Chapter 7
Anaerobic Bacteria and Archaea in Cold Ecosystems

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Contents

7.1 Introduction

Permanently cold environments are very common on Earth. For example, the average temperature in bottom waters of the largest fraction of the world oceans is 5°C or less and including terrestrial habitats about 80% of the Earth's biosphere is to be found in permanently cold habitats (Russell 1990). In addition to being cold, many of these environments are also oxygen-free, thus supporting exclusively facultative or obligately anaerobic microbial life. Anoxic permanently cold environments are very diverse and include the marine sea floor (Rysgaard et al. 1998; Sagemann et al. 1998; Bowman et al. 2003; Vandieken et al. 2006b), microbial mats (Mueller et al. 2005; Fernández-Valiente et al. 2007), endolithic communities in sandstones (Friedmann 1982), permafrost soils of the Arctic and Antarctic regions (Kobabe et al. 2004; Gilichinsky et al. 2005; Steven et al. 2007), and chilled food (Broda et al. 2000a, 2000b, 2002). It has also been demonstrated that these habitats harbor extensive microbial communities, including many microorganisms that are phylogenetically affiliated with obligately anaerobic organisms in culture (Ravenschlag et al. 1999; Purdy et al. 2003; Ganzert et al. 2007). Despite their ecological and economical significance very little is known about anaerobic bacteria and archaea

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that live in these environments and the mechanisms by which they thrive or survive under in situ conditions.

In this review paper, I have collected all accessible (to me) information on validly described obligately anaerobic psychrophilic bacteria and archaea. In addition, I have included information on selected psychrotolerant species. I have applied the definition of psychrophily introduced by Morita (1975) with modifications proposed by Scherer and Neuhaus (2006) for the definition of psychrotolerant microbes. Thus obligate psychrophiles are organisms with a minimal growth temperature <0°C, a temperature optimum for growth ≤15°C and a maximum temperature ≤20°C. Psychrotolerant organisms have a minimal growth temperature <7°C, a temperature optimum for growth ≤20°C and a maximum temperature ≤35°C. According to Scherer and Neuhaus (2006) the minimum temperature was set to <7°C instead of ≤0°C to cope with the variation in reporting on the growth of microorganisms in chilled food.

7.2 Bacteria

7.2.1 The genus Clostridium

The genus *Clostridium*, which contains obligately anaerobic fermenters, accounts at present for a large number of validly described species of psychrophilic and psychrotolerant obligate anaerobes within a single genus. This may mainly be due to the fact that the genus is defined by rather broad morphological and physiological traits, such as a Gram-positive cell wall, spore formation and energy generation by fermentation. On the basis of these characters, strains are clustered into the same genus, which are, based on 16S rRNA phylogeny, more than 10% different.

7.2.1.1 Psychrophilic clostridia

Of the nine psychrophilic clostridia, five were isolated from microbial mats in Antarctica (*C. frigoris, C. lacusfryxellense, C. bowmannii, C. psychrophilum* (Spring et al. 2003) and *C. vincentii* (Mountford et al. 1997), two from chilled meat (*C. estertheticum subsp. estertheticum*; Collins et al. 1992; and *C. gasigense*; Broda et al. 1999), one from cattle manure (*C. estertheticum* subsp. *laramiense*; Kotsyurbenko et al. 1995a) and one from overcooled water brine of arctic permafrost (*C. algoriphilum*; Shcherbakova et al. 2005). With the exception of *C. algoriphilum,* the publications on the other psychrophilic or psychrotolerant clostridia compile information on the isolates without specific focus on their ability to grow at low temperature (for details see http://www.bio.au.dk/KaiFinster/tables/). Therefore, the following summary of observed responses to temperature among clostridia is exclusively based on the study of *C. algoriphilum*.

