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Fatty acid composition of sulfate-reducing bacteria isolated from deep-sea hydrothermal vents (13° N, East Pacific Rise)

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Abstract

Five strains of vibrio-shaped, mesophilic sulfate-reducing bacteria were isolated from the deep-sea hydrothermal vent site at 13° N on the East Pacific Rise. Phospholipid analyses demonstrated a high percentage of branched-chain fatty acids, including the known biomarker for *Desulfovibrio*, in all five strains. The cell-wall lipids showed a fatty acid composition markedly different from the phospholipids. While straight-chain fatty acids were predominant the biomarker fatty acid was absent. Based on the morphological characteristics and the fatty acid composition, we tentatively have assigned the isolates to the genus *Desulfovibrio*.

Introduction

Phospholipid composition of sulfate-reducing bacteria (SRB) has been found to represent a tool for their identification. Several genera can be distinguished by the occurrence of specific fatty acids (BOON et al. 1977, DOWLING et al. 1986, TAYLOR and PARKES 1983).

Changes in the fatty acid composition of membrane lipids, however, play a crucial role in adaptation to temperature and pressure, as the fluidity of the cell membrane largely depends on the melting point of the structural fatty acids. Also, the availability of substrates, suitable as chain initiators in fatty acid biosynthesis, may influence the composition of bacterial membrane lipids, notably regarding the incorporation of branched-chain fatty acids (KANEDA 1977). Thus, to establish the significance of certain fatty acids as biomarkers it is important that the relevant organisms are examined under different environmental conditions.

In the present study we isolated five strains of SRB from the hydrothermal vent area at 13° N on the East Pacific Rise (DESBRUYERES et al. 1982) and examined the fatty acid composition of their phospholipids and cell-wall lipids. It was our interest to compare the fatty acid profiles to those of SRB isolated from coastal environments and to assess the taxonomic status of our isolates.

Material and methods

During the cruise *"Hydronaut"* (October - December, 1987) the hydrothermal vent site at 13° N was visited by the submersible *"Nautile"*. Samples of various origins (chimney fragments, invertebrate tissue, hydrothermal waters, immersed metal surfaces) were brought aboard the mothership *"N/O Nadir"* and treated for bacteriological studies as described by PRIEUR et al. (1989).

Enrichment: SRB were enriched on a modification of Postgate's lactate medium B (PFENNIG et al. 1981) supplemented with 15 mM acetate. Prior to inoculation, a sterile iron nail was added to the medium as proposed by ABD-EL-MALEK and RIZK (1958). Positive cultures were obtained at 20 °C under atmospheric pressure and were subcultured in fresh lactate-acetate medium. After 4 to 6 transfers, the positive cultures were inoculated in a basal medium containing 10 mM of either: acetate, benzoate, formate, isobutyrate or lactate (PFENNIG et al. 1981). Further enrichments on these media were carried out on microplaques (0.4 ml) handled in an anaerobic chamber with an atmosphere of $N_2/CO_2/H_2$ (90:5:5).

Isolation: Pure cultures were obtained by application of the agar shake method (PFENNIG et al. 1981). Before solidification of the agar medium, sterile dithionite was added to a final concentration of about 250 ^{1}M. The agar tubes were flushed with N_2 or N_2/CO_2 (80:20) and sealed with Iso-Versinic stoppers or Teflon-lined screw-caps. Purity of the isolates was checked microscopically after 3 weeks of growth in media containing yeast extract, pyruvate, fumarate and glucose as substrates.

Phospholipid composition: Strains were grown in 250 ml batch cultures at 28-30 °C in the media used for their isolation (see Table 1). Cultures in the stationary growth phase were harvested by centrifugation at 15,000 x g, washed twice in artificial seawater, and frozen until lipid extraction (within 1-2 days). Extraction and lipid analyses were performed according to the procedures previously described (WHITE et al. 1979). Briefly, lipids were separated in three general classes using silicic acid column chromatography and the polar lipids eluted were esterified by mild alkaline methanolysis. Fatty acids as FAME (fatty acid methyl esters) were analyzed by gas chromatography and gas chromatography-mass spectrometry. The double bond position in mono-unsaturated fatty acids was determined as described by NICHOLS et al. (1986).

