ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 3, pp. 266–273. © Pleiades Publishing, Inc., 2006.
Original Russian Text ©N.V. Grigor'eva, T.F. Kondrat'eva, E.N. Krasil'nikova, G.I. Karavaiko, 2006, published in Mikrobiologiya, 2006, Vol. 75, No. 3, pp. 320–328.

EXPERIMENTAL ARTICLES

Mechanism of Cyanide and Thiocyanate Decomposition by an Association of *Pseudomonas putida* and *Pseudomonas stutzeri* Strains

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Received December 12, 2005

Abstract—The intermediate and terminal products of cyanide and thiocyanate decomposition by individual strains of the genus *Pseudomonas, P. putida* strain 21 and *P. stutzeri* strain 18, and by their association were analyzed. The activity of the enzymes of nitrogen and sulfur metabolism in these strains was compared with that of the collection strains *P. putida* VKM B-2187^T and *P. stutzeri* VKM B-975^T. Upon the introduction of CN^- and SCN^- into cell suspensions of strains 18 and 21 in phosphate buffer (pH 8.8), the production of NH_4^+ was observed. Due to the high rate of their utilization, NH_3, NH_4^+, and CNO^- were absent from the culture liquids of *P. putida* strain 21 and *P. stutzeri* strain 18 grown with CN^- or SCN^-. Both *Pseudomonas* strains decomposed SCN^- via cyanate production. The cyanase activity was 0.75 μmol/(min mg protein) for *P. putida* strain 21 and 1.26 μmol/(min mg protein) for *P. stutzeri* strain 18. The cyanase activity was present in the cells grown with SCN^- but absent in cells grown with NH_4^+. Strain 21 of *P. putida* was a more active CN^- decomposer than strain 18 of *P. stutzeri.* Ammonium and CO_2 were the terminal nitrogen and carbon products of CN^- and SCN^- decomposition. The terminal sulfur products of SCN^- decomposition by *P. stutzeri* strain 18 and *P. putida* strain 21 were thiosulfate and tetrathionate, respectively. The strains utilized the toxic compounds in the anabolism only, as sources of nitrogen (CN^- and SCN^-) and sulfur (SCN^-). The pathway of thiocyanate decomposition by the association of bacteria of the genus *Pseudomonas* is proposed based on the results obtained.

DOI: 10.1134/S0026261706030052

Key words: cyanide, thiocyanate, *Pseudomonas putida, Pseudomonas stutzeri.*

Cyanides and their derivatives are extremely toxic compounds. They originate from both natural and anthropogenic sources. The galvanic, metallurgic, and gold-mining industries, coal gasification, and the production of coke, gas, synthetic fibers, and paints are the main anthropogenic sources of cyanide (CN^-) and thiocyanate (SCN^-). Environmental CN^- and SCN^- pollution caused by industrial activity is presently an urgent issue.

Microorganisms are able to utilize these toxic compounds, using them as sources of energy and sulfur (SCN^-) and/or nitrogen (CN^-, SCN^-) [1–3], employing a variety of pathways for cyanide decomposition [4]. This capacity was reported for bacteria of the genus *Pseudomonas* (*P. fluorescens, P. stutzeri*, and *P. putida*), *Bacillus pumilus*, and *Alcaligenes xylosooxidans* subsp. *denitrificans* [1, 2]. Thiocyanate decomposition was demonstrated for *Thiobacillus thioparus* [5] and other microorganisms: *Paracoccus thiocyanatus, Thioalkalivibrio thiocyanoxidans, Halomonas* sp. [3, 6].

At the Homestake plant (United States), an industrial technology is applied that uses microorganisms to detoxify effluents containing up to 12 mg/l cyanides, up to 100 mg/l thiocyanates, and their complexes with metals [7]. Other known biotechnological applications for CN^- and SCN^- removal are either unable to detoxify both compounds or have low decomposition rates [8, 9].

The goal of the present work was the investigation of the mechanism of cyanide and thiocyanate decomposition by the strains *P. putida* 21 and *P. stutzeri* 18, which comprise a bacterial association capable of CN^- and SCN^- decomposition when both toxicants are

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present in high concentrations, as in industrial waste and pulps.