C. algoriphilum was isolated from overcooled water brines, the so-called cryopegs, a habitat type that can be found in permafrost soil of marine origin. Cryopegs

are characterized by high salt concentration, which allows water to be in the liquid state even at in situ temperatures of about −10°C (Gilichinsky et al. 2005). *C. algoriphilum* has the lowest documented temperature limit of growth (−5°C) of all validly described psychrophilic obligately anaerobic bacteria and archaea. A transformation of the temperature dependence of growth rates using the Ratkovsky model (Ratkovsky et al. 1983) results in a predicted lowest growth temperature of −43°C, which is the lowest theoretical growth temperature yet determined. This indicates that *C. algoriphilum* may not only survive in its habitat but may also be metabolically active and even proliferate. However, incubation of cells of *C. algoriphilum* in original cryopeg water induced sporulation not growth. The strain shows several interesting physiological responses depending on the temperature at which it was grown. For example, a change in the optimum growth rate as a function of NaCl concentration of the growth medium was observed when the culture was grown at +5°C or −5°C, respectively. At −5°C, the highest grow rate was obtained with 1.0% NaCl in the growth medium and growth was observed between 0 and 10% NaCl, while at 5°C the culture grew best at 0.5% NaCl and the highest NaCl that permitted growth was 5%. The growth temperature also affected the fermentation pattern of glucose as well as the patterns of substrate production. Gilichinsky et al. (2005) also reported that the incubation temperature not only had an effect on the velocity at which the organism was able to grow on the specific substrate but whether it could grow on a specific compound at all. While glucose, sucrose, and trehalose supported growth at 18, 5 and −2°C, growth on L-glutamate was only observed at −2°C. The observation suggests that combinations of substrates should be employed during enrichment and isolation rather then single compounds, as specific substrate may incidentally not be used at a particular incubation temperature and consequently the enrichment may fail.

The composition of lipids that constitute the cell membrane of *C. algoriphilum* show clear adaptations to low temperature by a significant prevalence of short chain fatty acids ($C_{14:0}$ = 33%) and a high content of unsaturated fatty acids (60%). Both types of compounds increase the fluidity of the cell membrane at low temperature. Unfortunately, Shcherbakova et al. (2004) determined the composition of the lipids only at one temperature and thus the effect of temperature on the lipid composition of the membrane cannot be evaluated.

7.2.1.2 Psychrotolerant clostridia

The website http://www.bio.au.dk/KaiFinster/tables/ compiles data on several psychrotolerant clostridia. The table is incomplete as it only contains strains that were selected according to the following criteria: (1) the title of the publication includes the terms psychrotolerant (*C. algidixylanolyticum, C. frigidicarnis*) or psychroactive (*C. fimetarium)*, or (2) the species was mentioned in the description of a psychrophilic/psychrotolerant *Clostridium* as a strain expressing temperature patterns that would classify it as psychrotolerant sensu Scherer and Neuhaus (2006). Despite the fact that *C. schirmacherense* expressed the highest growth efficiency in the

5–10°C temperature range and for that reason is described as a psychrophile by Alam et al. (2006), it is here grouped among the psychrotolerant strains, as its upper limit for growth is 35°C and thus by far exceeds the upper limit for psychrophiles sensu Morita (1975). Only *C. schirmacherense* and *C. fimetarium* have been subjected to studies that addressed the strains response to different temperatures. With *C. schirmacherense* the protease activity was measured as a function of temperature. Alam et al. (2005) demonstrated that a purified protease from *C. schirmacherense* expressed 5–8% of its activity measured at T_{opt} (37°C) at 0°C. The membrane composition of *C. fimetarium* was determined with cultures grown at 6 and 25°C, respectively. The content of unsaturated compounds was slightly higher in 6°C cultures (55.1%) than in 25°C cultures (48.6%), and the content of short chain fatty acids ($C1_{14:0}$) increased from 4% at 25°C to 16% at 6°C. Both adaptations are typically reported for psychrotolerant microorganisms (see Sect. 7.2.2 below). The growth rate curve was also typical for psychrotolerant microbes, showing an optimum between 20 and 25°C. The growth rate at 6°C, which is the in situ temperature of the manure the strain was isolated from, was $0.026\,h^{-1}$, a 70% reduction compared to the rate obtained at T_{opt}. Apart from general descriptions, surprisingly little work has been done on food-spoiling clostridia such as *C. algidixylanolyticum, C. frigidicarnis, C. estertheticum subsp. estertheticum* and *C. gasigense* at in situ temperatures. More detailed studies on the temperature dependence of their physiological properties could have significant practical implications for the treatment and protection of meat products against contamination and further food spoilage.

