Babenzien, C., Babenzien, H.-D., 2003a. Sulfate reduction in the oligotrophic Lake Stechlin. Archiv für Hydrobiologie, Special Issues Advances in Limnology, 58, 37-52.

Sass, H., Rütters, H., Schledjewski, R., Freese, E., 2003b. The influence of seasonal changes on microbial communities in the Wadden Sea. Berichte Forschungszentrum Terramare, 12, 99-102.

Sasser, M., 2001. Identification of bacteria by gas chromatography of cellular fatty acids, Technical Note 101 MIDI Sherlock.

Schaechter, M., Maaløe, O., Kjelgaard, N.O., 1958. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimirium*. Journal of General Microbiology, 19, 592-606.

Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiology and Molecular Biology Reviews, 61, 262-280.

Schippers, A., Neretin, L.N., Kallmeyer, J., Ferdelman, T.G., Cragg, B.A., Parkes, R.J., Jørgensen, B.B., 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. Nature, 433, 861-864.

Schloter, M., Lebuhn, M., Heulin, T., Hartmann, A., 2000. Ecology and evolution of bacterial microdiversity. FEMS Microbiology Reviews, 24, 647-660.

Schouten, S., Hopmans, E.C., Baas, M., Boumann, H., Standfest, S., Könneke, M., Stahl, D.A., Sinninghe Damsté, J.S., 2008. Intact membrane lipids of "*Candidatus* Nitrosopumilus maritimus," a cultivated representative of the cosmopolitan mesophilic group I crenarchaeota. Applied and Environmental Microbiology, 74, 2433-2440.

Seo, H.J., Bae, S.S., Lee, J.-H., Kim, S.-J., 2005a. *Photobacterium frigidiphilum* sp. nov., a psychrophilic, lipolytic bacterium isolated from deep-sea sediments of Edison Seamount. International Journal of Systematic and Evolutionary Microbiology, 55, 1661-1666.

Seo, H.J., Bae, S.S., Yang, S.H., Lee, J.H., Kim, S.-J., 2005b. *Photobacterium aplysiae* sp. nov., a lipolytic marine bacterium isolated from eggs of the sea hare *Aplysia kurodai*. International Journal of Systematic and Evolutionary Microbiology, 55, 2293-2296.

Sieburth, J.M., 1967. Seasonal selection of estuarine bacteria by water temperature. Journal of Experimental Marine Biology and Ecology, 1, 98-121.

Simankova, M.V., Kotsyurbenko, O.R., Stackebrandt, E., Kostrikina, N.A., Lysenko, A.M., Osipov, G.A., Nozhevnikova, A.N., 2000. *Acetobacterium tundrae* sp nov., a new psychrophilic acetogenic bacterium from tundra soil. Archives of Microbiology, 174, 440-447.

Sinensky, M., 1974. Homeoviscous adaptation - homeostatic process that regulates viscosity of membrane lipids in *Escherichia coli*. Proceedings of the

National Academy of Sciences of the United States of America, 71, 522-525.

Skerratt, J.H., Bowman, J.P., Nichols, P.D., 2002. *Shewanella olleyana* sp. nov., a marine species isolated from a temperate estuary which produces high levels of polyunsaturated fatty acids. International Journal of Systematic and Evolutionary Microbiology, 52, 2101-2106.

Slomp, C.P., Thomson, J., de Lange, G.J., 2002. Enhanced regeneration of phosphorus during formation of the most recent eastern Mediterranean sapropel (S1). Geochimica et Cosmochimica Acta, 66, 1171-1184.

Smith, P.B.W., Snyder, A.P., Harden, C.S., 1995. Characterization of bacterial phospholipids by electrospray-ionization tandem mass-spectrometry. Analytical Chemistry, 67, 1824-1830.

Sohlenkamp, C., López-Lara, I.M., Geiger, O., 2003. Biosynthesis of phosphatidylcholine in bacteria. Progress in Lipid Research, 42, 115-162.

Sørensen, J., Jørgensen, B.B., Revsbech, N.P., 1979. Comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. Microbial Ecology, 5, 105-115.

Sørensen, J., Christensen, D., Jørgensen, B.B., 1981. Volatile fatty acids and hydrogen as substrates for sulfate reducing bacteria in anaerobic marine sediment. Applied and Environmental Microbiology, 42, 5-11.