MATERIALS AND METHODS

Bacterial strains. The strains *Pseudomonas putida* 21 and *P. stutzeri* 18 were isolated from the environments under anthropogenic contamination (soils contaminated with polychlorinated biphenyls and industrial effluent of a gold-enrichment plant). The type strains *P. putida* VKM B2187^T (ATCC 12633), *P. stutzeri* VKM B-975^T (ATCC 17588), and *Acidithiobacillus ferrooxidans* VKM B-458 were used for comparison of the genetic and biochemical characteristics.

Growth media and incubation conditions. The medium for the cultivation of bacteria capable of growth in the presence of CN^- and SCN^- contained (g/l) $K_2HPO_4 \cdot 3H_2O$, 3.0; $MgCl_2 \cdot 6H_2O$, 0.5; $NaHCO_3$, 0.3; $FeCl_3$, 0.01; $CaCl_2 \cdot 6H_2O$, 0.01; NaSCN, 0.5–6.0; KCN, 0.01–0.07; pH 8.8–9.0. Lactate (1.4 g/l), sucrose (2.0 g/l), or ethanol (10 g/l) were used as carbon sources. Ethanol and cyanide were added to the medium after sterilization.

For determination of the activity of cyanase and sulfur metabolism enzymes in the type strains, NaSCN was substituted with 1 g/l NH_4Cl as the nitrogen source; 0.5 g/l $MgSO_4 \cdot 7H_2O$ was used as a source of magnesium and sulfur.

For estimation of growth and determination of the activity of sulfur metabolism enzymes in the strains of the association, 1 g/l NH_4Cl was used as the nitrogen source and elemental sulfur S^0 (1%), $Na_2S_2O_3$ (0.5 g/l), or $MgSO_4 \cdot 7H_2O$ (0.5 g/l) were used as sulfur sources.

Cultivation on CN^--containing media was performed in rubber-stoppered 250-ml Erlenmeyer flasks with 100 ml of the medium at 26–28°C with shaking (160 rpm). Cultivation with thiocyanate was performed in the same flasks with cotton plugs at 28–30°C. The cultivation temperatures were chosen in accordance with the chemical properties of cyanide.

Analytical methods. Bacterial growth was monitored by direct cell count under a LYuMAM I1 light microscope or by measuring OD_{460} of the culture liquid with a KFK-3 spectrophotometer (Russia) in a 3-cm cuvette. Microbial protein content in cell-free extracts was determined by the Lowry method [10]. Excel software package was used to calculate the growth rates.

Thiocyanate was determined colorimetrically as ferrocyanate [11]. Cyanide was determined using Ionomer I-130 equipped with a Kritur 06-27 ion selective electrode (Czech Republic). Sulfite was determined colorimetrically with fuchsin [12]. Trithionate, tetrathionate, and thiosulfate were determined by cyanolysis [13]. Sulfate was determined on a Biotronik LC 500 liquid chromatograph (Czech Republic). Sulfide was determined as ZnS after precipitation with zinc chloride [14]. Ammonium was determined by the phenol-hypochlorite colorimetric method [15]. Cyanate ion was determined as NH_4^+ after acidification to pH 2–3 with 6 N HCl and heating in a boiling water bath for 1 min. Ethanol was determined using an M-3700 gas–liquid chromatograph.

The cyanase activity was determined in cell-free extracts obtained by treating resuspended cells with lysozyme in 0.07 M phosphate buffer (pH 7) for 30 min at 37°C and subsequent treatment with a UZDN-1 ultrasonic disintegrator at 22 kHz in the same buffer (pH 8.1) for 1 min under cooling. The lysate was then centrifuged at 8000 *g* for 10 min. The reaction was initiated by the addition of the extract to 2 mM KCNO with 2 mM HCO_3^-; the formation of NH_3 (NH_4^+) was determined every 15–30 min. Cyanase activity was expressed in μmol NH_4^+/(min mg protein). The pH optimum of the enzyme was determined using 0.07 M phosphate and carbonate buffers.

Bacteria in the late exponential growth phase were used for determination of the activity of the enzymes of sulfur metabolism. The cells were centrifuged for 10 min at 10000 *g* and washed twice with fresh medium without energy sources and then with 0.05 M Tris–HCl buffer (pH 7.4). The cells were sonicated at 22 kHz in the buffer for 3 × min with breaks for cooling. The homogenate was centrifuged for 25 min at 40000 *g*, and the enzymatic activity was determined in the supernatant using a Hitachi 200-20 spectrophotometer (Japan).

