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# Thiosulfate Oxidation and Mixotrophic Growth of *Methylobacterium goesingense* and *Methylobacterium fujisawaense*

**Anandham, R.[1,2], P. Indiragandhi[1], M. Madhaiyan[1], Jongbae Chung[3], Kyoung Yul Ryu[2], Hyeong Jin Jee[2], and Tongmin Sa[1]***

[1]*Department of Agricultural Chemistry, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Organic Farming Division, National Institute of Agricultural Science and Technology, Rural Development Administration (RDA), Suwon 441-707, Korea*
[3]*Division of Life and Environmental Sciences, Daegu University, Gyeongsan 712-714, Korea*

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**The mixotrophic growth with methanol plus thiosulfate was examined in nutrient-limited mixotrophic condition for *Methylobacterium goesingense* CBMB5 and *Methylobacterium fujisawaense* CBMB37. Thiosulfate oxidation increased the growth and protein yield in mixotrophic medium that contained 150 mM methanol and 20 mM sodium thiosulfate, at 144 h. Respirometric study revealed that thiosulfate was the most preferable reduced inorganic sulfur source, followed by sulfite and sulfur. *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 oxidized thiosulfate directly to sulfate, and intermediate products of thiosulfate oxidation such as polythionates, sulfite, and sulfur were not detected in spent medium and they did not yield positive amplification for tested *soxB* primers. Enzymes of thiosulfate oxidation such as rhodanese and sulfite oxidase activities were detected in cell-free extracts of *M. goesingense* CBMB5, and *M. fujisawaense* CBMB37, and thiosulfate oxidase (tetrathionate synthase) activity was not observed. It indicated that both the organisms use the "non-$S_4$ intermediate" sulfur oxidation pathway for thiosulfate oxidation. It is concluded from this study that *M. goesingense* CBMB5, and *M. fujisawaense* CBMB37 exhibited mixotrophic metabolism in medium containing methanol plus thiosulfate and that thiosulfate oxidation and the presence of a "Paracoccus sulfur oxidation" (PSO) pathway in methylotrophic bacteria are species dependant.**

**Keywords:** Thiosulfate oxidation, mixotrophic growth, rhodanese, sulfite oxidase, *soxB*, S4I, PSO

Microorganisms contribute to the biogeochemical cycling of sulfur. Various aerobic microorganisms have been described that utilize sulfur compounds obligately or facultatively as electron donors and oxidize them to sulfate [4, 9]. Two different biochemical pathways of thiosulfate oxidation appear to exist in those organisms [14]. The first one is the "$S_4$ intermediate" pathway ($S_4$I) includes formation and oxidation of conspicuous globules of polymeric, water-insoluble sulfur, sulfite, tetrathionate or trithionate or polythionate, from thiosulfate. The $S_4$I pathway occurs in many environmentally important sulfur oxidizers including the phototrophic green and purple sulfur bacteria and chemotrophic sulfur oxidizers such as *Beggiatoa* and *Thiothrix* [2]. The second pathway involves the oxidation of sulfur atoms of thiosulfate to sulfate without formation of sulfur deposits as intermediates. It occurs in a number of facultatively chemo- or photolithotrophic organisms such as *Paracoccus pantotrophus* or *Rhodovulum sulfidophilum* [2, 9] and this pathway is popularly known as the "Paracoccus sulfur oxidation" (PSO) pathway. In the PSO pathway, thiosulfate oxidation is carried out by a thiosulfate oxidizing multienzyme system (TOMES). In these, one enzyme is coded by *soxB*, which contains a prosthetic manganese cluster in the reaction center and is essential for thiosulfate oxidation by *Paracoccus pantotrophus* GB-17 [31]. Mixotrophic nutritional ability of the bacteria is defined as the concurrent utilization of the organic and inorganic substrates [20]. In nutrient-limited mixotrophic condition, *Xanthobacter tagetidis* and *Pseudaminobacter salicylatoxidans* showed increase in growth and cellular yield with succinate and thiosulfate [16, 22].

