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The Uptake and Accumulation of Iron by the Intestinal Bacterium *Desulfotomaculum acetoxidans* DSM 771

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Sulfate-reducing bacteria (e.g. *Desulfotomaculum acetoxidans*) exist in animal intestine. These bacteria are able to bind heavy metals (e.g. cadmium or lead). Comparative investigations on the composition of cellular walls of *Desulfotomaculum acetoxidans* - depending on the initial Fe^{2+} supplement in the medium (7.5, 57.5 and 507.5 M) were performed. Iron(II) was cumulated as FeS or as pyrite (FeS_2). However, if the initial amount of iron was higher, its majority (46% 85%) was transported onto the membrane. It was determined that the siderophore found in *Desulfotomaculum acetoxidans* was deferroxamine as in animals.

Key words: Accumulation, ferrous sulfides, cell wall, deferroxamine siderophore.

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Sulfate-reducing bacteria (SRB) are characterised by a specific pathway of sulfate reduction with the final dissimilation of hydrogen sulfide. This dissimilated product reacts with Fe^{2+} and forms FeS, which is responsible for the blackening of cultures so characteristic for these bacteria (POSTGATE 1984). In recent years the binding of other metals by ferromagnetic pyrite (FeS_2) produced by SRB has been reported (GADD 1992; PADO *et al*. 1994; WATSON & ELLWOOD 1994; WATSON *et al*. 1994).

SRB (especially *Desulfovibrio* sp.) exist in animal rumen (HOWARD & HUNGATE 1976) and *Desulfotomaculum* sp. occur in animal intestines (HOLT *et al*. 1999). The Gram-positive species (e.g. *Desulfotomaculum acetoxidans*) are easier to use for investigations on binding and transportation processes than Gram-negative species (e.g. *Desulfovibrio* sp.), therefore experiments were performed on *Desulfotomaculum acetoxidans*.

The aim of this study was to find out if an iron concentration greater than 7.5 μM added into bacterial media increased the quantity of iron sulfides binding toxic heavy metals in bacterial cell walls.

Materials and Methods

Bacteria culture

Cultures of *Desulfotomaculum acetoxidans* DSM 771 were grown at room temperature in three media differing in ferrous chloride ($FeCl_2.4H_2O$) content: 7.5 μM; 57.5 and 507.5 μM. The media also contained other chemical compounds as described previously (PADO & PAWŁOWSKA-ĆWIĘK 2004).

After 23 days and 17 h of incubation the samples were taken out and centrifuged at 2500 g for 20 min. The supernatants were concentrated at about 100 ml at 8°C. The sediments were centrifuged at 10 000 g for 15 min, washed with water and treated with 10 mM piperazine-N,N'-bis(2-ethane sulfonic acid) (PIPES) buffer pH 5.5, 0.1 M HCl and 0.5 M NaOH (for 24 h each and next centrifuged) (HANCOCK & POXTON 1988).

Chemical determination

The hydrogen sulfides were estimated by the methylene blue method (FAGO & POPOWSKY 1949) and ferrous ions were made after acidic hydrolysis (3 M HCl for 8 h at 95°C) with ferrozine according to the method of RICE-EVANS *et al*. (1991). The assay described by SCHWYN & NEILANDS (1987) was used for the determination of siderophores (deferroxamine) and absorption values at 685 nm were related to the initial absorbance culture. The spectrophotometric analysis was performed using a CECIL 8020 spectrophotometer.

All reagents were of the p.a. grade (Fluka, Sigma or Merck), while water was redistilled.

Results

The results showed that the highest amount of deferroxamine was in a 7-day sample at the lowest iron supplement (Fig. 1).