Sulfite dehydrogenase (sulfite: cytochrome *c* oxidoreductase, EC 1.8.2.1) and the thiosulfate-oxidizing enzyme (thiosulfate dehydrogenase, EC 1.8.2.2) were determined from ferricyanide reduction monitored at 420 nm or cytochrome *c* reduction in the presence of sulfite or thiosulfate monitored at 550 nm [16]. Adenylyl sulfate reductase (APS reductase, EC 1.8.99.2) was determined by ferricyanide reduction in the presence of sulfite and AMP [17]. Rhodanese (thiosulfate: cyanide sulfotransferase, EC 1.8.1.1) was determined by the rate of thiocyanate production from thiosulfate and cyanide [11]. Sulfur oxygenase (sulfur-oxidizing enzyme or sulfur: dioxygenase, EC 1.13.11.18) was determined by thiosulfate production from sulfur in the presence of reduced glutathione and 2,2'-dipyridyl [18]. Thiosulfate reductase (EC 2.8.1.3) was determined by hydrogen sulfide formation from thiosulfate in the presence of reduced glutathione and dithiothreitol [19]. Hydrogen sulfide was absorbed by filter paper soaked in 60% KOH and then determined by the dimethyl-*para*-phenylenediamine method.

The structure of the chromosomal DNA of the isolates and the type strains was studied by pulsed-field electrophoresis of the fragments obtained by treating native DNA with the *Xba* I restriction endonuclease. The methods of DNA isolation and digestion, as well as the electrophoresis conditions, were identical to those described for the acidophilic gram-negative chemolithoautotrophic bacterium *Acidithiobacillus ferrooxi-*

Table 1. The size (kb; accuracy, 4 kb) of the chromosomal DNA *Xba*I fragments of *Pseudomonas putida* strains B-2187 and 21

Strain 21	Strain B-2187
327	327
–	301
265	265
226	–
–	211
–	200
190	190
180	180
–	167

Note: A dash means the absence of a fragment.

Table 2. The size (kb; accuracy, 4 kb) of the chromosomal DNA *Xba*I fragments of *Pseudomonas stutzeri* strains B-975 and 18

Strain 18	Strain B-975
310	310
–	276
–	235
208	–
200	200
177	177
167	–
148	148
133	–
–	122

Note: A dash means the absence of a fragment.

dans, which, like *Pseudomonas*, has a DNA G+C content of over 50 mol % [20]. Denaturing gel electrophoresis (SDS-PAGE) of total cell polypeptide preparations was performed in 10% gel [21].

RESULTS

Characterization of the bacterial strains comprising the association capable of CN^- and SCN^- decomposition. According to their morphological, physiologo–biochemical, and molecular characteristics, strains 21 of *Pseudomonas putida* and 18 of *P. stutzeri* were previously demonstrated to be close to the type strains *P. putida* (VKM B-2187^T) and *P. stutzeri* (VKM B-975^T) [22]. However, since the strains of the association, unlike the type strains, were able to decompose high concentrations of cyanide and thiocyanate, the chromosome DNA structure of the type strains and of the technogenic mutants was studied by pulsed-field electrophoresis. This study revealed that treatment with *Xba*I restriction endonuclease resulted in different size and number of DNA fragments (Table 1, 2; Figs. 1, 2).

The tables and figures demonstrate that the environmental isolate of *P. putida* lacked the 301-, 211-, 200-, and 167-kb fragments present in the type strain; the 226-kb fragment was new. The *P. stutzeri* isolate lacked the 276-, 235-, and 122-kb fragments; the 208-, 167-, and 133-kb fragments were new (absent in the type strain). The *P. putida* and *P. stutzeri* strains differed in the number and size of the chromosomal DNA fragments.

These characteristics of the chromosome structure of the new *P. putida* and *P. stutzeri* strains indicate their variability in the natural toxicant-polluted environments (i.e., their microevolution) and can be used to control the culture purity under experimental conditions and to monitor the organisms in technological processes.

Growth of an association of the *P. stutzeri* strain 18 and *P. putida* strain 21 in synthetic medium with CN^-. The dynamics of cyanide decomposition by the association of the pseudomonad strains 18 and 21 was studied in batch culture on synthetic medium. The time of complete cyanide decomposition by the heterotrophic bacterial association depended on the initial cyanide concentration. For example, in typical experiments with batch culture on synthetic medium with lactate, complete decomposition of 30 and 40 mg/l cyanide occurred after 30 and 40 h, respectively (Fig. 3). The maximal initial decomposition rate was 2.0 mg/(l h). The maximal growth rate of bacteria decreased with increasing cyanide concentration from 0.07 h^{-1} at 30 mg CN^-/l to 0.02 h^{-1} at 53 mg CN^-/l. The final biomass yield correlated with the growth rate and was 0.7 and 0.3 OD units at μ 0.07 and 0.02 h^{-1}, respectively.