Sprott, G.D., Bakouche, L., Rajagopal, K., 2006. Identification of sulfoquinovosyl diacylglycerol as a major polar lipid in *Marinococcus halophilus* and *Salinicoccus hispanicus* and substitution with phosphatidylglycerol. Canadian Journal of Microbiology, 52, 209-219.

Stackebrandt, E., Goebel, B.M., 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. International Journal of Systematic Bacteriology, 44, 846-849.

Stal, L.J., 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. Geomicrobiology Journal, 20, 463-478.

Strömpl, C., Tindall, B.J., Jarvis, G.N., Lunsdorf, H., Moore, E.R.B., Hippe, H., 1999. A re-evaluation of the taxonomy of the genus *Anaerovibrio*, with the reclassification of *Anaerovibrio glycerini* as *Anaerosinus glycerini* gen, nov., comb, nov., and *Anaerovibrio burkinabensis* as *Anaeroarcus burkinensis* [corrig.] gen. nov., comb. nov. International Journal of Systematic Bacteriology, 49, 1861-1872.

Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry - new biomarkers for biogeochemistry and microbial ecology. Rapid Communications in Mass Spectrometry, 18, 617-28.

Süß, J., Engelen, B., Cypionka, H., Sass, H., 2004. Quantitative analysis of bacterial communities from Mediterranean sapropels based on cultivation-dependent methods. FEMS Microbiology Ecology, 51, 109-121.

Süß, J., Schubert, K., Sass, H., Cypionka, H., Overmann, J., Engelen, B., 2006. Widespread distribution and high abundance of *Rhizobium radiobacter* within Mediterranean subsurface sediments. Environmental Microbiology, 8, 1753-1763.

Suutari, M., Laakso, S., 1994. Microbial fatty acids and thermal adaptation. Critical Reviews in Microbiology, 20, 285-328.

Suzuki, D., Ueki, A., Amaishi, A., Ueki, K., 2007a. *Desulfopila aestuarii* gen. nov., sp. nov., a Gram-negative, rod-like, sulfate-reducing bacterium isolated from an estuarine sediment in Japan. International Journal of Systematic and Evolutionary Microbiology, 57, 520-526.

Suzuki, D., Ueki, A., Amaishi, A., Ueki, K., 2007b. Diversity of substrate utilization and growth characteristics of sulfate-reducing bacteria isolated from estuarine sediment in Japan. Journal of General and Applied Microbiology, 53, 119-132.

Suzuki, M.T., Giovannoni, S.J., 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Applied and Environmental Microbiology, 62, 625-630.

Takii, S., Hanada, S., Hase, Y., Tamaki, H., Uyeno, Y., Sekiguchi, Y., Matsuura, K., 2008. *Desulfovibrio marinisediminis* sp. nov., a novel sulfate-reducing bacterium isolated from coastal marine sediment via enrichment with casamino acids. International Journal of Systematic and Evolutionary Microbiology, 58, 2433-2438.

Tarpgaard, I.H., Boetius, A., Finster, K., 2006. *Desulfobacter psychrotolerans* sp. nov., a new psychrotolerant sulfate-reducing bacterium and descriptions of its physiological response to temperature changes. Antonie van Leeuwenhoek, 89, 109-124.

Taylor, C.J., Anderson, A.J., Wilkinson, S.G., 1998. Phenotypic variation of lipid composition in *Burkholderia cepacia*: a response to increased growth temperature is a greater content of 2-hydroxy acids in phosphatidylethanolamine and ornithine amide lipid. Microbiology, 144, 1737-1745.

Teske, A., Sørensen, K.B., 2008. Uncultured archaea in deep marine subsurface sediments: have we caught them all? ISME Journal, 2, 3-18.

Thiele, O.W., Biswas, C.J., Hunneman, D.H., 1980. Isolation and characterization of an ornithine-containing lipid from *Paracoccus denitrificans*. European Journal of Biochemistry, 105, 267-274.

Thompson, F.L., Thompson, C.C., Naser, S., Hoste, B., Vandemeulebroecke, K., Munn, C., Bourne, D., Swings, J., 2005a. *Photobacterium rosenbergii* sp. nov. and *Enterovibrio coralii* sp. nov., vibrios associated with coral bleaching. International Journal of Systematic and Evolutionary Microbiology, 55, 913-917.

Thompson, J.R., Randa, M.A., Marcelino, L.A., Tomita-Mitchell, A., Lim, E., Polz, M.F., 2004. Diversity and dynamics of a North Atlantic coastal *Vibrio* community. Applied and Environmental Microbiology, 70, 4103-4110.