In general, more studies dedicated to the effect of temperature on metabolism and growth of clostridia are needed to either validate or extend the sparse information that is currently available. In addition, the biotechnological potential of psychrophilic and psychrolerant clostridia has hardly been explored as yet. I only came across one publication (Akila and Chandra 2003) which reports on low temperature active xylanase and cellulase activity from a cold tolerant clostridium, which had a maximal activity at 20°C. This is surprising as energy saving is an important aspect of future processing and production. Furthermore, psychrophilic clostridia or other fermenting microorganisms from anoxic permanently cold marine sediments have only been described sporadically so far (Finne and Matches 1974). They may be important players in the anaerobic food chain in marine sediments (Arnosti and Jørgensen 2003; Arnosti et al. 2005) and may provide sulfate reducers and methanogens with their substrates (Schmitz et al. 2006).

7.2.2 Sulfate-reducing bacteria

7.2.2.1 Psychrophilic sulfate-reducing bacteria

The first psychrophilic sulfate reducer was described 10 years ago by Isaksen and Jørgensen (1996) and Isaksen and Teske (1996; http://www.bio.au.dk/KaiFinster/tables/). The sediment-inhabiting organism, designated *Desulforhopalus vacuolatus*,

contains large gas-vacuoles of as yet unknown function. The strain was subjected to detailed investigations focusing on its response to different temperatures. The temperature optimum of growth was significantly lower than the temperature optimum of sulfate reduction, which was determined in a short-term experiment with radiolabled sulfate (Isaksen and Jørgensen 1996). Sulfate reduction in contrast to growth involves a limited set of enzymes, which may all be relatively insensitive to higher temperature. The relatively high temperature maximum of sulfate reduction (28°C) would group *D. vacuolatus* among the mesophilic bacteria sensu Morita (Morita 1975), while the temperature optimum of growth (19°C) places it among the psychrotolerant bacteria. However, the Arrhenius plots of growth and sulfate reduction rate data showed a linear relationship between T_{opt} and T_{min} (28°C to −1.8°C for sulfate reduction and 18°C to 0°C for growth). Isaksen and Jørgensen (1996) interpret the linear response as an adaptation of the entire enzymatic machinery to low temperature. The observed linearity over the entire temperature range places *D. vacuolatus* among the psychrophiles. Thus, a single organism fits into three different temperature categories depending on the criteria that were used, which exposes the difficulties involved in grouping organisms according to the currently used definitions. With respect to growth yield (g biomass mol^{-1} substrate), *D. vacuolatus* expressed the highest and almost constant growth yield between 15°C and 0°C while the growth yield decreased towards the T_{opt} for growth. Both Arrhenius plot data and the growth yield data demonstrate that *D. vacuolatus* is particularly well adapted to low temperature (Isaksen and Jørgensen 1996). It is a weakness of this study and all the other studies of obligately anaerobic prokaryotes that they were carried out in batch cultures at high substrate concentrations. It would be very interesting to obtain information on the growth characteristics at low, and thus ecologically more relevant, substrate concentrations in combination with low temperatures.

D. vacuolatus was the only validly described psychrophilic sulfate reducer until Knoblauch et al. (1999a) reported the isolation of 19 psychrophilic sulfate reducers from permanently cold sediment from the coast of Spitsbergen, and published a detailed physiological study of three new genera of psychrophilic sulfate reducers (Knoblauch et al. 1999b; http://www.bio.au.dk/KaiFinster/tables/). The description was accompanied by comprehensive biogeochemical and molecular ecological investigations of samples from the sampling site from which the isolates were obtained, substantiating that the isolates very likely were biogeochemical key players in the system (Knoblauch et al. 1999a; Knoblauch and Jørgensen 1999; Sahm et al. 1999). All the isolates were metabolically active at in situ temperatures of −1.8°C and 2.6°C, respectively, and their relative growth rates at 0°C were >25% of the rates measured at T_{opt}. The latter observation distinguishes them from the psychrotolerant sulfate reducers, which suffer from a much more pronounced growth rate reduction at 0°C compared to growth rates at optimal growth temperature (Table 7.1). The growth yields determined by Knoblauch and Jørgensen (1999) in the low temperature range were comparable to yields measured with mesophilic sulfate reducers on the same substrates. The efficiency of an organism to transform a substrate into biomass and finally proliferate is crucial for its competitiveness in

Table 7.1 Cold adaptation among psychrophilic and psychrotolerant sulfate reducers