The presence of alternative nitrogen sources in the medium (NH_4^+, NO_3^-) prevented CN^- decomposition.

Ammonium formation was detected in the cell suspension of strains 18 and 21 in phosphate buffer without carbon sources after the addition of CN^-; formate production was not found. In accordance with the presently known pathways of cyanide decomposition [4], it can be concluded that NH_3 and CO_2 were the terminal products of decomposition and cyanide dioxygenase catalyzed this reaction.

Growth of the *P. stutzeri* strain 18 and *P. putida* strain 21 in synthetic medium with SCN^-. When *P. stutzeri* strain 18 and *P. putida* strain 21 were grown in batch culture on a synthetic medium with thiocyanate as the nitrogen and sulfur source and ethanol at pH 8.5–8.8, the thiocyanate/ethanol molar ratios for these strains were 1 : 3.4 and 1 : 5, respectively. In the process of SCN^- decomposition, NH_3, NH_4^+, and CNO^- were not detected in the culture liquid in any growth

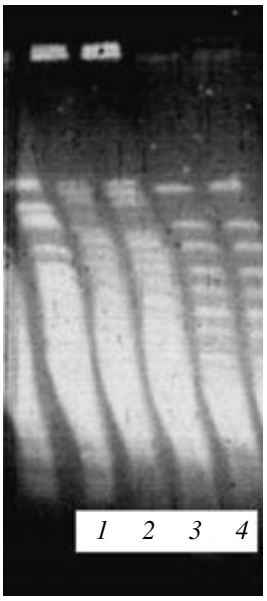

Fig. 1. *Xba*I restriction patterns of the chromosomal DNA of strains 21 (lane *1*), *P. putida* VKM B-2187 (lanes *2* and *3*), and the reference strain *Acidithiobacillus ferrooxidans* VKM B-458 (lane 4).

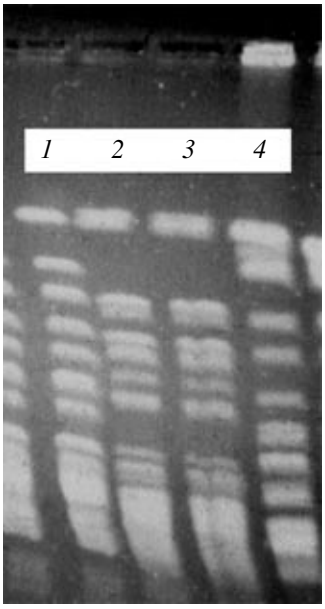

Fig. 2. *Xba*I restriction patterns of the chromosomal DNA of the reference strain *Acidithiobacillus ferrooxidans* VKM B-458 (lane *1*), strain 18 (lanes *2* and *3*) and strain VKM B-975 *P. stutzeri* (lane *4*).

phase. When washed cells of strain 18 and strain 21 were resuspended in phosphate buffer (pH 8.1) without carbon sources, NH_4^+ formation was detected upon the addition of SCN^-. The presence of other nitrogen sources in the medium (NH_3, NO_3^-) inhibited thiocyanate consumption.

The optimal pH value for culture growth and thiocyanate decomposition on synthetic medium was 8.5–8.7 for *P. stutzeri* and 8.4–8.5 for *P. putida*; both cultures were also capable of thiocyanate consumption at a higher pH 9.0–9.2.

At all initial SCN^- concentrations, its utilization as a nitrogen source by the strains of *P. stutzeri* and *P. putida* led to the accumulation of thiosulfate as the terminal product of sulfur oxidation in the exponential and early stationary growth phases (Figs. 4, 5). Thiosulfate was oxidized later in the stationary phase of *P. putida* 21 growth (Fig. 5). Tetrathionate and trithionate were detected in the medium at the end of the stationary growth phase (48 h); they were the terminal products of decomposition of the sulfur moiety of thiocyanate by *P. putida* (Fig. 6).

Sulfate, sulfite, tetrathionate, and trithionate were not detected in the medium as terminal products of thiocyanate oxidation by the *P. stutzeri* strain.

