MICROBIAL ECOLOGY

Microb Ecol (2001) 41:1–11
DOI: 10.1007/s002480000059

Sulfate Reduction Dynamics and Enumeration of Sulfate-Reducing Bacteria in Hypersaline Sediments of the Great Salt Lake (Utah, USA)

K.K. Brandt,* F. Vester,** A.N. Jensen,† K. Ingvorsen

Department of Microbial Ecology, Institute of Biological Sciences, University of Aarhus, DK-8000 Aarhus C, Denmark

Received: 1 December 1999; Accepted: 22 June 2000; Online Publication: 16 November 2000

ABSTRACT

Bacterial sulfate reduction activity (SRA) was measured in surface sediments and slurries from three sites in the Great Salt Lake (Utah, USA) using radiolabeled ^{35}S-sulfate. High rates of sulfate reduction (363 ± 103 and 6,131 ± 835 nmol cm^{-3} d^{-1}) were measured at two sites in the moderately hypersaline southern arm of the lake, whereas significantly lower rates (32 ± 9 nmol cm^{-3} d^{-1}) were measured in the extremely hypersaline northern arm. Bacterial sulfate reduction was strongly affected by salinity and showed an optimum around 5–6% NaCl in the southern arm and an optimum of around 12% NaCl in the more hypersaline northern arm of the lake. High densities of sulfate-reducing bacteria (SRB) ranging from 2.2×10^7 to 6.7×10^8 cells cm^{-3} were determined by a newly developed tracer MPN-technique (T-MPN) employing sediment media and ^{35}S-sulfate. Calculation of specific sulfate reduction rates yielded values comparable to those obtained in pure cultures of SRB. However, when using a conventional MPN technique with synthetic media containing high amounts of Fe(II), the numbers of SRB were underestimated by 1–4 orders of magnitude as compared to the T-MPN method. Our results suggest that high densities of slightly to moderately halophilic and extremely halotolerant SRB are responsible for the high rates of sulfate reduction measured in Great Salt Lake sediments.

* *Present address:* Royal Veterinary and Agricultural University, Department of Ecology, Section of Genetics and Microbiology, Thorvaldsensvej 40, DK-1871 Frederiksberg (Copenhagen), Denmark.
** *Present address:* Plougmann, Vingtoft & Partners, Sankt Annæ Plads 11, DK-1021 Copenhagen K, Denmark.
† *Present address:* Danish Veterinary Laboratory, Department of Microbiology, Bülowsvej 27, DK-1790 Copenhagen V, Denmark
Correspondence to: K. Ingvorsen; Fax: ++ 45 86 12 71 91; E-mail: Kjeld.Ingvorsen@biology.au.dk

Introduction

Bacterial sulfate reduction is of great ecological and biogeochemical importance in anoxic hypersaline sediments [25, 30] and in hypersaline photosynthetic microbial mats [4, 6, 18]. Indeed, some of the highest sulfate reduction rates (SRR) reported in the literature have been measured in hy-

persaline microbial mats with salinities up to 15–21% w/v NaCl [6, 18, 35]. Whereas many studies have dealt with ecological and ecophysiological aspects of sulfate reduction in hypersaline, photosynthetic microbial mats, relatively few studies have been concerned with sulfate reduction activity (SRA) in aphotic hypersaline sediments, where active sulfate reduction has been demonstrated at salinities up to 24–30% w/v NaCl [19, 39].

Although quite high viable counts of sulfate-reducing bacteria (SRB) have been reported from hypersaline microbial mats, the numbers of SRB were far too low to account for the extremely high SRA measured [6, 18]. This suggests that the use of culture-dependent enumeration techniques seriously underestimates the population size of SRB in hypersaline environments, as previously demonstrated in marine sediments [11, 17]. As judged from *in vitro* experiments, strains of SRB isolated so far from hypersaline sediments and microbial mats are relative poorly adapted to the high *in situ* salinities of their respective habitats [2, 3, 5, 20, 24, 32]. Whether these isolates or other SRB with similar or higher degrees of halophily and halotolerance are responsible for the high sulfate reduction rates often measured in hypersaline habitats is presently unknown. However, it has proved difficult to enrich and isolate SRB at extremely high salinities, and this is likely to be reflected in low viable counts, when SRB from hypersaline environments are enumerated in synthetic media with conventional MPN methods. Recently, an improved tracer-MPN technique employing sediment media has been developed in our laboratory [36].

One objective of the present investigation was to apply this newly developed MPN method for the first time to hypersaline environments of various salinities. A second objective was to quantify SRA in these environments and to investigate the effects of artificially induced changes of salinity and electron donor concentration on SRA. Sediments from different parts of the Great Salt Lake (Utah, USA) were chosen for this purpose.

Methods

Samples

Samples of surface sediment and bottom water from the Great Salt Lake were collected at two sites in the moderately hypersaline southern arm (Station AS2, Utah Geological Survey code, 40°49′51″N, 112° 15′26″W, and Station 20a on the northern shore of the Antelope Island causeway, 41°5′25″N, 112°10′36″W) and at one site in the extremely hypersaline northern arm (Station 27, right outside Little Valley Harbor, 41°15′6″N, 112°10′36″W). Sediment was collected by coring (Site 20a, upper 5 cm) or by Eckmann dredge (Site AS2 and 27) and stored at 0–5°C in completely filled glass bottles during transport. Samples of water overlaying the sediment were collected from all stations.

Sediment Handling

Approximately 500 ml of sediment from each station was homogenized anaerobically under a N_2 atmosphere by combined stirring and kneading in gas tight plastic bags (Ril-O-Ten) [21] and immediately used for experiments. Subsamples of homogenized sediment were taken for determination of sediment porosity and for analysis of porewater concentrations of sulfate and chloride.

Media for Most Probable Number (MPN) Counts of SRB

Enumerations of SRB were performed by two different MPN techniques at two different medium salinities for each station. The MPN techniques used were a conventional MPN count (normal MPN or N-MPN), in which the presence of SRB in MPN tubes was evaluated by the formation of black precipitates of ferrous sulfide, and a new radiotracer-based MPN technique (tracer MPN or T-MPN) employing media containing *in situ* sediment [36].