Species-specific physiological distinctions also pertain to the inequality of efficiency of energy conservation by sulfur oxidizers from the same substrate, different electron transport mechanisms, and distinct sulfur oxidation pathways and enzymes involved in the dissimilatory metabolism of sulfur [11]. Recently, Ghosh and Roy [11] successfully amplified the *soxB* gene from *Paracoccus pantotrophus*

*Corresponding author
Phone: 82-43-261-2561; Fax: 82-43-271-5921;
E-mail: tomsa@chungbuk.ac.kr

JT001 but not from *Paracoccus thiocyanatus* SST, and it could be inferred that a distinct thiosulfate oxidation pathway probably exists even at the species level. *Methylobacterium extorquens* AM1 is a sulfur oxidizing bacterium that utilizes thiosulfate and sulfur oxidizing (SOX) proteins, as deduced from its partial genome sequence, and hence the existence of the PSO pathway of thiosulfate oxidation is proposed in this organism [9]. In a previous study, we reported the thiosulfate oxidation, presence of non-$S_4$I pathway, and mixotrophic growth of *Methylobacterium oryzae* in a succinate plus thiosulfate medium [1]. In this study, we report the thiosulfate oxidation and mixotrophic growth of *Methylobacterium goesingense* CBMB5 and *Methylobacterium fujisawaense* CBMB37 in a mixotrophic medium containing methanol and thiosulfate, and the possible modes of thiosulfate oxidation by these organisms also investigated. Furthermore, that we propose the thiosulfate oxidation and presence of a PSO pathway in methylotrophic bacteria are species dependant, at least within the tested species of genus *Methylobacterium* used in this study.

## Materials and Methods

### Bacterial Strains, Media, and Culture Conditions

The methylotrophic bacteria used in this study are listed in Table 1. The pure cultures of bacterial strains were maintained in 50% glycerol at –80°C. Methylobacterial strains were grown in ammonium mineral salts medium (AMS) supplemented with 150 mM of methanol as described in Poonguzhali *et al.* [25]. The *Paracoccus pantotrophus* GB-17$^T$ and *Burkholderia vietnamiensis* CBMB40 were cultivated in nutrient medium (Difco Laboratories, Detroit, MI, U.S.A.).

### Thiosulfate Oxidation

Thiosulfate oxidation was examined in a mineral salts medium that contained (g/l) $Na_2S_2O_3{\cdot}5H_2O$, 5.0; $K_2HPO_4$, 0.2; $MgCl_2{\cdot}6H_2O$, 2.5; $NH_4Cl$, 1.0; bromocresol purple, 0.002; pH 7.5, and 150 mM of methanol was added just before plating. Plates were incubated at 30°C for 10 days and the experiments were replicated three times. The change in color from purple to yellow was indication of thiosulfate oxidation due to the production of sulfuric acid from thiosulfate. Additionally, control plates without thiosulfate were maintained.

### Growth Experiments

The bacterium was grown in 250-ml Erlenmeyer flasks containing 100 ml of mineral salts medium at 30°C in the shaking incubator at 120 rpm for 144 h. Measurement of bacterial growth was made by determining the optical density of the culture at 600 nm in a Shimadzu Spectrophotometer (UV-1601, Shimadzu, Japan). Biomass was estimated by determination of protein [17].

### Assay of Inorganic Sulfur Compounds

Batch culture experiments were performed to examine whether thiosulfate was oxidized directly to sulfate or accumulated as intermediate compounds. The bacterial strains were grown in 250-ml Erlenmeyer flasks containing 100 ml of mineral salts medium at 30°C in a shaking incubator at 120 rpm for 144 h. Thiosulfate consumption and formation of intermediate products such as tetrathionate, and trithionate were assayed spectrophotometrically by the cyanolytic method [13]. Accumulation of sulfur in the culture medium was determined as per the method of Sörbo [27], and sulfite was analyzed spectrophotometrically with *para*-rosaniline as the indicator [30]. Sulfate content in a culture supernatant was estimated turbidometrically [15].