Unlike the strains of the association, the type strains (*P. stutzeri* B-975 and *P. putida* B-2187) grown on synthetic media with various carbon sources (glucose, lactate, or ethanol) were unable to decompose thiocyanate and to utilize it as a nitrogen source.

Cyanase activity of the association of *P. putida* 21 and *P. stutzeri* 18. Thiocyanate decomposition by the *P. putida* and *P. stutzeri* strains was expected to occur via the cyanate pathway ($SCN \longrightarrow CNO \longrightarrow NH_3$). The activity of cyanase, the enzyme decomposing cyanate, to ammonium and carbon dioxide, was therefore determined.

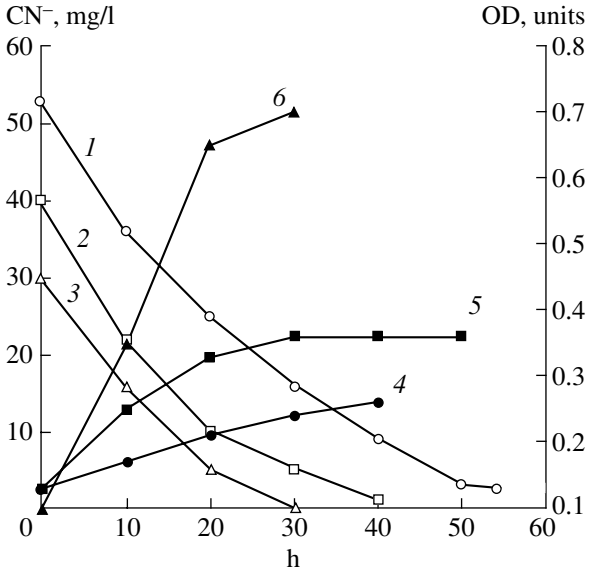

Fig. 3. Growth of the heterotrophic bacterial association on synthetic medium with lactate at initial CN^- concentrations of (*4*) 53, (*5*) 40, and (*6*) 30 mg/l and decomposition of CN^- at its initial concentrations of (*1*) 53, (*2*) 40, and (*3*) 30 mg/l.

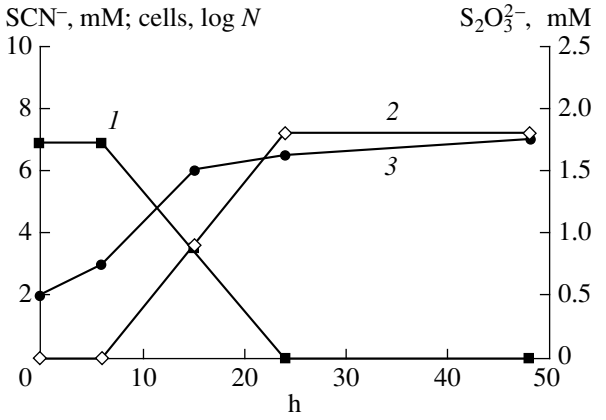

Fig. 4. Thiocyanate decomposition and thiosulfate formation by *P. stutzeri* 18 on synthetic medium with ethanol: *1*, SCN^-; *2*, $S_2O_3^{2-}$; *3*, cell number.

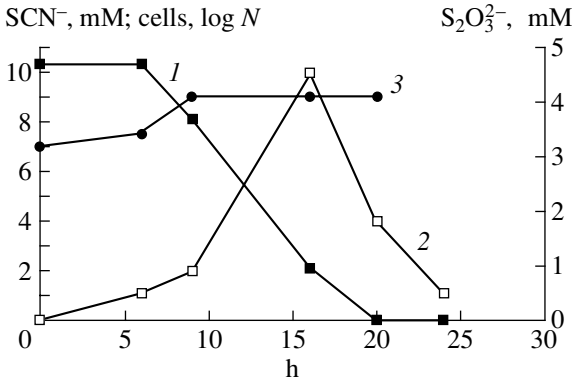

Fig. 5. Thiocyanate decomposition and thiosulfate formation by *P. putida* 21 on synthetic medium with ethanol: *1*, SCN^-; *2*, *2* – $S_2O_3^{2-}$; *3*, cell number.

