The utilization of thiocyanate as a nitrogen source by a heterotrophic bacterium: the degradative pathway involves formation of ammonia and tetrathionate

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A Gram-negative soil bacterium (isolate 26B) has been shown to utilize up to 100 mM thiocyanate as a source of nitrogen when supplied with glucose as the source of carbon and energy. During growth of isolate 26B with thiocyanate as the source of nitrogen, no ammonia, nitrate, nitrite, cyanide, cyanate, sulfate, sulfite, sulfide or carbonyl sulfide was detected in the growth medium. Growth of the bacterium on ^{14}C -labelled thiocyanate (1.6 μCi) and glucose, yielded ^{14}C -labelled carbon dioxide (0.9 μCi). The addition of 2.9 mM thiocyanate to a bacterial suspension in phosphate buffer (50 mM, pH 7.4) resulted in the utilization of 2.1 mM thiocyanate and the production of 2.0 mM ammonia. This activity was inducible and only occurred after growth of the bacterium with thiocyanate as the source of nitrogen. Tetrathionate (0.7 mM) was detected in the medium after the utilization of thiocyanate (2.4 mM) by a suspension of the bacterium in phosphate buffer, and thiosulfate (1.0 mM) was detected as an intermediate. The addition of sulfide or thiosulfate to the bacterial suspension also resulted in the formation of tetrathionate. The utilization of both of these compounds appeared to be constitutive. A pathway for thiocyanate utilization by isolate 26B is proposed which involves the hydrolysis of thiocyanate to produce cyanate and sulfide. The cyanate then undergoes further hydrolysis to form ammonia and carbon dioxide. The sulfide is ultimately oxidized to tetrathionate via a pathway which includes thiosulfate.

Keywords: thiocyanate, heterotrophic utilization, tetrathionate

INTRODUCTION

Thiocyanate is a toxic anion which arises from a diverse range of natural and industrial sources. In both animals and plants it occurs as the non-functional detoxification product from cyanide ingestion, or as a defence mechanism against microbial infection (Wood, 1975; Westley, 1981). Industrial sources of thiocyanate-containing effluents are widespread, but one of the major sources of such wastes is the carbonization of coal to produce coke. Cyanide reacts readily with sulfur to produce thiocyanate, and any industry with cyanide in its waste is a potential source of thiocyanate contamination. Such industries include steel manufacture, metal mining and electroplating.

Although thiocyanate is less toxic than cyanide, thio-

cyanate-containing wastes are produced in vast quantities. Thiocyanate must either be removed totally from effluents or have its concentration greatly reduced in wastes prior to their disposal as it can inhibit the degradation of other pollutants present in the waste and has a detrimental effect on aquatic flora and fauna.

Little is known about the microbial degradation of thiocyanate. Few isolates with the ability to utilize it have been described, and the biochemical pathways involved in thiocyanate degradation are poorly understood. The majority of organisms that have been studied were originally isolated from sludges which had previously been exposed to thiocyanate-containing wastes (Betts *et al.*, 1979).

Thiocyanate can support the autotrophic growth of some micro-organisms which utilize it as the sole source of

carbon and energy. *Thiobacillus thiocyanoxidans* (now reclassified as *T. thioparus*) was isolated from coke oven liquor and was reported to use thiocyanate as both a carbon and nitrogen source (Happold & Key, 1937). Another autotrophic bacterium, *Thiobacillus thioparus* strain TK-21, can utilize up to 50 mM thiocyanate (Katayama & Kuraishi, 1978). A consortium of *Bacillus* and *Pseudomonas* species has also been shown to utilize thiocyanate autotrophically (Paruchuri *et al.*, 1990).

Other bacteria, while not able to use thiocyanate as an energy source, have been reported to utilize it during heterotrophic growth. A *Pseudomonas stutzeri*-like organism (Stafford & Calley, 1969) and an *Arthrobacter* species (Betts *et al.*, 1979) can utilize thiocyanate as a source of nitrogen, whereas a strain of *Neisseria meningitidis* can utilize it as the sole source of sulfur (Port *et al.*, 1984).