N-MPN enumerations were performed in iron-rich synthetic minimal media (Medium N6, N12 and N26, respectively) supplemented with a mix of organic electron donors known to be important substrates for SRB in saline sediments. The media were prepared essentially as described by Widdel and Bak [38] and consisted of (per liter): NaCl, 60, 120, or 260 g (N6, N12, and N26, respectively); $MgSO_4 \cdot 7\ H_2O$, 13, 52, or 90 g (N6, N12, and N26, respectively); KCl, 2.5, 6, or 10 g (N6, N12, and N26, respectively); NH_4Cl, 2 g; KH_2PO_4, 0.5 g; $CaCl_2 \cdot 2\ H_2O$, 0.2 g; $MnCl_2 \cdot 2\ H_2O$, 0.04 g; trace element solution (TES3) [14], 2 ml; resazurin, 0.5 mg. The media were made anoxic, autoclaved, and dispensed into anoxic Hungate tubes as described previously [36]. The following medium constituents were subsequently added from anoxic sterile stock solutions to obtain the final enumeration media (final concentrations per liter): $FeCl_2 \cdot 4\ H_2O$, 0.2 g; $NaHCO_3$, 2.5 g; $Na_2S_2O_4$, 35 mg, and vitamins (solution 6, 7, and 8 of Widdel and Bak [38]), 1 ml of each. A mixture of sodium salts of acetate, propionate, butyrate, and lactate were added as electron donors (4 mM each). The pH of the media was finally adjusted to the *in situ* pH (see Table 1) by addition of NaOH.

The sediment media for tracer MPN enumerations were prepared according to Vester and Ingvorsen [36] by mixing one volume of sterile homogenized *in situ* sediment (autoclaved twice on two successive days) with two volumes of sulfate-free synthetic medium. Sulfate was omitted in order to increase the sensitivity of the tracer MPN-technique. The synthetic sulfate-free media were prepared as media N6, N12, and N26, respectively, with the modification that $MgSO_4 \cdot 7H_2O$ was replaced by $MgCl_2 \cdot 6H_2O$ (10, 40, or 70 g L^{-1}; Medium T6, T12, and T26, respectively). The sediment media were not amended with any synthetic energy sources.

For enumeration of SRB at Station 20a and AS2 (low salinity), media N6, N12, T6, and T12 were used, whereas media N6, N26,

Table 1. Characteristics of the three study sites from the Great Salt Lake

Study site	Salinity[b] (% NaCl w/v)		Sulfate (mM)		pH, water[c]	Porosity (ml cm^{-3}), sediment	Water depth (m)
	Water[c]	Sediment[d]	Water[c]	Sediment[d]			
Station 20a SA[a]	11.5	11.6	75.4	14.4	8.0	0.83	0.2
Station AS2 SA[a]	12.5	12.3	75.7	93.5	8.0	0.73	8–10
Station 27 NA[a]	27.0	27.4	202.1	208.7	7.5	0.64	3–5

[a] SA, southern arm of the Great Salt Lake; NA, northern arm
[b] Calculated from concentrations of chloride
[c] Water overlaying the sediment
[d] Sediment porewater extracted by centrifugation

T6, and T26 were employed for Station 27 (high salinity). When using medium T6, the final salinity of the MPN medium increased depending on the salinity of the sterile *in situ* sediment used for preparing the medium. The final salinities of medium T6 were thus around 8% w/v NaCl (Station 20a and AS2) and 10% NaCl (Station 27).

Enumeration of SRB by MPN Counts

Normal MPN (N-MPN) and tracer MPN enumerations (T-MPN) were carried out essentially as described by Vester and Ingvorsen [36]. A small fraction of the homogenized sediment from each station was diluted 1:1 with anoxic *in situ* bottom water to create a slurry, which subsequently served as inoculum for triplicate 10-fold MPN dilutions. After inoculation, carrier free $^{35}SO_4^{2-}$ (Isotope Laboratory, Risø, Denmark) was injected into each T-MPN tube to a final radioactivity of 20 kBq ml^{-1} sediment medium. All tubes including sterile blanks (one for each dilution series) were incubated in the dark at 30°C. During incubation, N-MPN tubes were monitored for formation of black FeS precipitates. At the end of the incubation period, 4 ml of sediment medium was withdrawn from T-MPN tubes and injected into 4 ml 20% (w/v) zinc acetate and frozen. The frozen samples were subsequently distilled using the single-step chromium reduction method to separate total reduced inorganic sulfur (TRIS) from sulfate [10]. Finally, the radioactivity of the TRIS fraction and of the sulfate fraction was measured by liquid scintillation counting (Packard, Tri-Carb, CA 2200). Tubes were considered to be positive for the presence of SRB if more than 0.1% of the added radiolabeled sulfur was recovered from the TRIS fraction. Calculation of MPN values and standard errors were performed using a computer program [13]. A Student *t*-test was used to determine whether the MPN values obtained differed significantly [8].

Measurement of Sulfate Reduction Rates in Sediments and Slurries

Sulfate reduction in raw and substrate amended sediments was measured as follows. Sediment (4 ml) was anaerobically transferred to 20 ml sterile serum vials containing 2 glass beads (diameter, 3–4 mm) and sealed under a nitrogen atmosphere. After 2–3 h of preincubation at 30°C, carrier-free ^{35}S sulfate was injected to obtain a radioactivity of approximately 50 kBq cm^{-3} sediment in each vial. The effects of adding common growth substrates of SRB on sulfate reduction rate were tested at all three stations. Acetate, propionate, and lactate were each added at a final concentration of 8 mM, butyrate at 4 mM, and molecular hydrogen (purity of 99.999%) as 50% v/v of the headspace. All incubations were carried out in the dark at 30°C, using incubation times ranging from a few hours to 5 weeks. Incubations were stopped by adding 4 ml of an anoxic 20% zinc acetate solution to each experimental vial. The vials were subsequently stored at −20°C for 1–2 weeks, until distillations were performed by the single-step procedure described in the preceding section. Sediment from Station AS2 was also distilled by the two step procedure of Fossing and Jørgensen [10] in order to separate the acid volatile sulfur (AVS; amorphous iron sulfides plus dissolved sulfide) and chromium reducible sulfur (CRS; elemental sulfur and pyrite) fractions. Sulfate reduction rates were calculated according to Jørgensen [16]: $SRR = a \cdot (A + a)^{-1} \cdot [SO_4^{2-}] \cdot t^{-1} \cdot 1.06$, where a is the radioactivity in the TRIS fraction, A is the radioactivity of the sulfate fraction, $[SO_4^{2-}]$ is the concentration of sulfate (nmol cm^{-3}), t is the incubation time (days), and 1.06 is a correction factor for the expected isotope fractionation. All sulfate reduction rates were calculated as the mean of at least four replicates.

