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**SHORT COMMUNICATIONS**

# Newly Discovered Properties of Spore-Forming Sulfate-Reducing Bacteria, *Desulfotomaculum* Strains 435 and 781

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In 1978, we described two strains (435 and 781) of thermophilic spore-forming sulfate-reducing bacteria (SRB), which on the basis of a limited number of properties were assigned to the species *Desulfotomaculum nigrificans* [1]. At that time, *Dt. nigrificans* was the only known thermophilic representative of the genus that was known to oxidize some organic substrates (not fatty acids) to acetate and $CO_2$ and to grow under lithoheterotrophic conditions at the expense of $H_2/CO_2$ and acetate [2], i.e., to realize incomplete oxidation of organic compounds, belonging thus to the first metabolic type.

New data concerning species composition of the genus *Desulfotomaculum* and the diversity of the physiological properties of its representatives belonging to different metabolic types (the first and the second metabolic types, implying incomplete and complete oxidation of organic compounds, respectively) [3–6] dictated a need for refinement of the description of metabolic properties of strains 435 and 781.

The aim of this work was to determine the metabolic type peculiar to the sulfate-reducing bacterial strains 435 and 781. Our main tasks were to elucidate the ability of these strains to oxidize organic compounds (with particular emphasis on the degradation of acetate, lactate, and butyrate during sulfate reduction) and to elucidate the capacity of the strains to grow under lithoautotrophic conditions at the expense of $H_2$ oxidation.

Strains 435 and 781 were isolated from the formation water of an oil field located in western Siberia [1].

The SRB were cultivated in Hungate tubes at 60°C in Widdel medium (brackwasser) containing NaCl, 1%; reducing agents ($Na_2S \cdot 9H_2O$, 500 mg/l and $Na_2S_2O_4$, 50 mg/l); and trace elements [7] and supplemented with (g/l) lactate, 2.0; caproate, 0.3; palmitate, 0.3; or other organic compounds, 1.0. Argon was used as the gas phase. To determine cell growth under lithoautotrophic conditions, a $H_2/CO_2$ (80 : 20) gas mixture was introduced into tubes with the above-specified mineral medium. Oxidation of acetate, butyrate, and propionate was assayed by GLC; methane production was determined by gas chromatography as described earlier [8]. The production of $H_2S$ was determined spectrophotometrically with the use of *N,N*-dimethyl-*p*-phenylenediamine [9].

The SRB under study produced sulfide when grown for 7–12 days in media containing lactate, pyruvate, malate, fumarate, succinate, formate, caproate, palmitate, methanol, ethanol, butanol, or yeast extract. After more prolonged incubation (for 30 days), sulfide production was determined in media with propionate, valerate, ethanol, and propanol. Accumulation of $H_2S$ occurred during growth in the media containing two reducing agents (sodium sulfide and sodium dithionite) or only sodium sulfide. No sulfate reduction was revealed in sulfate-containing media supplemented with citrate, tartrate, benzoate, glycerol, glucose, fructose, asparagine, glutamate, threonine, tryptophan, or phenylalanine. Fumarate was metabolized with the production of $H_2S$ and without acetate accumulation.

The SRB studied oxidized acetate as the sole source of carbon and energy and accumulated hydrogen sulfide; however, this process occurred very slowly, over 56 days of incubation (experiments 1 and 4, Table 1). The amount of acetate oxidized by cultures of strains 435 and 781 amounted to 1.76 and 1.28 mmol/l, respectively. Judging from the sulfide/acetate molar ratios determined (Table 1), it may be considered that the most part of acetate oxidized by strains 435 and 781 was oxidized via respiration. The cell number did not increase and did not exceed $\times 10^6$ cells/ml; a part of cells possibly died during the 56-day cultivation. Hampered oxidation of acetate by the SRB of the second metabolic type may be related to the energy expenditures for substrate activation [2].