The biochemical pathways involved during the autotrophic utilization of thiocyanate are not known. Youatt (1954) suggested a possible pathway for the utilization of thiocyanate by *T. thiocyanoxidans* in which thiocyanate is hydrolysed to produce cyanate and sulfide. The cyanate undergoes further hydrolysis to form ammonia and carbon dioxide, a step that involves the enzyme cyanase (EC 4.3.99.1). The sulfide produced is oxidized to form sulfate. It has been suggested that the oxidation of sulfide to sulfate is the only energy-yielding step allowing autotrophic growth (Kelly & Baker, 1990).

Recently, a second pathway for thiocyanate utilization by *T. thioparus* has been proposed which involves a thiocyanate hydrolase enzyme that produces carbonyl sulfide and ammonia (Katayama *et al.*, 1992). This hypothesis is supported by evidence that thiocyanate breakdown in the environment can yield carbonyl sulfide. Investigations by Banwart & Bremner (1976) showed that carbonyl sulfide was released when thiocyanate was incubated with soils and manures.

A pathway for thiocyanate utilization by heterotrophic micro-organisms has not been proposed. The present investigation concerns the utilization of thiocyanate by a novel heterotrophic soil isolate (26B). This bacterium has been shown to utilize unusually high concentrations of thiocyanate as a source of nitrogen when supplied with a carbon and energy source such as glucose (Dias, 1993). A potential pathway is presented for the utilization of thiocyanate by the isolate, which differs from the above pathways for thiocyanate utilization by autotrophic bacteria.

METHODS

Isolation of the bacterial isolate (26B). The bacterium, a Gram-negative short rod, was isolated from woodland soil by enrichment culture (Dias, 1993).

Growth of bacterial isolate 26B. Growth of isolate 26B was in a liquid medium consisting of M9 salts (Miller, 1972), 0.1% (v/v) trace elements (Bauchop & Elsdon, 1960), 10 mM glucose as the source of carbon and energy, and 3 mM KSCN (or NH_4Cl where indicated) as the source of nitrogen. Growth was in

250 ml conical flasks containing 100 ml medium or 2 l conical flasks containing 800 ml medium, incubated at 30 °C on an orbital shaker (200 r.p.m.). Growth was monitored by the optical density at 610 nm. Growth in sulfur-free mineral salts medium was as above but all sulfate salts were replaced by chloride salts.

Preparation of bacterial cell suspensions. Isolate 26B (800 ml) was grown to the mid-exponential phase of growth (OD_{610} 0.8). Bacteria were harvested by centrifugation at 15 000 g for 15 min at 4 °C. The cells were washed twice in phosphate buffer (pH 7.4, 50 mM), and finally resuspended in 90 ml of the same buffer. The resuspended cells were divided into 3 × 30 ml aliquots in 100 ml conical flasks, and 3 mM KSCN (or other compound under investigation, as indicated) was added. The flasks were incubated at 30 °C on an orbital shaker (200 r.p.m.).

Chemical analyses. At specified time intervals, either 2 or 3 ml medium was aseptically removed from the sample flasks. Bacteria were removed by centrifugation and the cell-free medium was assayed. Glucose was assayed using the Glucose GOD-PERID diagnostic reagents (Boehringer Mannheim). Thiocyanate was assayed by the method of Stafford & Calley (1969). Ammonia was determined by the assay of Fawcett & Scott (1960). Thiosulfate and tetrathionate were determined by the cyanolysis method of Kelly *et al.* (1969). Sulfate was determined by the method of Rand *et al.* (1975). Sulfide and sulfite were assayed by the method described by Truper & Schlegel (1964), and elemental sulfur by the method of Schedel & Truper (1980). Carbonyl sulfide was detected using a carbonyl sulfide analysis tube with a sensitivity of 2 to 200 p.p.m.

Analysis of the production of carbon dioxide from ^{14}C -labelled thiocyanate. Isolate 26B was grown in 250 ml conical flasks containing a centre well. The flasks contained 100 ml medium with 10 mM glucose, 3 mM KSCN and 1.6 μCi (59.2 kBq) ^{14}C -labelled KSCN. The centre well of the flasks contained 1 M NaOH (10 ml) as a CO_2 trap. The flasks were sealed with rubber septa and incubated at 30 °C, 200 r.p.m. At the end of the specified incubation period, 5 M HCl (5 ml) was added to the medium in the flasks to terminate biochemical activity and to release dissolved carbon dioxide. The flasks were shaken on the orbital shaker for a further 3 h to allow all of the CO_2 produced to enter the alkali trap. Aliquots (1 ml) of both the medium and the trap were added to 4 ml Optiphase 'Hisafe' 3 scintillation fluid and the radioactivity was measured in a liquid scintillation counter (Pharmacia, WALLAC 1410).