### Substrate-Dependant Oxygen Consumption

To test substrate-dependant oxidation of inorganic sulfur compounds, the cells were harvested by centrifugation, washed, and resuspended in a desired volume of sodium phosphate buffer (100 mM, pH 8.0). The assay mixture contained 300 µmol of sodium phosphate buffer (pH 8.0), cell suspension (200 µg of protein), an aliquot of a substrate solution, and water to final volume of 3 ml. Oxidation of reduced sulfur compounds by whole cells was then assayed polarographically with a biological oxygen monitor equipped with a Clarke-type oxygen electrode (Yellow Spring Instrument, Co. Inc., Yellow Springs, OH, U.S.A.). The concentrations of different substrates were as follows: 8 mM sodium thiosulfate, 4 mM sodium sulfite (dissolved in 5 mM EDTA), and 100 mg of elemental sulfur ($S^0$) as water-insoluble amorphous powder. Oxygen consumption was calculated on the basis of 256 nmol $O_2$/ml in air-saturated water at 30°C and expressed as nmol $O_2$ consumed/min/mg/protein. Oxygen consumption rates were corrected for chemical oxidation and endogenous respiration.

### Rhodanese, Thiosulfate Oxidase, and Sulfite Oxidase Activities

The *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 cultivated in mineral salts medium at 30°C in the shaking incubator at 120 rpm for 144 h, respectively, were harvested by centrifugation (4,000 ×*g*, 10 min, 4°C). The cells were washed and suspended in 100 mM potassium phosphate buffer (pH 7.4) and disrupted by sonication in a Bransonic sonicator (8510R-DTH; Branson Ultrasonic Corporation, U.S.A.) for 6×30 s at 250 W in ice. The cell debris was removed by centrifugation at 10,000 ×*g* for 30 min at 4°C, and clear supernatant was used for the assay of enzymes. The rhodanese (E.C. 2.8.1.1) assay was carried out as per the method described by Singleton and Smith [26]. Thiosulfate (E.C. 1.8.2.2) and sulfite oxidase (E.C. 1.8.2.1) assays were carried as per the methods described by Trudinger [29] and Charles and Suzuki [5], respectively.

### PCR Amplification of *soxB* Gene

A colony PCR was performed with live cells cultured on thiosulfate-containing solid mineral salts medium supplemented with 150 mM of methanol. PCR amplifications were performed with 3 *soxB* forward (432F-5'-GAYGGNGGNGAYACNTGG-3'; 693F-5'-ATCGGNCARGC-NTTYCCNTA-3'; 1164F-5'-TAYCGNCGNGGNAAYTT-3') and 4 *soxB* reverse primers (693B-5'-TANGGRAANGCYTGNCCGAT-3'; 1164B-5'-AARTTNCCNCGNCGRTA-3'; 1403B-5'-TTRTCNGCNACR-TCYTC-3'; 1446B-5'-CATGTCNCCNCCRTGYTG-3') in different combinations, as described by Petri *et al.* [24].

## Results

### Thiosulfate Oxidation in Solid Medium

The thiosulfate oxidizing ability of methylotrophic bacteria was examined in solid mineral salts thiosulfate medium. The change in the color of the plate from purple to yellow was observed in