Yeast extract at a concentration of 0.5 g/l produced virtually no effect on acetate degradation by strain 435 (Table 1) but increased acetate degradation (up to 2.90 mmol/l) by strain 781; in this case, the sulfide production and cell number also increased (experiment 5, Table 1). At least a part of sulfide in experiment 5 was formed during yeast extract metabolization, since, theoretically, according to the respiration equation (1) (Table 1), sulfide production at the expense of the oxidation of 1 mmol/l acetate should be equal to

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**Table 1.** Degradation of acetate by the sulfate-reducing bacterial strains 435 and 781

| Strain | Experiment no. | Cultivation time, days | Substrates | | Acetate oxidized, mmol/l | Sulfide production, mmol/l | | Sulfide/acetate oxidized | Cell number per 1 ml |
|---|---|---|---|---|---|---|---|---|---|
| | | | acetate, mM | yeast extract, g/l | | in experiments | theoretically*, from oxidized acetate | | |
| 435 | 1 | 56 | 4.6** | – | 1.76 | 1.60 | 1.76 | 0.90 | $3.7 \times 10^6$ |
| ″ | 2 | 56 | 4.6 | 0.5 | 1.85 | 1.72 | 1.85 | 0.92 | $4.0 \times 10^6$ |
| ″ | 3 | 10 | – | 0.5 | – | 1.74 | – | – | ND |
| 781 | 4 | 56 | 4.6 | – | 1.28 | 1.20 | 1.28 | 0.93 | $5.2 \times 10^6$ |
| ″ | 5 | 56 | 4.6 | 0.5 | 2.9 | 4.70 | 2.90 | 1.62 | $1.84 \times 10^7$ |
| ″ | 6 | 10 | – | 0.5 | – | 1.86 | – | – | ND |

Note: ND stands for "no data"; "–" means "acetate (or yeast extract) is absent from the initial medium."

* According to the respiration equation for *Dt. acetoxidans*: $CH_3COO^- + SO_4^{2-} \longrightarrow 2HCO_3^- + HS^-$ (1) [4].

** Mean data of four replicate experiments.

**Table 2.** Degradation of lactate by the sulfate-reducing bacterial strains 435 and 781 (10 days of cultivation)

| Strain | Experiment no.* | Substrates | | Production | | Acetate/sulfide | |
|---|---|---|---|---|---|---|---|
| | | lactate, g/l (mM) | yeast extract, g/l | acetate, mmol/l | sulfide, mmol/l | in experiments | theoretically** |
| 435 | 1 (K) | 2.0 (17.85 mM) | – | 0 | 0 | 0 | |
| | 2 | 2.0 (17.85 mM) | – | 15.67 | 5.21 | 3.01 | 2 |
| | 3 | 0.5 (4.45 mM) | – | 2.68 | 3.72 | 0.72 | 2 |
| | 4 | 0.5 (4.45 mM) | 0.5 | 1.63 | 3.92 | 0.41 | |
| 781 | 5 (K) | 2.0 (17.85 mM) | – | 0 | 0 | 0 | |
| | 6 | 2.0 (17.85 mM) | – | 12.8 | 5.24 | 2.44 | 2 |
| | 7 | 0.5 (4.45 mM) | – | 3.0 | 4.27 | 0.61 | 2 |
| | 8 | 0.5 (4.45 mM) | 0.5 | 1.68 | 4.86 | 0.39 | |

Note: (K) signifies control: storage in a refrigerator at 8°C; "–" means absence in the initial medium.

* Mean data of two replicate experiments.

** According to the respiration equation for *Desulfovibrio*: $2CH_3CHOHCOO^- + SO_4^{2-} \longrightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+$ (2) [2].

2.90 mmol/l, whereas actually it was 4.70 mmol/l. When grown in the medium containing yeast extract (0.5 g/l) as the sole substrate, the SRB under study produced sulfide without acetate accumulation (Table 1). Thus, the results obtained with strain 781 indicate that, to reveal acetate consumption by representatives of the genus *Desulfotomaculum*, it is necessary to perform prolonged cultivation of SRB in medium supplemented with a substrate (yeast extract in our case) that is easily assimilated by the cells without acetate production.