Chemicals. All chemicals were supplied by Sigma, except ^{14}C -labelled KSCN, which was supplied by Amersham, and Optiphase Scintillation fluid, which was supplied by LKB Scintillation Products. Carbonyl sulfide analysis tubes were supplied by GAS TEK.

RESULTS

Taxonomy and identification of isolate 26B

The novel soil bacterium 26B is a Gram-negative short rod that shows positive results to the catalase and oxidase tests. The colonies on solid medium are round, entire, smooth, shiny and convex (Dias, 1993). The metabolism of carbohydrates by isolate 26B was determined by measuring growth in an API 50 CH system incubated at 30 °C for 48 h. The organism oxidized 16 of the 48 carbohydrates tested including glycerol, arabinose, galactose, glucose, fructose, mannose, mannitol, maltose,

Fig. 1. Growth (○) of isolate 26B in mineral salts liquid medium with 9 mM glucose (●) as the carbon and energy source and 3 mM thiocyanate (□) as the nitrogen source. Growth was in 100 ml medium in 250 ml conical flasks at 30 °C, 200 r.p.m. No ammonia or sulfate was detected in the medium.

Fig. 2. Ammonia formation (■) from thiocyanate (□) by a bacterial suspension in phosphate buffer (50 mM, pH 7.4). Bacteria were grown in liquid medium with 10 mM glucose and 3 mM thiocyanate as described in Methods. Cells were harvested at mid-exponential growth, washed in phosphate buffer (50 mM, pH 7.4) and resuspended to 10% of the original volume in the same buffer. The bacterial suspension (30 ml) was incubated with 2.9 mM thiocyanate in 150 ml conical flasks at 30 °C, 200 r.p.m. Error bars represent the standard deviation ($n-1$) between three replicate samples.

fucose and gluconate. The bacterium was also analysed using an API 20E test system, a miniaturized assay containing 23 standard biochemical tests for the identification of *Enterobacteriaceae* and other Gram-negative bacteria. Isolate 26B showed positive results for arginine dehydrogenase, citrate utilization, and urease, but negative results for lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and metabolism, Voges-Proskauer and gelatin liquefaction. The API profile of the isolate identified it as a *Pseudomonas fluorescens*. However, this result seemed in doubt because the bacterium was non-motile, did not produce a fluorescent pigment and its morphology was atypical of a fluorescent *Pseudomonas*

species. The isolate was therefore sent to the National Collection of Industrial and Marine Bacteria, Aberdeen, UK (NCIMB) for identification, but after five stages of tests including gas chromatography of the fatty acid profile, the bacterium could not be confidently placed under any of the common bacterial genera (Dias, 1993). The major acids present in isolate 26B were hexadecanoic acid ($C_{16:0}$ 23.9%), hexadecanoic acid ($C_{16:1}$ 31%), octadecanoic acid ($C_{18:1}$ 12%) and possibly a cyclopropane acid ($C_{17:0}$ 15%).

Growth of 26B on thiocyanate

Isolate 26B was able to utilize thiocyanate as the sole source of nitrogen when supplied with glucose as the source of carbon and energy. Figure 1 shows the growth of isolate 26B on 9 mM glucose and 3 mM KSCN. Thiocyanate and glucose utilization occurred throughout growth of the bacterium after a short lag phase. On 3 mM thiocyanate, isolate 26B had a doubling time of 2.5 h. The bacterium could utilize concentrations of thiocyanate of up to 100 mM. No ammonia, nitrate, nitrite, cyanide or cyanate production was detected in the growth medium, no detectable odorous (sulfurous) gases, including carbonyl sulfide, were produced, and there was no appreciable change in pH of the growth medium.

Isolate 26B was also able to utilize thiocyanate as the source of nitrogen and sulfur, or as the sole source of sulfur when supplied with 3 mM ammonia as the source of nitrogen, but with a longer lag phase of up to 30 h. Thiocyanate was utilized throughout the growth of the bacterium and the doubling time was 8.8 h. Again, no ammonia, carbonyl sulfide, cyanide, cyanate or sulfate were detected in the growth medium.