The residual fraction of the Bligh and Dyer extraction was analyzed for lipopolysaccharide (LPS) fatty acids as described by EDLUND et al. (1985).

Results and discussion

Five pure cultures of SRB were isolated from samples originating from the deep-sea hydrothermal vent site at 13° N (Table 1). All strains were mesophilic SRB growing well at 30 °C under atmospheric pressure. The temperature growth range of strain H 2.5 was 5-44 °C with optimum at 37 °C (data not shown).

All isolates appeared as vibrio-shaped cells, the strain H 2.5 being larger than the others (Table 1). During the stationary growth phase, cells of the strain H 2.5 turned into coccoid, aberrant pleomorphs showing a plasmolyzed appearance with dark areas located at the periphery of the cells. Upon subculture, normal vibrios were recovered after a slightly prolonged lag phase.

Table 1. Origin and morphology of the isolates.

Strain	Origin	Isolation C-source	Form	Width µm	Length µm	Motility
H 2.5	Alvinella tube	Lactate	large vibrio	0,8-1,2	2,4-6,5	+
H 8.3	Hydrothermal water	Formiate	small vibrio	≈ 0,8	1,6-2,8	+
H 17.1	Chimney fragment	Acetate	"fat" vibrio	0,8-1,0	1,8-3,6	+
H 17.3	Chimney fragment	Formiate	vibrio	≈ 0,8	1,6-3,2	+
H 17.5	Chimney fragment	Lactate	vibrio	≈ 0,8	2,0-3,2	+

Table 2. Fatty acid composition of the phospholipids expressed as percentage.

	Strain No.				
Acid[a]	H 17,1	H 17,3	H 17,5	H 2,5[b]	H 8,3
14:0	0,6	0,4	0,8	0,1	0,8
i-15:1w7	-	0,9	0,3	0,9	2,4
a-15:1w7	-	-	0,1	1,0	-
i-15:0	9,2	9,6	9,9	15,5	20,9
a-15:0	-	2,2	3,5	2,2	3,6
15:0	0,4	-	0,1	0,2	0,5
i-16:1w7c	0,5	-	0,3	0,5	0,1
i-16:0	0,5	1,0	1,7	1,5	1,0
16:1w7c	11,7	2,0	6,1	0,5	1,6
16:1w5c	0,3	0,4	3,4	-	0,3
16:0	24,3	30,9	23,0	3,3	35,2
i-17:1w7c	20,6	10,6	18,0	30,0	6,3
a-17:1w7	5,3	1,5	4,3	3,9	1,0
17:1	-	-	0,9	0,1	0,2
i-17:0	4,8	14,2	7,2	24,1	13,0
a-17:0	2,3	3,9	3,0	8,4	3,0
17:0	0,6	0,8	0,2	1,4	0,4
i-18:0	0,2	0,9	0,2	-	0,2
18:1w9c	0,6	0,5	0,3	0,1	0,2
18:1w7c	8,7	4,3	8,4	1,3	1,7
18:1w5	-	0,5	0,2	-	-
18:0	4,7	9,3	5,4	1,4	6,5
i-19:1w7	0,3	-	0,3	0,7	0,1
a-19:1w7	0,2	-	0,2	0,4	0,1
i-19:0	-	-	0,1	0,4	-
a-19:0	-	-	-	0,1	0,1

[a] Abbreviations of fatty acid: numbers before and after the colon (:) correspond to the number of carbon atoms and double bonds, respectively. Positions of double bonds are given by their distance from the methyl end (w); cis configuration = c. Methyl branching occur as i = iso or a = anteiso. Example: i-15:1w7c is cis-13-methyltetradec-7-enoic acid.

[b] Culture appearing as coccoid bodies at the time of harvest.

The membrane phospholipids of all strains were characterized by the abundance of odd numbered branched-chain fatty acids (Table 2). Saturated branched-chain fatty acids accounted for 17 % to 52 % of the total fatty acids with the iso and anteiso C15:0 and C17:0 predominating. Mono-unsaturated fatty acids occured in all strains (13-48 %), with the lowest percentages recorded from the two strains isolated and grown on formate (H 8.3 and H 17.3). The monoenoic, branched-chain fatty acid i-$C_{17:1w7c}$ was present in all five isolates, but relatively low percentages were found in strain H 8.3 and H 17.3.