Genus and Species	Substrates	Rel. growth rate at 0 or [a]4°C vs. T_{opt} [%]	E_a [kJ mol^{-1}] and (Q_{10})	Temp. range [°C] used for calculation of [b]E_a and (Q_{10})	Growth yield [g dry weight mol^{-1}] (at °C)	T_{opt} for SRR	Growth
Psychrophilic							
Desulfofaba gelida[(1)]	Propionate	41	n.r.	n.r.	3.8 (5) and 3.2 (−1.8–5)	n.r.	7
Desulfofrigus oceanense[(1)]	Acetate	24	59 (2.5)	−2–17	4.9 (7) and 3.5 (−1.8)	17	10
Desulfotalea psychrophila[(1)]	Lactate	33	54.3 (2.3)	−4–12	4.2 (−1.8–15)	10	10
Moderately psychrophilic							
Desulfofrigus fragile[(1)]	Lactate	31	58.2 (2.4)	−4–22	7.2 (4) and 4.8 (18)	27	18
Desulfotalea arctica[(1)]	Lactate	29	54 (2.3)	−2–22	5.8 (0–4) and 2.8 (18)	23	18
Desulforhopalus vacuolatus[(1,5)]	Lactate	2	86.1 (3.4)	10–20	9.5 (0–12) and 5 (19)	28	19
Psychrotolerant							
Desulfobacter psychrotolerans[(2)]	Acetate	5	130.2 (8.0) and 64.3 (2.6)	(−3.6–6.4) and (6.4–16.4)	4.4 (5–15) and 3.5 (20)	26	20
Desulfobacter hydrogenophilus[(6)]	Acetate	8[(1)]	187 (18.4) and 59.9 (2.4)	(0–10) and (8–18)	n.r. n.r.	n.r.	n.r.
Desulfobacterium autotrophicum[(3)]	Lactate	3	n.r.	n.r.		n.r.	n.r.
Desulfovibrio cuneatus[(4)]	Lactate	22–26[a]	40 (1.75)[b]	(10–20)	3.4 (20) and 2 (28)	n.r.	28
Desulfovibrio litoralis[(4)]	Lactate	36[a]	38 (1.70)[b]	(10–20)	4.2 (10) and 3.2 (28)	n.r.	28

References: [(1)]Knoblauch and Jørgensen (1999);[(2)]Tarpgaard et al. (2006); [(3)]Rabus et al. (2002); [(4)]Sass et al. (1998); [(5)]Isaksen and Jørgensen (1996), [(6)]Bak (1988)

[a]Calculated at 4°C

[b]Calculated from values depicted from Sass et al. (1998)

n.r., not reported

E_a, Activation energy

SRR, sulfate reduction rate

nature and thus growth yield data are more informative then growth rate data, when using laboratory data to estimate the organisms' competitiveness in nature.

7.2.2.2 Psychrotolerant sulfate-reducing bacteria

The psychrophilic sulfate reducers can be distinguished from the psychrotolerant sulfate reducers when comparing the Arrhenius plots of growth and sulfate reduction rates of the two groups (http://www.bio.au.dk/KaiFinster/tables/). Though the material is currently still very limited it seems that the Arrhenius plot data of sulfate reduction and growth rates obtained with psychrophilic sulfate reducers were fitted best by one line ranging from T_{opt} to T_{min}, while the data obtained with psychrotolerant sulfate reducers could only be fitted with two lines resulting in two very different E_a values (Table 7.1; Bak 1988; Rabus et al. 2002; Tarpgaard et al. 2006). The point of infliction of the two lines in the Arrhenius plots of the psychrotolerant sulfate reducers is called the critical temperature, which indicates that cells are well adapted to temperature changes above $T_{critical}$, but only poorly to changes in temperature between $T_{critical}$ and T_{min}. The biochemical background for the bimodality has not yet been conclusively elucidated. Membrane fluidity may be an important factor, as this would influence transport processes including the supply with energy sources. Könneke and Widdel (2003), studying the effect of growth temperature on the fatty acid composition of the membrane of sulfate-reducing bacteria, reported significant differences in the response to different temperatures when psychrophiles were compared to psychrotolerant or mesophilic sulfate reducers. They observed that the proportion of cis-unsaturated fatty acids was high in psychrophiles and there was no significant change in the proportion with decreasing temperature. The latter was the case when psychrotolerant sulfate reducers were grown at decreasing temperatures. They conclude that the fatty acid patterns in psychrophiles were optimized to function in a permanently cold environment. The response of psychrotolerant sulfate reducers reported by Könneke and Widdel (2003) was also found by Rabus et al. (2002) investigating the effect of temperature change on the composition of the cellular fatty acids of *Desulfobacterium autotrophicum* as well as by Tarpgaard et al. (2006) studying *Desulfobacter psychrotolerans. D. autotrophicum* increased the relative fraction of unsaturated fatty acids as well as the relative fraction of short chain fatty acids, while *D. psychrotolerans* only increased the fraction of unsaturated fatty acids. The latter was also reported by Könneke and Widdel (2003) with both the psychrotolerant strain *D. hydrogenophilus*, which is closely related to *D. psychrotolerans*, and other mesophilic *Desulfobacter* species. During their investigation, Könneke and Widdel (2003) observed that the proportion of the unsaturated fatty acid *cis* 16:1(9) ranged from about 38% during late exponential growth to about 12% during stationary phase when cultures were grown at 28°C. In cultures grown at 12°C, the fraction of 16:1(9) steadily increased during exponential growth and reached a constant level of nearly 50% during late exponential growth. This observation indicates that the composition of the cellular membrane is not affected by temperature alone and care has to taken when the results are interpreted. Whether