Formation of ammonia from thiocyanate

Unlike the growing bacteria, when thiocyanate was added to a suspension, in phosphate buffer (50 mM, pH 7.4), of isolate 26B which had originally been grown with 3 mM thiocyanate as the source of nitrogen and harvested at the mid-exponential phase of growth ($OD_{610} = 0.8$), ammonia was produced (Fig. 2). The conversion of thiocyanate to ammonia was complete after approximately 4 h and 2.0 mM ammonia was produced from the utilization of 2.1 mM thiocyanate. However, when thiocyanate was added to a suspension of isolate 26B in phosphate buffer (50 mM, pH 7.4) which had originally been grown with 3 mM ammonia as the source of nitrogen, no thiocyanate utilization occurred and no ammonia formation was detected.

Formation of carbon dioxide from thiocyanate

The formation of carbon dioxide from thiocyanate was followed by the addition of ^{14}C -labelled KSCN to the thiocyanate (3 mM) in the growth medium as the source of nitrogen. Throughout growth of the bacterium, radioactivity in the medium decreased at a rate which paralleled the rate of thiocyanate utilization (Fig. 3). The

Fig. 3. Formation of ^{14}C -labelled carbon dioxide during growth ($\circ$) of isolate 26B in liquid medium on 10 mM glucose and 3 mM thiocyanate ($\square$) with ^{14}C -labelled thiocyanate added, as described in Methods. ^{14}C activity was monitored in cell-free medium ($\triangle$), in the sodium hydroxide trap ($\blacktriangle$) and in the bacterial cells (∇). Error bars represent SD ($n-1$) between three replicate samples.

Fig. 4. Thiocyanate ($\square$) utilization and tetrathionate ($\triangle$) and ammonia ($\blacksquare$) formation by a bacterial suspension in phosphate buffer (50 mM, pH 7.4). The bacterial suspension was prepared and incubated as indicated for Fig. 2. Thiosulfate ($\blacktriangle$) was detected in the medium as an intermediate. Error bars represent SD ($n-1$) between three replicate samples.

radioactivity detected in the alkali trap increased throughout growth, indicating that carbon dioxide was produced from thiocyanate. During growth, the radioactivity in the medium decreased from 1.6 to 0.1 μCi , whereas the amount of radioactivity recovered in the trap was 0.9 μCi . At no time was any ^{14}C detected in association with the washed bacterial cells, nor was it detected as another compound in the medium, suggesting that the major product was gaseous (carbon dioxide).

Formation of tetrathionate from thiocyanate

The utilization of thiocyanate by isolate 26B during growth or when suspended in phosphate buffer did not lead to formation of the sulfur-containing products expected in the medium. Products assayed for included sulfate, sulfide, sulfite, elemental sulfur and carbonyl

Table 1. Rate of utilization of reduced inorganic sulfur compounds by suspensions of isolate 26B

Bacteria were grown in liquid medium with 10 mM glucose and 3 mM thiocyanate or ammonia as nitrogen source, as described in Methods. They were harvested at the mid-exponential phase of growth, washed in phosphate buffer (50 mM, pH 7.4) and resuspended to 10% original volume in the same buffer. The inorganic sulfur compound (3 mM) was added to 30 ml bacterial suspension in 150 ml conical flasks and incubated at 30 °C, 200 r.p.m. At 30 min intervals (10 min intervals for sulfide), 2 or 3 ml samples were removed from the flasks, centrifuged to remove bacteria and the cell-free supernatants were assayed for the sulfur compound and tetrathionate as described in Methods. Sulfide utilization rates have been corrected for auto-oxidation of sulfide.

Sulfur compound	Activity [$\mu\text{mol S cpd removed h}^{-1}$ ($\text{mg cell dry wt}^{-1}$)] of:	
	Thiocyanate-grown cells	Ammonia-grown cells
SCN^-	0.27	0
S^{2-}	28.5	8.9
$\text{S}_2\text{O}_3^{2-}$	0.62	0.82
$\text{S}_4\text{O}_6^{2-}$	0	0

sulfide. The medium was therefore assayed for other sulfur-containing products including thiosulfate and tetrathionate.

