**Oxidation of Thiosulfate by a New Bacterium, **Bosea thiooxidans**(strain BI-42) gen. nov., sp. nov.: Analysis of Phylogeny Based on Chemotaxonomy and 16S Ribosomal DNA Sequencing

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A gram-negative bacterium which was capable of oxidizing reduced inorganic sulfur compounds was isolated from agricultural soil and designated BI-42. This new isolate grew on a wide range of organic substrates but was not able to grow autotrophically and lacked ribulose 1,5-bisphosphate carboxylase, a key enzyme of carbon dioxide fixation. These results suggested that strain BI-42 was a chemolithoheterotroph. Ammonia and nitrate were not used as sole nitrogen sources for growth, and strain BI-42 lacked glutamate synthase activity, which resulted in glutamate auxotrophy. The glutamate dehydrogenase activity of this organism was apparently insufficient for ammonia assimilation. On the basis of the results of additional biochemical tests, the G+C content of the DNA, the results of a respiratory ubiquinone analysis, the results of a 16S ribosomal DNA sequence analysis, the fatty acid composition, and the results of a membrane lipid analysis, strain BI-42 was identified as a phylogenetically and physiologically distinct taxon belonging to the alpha subclass of the **Proteobacteria**. **Bosea thiooxidans** gen. nov., sp. nov. is the name proposed for this taxon.

Because of the potential importance of chemolithoheterotrophs in soil, attempts were made to isolate chemolithoheterotrophic bacteria from soil by enrichment culture techniques. A single strain was isolated from agricultural field soil, and this strain was designated BI-42*. Strain BI-42 oxidizes reduced inorganic sulfur compounds only in the presence of some organic substrates. In this paper we describe the isolation of this organism, its morphological and biochemical characteristics, its phylogenetic position based on the results of a 16S ribosomal DNA (rDNA) sequence analysis, its chemotaxonomic properties, and its mode of thiosulfate utilization. In addition, we studied the properties of the nitrogen-metabolizing enzymes of strain BI-42, including glutamine synthetase (GS), glutamate dehydrogenase (GDH), and glutamate synthase (GOGAT), in order to characterize the glutamate auxotrophy of this organism.

**MATERIALS AND METHODS**

**Chemicals.** All of the chemicals used were analytical grade and were purchased from Sigma Chemical Co., St. Louis, Mo., E. Merck, Darmstadt, Germany, and SRL Pvt., Ltd., Bombay, India. Yeast extract, tryptone and agar-agar were obtained from Difco Laboratories, Detroit, Mich., and Dowex-1-chloride (50-100 dry msh) was obtained from Aldrich Chemical, Inc. NaH14CO3 was purchased from Bhabha Atomic Research Centre, Trombay, India.

**Enrichment and isolation.** Soil samples were collected from different agricultural fields around Calcutta, India. The soil samples (25 g) were moistened with 5 ml of sterile distilled water and enriched by adding sodium thiosulfate (100 mg), sodium sulfite (50 mg), or elemental sulfur (25 mg). After thorough mixing, the preparations were incubated in petri dishes at 30°C for 10 days. Further enrichment was carried out in mineral salts broth (pH 8.0) supplemented with phenol red (0.02 g/liter) and either Na2S·9H2O (100 mg), sodium sulfite (50 mg), or elemental sulfur (25 mg). After thorough mixing, the preparations were incubated in petri dishes at 30°C for 10 days. Further enrichment was carried out in mineral salts broth (pH 8.0) supplemented with phenol red (0.02 g/liter) and either Na2S·9H2O or thiosulfate plus yeast extract (5 g/liter). Portions (1 g) of the enriched soil samples were transferred into 250-ml conical flasks containing 50 ml of enrichment medium, and the resulting preparations were inoculated in a gyratory water bath shaker (model G76, New Brunswick) at 220 rpm and 30°C. After 2 days of incubation, one flask turned yellow, indicating that acid was formed. Serial dilutions of the contents of this flask were plated onto mineral salts-thiosulfate agar and onto mineral salts-thiosulfate-yeast extract agar plates. These colonies were purified by repeated streaking on the same medium. Thus, a purified culture was obtained, and this culture was designated strain BI-42.