The effect of major salts on sulfate reduction activity was tested in dilute slurries, which were prepared by mixing 1 volume of sediment slurry (made as 1:1 dilution of sediment with sterilized anoxic *in situ* water) with 4 volumes of anoxic synthetic minimal medium containing different amounts of NaCl, $MgCl_2$, and KCl. The synthetic media were prepared by mixing a sodium chloride–free medium (medium N0) with an extremely hypersaline medium (N30) in different proportions in an anaerobic glove chamber. Medium N30 contained (per liter) NaCl (300 g), $MgCl_2 \cdot 6H_2O$ (100 g), and KCl (11.5 g), whereas medium N0 did not contain any of these salts. Except for these major salts, media N0 and N30 were identical to media N6, N12 and N26. Sediment slurries were preincubated at 30°C in dinitrogen outgassed anaerobic culture tubes

Table 2. MPN-counts (SRB cm^{-3}) obtained from Great Salt Lake sediments after 4 months of incubation at 30°C

	T-MPN[b,c]			N-MPN[c]		
Locality	Medium T6	Medium T12	Medium T26	Medium N6	Medium N12	Medium N26
Station 20a (SA)[a]	4.8×10^8	4.8×10^{8}*		4.6×10^7#	5.5×10^{5}*,#	
Station AS2 (SA)[a]	4.8×10^7*	2.2×10^{8}*		4.6×10^{5}*	4.6×10^{5}*	
Station 27 (NA)[a]	2.2×10^{7}*,#		4.6×10^{4}*,#	2.9×10^{3}*,#		5*,#

[a] SA, southern arm; NA, northern arm

[b] The final salinities of the T-MPN media after mixing with sterile *in situ* sediment were approximately 8 and 12% (Station 20a and AS2) and approximately 10% and 26% (Station 27).

[c] T-MPN = tracer MPN using sediment media, N-MPN = normal MPN using synthetic media

* Significant differences within stations (*t*-test $P > 0.05$) between T-MPN and N-MPN

\# Significant differences within stations (*t*-test $P > 0.05$) between MPN counts obtained at different salinities using the same type of medium

(Bellco, 18 × 142 mm) for 2–3 h. Incubations were started by injection of radiolabeled ^{35}S-sulfate to a final radioactivity of 20 kBq ml^{-1} slurry and stopped after 48 h by injection of 5 ml 20% (w/v) zinc acetate followed by freezing at −20°C.

Analytical techniques

The concentrations of chloride and sulfate in sediment porewater, obtained by centrifugation, were determined by suppressed anion chromatography after appropriate dilution as described previously [2].

Statistics

The effects of substrate amendments on SRA were tested using a two-tailed Students *t*-test on the means of the measured sulfate reduction rates. The terms "significant" ($P < 0.05$), "very significant" ($P < 0.01$), and "highly significant" ($P < 0.001$) were used.

Results

Characteristics of the Study Sites

Some characteristics of the three sampling sites are listed in Table 1. The two stations in the southern arm (20a and AS2) both had salinities of around 12% w/v NaCl, whereas Station 27 in the northern arm had a salinity of 27% NaCl. Sulfate concentrations in the sediment porewater differed between the three study sites, and in one case (Station 20a) a high SRA in the uppermost sediment was indicated by a marked depletion of porewater sulfate (Table 1) and a strong smell of H_2S (the high SRA was confirmed by direct measurements; see below). The sediment at this station was very soft and contained high amounts of visible organic debris, further indicating a high organic content at this site.

Enumeration of Sulfate-Reducing Bacteria

Table 2 summarizes the results from the MPN-enumerations carried out with sediment from the three sampling sites. All incubations were carried out for at least 4 months. This was necessary in order to avoid serious underestimation of SRB numbers by the conventional MPN-technique (N-MPN), especially at the highest salinities investigated (data not shown). Longer incubation periods (up to 12 months) did not result in higher counts. When using the T-MPN technique longer incubation periods (up to 10 months) did not result in higher counts for Station AS2, but the T-MPN counts for Station 27 increased by one order of magnitude at the *in situ* salinity incubation (data not shown). When a T-MPN experiment was repeated a year later for Station 20a, using the original sediment sample which had been stored in the dark at 5°C, an almost identical MPN estimate of 6.7×10^8 SRB cm^{-3} sediment was obtained after 4 months of incubation (the corresponding value in Table 2 is 4.8×10^8 SRB cm^{-3}).

Densities of SRB obtained depended strongly on the chosen MPN-method (N-MPN versus T-MPN) and on the salinity of the enumeration medium (Table 2). The T-MPN technique thus yielded significantly higher counts (1 to 4 orders of magnitude) as compared to the N-MPN technique, the largest difference being observed at the site with the highest salinity (Station 27). At Station 27, the N-MPN and T-MPN counts both increased by 3 orders of magnitude

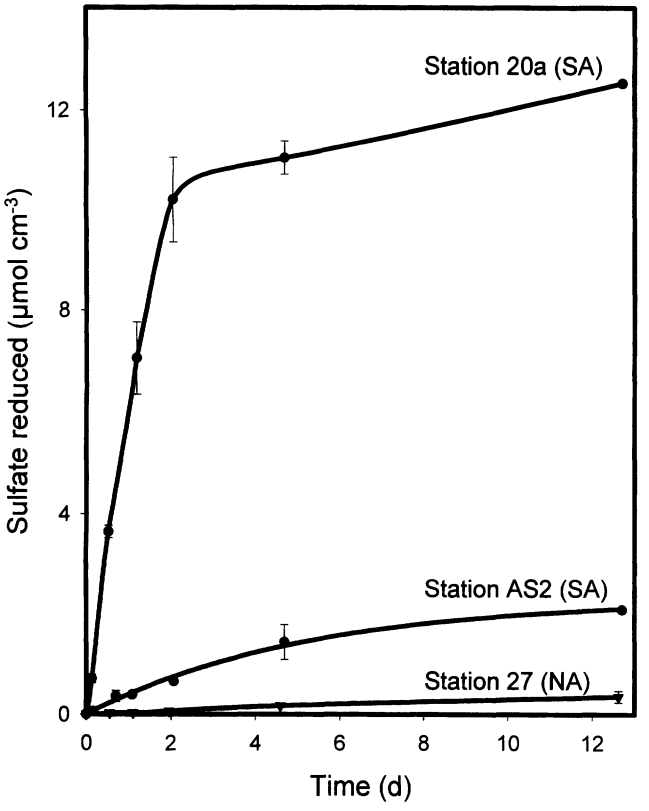

Fig. 1. Time-course experiments of sulfate reduction in homogenized sediment samples from the three study sites in the Great Salt Lake. SA, southern arm; NA, northern arm.

