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Reduction of Selenate and Selenite to Elemental Selenium by a *Pseudomonas stutzeri* Isolate

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A *Pseudomonas stutzeri* isolate rapidly reduced both selenite and selenate ions to elemental selenium at initial concentrations of both anions of up to 48.1 mM. Optimal selenium reduction occurred under aerobic conditions between pH 7.0 and 9.0 and at temperatures of 25 to 35°C. Reduction of both selenite and selenate was unaffected by a number of anions except for sulfite, chromate, and tungstate ions, which inhibited both growth and reduction.

Agricultural drainage, smelter effluents, fly ash, sewage sludge, and exhaust gases from the burning of fossil fuels are all sources of selenium (3, 5, 8, 10). A number of selenium compounds are very toxic; however, selenium is also an essential nutrient, and low concentrations of selenium are required by many microorganisms (3, 12). Various microorganisms are capable of mediating a number of transformations of inorganic selenium compounds, such as reduction (4, 6, 7), oxidation (11), and methylation (2, 4). Although microorganisms capable of reducing the selenate ion have been isolated (6, 7, 9), selenite is more easily reduced than is selenate (3). The objectives of this study were to characterize a *Pseudomonas stutzeri* isolate capable of reducing the selenate ion to elemental selenium and to evaluate the effect of various parameters on the reduction rate.

The strain used in this study is a motile gram-negative rod with one or two polar flagellae; it is oxidase positive and catalase positive, utilizes citrate, and reduces both nitrite and nitrate to nitrogen gas. The colonies are yellowish on tryptic soy agar (TSA) (Difco). This strain was tentatively identified in our laboratory as *Pseudomonas stutzeri*, and the identity was subsequently confirmed by Health and Welfare Canada (Ottawa). The isolate was maintained on TSA to which sodium selenate was added.

Prior to each experiment, the bacteria were transferred from solid medium to tryptic soy broth (TSB) for 24 h, and then a 5% inoculum was transferred to TSB in order to obtain a standard inoculum with which to begin each experiment. The inoculum plus filter-sterilized sodium selenite or selenate was then added to 250-ml Erlenmeyer flasks containing 100 ml of TSB. The cultures were incubated in triplicate on a rotary shaker (150 rpm) at 30°C. For soluble selenium analysis, 10 ml of the culture supernatant was filter sterilized (Nalgene units, 0.45-μm pore size), acidified with HCl to prevent subsequent growth, and kept at 4°C until analyzed by atomic absorption spectrophotometry (AAS).

The stoichiometry of selenite and selenate reduction was determined with a 24-h culture of the *P. stutzeri* isolate (2.53 mM Se, 30°C, pH 7.0, and 150 rpm). Ten milliliters of the culture was filtered for the determination of soluble selenium by AAS. Elemental selenium was extracted by a modification of the procedure of Oremland et al. (9). Twenty milliliters of the culture was centrifuged, and the cell pellet was washed and then suspended in 150 ml of a 1.5 M Na_2SO_3 solution containing 1.25 M H_2SO_4, which was shaken at 250 rpm under nitrogen for 48 h. The extract was filtered through a 0.45-μm Millipore filter, and 120 ml of concentrated HCl was added to the filtrate. After 30 min, the extract was filtered through a 0.45-μm Millipore filter, and the precipitated selenium was recovered. It was necessary to remove the selenium from the Na_2SO_3 solution because sodium interferes with selenium determination by AAS. The filter and adhering selenium were digested in 20 ml of concentrated HNO_3 for 2 h at room temperature. The digested material was filtered through Whatman no. 1 filter paper, made up to a 100-ml volume, and then analyzed for soluble selenium by AAS.