**Medium and growth conditions.** The mineral salts medium contained (per liter) 4.0 g of NaH2PO4, 1.5 g of KH2PO4, 0.1 g of MgCl2·6H2O, 1.0 g of...
were placed in the culture media to determine whether gas was formed. Whether glutamate, and 0.1 g of yeast extract; the pH of this medium was 8.0. GYM substrates (5 g/liter) or thiosulfate (5 g/liter) or both. Inverted Durham tubes performed as described by Holliday (10). All other routine biochemical tests and procedures were performed as described previously (3, 43).

Utilization of amino acids as sole nitrogen sources or sole nitrogen and carbon sources was determined in mineral salts medium lacking NH₄Cl. Utilization of organic compounds other than amino acids was determined in GYM medium containing substrates at concentrations of 1 to 5 g/liter.

Utilization of inorganic sulfate compounds was determined in GYM medium supplemented with potassium thiosulfate or both thiosulfate and succinate.

Flagellum staining and electron microscopy. The presence of flagella was determined by using cells that were negatively stained with 2% phosphoric acid.

Extraction of respiratory lipooligopeptides and polar lipids. Respiratory lipooligopeptides and polar lipids were extracted from 100-ng portions of freeze-dried cells by using the two-stage method described by Tindall (47, 48). Cells were homogenized in 0.1 M Tris-HCl buffer (pH 8.5), 50 mM NaCl, 1 mM 2-oxoglutarate, 0.1 mM NADH, and the cell extract. The assay was performed at 30°C by measuring the rate of oxidation of NADH at 340 nm with a Shimadzu UV-visible recording spectrophotometer (model UV 240). Similarly, the NADH-dependent GDH activity was measured by replacing NADH with 1 mM NADPH and increasing the concentration of 2-oxoglutarate to 10 mM. After incubation for 3 min at 30°C, the reaction was stopped by adding 6.5 ml of 100 mM NaOH, and the A₅₅₀ was determined. The oxidative deamination of glutamate by GDH was assayed by measuring the formation of NADPH at 340 nm by using an assay mixture containing 100 mM Tris-HCl (pH 8.0), 25 mM glutamate, and 0.5 mM NADP in a final volume of 1 ml.

GOGAT activity was determined by using 2-ketoglutarate and determining glutamine-dependent oxidation of NADPH as described by Meers et al. (27). GS activity was measured by the g-glutamyl transpeptidase method described by Miller et al. (29). In every case, the g-glutamyl transferase activities in the presence and absence of 60 mM MgCl₂ were measured to determine the active state of the enzyme. The reaction mixture was incubated for 10 min at 30°C, and the amount of g-glutamyl hydroylase formed was determined at 553 nm by using a reagent blank for comparison. Specific activity was expressed as the amount of enzyme which catalyzed the formation or disappearance of 1 μmol of product or substrate per min.

The RuBP carboxylase activity in the cell extract was determined as described by Kelly et al. (16). The final volume of the assay mixture, which contained cell extract (0.1 to 0.4 g of protein), was 0.3 ml. The reaction was initiated with RuBP and was terminated after 0.5, 1, 1.5, 2, 3, and 4 min by adding 0.2 ml of acetic acid. Zero-time and RuBP-free controls were included. Each reaction mixture was dried in its vial, 5 ml of scintillation fluid, which contained 10 g of 2,5-di-phenylxazole per liter, 0.25 g of 1,4-bis-(5-phenyl-2-oxazolyl)benzene (POPPOP) per liter, and 100 g of naphthalene per liter in 1,4-dioxane, was added, and 14CO₂ incorporation was determined by a liquid-scintillation counting method. 

Analytical methods. DNA base composition was determined by the thermal denaturation method of Marmur and Doty (25). The growth of cells was monitored by measuring optical density at 660 nm. Protein contents were estimated by using the methods of Lowry et al. (22) and bovine serum albumin as the standard. The cells were sonicated and the supernatant was removed from the plate and analyzed by high-performance liquid chromatography (HPLC). The HPLC analysis was carried out with an LDC analytical HPLC (Thermosteril, Darmstadt, Germany) equipped with a reverse-phase column (2 by 125 mm; 3 μm; RP-18 [Macherey-Nagel]) by using methanol as the eluant. The respiratory lipooligopeptides were detected at 269 nm.