when carried out at a lower salinity (6 and 10% final salinity, respectively) as compared to counts performed at the *in situ* salinity of 27%. Likewise, the N-MPN count at Station 20a increased by 2 orders of magnitude, if the salinity of the enumeration medium was decreased from 12% to 6% (Table 2). It is noteworthy that T-MPN counts for Station 20a were not affected by reducing the salinity of the T-MPN medium. At Station AS2 no significant differences were observed between MPN counts obtained at the different salinities for either of the MPN methods (Table 2).

Sulfate Reduction in Sediments and Slurries

SRA in sediment samples was determined in time course experiments for each station (Fig. 1). Sulfate reduction at Station 20a was very high and proceeded linearly (R^2 = 0.982) during the first 2 days of incubation, after which it continued at a significantly lower rate probably due to sulfate and/or electron donor limitation. After 13 days of incubation more than 99% of the radioactivity added was present in the TRIS fraction. The sulfate reduction curve for Station AS2 was linear (R^2 = 0.965) up to at least 5 days of incubation before leveling off, whereas at Station 27 the progress curve of sulfate reduction was linear (R^2 = 0.989) over the entire 34 days of incubation. Bacterial sulfate reduction at Station AS2 and 27 was not limited by sulfate, since more than 97% of the radioactivity remained in the sulfate fraction at the end of the experiments (data not shown). Sulfate reduction rates, as calculated from the initial slope of the time courses shown in Fig. 1, differed drastically between the three stations amounting to (mean value ± standard deviation) 32 ± 9, 363 ± 103, and 6131 ± 835 nmol cm^{-3} d^{-1} for Stations 27, AS2 and 20a, respectively.

Stimulation of sulfate reduction was investigated by adding different electron donors known to be common substrates for SRB (Fig. 2). The results revealed a considerable difference in the metabolic responses of the SRB populations at the three study sites. In the organic-rich sediment from Station 20a, butyrate was the only electron donor tested that caused a significant ($P < 0.05$) albeit small stimulation of sulfate reduction. At Station AS2, the addition of acetate, propionate, and butyrate caused a significant stimulation, whereas lactate and molecular hydrogen caused a highly significant ($P < 0.001$) stimulation of SRA. In the sediment from Station 27, SRA was significantly enhanced only by the addition of hydrogen and lactate, and interestingly, SRA was reduced slightly (although not significantly; $P = 0.13$), when acetate was added.

The effect of salinity on sediment SRA is shown in Fig. 3. The populations of SRB at the two southern arm stations (20a and AS2) responded very similarly to the imposed changes of salinity. Thus for both stations, SRA was maximal at a salinity of 5–6%, corresponding to about half the *in situ* salinity (Table 1). Also sulfate reduction rates at Station 20a and AS2 were progressively inhibited by increasing salinities (Fig. 3). At the highest salinity tested (25% w/v), a SRA of only 3% of the maximum SRA was measured at Station 20a as compared to 13% at Station AS2, indicating a slightly more halotolerant population of SRB at Station AS2. Likewise, the results obtained with sediment from the sampling site in the northern arm (Station 27) demonstrated the presence of a sulfate-reducing population, which was metabolically stimulated, when the salinity was decreased. At this station the SRA determined at the *in situ* salinity amounted to only 20% of the maximal activity at 10–15% NaCl. It is noteworthy that all salinity-response curves depicted in Fig. 3 showed a weak biphasic tendency, indicating the possible existence of distinct halophilic subpopulations of SRB at the three sites.

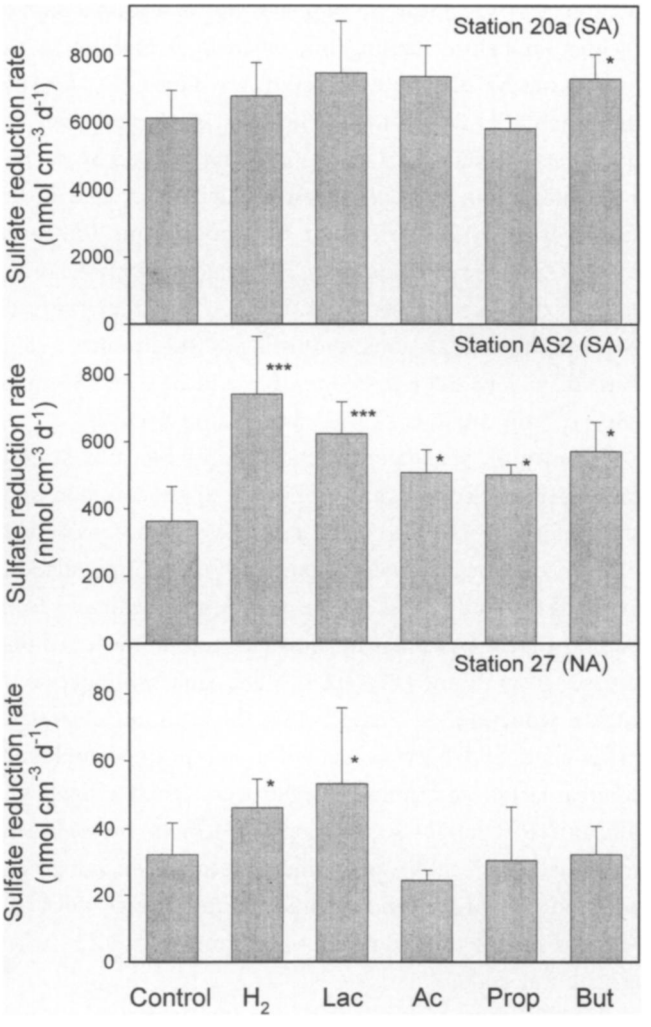

Fig. 2. Effect of substrate additions on sulfate reduction activity (SRA) in homogenized sediment samples from different parts of the Great Salt Lake. The added substrates are indicated as follows: Control, no substrates added; H_2, molecular hydrogen; Lac, lactate; Ac, acetate; Prop, propionate; But, butyrate. Standard deviations are depicted as error bars. Significant stimulation of SRA by addition of subtrates is indicated on top of the bars; *, significant ($P < 0.05$); **, very significant ($P < 0.01$); ***, highly significant ($P < 0.01$). SA, southern arm; NA, northern arm.