**Table 1.** Thiosulfate oxidation by methylotrophic bacteria.

| Strain | Taxon | GenBank Accession No. | Thiosulfate oxidation in liquid medium | | | Source/Reference |
|---|---|---|---|---|---|---|
| | | | Thiosulfate consumed (µg/ml) | Sulfate (µg/ml) | pH[b] | |
| CBMB5[a] | *Methylobacterium goesingense* | EF126739 | 31.2±0.5 | 62.3±1.0 | 5.2±0.2 | Madhaiyan |
| CBMB37[a] | *Methylobacterium fujisawaense* | EF126742 | 57.0±1.0 | 114±2.0 | 5.6±0.6 | *et al.* unpublished |
| CBMB20[T] | *Methylobacterium oryzae* | AY683045 | 12.8±0.6 | 25.7±1.2 | 5.2±0.2 | [18] |
| CBMB110 | *Methylobacterium oryzae* | AY683046 | 14.0±1.0 | 28.0±2.0 | 5.3±0.3 | |
| CBMB120 | *Methylobacterium suomiense* | AY683047 | – | – | 7.5±0.5 | [25] |
| CBMB130 | *Methylobacterium rhodinum* | AY683048 | – | – | 7.5±0.5 | |
| CBMB40 | *Burkholderia vietnamiensis* | AY683043 | – | – | 7.5±0.1 | [19] |
| ALL/SCN-P[T] | *Methylobacterium thiocyanatum* | | 1413±10 | 2826±20 | 4.4±0.4 | A.P. Wood, King's College, London, U.K. |
| DSM1337[T] | *Methylobacterium extorquens* | | 29.8±1.0 | 59.7±2.0 | 5.2±0.2 | C.G. Friedrich, |
| GB17[T] | *Paracoccus pantotrophus* | | 39.0±1.0 | 78.0±2.0 | 5.5±0.5 | University of Dortmund, Germany |

[a] Strains were identified according to their 16S rDNA sequences, submitted to GenBank (NCBI).

[b] Initial pH of the medium was 7.50. Thiosulfate oxidation of methylobacterial strains was observed on day 4 in mineral salts medium supplemented with 150 mM methanol with thiosulfate (20 mM).

[T] Type strains; values are the mean of three replications of three experiments±standard deviation (SD).

the case of *Methylobacterium goesingense* and *M. fujisawaense*. Moreover, these strains were able to grow in the mineral salts medium containing methanol without thiosulfate and no change in color was noted. Among our isolates, *M. fujisawaense* CBMB37 and *M. goesingense* CBMB5 consumed the highest amount of thiosulfate (57.0 and 31.2 µg/ml) and accumulated sulfate (114.0 and 62.3 µg/ml), and concomitant reduction in pH of the spent medium was noted (Table 1). Hence, these two strains were selected for further studies.

### Growth and Thiosulfate Oxidation in Broth Assay

The metabolic benefits due to thiosulfate oxidation by *M. fujisawaense* CBMB37 and *M. goesingense* CBMB5 were examined in liquid mixotrophic medium containing 150 mM of methanol and 20 mM of thiosulfate. The results showed that in *M. goesingense* CBMB5, protein yield was increased with methanol in the presence of thiosulfate from 10.4 µg/ml to 16.9 µg/ml. In methanol mixotrophic medium, thiosulfate was oxidized to sulfate, and intermediate products of thiosulfate oxidation such as tetrathionate, trithionate, polythionate, sulfite and sulfur were undetectable in spent medium. In methanol mixotrophic medium, *M. goesingense* CBMB5 consumed 70.3 µg/ml of thiosulfate and accumulated 140.7 µg/ml of sulfate (sulfuric acid) (Table 2). The growth coupled with oxidation of thiosulfate was investigated in mixotrophic medium containing 150 mM of methanol and 20 mM of sodium thiosulfate over a total incubation period of 144 h (Fig. 1). The results revealed that the bacterial growth was consistently higher in medium containing methanol plus thiosulfate as compared with medium unamended with thiosulfate. During the time course of thiosulfate oxidation by methylotrophic bacteria in mixotrophic medium, thiosulfate was oxidized directly to sulfate, and intermediates such as tetrathionate, trithionate,

**Table 2.** Growth and biomass production of *Methylobacterium goesingense* CBMB5 and *Methylobacterium fujisawanse* CBMB37.

| Bacteria/growth condition | Growth ($OD_{600nm}$) | Protein content (µg/ml) | Thiosulfate consumed (µg/ml) | Sulfate (µg/ml) | pH[a] |
|---|---|---|---|---|---|
| *Methylobacterium goesingense* CBMB5 | | | | | |
| Methanol+thiosulfate | 0.682±0.03 | 16.9±0.44 | 70.3±0.34 | 140.7±0.67 | 5.1±0.17 |
| Methanol | 0.519±0.02 | 10.4±0.43 | – | – | 7.4±0.50 |
| *Methylobacterium fujisawaense* CBMB37 | | | | | |
| Methanol+thiosulfate | 0.476±0.03 | 11.8±0.52 | 54.2±0.20 | 108.4±0.40 | 5.6±0.60 |
| Methanol | 0.096±0.01 | 1.3±0.05 | – | – | 7.4±0.50 |

Growth and biomass production of methylobacterial strains were observed in mineral salts medium on day 6, supplemented with 150 mM methanol with or without thiosulfate (20 mM). Values are the mean of three replications of three experiments ± standard deviation (SD).