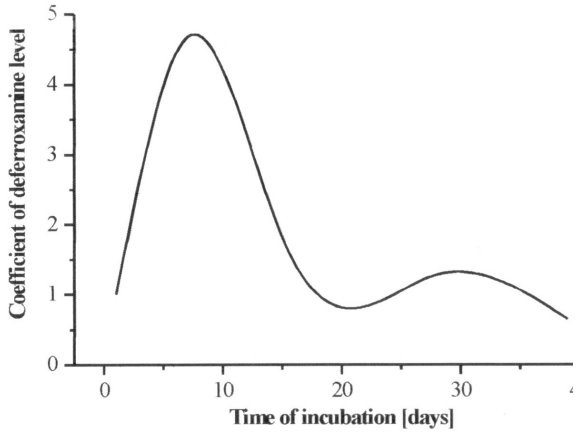

Fig. 1. The deferroxamine level in supernatants from the cultures after different times of incubation of *Desulfotomaculum acetoxidans*

that the process of binding iron in the early period of bacterial growth is of a strictly chemical character.

The results of iron determination indicate that this metal is shifted in cell walls during the growth of *Desulfotomaculum acetoxidans*. After 23 days of incubation even a high supplement of iron (507.5 μM) was transported into the cellular membrane in approximately 85% (while in the case of a mean culture in nearly 46%). This was proved by an intensively black colour of the biomass.

The results of H_2S (and /or other sulfides) determination indicate that the final product of sulfate reduction is cumulated in cell walls (Table 2). The level of sulfides is clearly dependent on the amount of iron introduced into the medium. This would indicate an occurrence of ferrous sulfide (FeS) or pyrite (FeS_2) in cellular walls, which is more probable in view of the molar ratios of iron (mmoles) to sulfide (mmoles).

Table 1

Iron [μM] concentration in PIPES, HCl and NaOH extracts and supernatants

Initial Fe^{2+} concentrat	17 h					23 days				
	PIPES	HCl	NaOH	Supernatant	Total	PIPES	HCl	NaOH	Supernatant	Total
7.5 μM	0.23	0.80	0	1.20	2.23	1.13	0.38	0.30	0	1.81
57.5 μM	0.45	8.13	0	19.00	27.58	9.56	21.00	0.05	0.50	31.11
507.5 μM	0.89	202.30	0.05	285.90	489.14	22.60	51.90	0.05	0.90	75.45

Table 2

The sulfides [mM] concentration in PIPES, HCl and NaOH extracts and supernatants

Initial Fe^{2+} concentrat	17 h					23 days				
	PIPES	HCl	NaOH	Supernatant	Total	PIPES	HCl	NaOH	Supernatant	Total
7.5 μM	0.396	0.350	2.240	0.05	3.036	4.775	0.613	0.812	0.05	6.25
57.5 μM	0.012	2.314	4.624	0	6.950	2.813	5.847	5.84	0	13.9
507.5μM	0.003	5.299	6.875	0	12.177	0.518	8.385	10.951	0	19.854

In agreement with our expectations the obtained results prove that *Desulfotomaculum acetoxidans* strains are able to cumulate iron in structures of their cell walls and that the degree of accumulation is proportional to the Fe^{2+} concentration in the initial medium (Table 1). However, the rate of cumulative processes, contrary to the observed sudden blackening dependent on the concentration of iron, was not very high. In all three cultures after 17 h of incubation more than 50% of the introduced Fe^{2+} was still left in supernatants. These kinetics show

Discussion

Previous studies indicated that SRB were able to detoxify heavy metals (GADD 1992; PADO *et al*. 1994). These bacteria can bind heavy metals by FeS and FeS_2 (WATSON & ELLWOOD 1994; WATSON *et al*. 1994). The present experiments show that the amount of iron sulfides accumulating in cellular walls of *Desulfotomaculum acetoxidans* depends on the initial level of Fe in the medium or the environment (Tables 1& 2). Most

probably, the quantity of bound toxic heavy metals (e.g. Cd) depends on the quantity of accumulated iron sulfides, especially piryte, by these bacteria. The iron sulfides are formed in an early growth period when deferroxamine secretion does not yet occur (Fig. 1). Similar detoxification processes can take place with SRB participation in animal rumen and/or intestines. The bound toxic metals (e.g. Cd) cannot be absorbed by rumen and/or intestine efflux transporters and be included into subsequent metabolic pathways, so they cannot be incorporated into any metabolite. These processes, even if only partial, would explain the negative correlation between the Fe level and Cd concentration in the bank vole *Clethrionomys glareolus* found by BAŁOŃSKA *et al*. (2002).

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