The effects of selenium concentration, temperature, and pH on selenium reduction were evaluated for both oxyanions by the above-described procedure. HCl was added to TSB to obtain pHs below 7.0, and NaOH was used to obtain pHs above 7.0. Solutions of a number of salts were filter sterilized and added to 250-ml Erlenmeyer flasks containing 100 ml of TSB medium and either sodium selenite or

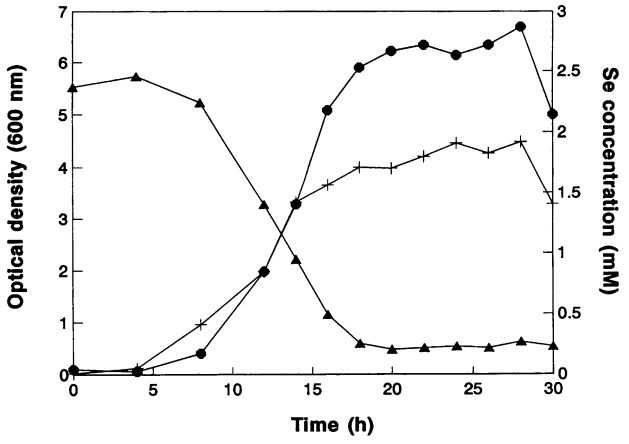

FIG. 1. Reduction of the selenite ion by *P. stutzeri*. The OD_{600} was measured for cultures grown without selenium (+) and with 2.53 mM SeO_3 (●); the SeO_3 concentration was measured over time (▲).

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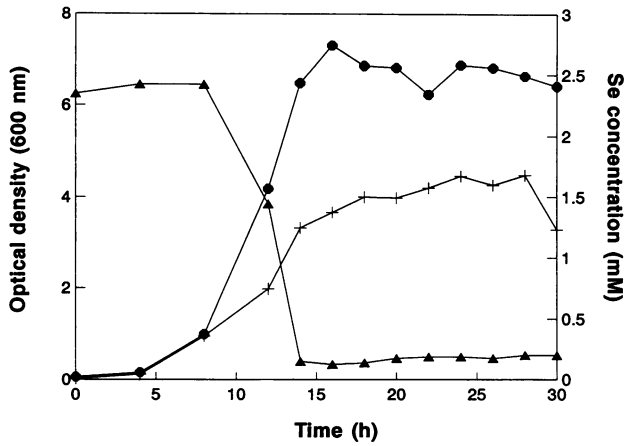

FIG. 2. Reduction of the selenate ion by *P. stutzeri*. Experimental conditions and symbols are the same as in Fig. 1 except that SeO_4 was used and measured.

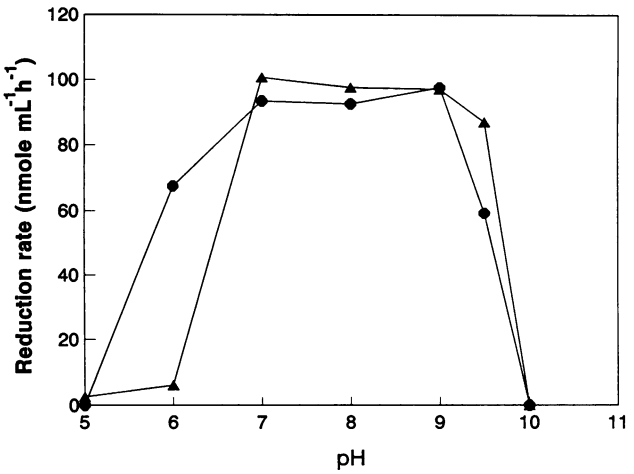

FIG. 4. Effect of pH on the reduction of selenite (●) and selenate (▲). Cultures were grown with 2.53 mM Se at 30°C and 150 rpm.

selenate. Controls contained only the selenium oxyanions, and other flasks contained only the salts. The optical density at 600 nm (OD_{600}) was measured in triplicate in 2,800-ml Erlenmeyer flasks containing 1 liter of TSB in the presence of selenite or selenate and in the absence of both selenium salts.