psychrophilic or psychrotolerant sulfate reducers have other physiological adaptations, such as low temperature active enzymes or transport proteins, is currently not known. Clues may be obtained from genome sequence data from psychrophiles. Recently, the genome of *Desulfotalea psychrophila* was fully sequenced (Rabus et al. 2004). In contrast to results obtained with psychrophilic methanogens, where the analysis of proteins revealed a high content of non-charged polar amino acids and a lower content of hydrophobic amino acids than found with mesophilic and hydrophilic archaea and which were interpreted as adaptations to low temperatures (Saunders et al. 2003), no such patterns were obtained from the genome analysis of *D. psychrophila*. The presence of genes encoding cold-shock proteins that are involved in regulation and DNA processing were identified. However, it cannot be concluded from these data that the cold-shock genes are particularly important for the strain's ability to grow at low or at high temperature. In addition, Rabus et al. (2004) reported the presences of at least 12 tRNA modifying enzymes, which might be important for cold adaptation of the translation process and they also identified a new transcriptional regulation mechanism, which may be relevant for growth at low temperatures. Finally, they reported on the presence of a type of helicases that had been shown to enable bacteria to survive cold shock and grow at low temperature (Lim et al. 2000). Overall at present, mining of the genome sequence data is far from being completed and thus more information may be extracted with respect to the strain's psychrophilic nature.

7.2.3 Sulfur- and iron-reducing bacteria

Recently, the first obligately anaerobic sulfur and iron reducers have been isolated that expressed cold adaptation (Holmes et al. 2004; Nevin et al. 2005; Vandieken et al. 2006a). In particular from an ecological point of view, the isolation of cold-adapted iron reducers is very interesting because iron reduction has been identified as an important process in carbon cycling in many cold marine and freshwater environments (Lovley et al. 2004; Vandieken et al. 2006b). *Geopsychrobacter electrodipilus* was isolated from the surface of an anaerobic electrode in a laboratory-incubated marine sediment fuel cell, used to extract electro-chemical energy from anoxic sediments (Holmes et al. 2004). With acetate and Fe(III) oxide as substrates the growth rates were determined between 4 ($\mu = 0.006\,h^{-1}$) and 30°C ($\mu = 0.004\,h^{-1}$). The strain grew optimally at 22°C ($\mu = 0.011\,h^{-1}$). Since the strain grew at all temperatures tested, the lower and upper limits for growth have not yet been determined. However, the preliminary documented temperature regime would group *G. electrodipilus* among the psychrotolerant microorganisms. Organisms like *G. electrodipilus* may have interesting technical applications due to their capacity to transfer chemical energy to electrodes. It would thus be interesting to explore the temperature dependence of this mechanism.

Nevin et al. (2005) reported on the isolation of three closely related strains that were grouped into the species *Geobacter psychrophilus*. The iron reduction rate at 4°C was

about one-third the rate measured at 30°C. No iron reduction could be observed at 37°C. Thus, the strain's lower limit for iron reduction has not been determined yet.