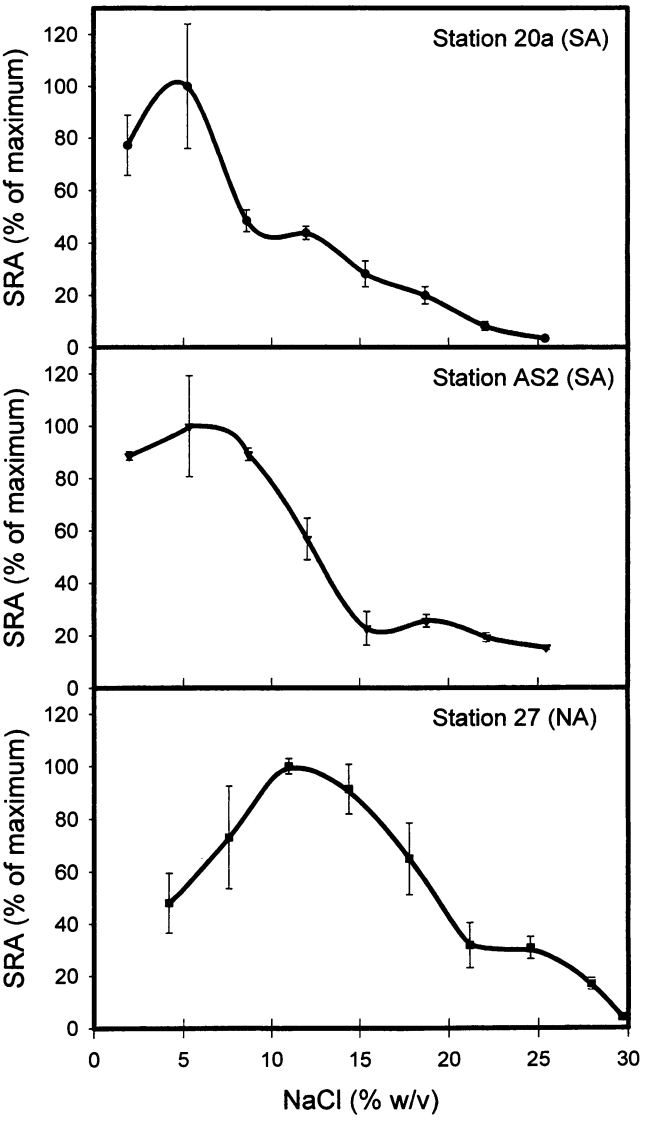

Fig. 3. The effect of salinity on sulfate reduction activity (relative values) in dilute slurries made from homogenized sediment samples from different parts of the Great Salt Lake. The results are showed as means of duplicate measurements with standard deviations depicted as error bars. SA, southern arm; NA, northern arm.

The relative distribution of radioactivity in the acid volatile sulfur (AVS) and in the chromium reducible sulfur (CRS) fraction was investigated with sediment from Station AS2. This was done in order to allow for a comparison between sediment sulfate reduction rates obtained in this study and those measured previously at the same location [39]. It was found that the radioactivity in the AVS fraction constituted (81 ± 11)% of the radioactivity present as total reduced inorganic sulfur (TRIS), where TRIS = AVS + CRS.

Discussion

Sulfate Reduction Rates

Sulfate reduction rates in the surface sediments at the three study sites in the Great Salt Lake differed greatly. By far, sulfate reduction was most active in the organic rich sediment at Station 20a, yielding an extremely high SRA of 6,131 ± 835 nmol SO_4^{2-} cm^{-3} d^{-1} (average value for the uppermost 5 cm of the sediment). Tentatively assuming that all sulfate reduction at Station 20a occurs at this rate only in the

sampled uppermost 5 cm of the sediment, this corresponds to a SRA of 307 mmol SO_4^{2-} m^{-2} d^{-1}. Although this value probably seriously underestimates the true area-based sulfate reduction rate at this site, it is still among the highest ever reported for natural environments [6, 30]. It must be stressed, however, that our ^{35}S-radiotracer incubations were carried out with homogenized surface sediment (top 5 cm), and that our results therefore do not represent *in situ* rates as precisely as those obtainable by the sediment core injection method [16], although the two measurements have been shown to give comparable values for SRA in nonlaminated sediments [7].

Significantly lower sulfate reduction rates of 363 ± 103 nmol SO_4^{2-} cm^{-3} d^{-1} were measured at Station AS2. These rates are approximately twofold higher than those of 175 ± 22 nmol SO_4^{2-} cm^{-3} d^{-1} previously reported from a nearby site at the time when the sediment was covered by a sulfide-containing fetid brine of about 24% salinity [39]. The different sulfate reduction rates measured is probably best explained by this change in salinity, although sediment heterogeinity or other factors cannot be ruled out either. Sediment sulfate reduction rates determined in 1980 by Ingvorsen and by Zeikus [39] were not corrected for the formation of ^{35}S-CRS, which was negligible, since approximately 95% of the added tracer was recovered as ^{35}S-sulfate plus ^{35}S-AVS (Ingvorsen, unpublished data). However, in the present study significant quantities of the ^{35}S-TRIS (about 19%) were recovered as ^{35}S-CRS at Station AS2 after a 24-h incubation period. This difference most likely can be ascribed to changes in the physicochemical characteristics of the sediment that have occurred after the disappearance of the dense brine layer in 1991.