The SRB grown in lactate-containing media produced acetate (Table 2); in this case, sulfide accumulation was observed and the cell number increased by two orders of magnitude. When strains 435 and 781 were grown in yeast extract–containing medium with a decreased lactate concentration, the acetate/sulfide molar ratio decreased to values below 1 (Table 2), although, theoretically, for the SRB exhibiting the first metabolic type, this value should be 2 (equation (2), Table 2). According to Widdel [2], upon oxidation of some organic compounds by SRB exhibiting the second metabolic type, the production of acetyl-CoA proceeds more rapidly than its terminal oxidation to $CO_2$, and the excess of acetyl-CoA is converted to acetate. Therefore, bacteria of the second metabolic type can metabolize acetyl-CoA to $CO_2$ and acetate.

Strains 435 and 781 oxidized 3.9 and 4.0 mmol/l butyrate, respectively, out of its initial concentration of 7 mmol/l and produced 6.5 and 6.0 mmol/l sulfide, respectively. Acetate (0.3 mmol/l) was revealed in a 4-day culture of strain 781; it was an intermediate product and disappeared during further cultivation. In the case of complete metabolization of butyrate according to equation (3) (Table 3), the molar ratio of sulfide to butyrate oxidized should equal 2.5, while in our experiments it was 1.66, which is apparently due to partial

**Table 3.** Degradation of butyrate by the sulfate-reducing bacterial strains 435 and 781

| Strain | Experimental variant | Cultivation time, days | Butyrate, mmol/l | | Production, mmol/l | | Sulfide/butyrate oxidized | |
|---|---|---|---|---|---|---|---|---|
| | | | initial | oxidized | acetate | sulfide | in experiments | theoretically* |
| 435** | 1 | 10 | 7.0 | 3.9 | – | 6.5 | 1.66 | 2.5 |
| 781 | 1 | 4 | 7.0 | 0.6 | 0.3 | 1.0 | 1.50 | |
| ″ | 2 | 10 | 7.0 | 4.0 | – | 6.0 | 1.66 | 2.5 |

Note: "–" means absence of acetate.
* According to the respiration equation for SRB oxidizing butyrate completely to $CO_2$: $2CH_3(CH_2)_2COO^- + 5SO_4^{2-} \longrightarrow 8HCO_3^- + 5HS^- + H^+$ (3) [2].
** Mean data of two replicate experiments.

utilization in constructive metabolism of the acetyl-CoA formed.

Both SRB strains fermented pyruvate at a concentration of 1 g/l. In the culture liquid of strain 435, acetate (9 mmol/l) and propionate (0.5 mmol/l) were detected. No lactate fermentation by these strains was revealed.

Strains 435 and 781 produced hydrogen sulfide (10.4 and 9.4 mmol/l, respectively) during lithoautotrophic growth at the expense of $H_2/CO_2$; in this case, the cell number increased from $10^6$ to $10^8$ cells/ml. Low concentrations of $CH_4$ were detected in the gas phases of strain 435 and 781 cultures: 122 and 118 nmol/100 ml, respectively (so-called mini-methane [10]).

The results obtained make it possible to conclude that the SRB strains 435 and 781 differ from *Dt. nigrificans* in a number of properties, such as oxidation of a wide range of organic compounds, including formate, acetate, and higher fatty acids; metabolization of butyrate without acetate accumulation; and reduction of sulfates under autotrophic conditions at the expense of $H_2$ oxidation with mini-methane production. According to these properties, the strains under study are closely related to the species of the genus *Desulfotomaculum* that exhibit the second type of metabolism, such as *Dt. acetoxidans* [3, 10], *Dt. sapomandens* [4], and, among thermophiles, *Dt. kuznetsovii* [5] and *Dt. thermoacetoxidans* [6].

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