Two psychrophilic iron reducers, designated *Desulfuromonas svalbardiense* and *Desulfuromusa ferrireducens*, were isolated by Vandieken et al. (2006a) from permanently cold sediment close to Svalbard. The two species expressed psychropilic temperature adaptations and grew well at the in situ temperature of their natural environment (−2°C). Both grew at −2°C and had a temperature optimum for growth at round 15°C, but differed slightly in their respective maximum growth temperature. While *D. svalbardiense* did not grow above 20°C, the upper temperature limit of *D. ferrireducens* was 23°C.

7.2.4 Acetogenic bacteria

Studies by Conrad and coworkers indicate that homoacetogens are important hydrogen consumers in cold anoxic sediments (Conrad et al. 1989 Schulz and Conrad 1996; Kotsyurbenko et al. 2001) and several cold-adapted strains of acetogens have recently been obtained in pure culture (Kotsyurbenko et al. 1995b; Simankova et al. 2000; Paarup et al. 2006). The isolates were obtained from habitats as diverse as paper-mill wastewater (*Acetobacterium bakii*), fen sediment (*A. paludosum*), digested manure (*A. fimetarium*), tundra soil (*A. tundrae*) and fjord sediment (*A. carbinolicum* subsp. *kysingense*). A phylogeny of the strains based on 16S rRNA gene sequence comparison indicates that they are closely related.

Despite the fact that the Kotsyurbenko and the Simankova publications characterize the isolates as psychrophilic, none of the isolates fits the definition sensu Morita (1975). The four strains, designated *Acetobacterium bakii*, *A. paludosum*, *A. fimetarium* (Kotsyurbenko et al. 1995b) and *A. tundrae* (Simankova et al. 2000), grew between 1 and 30°C with temperature optima for growth at about 20°C. On the basis of their cardinal temperatures they should thus be grouped with the psychrotolerant bacteria. The growth curves of the Kotsyurbenko isolates differed considerably. *A. fimetarium* grew fastest at 30°C ($\mu = 0.15\,h^{-1}$). The growth rate decreased rapidly down to 15°C ($\mu = 0.035\,h^{-1}$), hereafter it decreased slightly towards 1°C ($\mu = 0.02\,h^{-1}$), which was the lowest temperature at which growth was measured. The growth curve of *A. paludosum* peaked at 20°C ($\mu = 0.2\,h^{-1}$) and decreased steadily to 1°C ($\mu = 0.04\,h^{-1}$). *A. bakii* like *A. paludosum* peaked at 20°C ($\mu = 0.11\,h^{-1}$) but the growth rate was only about half the rate of *A.paludosum*. While the rate decreased very rapidly towards T_{max} at 30°C, it decreased very slowly towards T_{min} and was still about two-thirds the rate determined at T_{opt}. Thus, all three strains expressed relatively high growth rates at the lowest temperature at which the rates were measured while the lower limit for growth had consequently not yet been determined. The different growth curves reflect interesting underlying physiologies, which await further elucidation.

In a detailed study on the competition between acetogens and methanogens for hydrogen at low temperature, Kotsyurbenko et al. (2001) measured H_2 consumption

kinetics (V_{max}, K_m and the hydrogen threshold) of *Acetobacterium bakii*, *A. paludosum*, *A. fimetarium* and *A. tundrae*. The hydrogen threshold decreased with decreasing temperature in cultures of *A. bakii* and *A. tundrae*, while in *A. paludosum* and *A. fimetarium* thresholds increased again below 10–15°C. This observation indicates that *A. baki* and *A. tundrae* would out-compete *A. paludosum* and *A. fimetarium* at low temperatures and low hydrogen partial pressure.

7.2.5 Anoxygenic phototrophic bacteria

Herbert and coworkers published first results on isolated anoxygenic phototrophic bacteria from Antarctica in the mid-1970s (Herbert 1976; Herbert and Tanner 1977). Herbert (1976) reported that the isolated strains grew slowly between 0 and 5°C and expressed optimal growth at 25°C, which fits well with a psychrotolerant temperature regime. The strains survived well repeated slow freezing and thawing and survived long periods (2 years) of permanent freezing. Herbert (1976) concluded that they were able to withstand long periods of darkness and cold, as it is the case during the Antarctic winter.