Analysis of polar lipids. Polar lipids were separated by two-dimensional silica gel thin-layer chromatography (catalog no. 805 023; Macherey-Nagel, Düren, Germany), using hexane-tert-butylmethyl ether (9:1, vol/vol) as the solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and analyzed by high-performance liquid chromatography (HPLC). The HPLC analysis was carried out with an LDC analytical HPLC (Thermosteril, Darmstadt, Germany) equipped with a reverse-phase column (2 by 125 mm; 3 μm; RP-18 [Macherey-Nagel]) by using methanol as the eluant. The respiratory lipooligopeptides were detected at 269 nm.

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Analysis of respiratory lipooligopeptides. The respiratory lipooligopeptides were separated into different classes (menaquinones and ubiquinones) by thin-layer chromatography on silica gel thin layers (catalog no. 805 023; Macherey-Nagel, Düren, Germany), using hexane-tert-butylmethyl ether (9:1, vol/vol) as the solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and analyzed by high-performance liquid chromatography (HPLC). The HPLC analysis was carried out with an LDC analytical HPLC (Thermosteril, Darmstadt, Germany) equipped with a reverse-phase column (2 by 125 mm; 3 μm; RP-18 [Macherey-Nagel]) by using methanol as the eluant.

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RESULTS

Morphological and biochemical features of strain BI-42. Colonies of the organism which we isolated grown on Luria agar or on thiosulfate-yeast extract agar were circular, 1 to 1.5 mm in diameter, smooth, mucoid, round, and creamy colored. Single colonies appeared within 4 days at 30°C. The cells stained gram negative and were motile, rod shaped, 0.85 μm wide, and 1.4 to 1.6 μm long. The cells occurred mostly singly, did not form spores, and were motile by means of a single polar flagellum (Fig. 1). Cells grown on GYM medium containing thiosulfate and yeast extract or on mixed-substrate agar containing thiosulfate and succinate did not form sulfur deposits on the colony surfaces. The isolate which we obtained was designated BI-42.

Strain BI-42 did not grow anaerobically in the presence or absence of nitrate with any of the substrates tested. However, under microaerobic conditions, strain BI-42 formed gas in GYM medium containing malate, succinate, glucose, or sucrose. When thiosulfate was added to the media, gas was not produced. The gas produced was not absorbed by NaOH. Thus, the gas was identified as N₂ or N₂O, the products of denitrification. Strain BI-42 exhibited catalase, oxidase, and nitrate reductase activities and could grow on Simmons citrate agar and MacConkey agar. Methyl red, urease, starch hydrolysis, indole production, H₂S production, Voges-Proskauer, gelatin hydrolysis, and pigment production tests were negative. The optimum temperature for growth was 30 to 32°C. The pH range for growth was 6.0 to 9.0, and the optimum pH was 7.5 to 8.0.

Utilization of organic compounds. Glutamate, glutamine, proline, cysteine, aspartic acid, serine, asparagine, alanine, and lysine were used as both nitrogen and carbon sources, while arginine and histidine were used as nitrogen sources. In contrast, leucine, glycine, isoleucine, methionine, tyrosine, tryptophan, and phenylalanine did not support growth.

Strain BI-42 grew heterotrophically in GYM medium supplemented with glucose, fructose, rhamnose, xylose, sorbose, ribose, galactose, citrate, gluconate, acetate, pyruvate, arabinose, succinate, and malate. Glycerol, raffinose, mannitol, formate, lactate, glyoxylate, propionate, salicylate, butyrate, cyclohexanol, p-aminobenzoate, and methanol did not support the growth of this organism.

Growth and oxidation of sulfur compounds. In GYM medium supplemented with thiosulfate the growth yield of strain BI-42 was almost the same as the growth yield in GYM medium alone. No stimulation of growth occurred, although a considerable amount of thiosulfate was consumed (data not shown). However, marked stimulation of growth was observed when GYM medium was supplemented with sodium succinate in addition to thiosulfate. The growth yield varied with the concentration of thiosulfate (Fig. 2); the greatest growth (A₆₆₀ 1.2 to 1.3) was observed at a concentration of 5 g/liter. Under these conditions, conversion of thiosulfate to sulfate was stoichiometric, and the pH of the medium decreased from 8.0 to 6.6 (Fig. 3). Thiosulfate added to the medium was almost
entirely consumed within 36 h. Interestingly, the pH of the medium increased after 36 to 40 h of growth. Like thiosulfate, tetrathionate also stimulated growth in the presence of succinate, but NaSCN, Na$_2$SO$_3$, and Na$_2$SO$_4$ inhibited growth (data not shown).