At Station 27 in the northern arm, sediment sulfate reduction rates of 32 ± 9 nmol SO_4^{2-} cm^{-3} d^{-1} were measured. It is noteworthy that sulfate reduction in unamended sediment from this site was linear for more than a month (Fig. 1, data not shown). To our knowledge, these data represent the first direct measurements of sulfate reduction in this the most extreme part of the Great Salt Lake showing that some of the sulfide present in this sediment [28] derives from dissimilatory reduction of sulfate.

Sediment sulfate reduction at the three sampling sites responded very differently to the addition of electron donors known to be important substrates for SRB in saline sediments [7, 27, 31, 33]. All electron donors tested significantly stimulated SRA in sediment from Station AS2 (Fig. 2), indicating that the natural population of SRB was carbon limited. Results obtained in 1984 from a nearby site during stratification of the water column (24% NaCl in the bottom water) showed stimulation by lactate and hydrogen, but not by acetate [22]. The different responses to acetate addition observed in the two studies could be due to an extremely low rate of acetate oxidation by SRB and/or a low number of acetate-oxidizing SRB in the sediment during the stratification period when the bottom water salinity was around 24% NaCl. There are several reports, that acetate metabolism by SRB and other anaerobes is severely inhibited at extreme salinities (above 15-20%), causing acetate to accumulate to high levels [2, 19, 23, 25, 40].

In the extremely hypersaline sediment from Station 27, only addition of lactate and hydrogen was found to stimulate sulfate reduction, whereas acetate, propionate, and butyrate had no stimulatory effect on the SRA. Indeed, acetate amendment caused a slight (albeit not significant; $P = 0.13$) decrease in SRA (Fig. 2), which may be explained by product inhibition. It is hence believed that most SRB active in extremely hypersaline environments are incomplete oxidizers producing acetate as a major end product [23, 26], and the huge energetic cost of coping with salt stress probably renders SRB very susceptible to energetic constraints [26].

In contrast to what was observed at Stations AS2 and 27, the SRA in the organic-rich sediment at Station 20a was only slightly affected by the addition of substrates (potential electron donors for SRB). Despite the extremely high SRA at this site, the indigenous populations of hydrolytic, fermentative bacteria were evidently able of supplying these electron donors to the SRB at a non-limiting rate. The time-course experiment shown in Fig. 1 supports this notion, since virtually all (>99%) radiolabeled sulfate added was reduced after 13 days of incubation, clearly indicating a surplus of available electron donor compared to sulfate (electron acceptor) in this very dynamic anaerobic community. Indeed, the SRA already started to decrease after 2 days, when 85% of the labeled sulfate had been transformed into ^{35}S-TRIS, leaving about 2 mM sulfate in the sediment porewater (data not shown). Sulfate limitation could be the reason for this decrease in SRA, since half-saturation constants for sulfate uptake have been reported to be as high as 1–4 mM in some marine sediments [1, 37]. On the other hand, other investigators have reported much lower half-saturation constants (in the micromolar range) for SRA of pure cultures of marine SRB [14, 15], and at present this discrepancy has not been adequately solved. However, it should be noted that because of energetical and physiological constraints, SRB in

半饱和常数：反应速率降至最大速率一半时的介质浓度

Table 3. Cell-specific sulfate reduction rates obtained from this study

	qSO_4^{2-} (mol d^{-1} $cell^{-1}$)					
	T-MPN			N-MPN		
Locality	Medium T6	Medium T12	Medium T26	Medium N6	Medium N12	Medium N26
Station 20a	1.3×10^{-14}	1.3×10^{-14}		1.3×10^{-13}	1.1×10^{-11}	
Station AS2	7.6×10^{-15}	1.7×10^{-15}		7.9×10^{-13}	7.9×10^{-13}	
Station 27	1.5×10^{-15}		7.0×10^{-14}[a]	1.1×10^{-11}		6.4×10^{-9}

[a] Based on T-MPN value after 10 months of incubation. If based on the T-MPN value after 4 months of incubation, a cell-specific SRA of 7.0×10^{-13} mol d^{-1} $cell^{-1}$ was obtained.

hypersaline habitats may have lower affinity for sulfate uptake than their counterparts in less extreme and relatively sulfate-poor environments. At Station AS2 sediment sulfate reduction was limited by the amount of available electron donor and not by sulfate (Figs. 1 and 2). At Station 27, the sulfate reduction rate was constant over the entire incubation period (34 days), indicating the presence of a functionally very stable anaerobic degrader community.

MPN Enumerations of SRB

Our results clearly demonstrate that the newly developed MPN technique employing sediment media and radiolabeled sulfate is much superior to the conventional MPN-technique employing synthetic media for enumeration of SRB in Great Salt Lake sediments (Table 2). It is thus evident that the most abundant SRB were unable to grow or grew poorly in the synthetic medium used for N-MPN. Another explanation could be that a larger inoculum size is needed for initiation of growth in the synthetic medium as compared to the sediment medium used for T-MPN. The sediment medium used for T-MPN much more closely mimics the natural sediment environment, providing surfaces for attachment and a broad range of natural growth substrates at low noninhibiting concentrations, this probably being the reasons for the increased MPN counts obtained by this method [36]. The difference between counts obtained by N-MPN and T-MPN was most pronounced (4 orders of magnitude) at the station with the highest *in situ* salinity (Station 27), suggesting that the T-MPN technique is especially favorable when salt-stressed SRB are to be enumerated. The inadequacy of N-MPN techniques to estimate population sizes of SRB seems to be a general phenomenon, and especially so when the method is used on SRB from extreme environments [17, 34]. The T-MPN approach might thus be the method of choice for enumeration of SRB in extreme environments such as thermal and/or hypersaline oil wells. In less extreme environments N-MPN estimates may occasionally approach realistic values [12], although this appears to be the exception rather than the rule [11, 36].