The first validly described psychrotolerant anoxygenic phototroph was published by Madigan et al. (2000) and given the name *Rhodoferax antarcticus. R. antarcticus,* a member of to the purple non-sulfur bacteria, grew at all temperatures it was tested at between 0°C ($\mu = 0.003\,h^{-1}$) and 25°C ($\mu = 0.011\,h^{-1}$) and had a temperature optimum for growth between 12 and 18°C ($\mu = 0.03\,h^{-1}$). Cells survived temperatures above 25°C for at least 1 week but were not able to proliferate. Between 5 and 24°C, the growth rate varied by a factor of 2 while it decreased by a factor of 3 when the temperature dropped from 5 to 3°C. The cause or causes for this dramatic response in growth rate to a minor change in temperature was not investigated. Madigan et al. (2000) may have succeeded in the isolation of phototrophs growing at lower temperatures then the strains isolated by Herbert (1976) because they kept the inoculum and the enrichment cultures at 5°C, which may have prevented psychrotolerant microbes from out-competing psychrophilic once (Harder and Veldkamp 1971).

7.2.6 Miscellaneous

A few studies can be found in the literature which report on results obtained with unidentified (named) anaerobic heterotrophs that can be grown at low temperature. Dyrset et al. (1984) reported on an anaerobic bacterium designated strain B6 that shared phenotypic traits with *Bacteroides*. The isolate had the highest growth rate at 15°C and did survive but not grow at temperatures above 21°C. A fermenting coil-shaped psychrophilic bacterium was isolated by Franzmann and Rohde (1991) from anoxic water samples from the Antarctic meromictic Ace Lake. Prior to isolation,

the strain was part of a coculture with a trimethylamine-utilizing methanogen (*Methanococcoides burtonii*, see below). The strain grew well at the in situ temperature of Ace Lake (1.7°C; $\mu = 0.013\,h^{-1}$), had a temperature optimum between 15–16°C ($\mu = 0.12\,h^{-1}$) and did not grow at 22°C. Morphologically similar cells were observed in the anoxic hypolimnion of nearby Burton Lake at considerable abundance (10^5 cells ml^{-1}; McGuire et al. 1987). However, Franzmann and Rohde (1991) did not demonstrate that the isolate and the cells in MPN cultures, apart from sharing a common morphology, were related. Franzmann and Rohde (1991) also reported on the isolation of a cell wall-less anaerobic bacterium from the hypolimnion of Ace Lake, which affiliated with the genus *Spirochaeta* (Franzmann and Dobson 1992, 1993). The strain was psychrophilic with an optimum temperature for growth between 12 and 13°C (growth rate not reported). The growth rate at in situ temperature (1.7°C) was $0.013\,h^{-1}$. The strain grew well under proxy in situ conditions (salinity, pH, temperature) in the laboratory. Recently, the isolation of the first psychrotolerant syntrophic bacterium was reported by Kendall et al. (2006) from marine sediment in Skan Bay, Alaska. *Algorimarina butyrica* oxidized butyrate syntrophically in defined coculture with a hydrogen using methanogen. The presence of butyrate oxidizing syntrophic bacteria in marine sediment is surprising, as one would expect them to being out-competed by sulfate reducers such as the psychrophilic sulfate reducer of the genus *Desulfofrigus* or *Desulfofaba* (Knoblauch et al. 1999b). *A. butyrica* grew extremely slowly and colonies were not observed before 6–7 months at a growth temperature of 15°C. *A. butyrica* did not grow above 25°C, while the methanogenic coculture grew well above that temperature. *A. butyrica* grew at 10°C but was not tested at lower temperatures. Growth rates were not determined.

7.3 Archaea

Studies on cold-adapted obligately anerobic archaea are restricted to methanogens. Methanogens play a quantitatively very important role as terminal consumers in anoxic permanently cold environments such as lake sediments (Nozhevnikova et al. 2001), tundra soil (Kobabe et al. 2004) or permafrost (Ganzert et al. 2007). Hitherto, five psychrophilic and psychrotolerant methanogens have been isolated: *Methanococcoides burtonii* (Franzmann et al. 1992), *Methanogenium frigidum* (Franzmann et al. 1997), *Methanosarcina lacustris* (Simankova et al. 2001), *Methanosarcina baltica* (von Klein et al. 2002), and *Methanococcoides alaskense* (Singh et al. 2005) (http://www.bio.au.dk/KaiFinster/tables/). In addition, Simankova et al. (2003) have reported on the isolation of cold-adapted methanogenic strains that were closely related to validly described psychrotolerant and mesophilic species. All isolates of the Simankova study grew at 1–5°C. However, a temperature optimum of 25–35°C classifies them as psychrotolerant.