**CO$_2$ fixation.** No RuBP carboxylase activity was detected in strain BI-42.

**Susceptibility to antibiotics.** The MICs of several antibiotics for strain BI-42 were determined. Growth of this organism was inhibited by 120 µg of streptomycin per ml, 10 µg of tetracycline per ml, 40 µg of neomycin per ml, 20 µg of chloramphenicol per ml, and 15 µg of rifampin, per ml. Even ampicillin did not inhibit growth even at a concentration of 200 µg/ml.

**GS, GDH, and GOGAT activities.** Strain BI-42 cells contained readily detectable levels of GS and GDH (Table 1). Addition of NH$_4$Cl in the exponential phase of growth reduced the GS activity. The GS activity measured in the presence of Mn$^{2+}$ alone was significantly higher than the GS activity measured in the presence of both Mn$^{2+}$ and Mg$^{2+}$. NADP$^+$- and NADH-dependent GDH activities were not detected. However, considerable activity in the assimilatory direction was observed when NADPH was the cofactor. Addition of ammonium chloride did not influence the GDH activity (Table 1). GOGAT activity was not observed in this organism.

**DNA base composition.** The G+C content of the DNA of strain BI-42 was 68.2 mol%.

**Ubiquinones.** Ubiquinone was the sole respiratory quinone present. The major ubiquinone contained 10 isoprenoid units (ubiquinone 10).

**Fatty acid composition.** The cellular fatty acid composition of strain BI-42 is shown in Table 2. The fatty acids identified were the saturated straight-chain acids C$_{12}$:0, C$_{16}$:0, C$_{17}$:0, C$_{18}$:0, and C$_{19}$:0 10-methyl; the unsaturated straight-chain acids C$_{16}$:1 ω7c, C$_{17}$:1 ω9c, and C$_{18}$:1 ω7c; and the nonhydroxy acids C$_{17}$:1 cyclopropane and C$_{19}$:1 ω8c. cis-Octadec-9-enoic acid (C$_{18}$:1 ω7c) was the predominant compound among the other nonhydroxy acids. The major hydroxylated fatty acids found were C$_{15}$:0 3-OH, C$_{16}$:0 3-OH, and C$_{17}$:0 3-OH.

**Polar lipids.** The polar head groups consisted of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, diphosphatidylglycerol, and an unidentified amino lipid.

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## DISCUSSION

A great deal of work has been performed to increase our understanding of the oxidation of inorganic sulfur compounds by obligately or facultatively chemolithotrophic bacteria, especially bacteria belonging to the genus *Thiothricus* (44). These bacteria oxidize inorganic sulfur compounds under autotrophic

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**TABLE 1.** Effect of NH$_4^+$ on GS, GDH, and GOGAT activities of strain BI-42

<table>
<thead>
<tr>
<th>Addition to medium</th>
<th>Sp act (nmol/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GS</td>
</tr>
<tr>
<td></td>
<td>Mn$^{2+}$</td>
</tr>
<tr>
<td>None</td>
<td>1,347</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>827</td>
</tr>
</tbody>
</table>

* NH$_4^+$ was added to a concentration of 0.1% during the exponential phase of growth. The preparation was incubated for 3 h, and then the enzyme activities were determined.

**TABLE 2.** Cellular fatty acid composition of strain BI-42

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Designation</th>
<th>% in strain BI-42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonhydroxylated acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentadecanoic acid</td>
<td>C$_{15}$:0</td>
<td>2.33</td>
</tr>
<tr>
<td>cis-Heptadec-9-enoic acid</td>
<td>C$_{16}$:1</td>
<td>3.70</td>
</tr>
<tr>
<td>Hexadecanoic acid</td>
<td>C$_{16}$:0</td>
<td>7.58</td>
</tr>
<tr>
<td>cis-Hepta-9-enoic acid</td>
<td>C$_{17}$:1</td>
<td>3.56</td>
</tr>
<tr>
<td>cis-9,10-Methylene hexadecanoic acid</td>
<td>C$_{17}$:0</td>
<td>2.08</td>
</tr>
<tr>
<td>Heptadecanoic acid</td>
<td>C$_{17}$:0</td>
<td>4.82</td>
</tr>
<tr>
<td>cis-Octadec-9-enoic acid</td>
<td>C$_{18}$:1</td>
<td>61.55</td>
</tr>
<tr>
<td>Octadecanoic acid</td>
<td>C$_{18}$:0</td>
<td>0.67</td>
</tr>
<tr>
<td>cis-Cyclo-10,11-methylene octadecanoic acid</td>
<td>C$_{19}$:0</td>
<td>8.35</td>
</tr>
<tr>
<td>10-Methyl octadecanoic acid</td>
<td>C$_{19}$:0 10-methyl</td>
<td>0.71</td>
</tr>
<tr>
<td>Hydroxylated acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Hydroxypentadecanoic acid</td>
<td>C$_{15}$:0 3-OH</td>
<td>0.49</td>
</tr>
<tr>
<td>3-Hydroxyhexadecanoic acid</td>
<td>C$_{16}$:0 3-OH</td>
<td>3.25</td>
</tr>
<tr>
<td>3-Hydroxyheptadecanoic acid</td>
<td>C$_{17}$:0 3-OH</td>
<td>0.90</td>
</tr>
</tbody>
</table>