[a] Initial pH of the medium was 7.50.

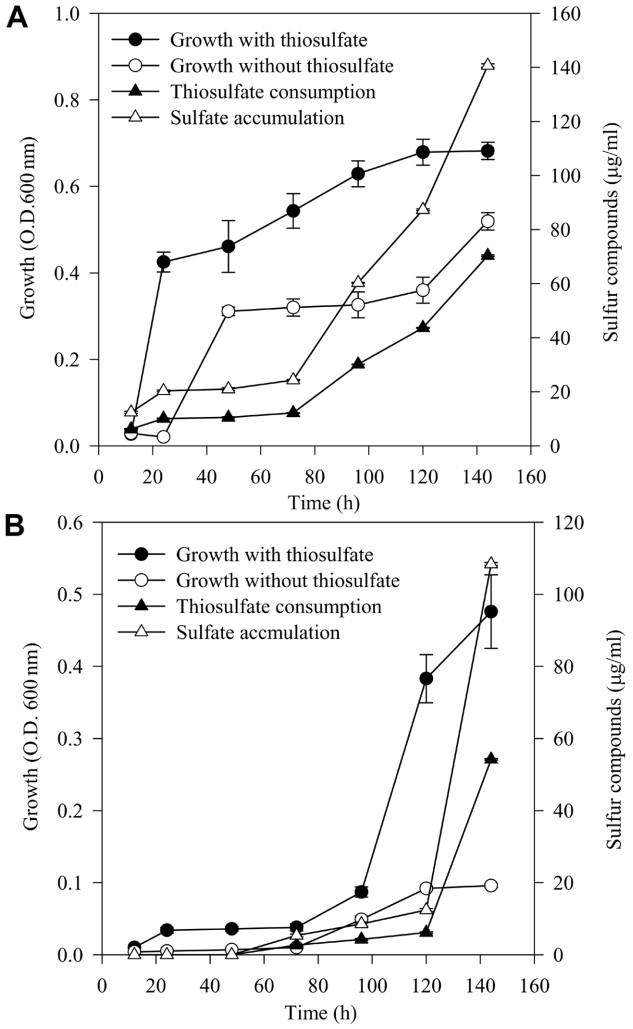


**Fig. 1.** Mixotrophic growth coupled with thiosulfate consumption and sulfate accumulation of *M. goesingense* CBMB5 (**A**) and *M. fujisawanse* CBMB37 (**B**) in mixotrophic medium containing 150 mM of methanol and 20 mM of sodium thiosulfate. The cells were incubated at 30°C in a shaking incubator operating at 120 rpm for 144 h. Values are the mean±SD of 5 determinations. Error bars are shown when larger than the symbol.

sulfite and sulfur, were not detected in spent medium on the tested time intervals (Fig. 1).

The substrate-dependant oxidation (*i.e.*, oxygen consumption in the presence of) of thiosulfate, sulfur, and sulfite by mixotrophically grown washed whole cells of *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 confirmed the chemolithotrophic capabilities of these organisms. The *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 oxidized thiosulfate the fastest, and sulfite and sulfur were oxidized at the rates of 70–75% and 60% of thiosulfate oxidation rate, respectively. Thiosulfate metabolizing enzymes such as rhodanese, sulfite oxidase, and thiosulfate oxidase were examined in cell-free extracts of *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37. The maximum rhodanese (16.6 nM $SCN^-$ formed/min/mg protein) and sulfite oxidase (186.1 µmol ferricyanide reduced/min/mg protein) activities were found in *M. goesingense* CBMB5. Thiosulfate oxidase (tetrathionate synthase) activity was not observed in crude enzyme extracts of both methylotrophic bacteria (Table 3).