To validate the efficiency of our MPN-estimations, cell specific sulfate reduction rates (qSO_4^{2-}) were calculated for each sediment and MPN-technique employed (Table 3). The qSO_4^{2-} values, calculated from the sulfate reduction rates and the T-MPN estimates obtained in this study, were generally in the same range (10^{-15} to 10^{-14} mol SO_4^{2-} $cell^{-1}$ d^{-1}) as reported for pure cultures of SRB [14, 15, 17, 36] (Brandt, unpublished results). However, the T-MPN counts determined at Station 27, employing sediment medium of *in situ* salinity, still yielded counts somewhat too low as evidenced by an unrealistically high qSO_4^{2-} value of 7.0×10^{-13} mol $cell^{-1}$ d^{-1}. By reducing the salinity of the T-MPN counting media to 10% NaCl (final salinity), we obtained higher and more realistic MPN-estimates and consequently lower qSO_4^{2-} values of around 1.5×10^{-15} mol $cell^{-1}$ d^{-1} (Table 3). The observed inhibitory effect of high salinity on the recovery of SRB by T-MPN at Station 27 could be overcome, although only to some extent, by increasing the incubation time (Table 3). Thus, the T-MPN value obtained using sediment medium containing 26% w/v NaCl increased by one order of magnitude, when the incubation period was extended from 4 to 10 months.

Although the T-MPN technique yielded much higher and more realistic MPN estimates as compared to the N-MPN technique, it is important to note that all MPN methods inherently tend to underestimate the true number of target cells due to factors such as clumping and attachment of cells to particles. The cell-specific sulfate reduction rates calculated from our T-MPN and SRA data are thus most likely still too high (probably at least by one order of magnitude), since it cannot be expected that sulfate reduction rates of individual cells in many natural environments will generally

be as high as for pure cultures grown in laboratory media [12, 29]. This probably holds true especially for the extremely saline sediments at Station 27 studied in our work. On the other hand, it could be argued that in some high-nutrient environments *in situ* cell specific sulfate reducing activity may approach values obtained in pure cultures because of the absence of substrate and product inhibition and beneficial microbial interactions. Enumeration of SRB by cultivation-independent methods based on the quantification of SRB rRNA or rDNA may provide an attractive alternative to MPN techniques. These methods, however, may also suffer from inherent shortcomings related to reproducibility, oligonucleotide probe specificity, and uncertainties concerning genome size, genome copy number, or rRNA-content per sulfate-reducing bacterium [9, 29].

Salinity and SRB

The populations of SRB in the sediments of the northern and southern arm of GSL showed the same general response with respect to SRA, when the salinity was changed artificially in slurries incubated with ^{35}S-sulfate (Fig. 3). Thus at all three stations, sulfate reduction rates increased when the salinity was reduced relative to the respective *in situ* salinities. However, the optimum NaCl concentrations for sediment sulfate reduction were higher in sediment from the northern arm (10–15% NaCl) as compared to sediments from the southern arm (3–9% NaCl). This strongly suggests that as a whole the population of SRB from the northern arm was better adapted to high salt concentrations. This raises the intriguing question of why the more halophilic types of SRB present in the northern arm (salinity optimum between 10 and 15% NaCl; Fig. 3) apparently are missing or at least not dominantly present in the sediments of the southern arm, where the *in situ* salinity is presently around 11–14%. So far enrichments and isolations of SRB from the southern arm of the Great Salt Lake, using media containing $\geq$10% NaCl (w/v), have resulted only in strains exhibiting optimal growth at 2–5% NaCl. These strains thus display a salinity–growth response very similar to that observed for the SRA of the natural populations of SRB at Stations 20a and AS2 in the southern arm (Fig. 3). The only described sulfate-reducing bacterium (*Desulfohalobium retbaense*) isolated so far from an extremely hypersaline environment (Lake Retba in Senegal containing 34% w/v of total salts) grows optimally *in vitro* with 10% NaCl in the medium [24]. *D. retbaense* hence resembles the natural populations of SRB from the northern arm of the Great Salt Lake regarding its growth response to salinity. Interestingly, *D. retbaense* was not able to grow in media of the same high salinity as found in the habitat from which it was isolated. Our MPN data (Table 2) suggest the presence of a severely salt stressed population of SRB in the northern arm of the Great Salt Lake. Thus significantly higher MPN-counts (3 orders of magnitude) were obtained at Station 27 when the salinity of the counting media for both T-MPN and N-MPN was reduced from 26% to 10% final salinity (Table 2). However, the biphasic nature of the salinity–response curves found for dissimilatory sulfate reduction at Station AS2 and 27 might indicate the presence of subpopulations of SRB having salinity optima around or even above 20% NaCl (Fig. 3).

In conclusion, our data suggest that the high sulfate reduction rates measured in sediments of the southern arm of the Great Salt Lake result from the activity of dense populations of slightly to moderately halophilic and extremely halotolerant SRB. Our results further indicate that most of the SRB present in the northern arm exist under extreme conditions of salt stress, probably resulting in very low *in situ* growth rates. This might indeed be due to the bioenergetic burden of osmoadaptation as compared to the relatively low amount of energy liberated from sulfate respiration as recently discussed by Oren [26].

Acknowledgments

The authors thank Dr. Wallace Gwynn from Utah Geological Survey and Richard Denton from Department of Environmental Quality, State of Utah (both at Salt Lake City, Utah, USA) for valuable help during sampling. In addition, we thank Dorthe Ganzhorn and Tove Wiegers for performing some of the sulfur distillations. Finally, we thank Dr. Ian M. Head for useful suggestions and criticism. This study was supported in part by grant 9701310 from the Danish Technical Research Council.

References

1. Boudreau BP, Westrich JT (1984) The dependence of bacterial sulfate reduction on sulfate concentrations in marine sediments. Geochim Cosmochim Acta 48:2503–2516
2. Brandt KK, Ingvorsen K (1997) *Desulfobacter halotolerans* sp nov, a halotolerant acetate-oxidizing sulfate-reducing bacterium isolated from sediments of Great Salt Lake, Utah. Syst Appl Microbiol 20:366–373
3. Brandt KK, Patel BKC, Ingvorsen K (1999) *Desulfocella halo-*

phila gen nov, sp nov, a halophilic fatty acid-oxidizing sulfate-reducing bacterium isolated from sediments of the Great Salt Lake. Int J Syst Bacteriol 49:193–200