* Percentages of the total fatty acids.
or mixotrophic growth conditions (19). However, it has also been reported that *Thiobacillus* sp. strain Q (8) and *C. thio- 
clyclus* (39) oxidize sulfur compounds to yield metabolically use-
ful energy under lithoheterotrophic growth conditions. In this 
context, the stimulation of strain BI-42 growth by thiosulfate 
observed in an organic culture medium is very similar to the 
chemolithoheterotrophic growth of *Thiobacillus* sp. strain Q 
described by Gomers and Kuenen (8).

The following major physiological characteristics of strain 
BI-42 distinguish it from similar gram-negative bacteria, in-
cluding *Thiobacillus novellus* (13), *Thiobacillus versutus* (46),  
*Thiobacillus* sp. strain Q (8), *Thiophasera pantotropha* (32),  
*Pseudomonas aeruginosa* (35), *Achromobacter stutzeri* (41), and  
*Pseudomonas* sp. strain 16B (50), which are capable of oxidiz-
ing inorganic sulfur compounds: (i) strain BI-42 cannot grow in 
chemically defined medium containing ammonia or nitrate as 
the sole nitrogen source; (ii) strain BI-42 requires trace 
amounts of yeast extract as a growth factor; and (iii) strain 
BI-42 is auxotrophic for glutamate.

A large number of organic compounds supported heterotro-
phic growth of strain BI-42 but this organism was not able to 
utilize aromatic compounds and alcohol. Unlike *Thiobacillus*  
sp. strain Q (8), strain BI-42 grown on mixed-substrate agar 
did not produce sulfur deposits on the colony surfaces. Even 
though both strain BI-42 and *Thiobacillus versutus* (46) deni-
trify under heterotrophic growth conditions, the absence of 
denitrification under mixed-substrate growth conditions differ-
etiates strain BI-42 from *Thiophasera pantotropha* and *Thio-
bacillus versutus* (32). This may be due to the inhibitory effect 
of thiosulfate for denitrifying activity under mixed-substrate 
growth conditions.

Despite the presence of an assimilatory GDH, strain BI-42 
was not able to grow in culture medium containing ammonia as 
the sole nitrogen source. Thus, the assimilatory GDH may not 
have been sufficient for assimilation of ammonia.

The fatty acid composition of BI-42 was distinct from the 
fatty acid compositions of other autotrophic and facultatively 
chemolithoautotrophic sulfur oxidizers (14). The uniqueness 
of this isolate was supported by the presence of hydroxy fatty 
acids along with 10-methyl and cyclic fatty acids, which are not 
found in any other species belonging to the alpha subclass of 
the class *Proteobacteria* (4, 23, 33).

The respiratory quinone present in strain BI-42 was a 
ubiquinone with 10 isoprenoid units (ubiquinone 10), which 
indicates that this organism is closely related to members of 
the alpha subclass of the *Proteobacteria*. The lipids of the polar 
head groups of strain BI-42 were not unique but were similar 
to those found in the obligate or restricted facultative methy-
lotrophs (11). The presence of ubiquinone 10 and phosphat-
dylycholine and the high level of the unsaturated nonhydroxy 
fatty acid C\(_{18:1}\) \(\alpha\)7C resembles characteristics of the genus 
*Methylobacterium* (9, 11).