Tetrathionate was produced from thiocyanate by thiocyanate-grown suspensions of isolate 26B in phosphate buffer (50 mM, pH 7.4), and thiosulfate (up to 1.0 mM) was detected as an intermediate (Fig. 4). The utilization of 2.4 mM thiocyanate gave rise to 0.7 mM tetrathionate, suggesting that there is a high degree of conversion to tetrathionate (i.e. $4 \times \text{SCN}^- \rightarrow 1 \times \text{S}_4\text{O}_6^{2-}$). Stoichiometric conversion of thiocyanate to tetrathionate was also shown by growing cells of isolate 26B, and tetrathionate was produced from thiocyanate utilized as a source of sulfur by the bacterium.

Utilization of reduced sulfur compounds

The rates of utilization of thiocyanate, thiosulfate, tetrathionate and sulfide by suspensions of bacteria that had originally been grown with either thiocyanate or ammonia as the source of nitrogen are shown in Table 1. Thiocyanate, thiosulfate and sulfide were all converted to tetrathionate by bacterial suspensions in phosphate buffer (50 mM, pH 7.4). Thiocyanate was utilized at a rate of 0.27 $\mu\text{mol thiocyanate h}^{-1}$ ($\text{mg cell dry wt}^{-1}$) by cells that had originally been grown on thiocyanate, but was not utilized by cells originally grown on ammonia. Thiosulfate (3.8 mM) was converted to tetrathionate (2.0 mM) at similar rates of 0.62 and 0.82 $\mu\text{mol thiosulfate h}^{-1}$ ($\text{mg cell dry wt}^{-1}$) by bacteria grown on either thiocyanate or ammonia. The rate of sulfide utilization by thiocyanate grown cells was 28.5 $\mu\text{mol sulfide h}^{-1}$ ($\text{mg cell dry wt}^{-1}$), more than threefold higher than for ammonia-grown

cells: $8.9 \mu\text{mol sulfide h}^{-1} (\text{mg cell dry wt})^{-1}$. A concentration of 0.7 mM tetrathionate was produced from 2.1 mM sulfide by the bacterial suspension and thiosulfate (0.4 mM) was detected as an intermediate.

Tetrathionate was not utilized when added to suspensions of bacteria that had been grown on either thiocyanate or ammonia, although the addition of 5 mM tetrathionate to thiocyanate-grown bacterial suspensions did not inhibit the utilization of 3 mM thiocyanate, thiosulfate or sulfide.

DISCUSSION

Isolate 26B has been shown to utilize thiocyanate during heterotrophic growth as the sole source of either nitrogen and/or sulfur when supplied with a carbon and energy source such as glucose. During the growth of this isolate on thiocyanate as the source of nitrogen none of the compounds (ammonia, cyanate, sulfide, sulfate or carbonyl sulfide) suggested by Youatt (1954) or Katayama *et al.* (1992) as possible products from the utilization of thiocyanate by autotrophs were detected in the growth medium. This was unusual, as Happold *et al.* (1954) showed ammonia formation from thiocyanate by a *Thiobacillus* species and Paruchuri *et al.* (1990) observed an 89% conversion of thiocyanate-nitrogen to ammonia by a microbial consortium utilizing thiocyanate autotrophically. Sulfate was detected in the medium after the growth of a heterotrophic *Pseudomonas stutzeri*-like organism on thiocyanate (Stafford & Calley, 1969) and a 92% conversion of thiocyanate-sulfur to sulfate was shown by the above consortium (Paruchuri *et al.*, 1990).

Unlike growing bacteria, when thiocyanate was added to a bacterial suspension in phosphate buffer (50 mM , $\text{pH } 7.4$) that had originally been grown with 3 mM thiocyanate as the source of nitrogen, ammonia formation was detected. The production of ammonia from thiocyanate was stoichiometric. In contrast, the addition of thiocyanate to a cell suspension that had originally been grown with 3 mM ammonia as the source of nitrogen did not result in any thiocyanate utilization or ammonia formation showing that thiocyanate utilization by isolate 26B is inducible.

Carbon dioxide has also been shown to be a degradation product of thiocyanate by isolate 26B. Only 57% of the initial ^{14}C added to the growth medium in the form of labelled thiocyanate was recovered in the carbon dioxide trap. However, at no time was any ^{14}C detected either associated with washed bacterial cells or as another component of the medium. The reason for the discrepancy in ^{14}C recovery is not clear, but could be due to incomplete carbon dioxide trapping.