In most studies of selenate reduction, mixed cultures or sediment samples rather than pure cultures are used to obtain selenate reduction (5, 10, 14). Maiers et al. (7) isolated a bacterium that reduced selenate to selenite, but 1 week was required for the complete reduction of 1.27 mM of selenium in solution. Our isolate was capable of reducing up to 6.3 mM of selenate in 24 h. Approximately 79 and 68% of the added selenite and selenate, respectively, were recovered as elemental selenium. The deep red color of the medium during growth also confirms this finding. In an additional series of experiments, total selenium recoveries were in excess of 98% (data not shown).

Aeration was found to be necessary for selenite and selenate reduction (data not shown). Selenium reduction occurred concurrently with the exponential growth phase for both selenium oxyanions (Fig. 1 and 2). Phase microscopy of these preparations shows large numbers of refractile crystals, some of which tend to associate with the tips of the bacterial cells, while others are free in the culture medium. The reduction rate increased with increasing selenite and selenate concentrations up to a selenium concentration of 19.0 mM (Fig. 3). Selenate reduction rates remained constant for selenium concentrations between 19.0 and 48.1 mM, but selenite reduction decreased at concentrations above 19.0 mM. The higher toxicity of the selenite ion may be responsible for the decrease in the rate of selenite reduction at higher selenium concentrations (Fig. 3).

The increased optical density which was observed in the flasks containing the selenium oxyanions compared with the controls was due to the contribution of the elemental selenium granules produced during reduction. The reduction of selenate into elemental selenium is at least a two-step reaction, in which selenate is reduced to selenite and then possibly to Se(II) and eventually to red amorphous granules of elemental selenium (1).

Little or no selenite reduction occurred below pH 5.5 or above pH 9.5, and the lower limit for selenate reduction was pH 6.5 and the upper limit was pH 9.5 (Fig. 4). The reduction

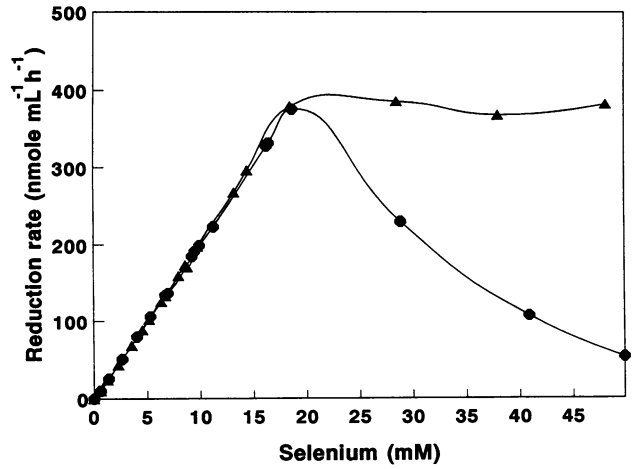

FIG. 3. Effect of selenium concentration on the reduction of selenite (●) and selenate (▲). Cultures were grown at pH 7.0, 30°C, and 150 rpm.

TABLE 1. Effect of temperature on selenium reduction by *P. stutzeri*[a]

Selenium salt	Temp (°C)	Mean reduction rate (nmol of Se/ml/h) ± SD
Na_2SeO_3	10	4.18 ± 2.91
	20	14.05 ± 3.04
	25	88.48 ± 0.25
	30	93.16 ± 2.15
	35	87.72 ± 2.15
	40	87.59 ± 1.39
Na_2SeO_4	10	0
	20	44.68 ± 0.63
	25	93.67 ± 2.53
	30	91.77 ± 2.28
	35	89.62 ± 2.66
	40	51.01 ± 10.76

[a] Initial selenium concentration, 2.53 mM.