Oxidation of thiosulfate in GYM medium was gratuitous 
since it did not increase the growth yield. Despite thiosulfate 
oxidation, the low cell yield clearly indicated that like other 
chemolithoheterotrophic sulfur oxidizers, strain BI-42 could 
not assimilate carbon dioxide (8, 31). The failure to detect 
RuBP carboxylase is consistent with this conclusion. In con-
trast, the increased growth yield obtained with thiosulfate 
and succinate implies that thiosulfate oxidation is used to enhance 
heterotrophic carbon assimilation. Under these growth condi-
tions, the stoichiometry for thiosulfate oxidation to sulfate by 
strain BI-42 is very similar to the stoichiometry reported for 
*Thiobacillus thioparus* (52) and *Thiobacillus novellus* (34).

As determined by a 16S rDNA sequence analysis, biochem-
ical tests, and a chemical composition analysis, strain BI-42 
represents a novel genus and species in the alpha subclass of 
the *Proteobacteria*, for which the name *Bosea thiooxidans* is 
proposed.

**Description of Bosea gen. nov. Bosea** (Bos' e.a. M. L. gen. n. 
*Bosea*, of J. C. Bose the founder of the Bose Institute, where 
The organism was isolated). Cells are gram-negative rods that 
occur mostly as single cells. Strictly aerobic. The optimum 
temperature for growth is 30 to 32°C. No growth occurs at 
temperatures below 20°C and above 37°C. Oxidase and cata-
lase positive. Reduced inorganic sulfur is oxidized in the pres-
ence of organic carbon sources. No autotrophic growth occurs.

Phosphatidylglycerol, phosphatidylethanolamine, phosphati-
dylycholine, diphasphatidylethanol, and an amino lipid are the 
major lipid constituents of the polar head groups. The abun-
dant fatty acids are cis-octadec-9-enoic acid (C\(_{18:1}\) \(\alpha\)7C), 
cis-cyclo-10,11-methylene octadecanoic acid (C\(_{18:0}\) cyclo \(\alpha\)8C), 
and hexadecanoic acid (C\(_{16:0}\)). Hydroxylated fatty acids are also 
present. A 16S rDNA sequence analysis of strain BI-42 re-
vealed that the genus branches intermediate to the genus 
*Methylobacterium*, the genus *Beijerinckia*, *R. palustris*, and 
related taxa. The type species is *Bosea thiooxidans*.

**Description of Bosea thiooxidans** sp. nov. *Bosea thiooxidans* 
(thi.o. ox'i. dans. Gr. n. *thion*, sulfur; M.L.v. *oxido*, make acid, 
oxidize; M.L. part. adj. *thiooxidans*, oxidizing sulfur). This bac-
terium was isolated from agricultural soil. Cells are straight 
rods that are 0.85 µm wide by 1.4 to 1.6 µm long. Cells occur 
singly. Motile by means of a single polar flagellum. Strictly 
aerobic. Spores are not formed. Colonies on agar containing 
thiosulfate and succinate or yeast extract are smooth, mucoid, 
round, and cream colored; these colonies are about 1 to 1.5 
mm in diameter after 4 days of incubation. No sulfur deposi-
tion occurs on the colony surfaces. Glutamate, glutamine, 
and aspartate, but not NH\(_3\), NO\(_3\)\(^-\), and urea, can serve as nitro-
gen sources. This organism can denitrify and produce gas only 
in heterotrophic growth medium containing nitrate. It has glu-
tamate auxotrophy and requires yeast extract (0.1 g/liter) as a

![Phylogenetic tree based on dissimilarity values, showing the relationships between strain BI-42 and related reference organisms. Bar = 5% nucleotide difference.](image-url)
growth factor. The pH range for growth is 6.0 to 9.0, and the optimum pH is 7.5 to 8.0. A variety of organic compounds support heterotrophic growth in GYM medium. These compounds include glucose, fructose, rhamnose, xylose, sorbose, ribose, arabinose, galactose, citrate, gluconate, succinate, malate, acetate, glutamate, glutamine, proline, cysteine, aspartic acid, serine, asparagine, alanine, lysine, common hexoses, pentoses, and organic acids, pyruvate, and some amino acids.

This species is a chemolithoheterotroph. Thiolsulfate stimulates growth in the presence of succinate. Tetrathionate is oxidized slowly. Sulfitic, thiocyanate, and elemental sulfur do not support growth.

The G+C content is 68.2 mol%. Ubiquinone 10 is the major ubiquinone. A culture of the type strain has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 9653.

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