Thompson, J.R., Pacocha, S., Pharino, C., Klepac-Ceraj, V., Hunt, D.E., Benoit, J., Sarma-Rupavtarm, R., Distel, D.L., Polz, M.F., 2005b. Genotypic diversity within a natural coastal bacterioplankton population. Sciene, 307, 1311-1313.

Tison, D.L., Pope, D.H., Boylen, C.W., 1980. Influence of seasonal temperature on the temperature optima of bacteria in sediments of Lake George, New York. Applied and Environmental Microbiology, 39, 675-677.

Toffin, L., Webster, G., Weightman, A.J., Fry, J.C., Prieur, D., 2004. Molecular monitoring of culturable bacteria from deep-sea sediment of the Nankai Trough, Leg 190 Ocean Drilling Program. FEMS Microbiology Ecology, 48, 357-367.

Traore, A.S., Hatchikian, C.E., LeGall, J., Belaich, J.P., 1982. Microcalorimetric studies of growth of sulfate-reducing bacteria: comparison of the growth parameters of some *Desulfovibrio* species. Journal of Bacteriology, 149, 606-611.

Treude, N., Rosencrantz, D., Liesack, W., Schnell, S., 2003. Strain FAc12, a dissimilatory iron-reducing member of the *Anaeromyxobacter* subgroup of *Myxococcales*. FEMS Microbiology Ecology, 44, 261-269.

Uda, I., Sugai, A., Itoh, Y.H., Itoh, T., 2001. Variation in molecular species of polar lipids from *Thermoplasma acidophilum* depends on growth temperature. Lipids, 36, 103-105.

Ueki, A., Suto, T., 1979. Cellular fatty acid composition of sulfate-reducing bacteria. Journal of General and Applied Microbiology, 25, 185-196.

Unell, M., Kabelitz, N., Jansson, J.K., Heipieper, H.J., 2007. Adaptation of the psychrotroph *Arthrobacter chlorophenolicus* A6 to growth temperature and the presence of phenols by changes in the *anteiso*/*iso* ratio of branched fatty acids. FEMS Microbiology Letters, 266, 138-143.

Vainshtein, M., Hippe, H., Kroppenstedt, R.M., 1992. Cellular fatty acid composition of *Desulfovibrio* species and its use in classification of sulfate-reducing bacteria. Systematic and Applied Microbiology, 15, 554-566.

Valentine, D.L., 2007. Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nature Reviews Microbiology, 5, 316-323.

Van Mooy, B.A.S., Rocap, G., Fredricks, H.F., Evans, C.T., Devol, A.H., 2006. Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. Proceedings of the National Academy of Sciences of the United States of America, 103, 8607-8612.

Van Mooy, B.A.S., Fredricks, H.F., Pedler, B.E., Dyhrman, S.T., Karl, D.M., Koblížek, M., Lomas, M.W., Mincer, T.J., Moore, L.R., Moutin, T., Rappé, M.S., Webb, E.A., 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature, 458, 69-72.

Vancanneyt, M., Witt, S., Abraham, W.R., Kersters, K., Fredrickson, H.L., 1996. Fatty acid content in whole-cell hydrolysates and phospholipid fractions of pseudomonads: a taxonomic evaluation. Systematic and Applied Microbiology, 19, 528-540.

Versalovic, J., Koeuth, T., Lupski, J.R., 1991. Distribution of repetitive DNA-sequences in *Eubacteria* and application to fingerprinting of bacterial genomes. Nucleic Acids Research, 19, 6823-6831.

Vezzi, A., Campanaro, S., D'Angelo, M., Simonato, F., Vitulo, N., Lauro, F.M., Cestaro, A., Malacrida, G., Simionati, B., Cannata, N., Romualdi, C., Bartlett, D.H., Valle, G., 2005. Life at depth: *Photobacterium profundum* genome sequence and expression analysis. Science, 307, 1459-1461.

Villanueva, L., Navarrete, A., Urmeneta, J., Geyer, R., White, D., Guerrero, R., 2007. Monitoring diel variations of physiological status and bacterial diversity in an estuarine microbial mat: an integrated biomarker analysis. Microbial Ecology, 54, 523-531.