Methanococcoides burtonii, isolated from cold anoxic button waters of Ace Lake, is the best studied of all the cold-adapted methanogens and has been subjected

to detailed biochemical/proteome (Nichols and Franzmann 1992; Thomas and Cavicchioli 1998; Goodchild et al. 2004a, 2004b) and genome analysis (Saunders et al. 2003). These studies focused on cold-adaptations in that strain. The cellular adaptations of this organism to cold are nicely summarized by Cavicchioli (2006). They include cold-related modification both on the structural as well as on the process level. Nichols and Franzmann (1992) demonstrated a large fraction of unsaturated diether lipids (57%) in membrane components of *M. burtonii*, which they interpret as an adaptation to low temperature. In comprehensive studies of the proteome of *M. burtonii*, Goodchild et al. (2004a, 2004b) investigated protein patterns from cultures grown at 4 and 23°C, respectively. Apart from a significant difference in the expression of a large number of proteins as a function of temperature also on the mRNA level, the authors report the interesting observation that heat shock protein DnaK was expressed at much higher levels at $T_{opt,}$ than at 4°C. This may indicate, according to Goodchild et al. (2004a), that life at optimal temperature was stressful to the organism, a feature that was also discussed by Feller and Gerday (2003). This observation challenges the view of psychrophiles being poorly adapted to the low temperature of their habitat, because T_{opt} is usually much higher than $T_{in\ situ}$. The study on *M. burtonii* demonstrates nicely how new technologies (genomics in combination with proteonomics) can be deployed to study the biochemistry/physiology of difficult to grow microorganisms and should be extended to other psychrophiles.

Studies on the other methanogens are more on a descriptive level and have only generated information on the temperature regime of the isolates (http://www.bio.au.dk/KaiFinster/tables/). Data on yields at different temperatures, as it was the case for the sulfate reducers have not been published yet.

7.4 Conclusions

First, despite the fact that permanently cold anoxic environments are widely distributed around the globe, our knowledge about obligately anaerobic psychrophilic bacteria and archaea is very sparse. This is very likely a consequence of the intrinsic difficulties in working with these kinds of microbes. They are both difficult to isolate and grow, and enzymatic studies of their metabolic pathways are notoriously tedious and technically demanding. A combination of different "omics" is very promising and could help overcome the methodological problems with culturing the organisms. Nevertheless, there is also an urgent need for the isolation of more obligately anaerobic psychrophilies to understand the mechanisms by which they have adapted to the cold. Results obtained from the studies may also contribute to "Search for Life missions" to Mars or Europa, both cryo-environments with a potential for active life forms.

Second, and interestingly, the world of psychrophilic obligately anaerobic archaea is still very little explored. Considering the large reservoirs of methane in the sea floor and the large potential for methane productions in permafrost soil,

there is an urgent need to gain more insight into the physiology of these organisms. As producers of a very strong green house gas these organisms may have a very important impact on the prevalence and geographic distribution of permanently cold environments in the future.

Third, concerning the publications on cold-adapted microbes, more comprehensive efforts are needed that elucidate the strategies of the different microbes to cope with the cold. Often, little information on cold adaptation of the specific isolates is provided including activation energies and Q_{10}, and growth yield data. It would also be helpful to include square root transformed growth rate data to obtain comparable theoretical T_{min} values. Also, the often-observed large discrepancy between T_{opt} and $T_{in\ situ}$ should be addressed in more detail and studies should include investigations at $T_{in\ situ}$. Quoting from Cavicchioli (2006): "In the view of the ecological data and a range of physiological indicators (for example enzyme secretion, macromolecular synthesis, membrane permeability, viability and growth yield), the molecular indicators clearly show that T_{opt} is a poor measure of cold adaptation". T_{opt} may not only be a poor but even a misleading indicator.

Last but not least, there is a strong need for a clear unifying definition of the terms "psychrophilic", "psychroactive", "psychrotrophic" and "psychrotolerant" and an agreement on which terms should be used. Currently it seems to be up to the individual scientist to decide whether a specific isolate fits into one regime or the other. This creates a lot of confusion and makes it difficult to compare different organisms.

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