TABLE 2. Effect of different anions on selenite reduction by *P. stutzeri*[a]

Anion	Concn (M)	Mean reduction rate (nmol of Se/ml/h) ± SD	Mean OD_{600} after 24 h ± SD
None	0	93.16 ± 2.15	4.96 ± 0.76
SO_4	1×10^{-2}	90.50 ± 2.03	6.34 ± 0.10
	5×10^{-2}	75.06 ± 3.42	5.12 ± 0.46
SO_3	1×10^{-4}	62.78 ± 10.76	5.06 ± 0.52
	1×10^{-3}	8.73 ± 2.15	2.46 ± 0.08
NO_3	1×10^{-3}	86.96 ± 6.08	6.24 ± 0.12
	1×10^{-2}	82.91 ± 10.89	5.38 ± 0.42
NO_2	1×10^{-3}	92.78 ± 5.57	5.84 ± 0.28
	1×10^{-2}	86.45 ± 5.06	5.86 ± 0.16
WO_4	1×10^{-3}	95.06 ± 5.44	6.86 ± 0.36
	1×10^{-2}	86.33 ± 2.15	6.26 ± 0.30
CrO_4	1×10^{-4}	90.25 ± 3.80	5.86 ± 0.06
	1×10^{-3}	0	0.24 ± 0.10
MoO_4	1×10^{-3}	93.16 ± 7.09	6.06 ± 0.40
	1×10^{-2}	83.92 ± 3.16	5.70 ± 0.34

[a] Initial selenium concentration, 2.53 mM.

rate of both anions increased rapidly between 20 and 25°C, with the optimum temperature range being between 25 and 30°C (Table 1). Substrate limitation may have been responsible for the leveling off of the reduction rate above 25°C. After 24 h at 25°C, 84 and 89% of the selenite and selenate, respectively, had been reduced by the *P. stutzeri* isolate.

The sulfate ion had no effect on either selenite or selenate reduction by *P. stutzeri* (Tables 2 and 3). Higher concentrations of sulfite and chromate inhibited both growth and selenium reduction (Tables 2 and 3). The molybdate ion had little effect on selenite or selenate reduction. The molybdate and chromate anions, which are known to inhibit the reduction of sulfur oxyanions, did not completely inhibit selenate reduction in our studies. Oremland et al. (9) concluded in their studies that Se and S have different reductive biogeochemical cycles, which is also in agreement with our results. In the studies of Oremland et al. (9, 13), chromate, tungstate, molybdate, nitrite, and nitrate inhibited selenate reduction. They postulated that denitrification and selenate reduction may proceed by similar pathways, because nitrate was found to inhibit selenate reduction (13). However, the reduction of both selenium oxyanions by the *P. stutzeri* isolate was unaffected by either nitrite or nitrate.

TABLE 3. Effect of different anions on selenate reduction by *P. stutzeri*[a]

Anion	Concn (M)	Mean reduction rate (nmol of Se/ml/h) ± SD	Mean OD_{600} after 24 h ± SD
None	0	91.77 ± 2.28	5.08 ± 0.42
SO_4	1×10^{-2}	88.73 ± 2.91	4.98 ± 0.20
	5×10^{-2}	83.04 ± 1.65	4.20 ± 0.40
SO_3	1×10^{-3}	82.66 ± 13.80	5.18 ± 0.10
	1×10^{-2}	0	0.38 ± 0.08
NO_3	1×10^{-2}	90.88 ± 4.56	5.60 ± 0.40
NO_2	1×10^{-2}	90.88 ± 2.41	5.84 ± 1.00
WO_4	1×10^{-4}	0	1.84 ± 0.40
CrO_4	1×10^{-4}	36.71 ± 20.13	3.26 ± 1.00
	1×10^{-3}	0	0.24 ± 0.12
MoO_4	1×10^{-3}	89.62 ± 6.08	5.04 ± 0.40
	1×10^{-2}	73.29 ± 3.42	4.02 ± 0.20

[a] Initial selenium concentration, 2.53 mM.

The inhibition of growth and selenate reduction by 10^{-4} M tungstate is unusual (Table 3). Since no effect of tungstate was observed in the presence of selenite, there seems to be a specific interaction between tungstate and selenate that prevents growth of the organism. Because there appears to be no evidence for dissimilatory selenium reduction in this isolate, the reductive pathway in this case may function as a system for the detoxification of selenium oxyanions.

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