Volkman, J.K., Rohjans, D., Rullkötter, J., Scholz-Böttcher, B.M., Liebezeit, G., 2000. Sources and diagenesis of organic matter in tidal flat sediments from the German Wadden Sea. Continental Shelf Research, 20, 1139-1158.

von Wintzingerode, F., Göbel, U.B., Stackebrandt, E., 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiology Reviews, 21, 213-29.

Wada, H., Murata, N., 2007. The essential role of phosphatidylglycerol in photosynthesis. Photosynthesis Research, 92, 205-215.

Wada, M., Fukunaga, N., Sasaki, S., 1987. Effect of growth temperature on phospholipid and fatty acid compositions in a psychrotrophic bacterium, *Pseudomonas* sp. strain E-3. Plant and Cell Physiology, 28, 1209-1217.

Wagner, M., Roger, A.J., Flax, J.L., Brusseau, G.A., Stahl, D.A., 1998. Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. Journal of Bacteriology, 180, 2975-2982.

Wanner, U., Egli, T., 1990. Dynamics of microbial growth and cell composition in batch culture. FEMS Microbiology Reviews, 75, 19-44.

Webster, G., Parkes, J.R., Fry, J.C., Weightman, A.J., 2004. Widespread occurrence of a novel division of bacteria identified by 16S rRNA gene sequences originally found in deep marine sediments. Applied and Environmental Microbiology, 70, 5708-5713.

Webster, G., Yarram, L., Freese, E., Köster, J., Sass, H., Parkes, R.J., Weightman, A.J., 2007. Distribution of candidate division JS1 and other *Bacteria* in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE. FEMS Microbiology Ecology, 62, 78-89.

Webster, G., Blazejak, A., Cragg, B.A., Schippers, A., Sass, H., Rinna, J., Tang, X., Mathes, F., Ferdelman, T., Fry, J.C., Weightman, A.J., Parkes, J.R., 2009. Subsurface microbiology and biogeochemistry of a deep, cold-water carbonate mound from the Porcupine Seabight (IODP Expedition 307). Environmental Microbiology, 11, 239-257.

Weissenmayer, B., Gao, J.-L., López-Lara, I.M., Geiger, O., 2002. Identification of a gene required for the biosynthesis of ornithine-derived lipids. Molecular Microbiology, 45, 721-733.

Wellsbury, P., Mather, I., Parkes, R.J., 2002. Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean. FEMS Microbiology Ecology, 42, 59-70.

Werner, U., Bird, P., Wild, C., Ferdelman, T., Polerecky, L., Eickert, G., Jonstone, R., Hoegh-Guldberg, O., de Beer, D., 2006. Spatial patterns of aerobic and anaerobic mineralization rates and oxygen penetration dynamics in coral reef sediments. Marine Ecology Progress Series, 309, 93-105.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia, 40, 51-62.

White, D.C., 1997. Signature lipid biomarker analysis for quantitative assessment in situ of environmental microbial ecology. In: Eganhouse, R. P. (Hrsg.), Molecular markers in environmental geochemistry Vol. 671, ACS Symposium Series, American Chemical Society, Washington D. C., 22-34.

White, P.L., Wynn-Williams, D.D., Russell, N.J., 2000. Diversity of thermal responses of lipid composition in the membranes of the dominant culturable members of an Antarctic fellfield soil bacterial community. Antarctic Science, 12, 386-393.

Widdel, F., 1980. Anaerober Abbau von Fettsäuren und Benzoesäure durch neu isolierte Arten sulfatreduzierender Bakterien. Dissertation, Georg-August-Universität, Göttingen.

Widdel, F., 1988. Microbiology and ecology of sulfate-reducing and sulfur-reducing bacteria. In: A.J.B. Zehnder (Hrsg.), Biology of Anaerobic Microorganisms, S. 469-585. John Wiley and Sons Inc., New York.

Widdel, F., Bak, F., 1992. Gram-negative mesophilic sulfate-reducing bacteria. In: A. Balows, H.G. Trüper, M. Dworkin, W. Harder, K.-H. Schleifer (Hrsg.), The prokaryotes, second edition, S. 3352-3372. Springer, Heidelberg.

Wieringa, E.B.A., Overmann, J., Cypionka, H., 2000. Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. Environmental Microbiology, 2, 417-427.

Wilkinson, B.J., Sment, K.A., Mayberry, W.R., 1982. Occurrence, localization, and possible significance of an ornithine-containing lipid in *Paracoccus denitrificans*. Archives of Microbiology, 131, 338-343.