The cells of *P. putida* and *P. stutzeri* were grown with thiocyanate (0.5 g/l) until the late exponential growth phase. Cyanase activity was determined in cell-free extracts in a phosphate or carbonate buffer at different pH values (7.0, 8.1, and 9.1) in the presence of 2 mM HCO_3^-. Both strains exhibited the highest cyanase activity at pH 8.1 (phosphate buffer); it was 1.26 μmol/(min mg protein) for *P. stutzeri* 18 and 0.75 μmol/(min mg protein) for *P. putida* 21. The enzyme was inactive at pH 7.0; at pH 9.1, its activity was 15.8–16.0% of the maximum for both strains. No cyanase activity was found in the strains of the association grown on the medium with ammonium; this finding confirms the inducible synthesis of the enzyme. Denaturing gel electrophoresis of the total protein of cells grown with different nitrogen sources (NH_4^+, CNO^-, SCN^-) revealed new bands on media with CNO^- (26.5 and 25 kDa) and with SCN^- (22.2 kDa); their existence correlated with the cyanase activity and thiocyanate hydrolysis by strain 18 (Fig. 7).

Activity of the enzymes of sulfur metabolism in *P. putida* strain 21 and *P. stutzeri* strain 18. The activity of the enzymes of sulfur metabolism that might be involved in thiocyanate metabolism of the strains *P. putida* 21 and *P. stutzeri* 18 was studied. The enzymes from late-exponential-phase cells grown on synthetic medium with thiocyanate and ethanol were studied. The cells grown in the medium without sulfur sources and with SCN^- replaced by an equimolar amount of NH_4Cl as the nitrogen source were used as control. No additional nitrogen or sulfur sources were introduced into the SCN^--containing medium.

The following enzymes of the metabolism of inorganic sulfur compounds were detected in thiocyanate-grown bacteria: thiosulfate dehydrogenase, rhodanese, sulfite dehydrogenase, and sulfur oxygenase. No activity of APS reductase or thiosulfate reductase was detected in these experiments. The activities of rhodanese, sulfite dehydrogenase, and sulfur oxygenase were similar in the two strains (Table 3). The thiosulfate dehydrogenase activity in *P. putida* 21 was 4.5 times higher than in *P. stutzeri* 18.

Only rhodanese and sulfite dehydrogenase activities of 5.0 and 5.2 nmol/(min mg protein), respectively, were detected in cells grown without sulfur sources.

Thus, the strains of the *putida* 18 and *P. putida* 21 association differed in the activity of the enzymes of thiosulfate metabolism.

It was found that the activities of the enzymes of sulfur metabolism in the type strains *P. putida* B-2187 and *P. stutzeri* B-975 were close to those of *P. putida* 21 and *P. stutzeri* 18 on the medium with sulfate and NH_4^+.

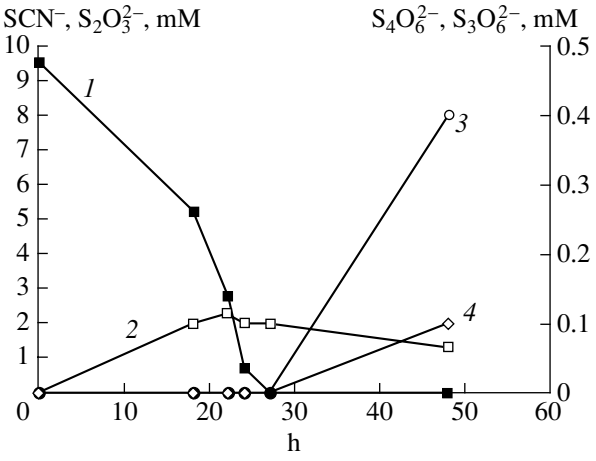

Fig. 6. Thiocyanate decomposition and polythionate formation by *P. putida* 21 on synthetic medium with ethanol: *1*, thiocyanate SCN^-; *2*, thiosulfate $S_2O_3^{2-}$; *3*, tetrathionate $S_4O_6^{2-}$; *4*, trithionate $S_3O_6^{2-}$.

DISCUSSION

Since cyanide and thiocyanate are constantly produced in nature, the problem of their removal from industrial waste was bound to have an environmental solution. Cyanogenic bacteria (*Chromobacterium violaceum, Pseudomonas aeruginosa*), algae (*Chlorella vulgaris*, *Nostoc muscorum*), pathogenic fungi (*Marasmius oreandes*, *Stemphyllium loti*, *Gloeocercospora sorhii*, *Snon moulds*), and higher plants (lima beans, almond, cassava) are among the natural sources of cyanides [4]. Cyanogenic microorganisms are known to possess the enzyme systems for detoxification. The most common pathways of cyanide detoxication are either transformation to cyanate by cyanide monooxygenase with the subsequent cyanate hydrolysis to ammonium and carbon dioxide by cyanase [4], or direct cyanide hydrolysis to ammonium and carbon dioxide (by cyanide dioxygenase) or to ammonium and formate (cyanidase) [6]. These decomposition pathways were reported for representatives of the genera *Pseudomonas, Bacillus*, and *Alcaligenes*.