The ultimate product of the sulfur moiety of the thiocyanate utilized as a source of nitrogen by this organism is tetrathionate. Tetrathionate was produced from thiocyanate by both growing cells and suspensions of bacteria that had been grown on thiocyanate, and thiosulfate was detected as an intermediate. To our knowledge, there have been no previous reports of tetrathionate production as the final product of thio-

cyanate utilization by bacteria. Sulfate has been suggested (Youatt, 1954; Stafford & Calley, 1969) as the final product, as has carbonyl sulfide (Katayama *et al.*, 1992). Tetrathionate was also produced from thiocyanate utilized as a source of sulfur by the bacterium; however at present it is not known whether the pathway of utilization of thiocyanate as a S-source is the same as the pathway of its utilization as an N-source. The addition of either sulfide or thiosulfate to suspensions of the isolate grown on thiocyanate as the source of nitrogen also resulted in the formation of tetrathionate, suggesting that both compounds could be intermediates in the production of tetrathionate from thiocyanate. Thiosulfate is an intermediate as it was detected in the medium during thiocyanate utilization. A study of the utilization rates of these sulfur compounds by suspensions of the bacterium suggested that thiocyanate utilization is inducible, whereas the ability to oxidize thiosulfate to tetrathionate is constitutive. The conversion of sulfide to tetrathionate is also greater in thiocyanate grown cells than in ammonia grown cells.

It has been suggested that thiosulfate and tetrathionate could be intermediates in the oxidation of thiocyanate to sulfate by an autotrophic consortium (Paruchuri *et al.*, 1990), although this pathway was not demonstrated. Thiosulfate can be utilized by thiocyanate-utilizing organisms (Paruchuri *et al.*, 1990; Happold *et al.*, 1954; Youatt, 1954), and Katayama & Kuraishi (1978) reported that thiosulfate inhibited thiocyanate utilization by *T. thio-parus*. However, in these cases sulfate was suggested as the final product of the sulfur moiety of thiocyanate. The ability to oxidize thiosulfate to sulfate via tetrathionate has been demonstrated in many organisms, especially the thiobacilli. Wood & Kelly (1986) and Lu & Kelly (1988) showed oxidation of thiosulfate to sulfate with the obligatory formation of tetrathionate as an intermediate in *T. tepidarius*. They showed that thiosulfate oxidation to tetrathionate occurs in the periplasm whereas further oxidation of tetrathionate to sulfate occurs after transport to the cytoplasmic side of the membrane. Thiosulfate conversion to tetrathionate without further oxidation to sulfate has been shown to occur in a variety of heterotrophic bacteria (Mason & Kelly, 1988).

The physiological function of tetrathionate formation by isolate 26B is unclear. It would appear that this isolate lacks the enzymes to enable it to further oxidize tetrathionate to sulfate. This could be a membrane transport effect as it has been suggested that thiocyanate inhibits the oxidation of tetrathionate to sulfate in *T. tepidarius* by dissipating the transmembrane electrochemical potential (Lu & Kelly, 1988). The ability of heterotrophic microorganisms to oxidize thiosulfate to tetrathionate has been proposed to have energetic or ecological advantages (Mason & Kelly, 1988).

Thiocyanate degradation by the novel isolate 26B has been previously shown (Stratford *et al.*, 1992) to be associated with a stimulation in the cyanase activity of this organism, suggesting that the degradation pathway is via cyanate (Fig. 5). Thiocyanate is hydrolysed to cyanate and sulfide. The cyanate then undergoes hydrolysis to form

Fig. 5. Proposed pathway for thiocyanate utilization by the heterotrophic bacterium 26B. Thiocyanate is hydrolysed to cyanate and sulfide by an inducible enzyme. Cyanate is hydrolysed further to carbon dioxide and ammonia by cyanase (EC 4.3.99.1). Cyanase activity in isolate 26B is also inducible and has been shown to hydrolyse cyanate under anaerobic conditions. Sulfide is oxidized to produce tetrathionate via the formation of thiosulfate. The ability to convert thiosulfate to tetrathionate is constitutive. No further oxidation of tetrathionate was observed.

ammonia and carbon dioxide. The sulfide is oxidized to produce tetrathionate via thiosulfate. The ability to utilize thiocyanate, and cyanase activity in this bacterium is inducible (Stratford *et al.*, 1992), but the oxidation of thiosulfate appears to be constitutive. This pathway is similar to that proposed by Youatt (1954) in that cyanate is an intermediate; however, tetrathionate is the final product of the sulfur moiety of thiocyanate.

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