4. Canfield DE, Des Marais DJ (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim Cosmochim Acta 57:3971–3984
5. Caumette P, Cohen Y, Matheron R (1991) Isolation and characterization of *Desulfovibrio halophilus* sp nov, a halophilic sulfate-reducing bacterium isolated from Solar Lake (Sinai). Syst Appl Microbiol 14:33–38
6. Caumette P, Matheron R, Raymond N, Relexans J-C (1994) Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de-Giraud, France). FEMS Microbiol Ecol 13:273–286
7. Christensen D (1984) Determination of substrates oxidized by sulfate reduction in intact cores of marine sediments. Limnol Oceanogr 29:189–192
8. Cochran WG (1950) Estimation of bacterial densities by means of the "most probable number." Biometrics 6:105–116
9. Edgcomb VP, McDonald JH, Devereux R, Smith DW (1999) Estimation of bacterial cell numbers in humic acid–rich salt marsh sediments with probes directed to 16S ribosomal DNA. Appl Environ Microbiol 65:1516–1523
10. Fossing H, Jørgensen BB (1989) Measurement of bacterial sulfate reduction in sediments: Evaluation of a single-step chromium reduction method. Biogeochem 8:205–222
11. Gibson GR, Parkes RJ, Herbert RA (1987) Evaluation of viable counting procedures for the enumeration of sulfate-reducing bacteria in estuarine sediments. J Microbiol Meth 7:201–210
12. Hines ME, Evans RS, Sharak GB, Willis SG, Friedman S, Rooney-Varga JN, Devereux R (1999) Molecular phylogenetic and biogeochemical studies of sulfate-reducing bacteria in the rhizosphere of *Spartina alterniflora*. Appl Environ Microbiol 65:2209–2216
13. Hurley MA, Roscoe ME (1983) Automated statistical analysis of microbial enumeration by dilution series. J Appl Bacteriol 55:159–164
14. Ingvorsen K, Jørgensen BB (1984) Kinetics of sulfate uptake by freshwater and marine species of *Desulfovibrio*. Arch Microbiol 139:61–66
15. Ingvorsen K, Zehnder AJB, Jørgensen BB (1984) Kinetics of sulfate and acetate uptake by *Desulfobacter postgatei*. Appl Environ Microbiol 47:403–408
16. Jørgensen BB (1978) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. Geomicrobiol J 1:11–27
17. Jørgensen BB (1978) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. III. Estimation from chemical and bacteriological field data. Geomicrobiol J 1:49–63
18. Jørgensen BB, Cohen Y (1977) Solar Lake (Sinai). 5. The sulfur cycle of the benthic cyanobacterial mats. Limnol Oceanogr 22:657–666
19. Klug M, Boston P, Francois R, Gyure R, Javor B, Tribble G, Vairavamurthy A (1985) Sulfur reduction in sediments of marine and evaporite environments. In: Sagan D (ed) The Global Sulfur Cycle. NASA Technical Memorandum 87570, National Aeronautics and Space Administration, Washington D.C., pp 128–157
20. Krekeler D, Sigalevish P, Teske A, Cypionka H, Cohen Y (1997) A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai), *Desulfovibrio oxyclinae* sp nov. Arch Microbiol 167:369–375
21. Kruse B (1993) Measurement of plankton O_2 respiration in gas-tight plastic bags. Mar Ecol Prog Ser 94:155–163
22. Lupton FS, Phelps TJ, Zeikus JG (1984) Methanogenesis, sulphate reduction and hydrogen metabolism in hypersaline anoxic sediments of the Great Salt Lake, Utah. Annual Report 1983, Baas Becking Geobiological Laboratory, Bureau of Mineral Resources, Canberra, Australia, pp 42–48
23. Ollivier B, Caumette P, Garcia J-L, Mah RA (1994) Anaerobic bacteria from hypersaline environments. Microbiol Rev 58:27–38
24. Ollivier B, Hatchikian CE, Prensier G, Guezennec J, Garcia J-L (1991) *Desulfohalobium retbaense* gen nov, sp nov, a halophilic sulfate-reducing bacterium from sediments of a hypersaline lake in Senegal. Int J Syst Bacteriol 41:74–81
25. Oren A (1988) Anaerobic degradation of organic compounds at high salt concentrations. Antonie van Leeuwenhoek 54:267–277
26. Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 63:334–348
27. Parkes RJ, Gibson GR, Mueller-Harvey I, Buckingham WJ, Herbert RA (1989) Determination of the substrates for sulphate-reducing bacteria within marine and estuarine sediments with different rates of sulphate reduction. J Gen Microbiol 135:175–187
28. Post FJ (1981) Microbiology of the Great Salt Lake north arm. Hydrobiologia 81:59–69
29. Sahm K, MacGregor BJ, Jørgensen BB, Stahl DA (1999) Sulphate reduction and vertical distribution of sulphate-reducing bacteria quantified by rRNA slot-blot hybridization in a coastal marine sediment. Environ Microbiol 1:65–74
30. Skyring GW (1987) Sulfate reduction in coastal environments. Geomicrobiol J 5:295–374
31. Skyring GW (1988) Acetate is the main energy substrate for the sulfate-reducing bacteria in Lake Eliza (South Australia) hypersaline sediments. FEMS Microbiol Ecol 53:87–94
32. Skyring GW, Jones HE (1977) The taxonomy of some isolates of dissimilatory sulfate-reducing bacteria. Can J Microbiol 23:1415–1425
33. Sørensen J, Christensen D, Jørgensen BB (1981) Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. Appl Environ Microbiol 42:5–11
34. Tardy-Jacquenod C, Caumette P, Matheron R, Lanau C, Arnauld O, Magot M (1996) Characterization of sulfate-reducing bacteria from oil-field waters. Can J Microbiol 42:259–266
35. Teske A, Ramsing NB, Habicht K, Fukui M, Küver J, Jør-

gensen BB, Cohen Y (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl Environ Microbiol 64:2943–2951

36. Vester F, Ingvorsen K (1998) Improved most-probable-number method to detect sulfate-reducing bacteria with natural media and a radiotracer. Appl Environ Microbiol 64:1700–1707
37. Westrich JT (1983) The consequences and controls of bacterial sulfate reductions in marine sediments. Ph.D. thesis, Yale University
38. Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The Prokaryotes. Springer-Verlag, New York, pp 3353–3378
39. Zeikus JG (1983) Metabolic communication between biodegradative populations in nature. In: Slater JH, Whittenbury JH, Wimpenny JWT (eds) Microbes in their Natural Environments. Cambridge University Press, Cambridge, UK, pp 423–462
40. Zhilina TN, Zavarzin GA (1990) Extremely halophilic, methylotrophic, anaerobic bacteria. FEMS Microbiol Rev 87:315–322