Thiocyanates in the environment are usually a result of the interaction between cyanide and sulfur compounds (polysulfides, polythionates). Two pathways of microbial thiocyanate decomposition are known [23]. The first of these pathways, which involves the formation of the carbonyl sulfide COS as an intermediate product, is known only for autotrophic bacteria. The existence of a specific enzyme, thiocyanate hydrolase, was shown, which performs thiocyanate hydrolysis to COS and NH_3; sulfide is further oxidized to sulfate.

The second pathway is characteristic of both autotrophic (*Paracoccus thiocyanatus, Thioalkalivibrio thiocyanoxidans*) and heterotrophic bacteria (*Pseudomonas putida, Halomonas sp.*) [3, 6]. In this pathway, cyanate is formed as an intermediate product. The S–C bond of $S–C{\equiv}N^-$ is initially affected (the enzyme has not been isolated), with the formation of CNO^- and HS^-. Autotrophic bacteria can utilize sulfide as an electron donor and energy source; cyanate is converted to NH_3 and CO_2 by cyanase. Ammonium is then utilized as a nitrogen source.

Heterotrophic bacteria that hydrolyze thiocyanate via cyanate utilize it as a source of nitrogen and sulfur.

The sulfur not involved anabolism was reported to be converted to sulfate [24]. However, depending on the amount of oxygen, other sulfur-containing terminal products are also possible:

$$SCN^- + 2H_2O + 2O_2 = CO_2 + SO_4^{2-} + NH_4^+,$$

$$SCN^- + 3H_2O + 1/2O_2 = CO_2 + S^0 + NH_4^+ + 2OH^-,$$

$$SCN^- + 2H_2O = CO_2 + S^{2-} + NH_4^+ \text{ (anaerobic conditions).}$$

In the present work, strains capable of decomposing CN^- and SCN^- at their high concentrations were isolated from an anthropogenically polluted environment. The strains resistant to 10–50 mg/l cyanide were selected by treatment of soil and industrial waste samples with 0.01% KCN solution. The screening resulted in the isolation of an association of two strains (*P. putida* strain 21 and *P. stutzeri* strain 18). These strains were able to perform CN^- and SCN^- decomposition at high concentrations independently and in association, in solutions and in dense pulps. In the course of CN^- and SCN^- decomposition under different conditions (synthetic media, industrial pulps and effluents), microscopy and plating revealed the prevalence of *P. putida* 21 during cyanide decomposition (95% of the total cell number). During thiocyanate decomposition, *P. stutzeri* 18 prevailed (80%) [25]. The strains utilized these compounds as sources of nitrogen (CN^-, SCN^-) and sulfur (SCN^-). On liquid synthetic media (pH 9.2–9.4), the strains decomposed cyanide at concentrations of 70 mg/l or less. If both cyanide and thiocyanate were

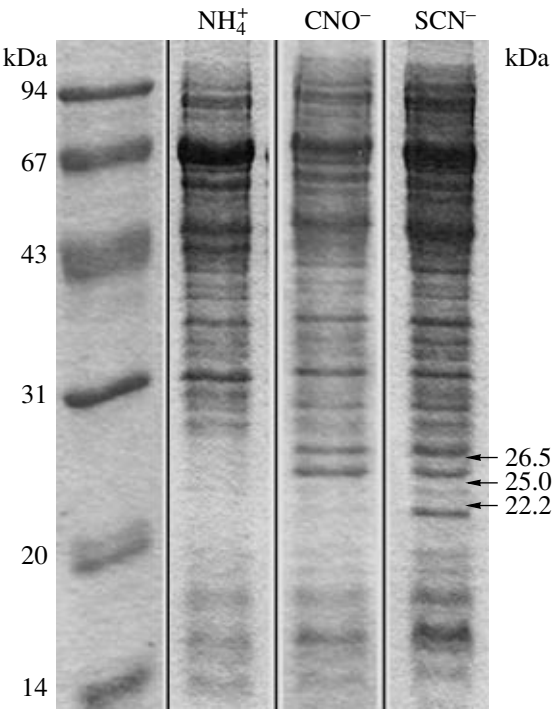

Fig. 7. Denaturing gel electrophoresis of total proteins of *P. stutzeri* 18 after growth on media with different nitrogen sources (NH_4Cl, KCNO, KSCN).