Table 3. Fatty acid composition of the cell-wall lipids expressed as percentage.

	Strain No.			
Acid[a]	H 17,1	H 17,5	H 2,5[b]	H 8,3
14:0	3,5	1,2	2,5	0,6
i-15:1w7	0,8	2,1	1,9	0,5
i-15:0	4,0	3,5	5,1	0,1
a-15:0	2,0	1,4	2,0	-
15:0	1,9	0,2	1,0	0,4
i-16:0	0,4	0,6	0,2	0,1
16:1w7c	4,5	12,3	4,1	21,7
16:1w5c	1,5	1,0	-	1,6
16:0	46,7	35,3	41,8	22,6
i-17:1w7c	1,7	-	0,3	-
a-17:1w7	1,2	-	-	-
17:1	0,7	-	-	-
i-17:0	3,0	4,6	2,9	3,1
a-17:0	0,9	1,1	1,1	0,4
17:0	1,1	0,8	0,9	0,4
i-18:0	0,3	0,4	-	-
18:1w9c	0,9	-	-	0,4
18:1w7c	3,3	18,9	7,0	37,0
18:1w5	0,5	0,3	-	-
18:0	14,2	7,3	12,8	1,0

[a,b] see Table 2

The cell-wall lipids (Table 3) were found to exhibit a fatty acid composition quite different from the phospholipids. Thus, i-$C_{17:1w7c}$ was only found in trace amounts in the LPS while straight-chain fatty acids were very predominant (68-85 %). Except for strain H 8.3, the total percentages of unsaturated fatty acids (UFA) were markedly reduced in the cell-wall lipids.

The LPS analyses further revealed significant amounts of β-hydroxy fatty acids, with β-OH iC17:0 predominating for all strains (49-63 %, data not shown).

The occurrence of pleomorphic, coccoid bodies, as observed in the strain H 2.5, has previously been reported from old cultures of *Desulfovibrio* (TRÜPER et al. 1969, SKYRING et al. 1977) where they are thought to be favoured by high concentrations of hydrogen sulfide (POSTGATE 1984).

Epifluorescence counts of H 2.5 revealed that more than 99 % of the population turned into pleomorphs within a few days after cessation of growth when [H_2S] had reached about 9 mM (data not shown).

Structural fatty acids can be used as good indicators for the presence of SRB in the natural environment (PARKES 1987). Thus, i-$C_{17:1w7c}$ has been identified as a signature for *Desulfovibrio* spp. and constitute 20-40 % of the phospholipid fatty acids in this group.

The results of our phospholipid analyses indicate that the strains isolated belong to the genus *Desulfovibrio*. The presence of β-iOH C17:0 in the LPS tend to support this hypothesis. The phospholipids of strain H 8.3 and H 17.3 have relatively low contents of i-$C_{17:1w7c}$, as well as other UFA, but not fatty acid biomakers representing other groups of SRB are present. Undoubtly the different carbon source influence the fatty acid distribution and it seems that the formate growth medium causes a shift in the i-$C_{17:1w7c}$ content as compared to acetate or lactate growth medium. However, further experiments are required to confirm these findings.

The significant proportion of branched-chain fatty acids (BCFA) in the membranes of all strains indicates the occurrence of a biosynthethic pathway involving branched-chain fatty acid synthetase (KANEDA 1977), previously ascribed to *Desulfovibrio* by TAYLOR and PARKES (1983). Except for the strain H 2.5, the percentages of BCFA in our strains (44-51 %) are, however, rather low as compared to the percentages (55-85 %) reported in coastal isolates of *Desulfovibrio* (BOON et al. 1977, TAYLOR and PARKES 1983, EDLUND et al. 1985). In comparison, the proportion of UFA is in the same range or lower (strain H 8.3 and H 17.3) than in these previous reports.

It might be speculated that the bacterial membranes *in situ* could have a higher percentage of BCFA which would be consistent with the enzymatic pathway and the physiological constraints of the deep-sea hydrothermal environment. Future investigations will include fatty acid analyses of cells grown at high pressure to evaluate the influence of pressure.

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