Wilkinson, S.G., 1972. Composition and structure of ornithine-containing lipid from *Pseudomonas rubescens*. Biochimica et Biophysica Acta, 270, 1-17.

Wilkinson, S.G., 1988. Gram-negative bacteria. In: C. Ratledge, S.G. Wilkinson (Hrsg.), Microbial Lipids, 1, S. 299-488. Academic Press, London.

Wilms, R., Köpke, B., Sass, H., Chang, T.S., Cypionka, H., Engelen, B., 2006a. Deep biosphere-related bacteria within the subsurface of tidal flat sediments. Environmental Microbiology, 8, 709-719.

Wilms, R., Sass, H., Köpke, B., Köster, J., Cypionka, H., Engelen, B., 2006b. Specific bacterial, archaeal, and eukaryotic communities in tidal-flat sediments along a vertical profile of several meters. Applied and Environmental Microbiology, 72, 2756-2764.

Wilms, R., Sass, H., Köpke, B., Cypionka, H., Engelen, B., 2007. Methane and sulfate profiles within the subsurface of a tidal flat are reflected by the distribution of sulfate-reducing bacteria and methanogenic archaea. FEMS Microbiology Ecology, 59, 611-621.

Wuchter, C., Schouten, S., Coolen, M.J.L., Sinninghe Damsté, J.S., 2004. Temperature-dependent variation in the distribution of tetraether membrane lipids of marine Crenarchaeota: implications for TEX86 paleothermometry. Paleoceanography, 19, 1-10.

Xie, C.-H., Yokota, A., 2004. Transfer of *Hyphomicrobium indicum* to the genus *Photobacterium* as *Photobacterium indicum* comb. nov. International Journal of Systematic and Evolutionary Microbiology, 55, 335-339.

Yano, Y., Nakayama, A., Ishihara, K., Saito, H., 1998. Adaptive changes in membrane lipids of barophilic bacteria in response to changes in growth pressure. Applied and Environmental Microbiology, 64, 479-485.

Yayanos, A.A., 1995. Microbiology to 10,500 meters in the deep sea. Annual Review of Microbiology, 49, 777-805.

Yoon, J.-H., Lee, J.-K., Kim, Y.-O., Oh, T.-K., 2005. *Photobacterium lipolyticum* sp. nov., a bacterium with lipolytic activity isolated from the Yellow Sea in Korea. International Journal of Systematic and Evolutionary Microbiology, 55, 335-339.

Yuasa, J., Nakagawa, Y., Kotani, Y., Shimohata, A., Matsuyama, T., 2002. *Pseudomonas aeruginosa* under phosphorus poor culture conditions: phospholipid-poor bacterial membranes, and susceptibility to antibacterial chemicals, high temperature and low pH. Microbes and Environments, 17, 75-81.

Zeikus, J.G., Fuchs, G., Kenealy, W., Thauer, R.K., 1977. Oxidoreductases involved in cell carbon synthesis of *Methanobacterium thermoautotrophicum*. Journal of Bacteriology, 132, 604-613.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils, 29, 111-129.

Zhang, X., Ferguson-Miller, S.M., Reid, G.E., 2009. Characterization of ornithine and glutamine lipids extracted from cell membranes of *Rhodobacter sphaeroides*. Journal of the American Society for Mass Spectrometry, 20, 198-212.

Zhang, Y.M., Rock, C.O., 2008. Membrane lipid homeostasis in bacteria. Nature Reviews Microbiology, 6, 222-233.

Zink, K.G., Wilkes, H., Disko, U., Elvert, M., Horsfield, B., 2003. Intact phospholipids - microbial "life markers" in marine deep subsurface sediments. Organic Geochemistry, 34, 755-769.

Zink, K.G., Mangelsdorf, K., 2004. Efficient and rapid method for extraction of intact phospholipids from sediments combined with molecular structure elucidation using LC-ESI-MS-MS analysis. Analytical And Bioanalytical Chemistry, 380, 798-812.

Zink, K.G., Mangelsdorf, K., Granina, L., Horsfield, B., 2008. Estimation of bacterial biomass in subsurface sediments by quantifying intact membrane phospholipids. Analytical and Bioanalytical Chemistry, 390, 885-896.

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Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbständig angefertigt habe und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Zusätzlich erkläre ich, dass diese Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vorliegt oder vorgelegen hat.

Oldenburg, den 11. Mai 2009

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