Table 3. Activity (nmol/(min mg protein)) of the enzymes of sulfur metabolism in *P. stutzeri* strain 18 and *P. putida* strain 21 grown on synthetic medium with thiocyanate and ethanol, pH 8.8

Enzymes	Strain 18	Strain 21
Rhodanese, EC 1.8.1.1	8.6	8.7
Thiosulfate dehydrogenase, EC 1.8.2.2	13.0	60.0
Sulfite dehydrogenase, EC 1.8.2.1	37.5	37.7
Sulfur oxygenase, EC 1.13.11.18	0.03	0.23

present in the medium, the bacterial association initially decomposed cyanide; thiocyanate decomposition commenced afterwards. This was either the result of higher availability of cyanide as the nitrogen source or of inhibition of thiocyanate decomposition by cyanide. Unlike the known pathways of thiocyanate decomposition, the investigated heterotrophic bacterial association did not form sulfate as the terminal product. In the course of thiocyanate decomposition by strains *P. putida* 21 and *P. stutzeri* 18, we detected thiosulfate either as an intermediate product of *P. putida* 21 or as a terminal product of *P. stutzeri* 18. In the case of strain 21, polythionates (tetrathionate and trithionate) were the terminal products of the thiocyanate sulfur. Thiosulfate accumulation in the medium caused a decrease in the rate of SCN^- decomposition by *P. stutzeri* strain 18. The relations between the two strains in the association were most probably mutualistic.

Inhibition of enzymatic tetrathionate oxidation to sulfate by thiocyanate was previously supposed to occur [26]. We found that thiosulfate oxidation to tetrathionate occurred during the stationary growth phase, when thiocyanate was present in trace amounts (1 mM). The terminal products of SCN^- decomposition were revealed at the end of the stationary growth phase, when no thiocyanate was present in the medium and enzyme inhibition by thiocyanate could be ruled out.

Both strains decomposed SCN^- via the cyanate pathway. The presence of an inducible cyanase in both strains and the differences in the protein profiles of *P. stutzeri* 18 cells grown with different nitrogen sources (NH_4^+, CNO^-, SCN^-) confirmed this fact indirectly. The new specific bands corresponding to 26.5- and 25.0-kDa proteins appeared on media with cyanate, but not on the medium with ammonium. These bands were also present on the medium with thiocyanate; in this case, however, an additional band corresponding to a 22.2-kDa protein appeared.

The pathways of cyanide and thiocyanate decomposition by the strains isolated can be presented as follows:

$$CN^- + H_2O \longrightarrow NH_3 + CO_2 \text{ (}P.\ putida\text{ strain 21, }P.\ stutzeri\text{ strain 18)}$$

$$SCN^- + H_2O \rightarrow HS^- + CNO^- \xrightarrow{\text{cyanase}} NH_3 + CO_2 \text{ (}P.\ stutzeri\text{ strain18, }P.\ putida\text{ strain 21)}$$

$$\downarrow$$

$$S_2O_3^{2-} \text{ (}P.\ stutzeri\text{ strain 18, }P.\ putida\text{ strain 21)}$$

$$\downarrow \text{ thiosulfate dehydrogenase}$$

$$S_3O_6^{2-} \leftarrow\cdots S_4O_6^{2-} \text{ (}P.\ putida\text{ strain 21).}$$

Strain *P. putida* 21 was unable to perform oxidation to sulfate; the thiocyanate sulfur was oxidized to tetrathionate (trithionate was produced via alkaline hydrolysis of tetrathionate). Strain *P. stutzeri* 18 performed oxidation to thiosulfate. No ability to decompose SCN^- was found in the type strains of *P. stutzeri* and *P. putida*, probably because of the inactivity of the required enzymes (primary hydrolases).

Since the organisms of one species do not always share the same physiological capacities, a detailed study of the characteristics of strains is required for the biotechnological application of microbial decomposers of various compounds. This research was necessary for the development of the technology for detoxification of cyanide- and thiocyanate-containing industrial effluents [25].

ACKNOWLEDGMENTS

The work was supported by the State Program for Science and Technology, grant no. 43.073.1.1.2515.

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