### Amplification of *soxB* Gene

The positive amplification of the *soxB* gene was attributed for existence of the PSO pathway of thiosulfate oxidation in sulfur oxidizers. Hence, the presence of the *soxB* gene was examined in *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37, but no positive amplification was observed when primers were used in different combinations (data not shown).

## Discussion

Thiosulfate is a rather stable and environmentally abundant sulfur compound of the intermediate oxidation state and fulfils an important role in the natural sulfur cycle. Most phototrophic and chemotrophic sulfur oxidizers are able to use thiosulfate, and hence it is most suitable for the investigations of sulfur lithotrophic process [11]. In this study, thiosulfate oxidation was observed in *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37. In a previous study, we reported the absence of thiosulfate oxidation in pink-pigmented facultative methylotrophic bacteria such as *Methylobacterium suomiense* CBMB120, *Methylobacterium*

**Table 3.** Substrate-dependant oxygen consumption of washed whole cells of methylobacteria and rhodanese and sulfite oxidase activities.

| Bacteria | Oxygen consumption (nmol $O_2$/min/mg protein) | | | Rhodanese[a] | Sulfite oxidase[b] |
|---|---|---|---|---|---|
| | Thiosulfate | Sulfite | Sulfur | | |
| *M. goesingense* CBMB5 | 17.7±1.15 | 13.3±1.15 | 10.6±0.58 | 16.6±0.57 | 186.1±0.13 |
| *M. fujisawaense* CBMB37 | 13.9±0.06 | 9.7±1.15 | 9.00±1.15 | 10.4±0.39 | 129.9±0.37 |

Substrate-dependant oxygen uptake rate was determined with different reduced inorganic sulfur compounds (8 mM of sodium thiosulfate, 4 mM of sodium sulfite in 5 mM of EDTA, and 100 mg of elemental sulfur) in a biological oxygen monitor.

[a]Rhodanese activity is expressed as nM of thiocyanate formed/min/mg protein.

[b]Sulfite oxidase activity is expressed as µmol ferricyanide reduced/min/mg protein. The mean±standard deviations (SD) are shown. Data are the means of three replications of three experiments. Both of the methylotrophic bacteria did not show thiosulfate oxidase (tetrathionate synthase) activity.

*rhodinum* CBMB130, and *Burkholderia vietnamiensis* CBMB40 [1], whereas Jung *et al.* [12] observed positive thiosulfate oxidation in *Burkholderia* spp. It indicated that thiosulfate oxidation in methylotrophic bacteria is species dependant, at least within the species of *Methylobacterium* used in this study. The plate test did not distinguish between thiosulfate oxidation and chemolithotrophic growth with thiosulfate [1], so in the present study, thiosulfate oxidation was examined in mixotrophic liquid medium.

The definite growth of *P. pantotrophus*, *M. extorquens,* and *M. thiocyanatum* included as control strains confirmed that the presently used medium was suitable for sulfur oxidizing bacteria and lithotrophic growth. In mixotrophic medium with methanol, higher protein yields were observed when thiosulfate was added (Table 2). It indicated that *Methylobacterium* gains metabolically useful energy from thiosulfate oxidation. This was attributable to energy from thiosulfate oxidation enabling greater heterotrophic carbon assimilation [1, 22]. In a separate growth experiment, growth-coupled thiosulfate oxidation was observed for *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 (Fig. 1). Similar results of growth-coupled thiosulfate oxidation and increased cellular yields were reported in *Starkeya novella* and *Thioclava pacifica* grown in mixotrophic medium containing glucose/glutamate/acetate and thiosulfate [10, 28]. To date, this is the first study to report the thiosulfate oxidation and mixotrophic growth of *M. goesingense* and *M. fujisawaense*. In this study, the ratio of sulfate accumulation and thiosulfate consumption was around 2. A similar result has been reported in *S. novella* and *M. oryzae* by Perez and Matin [23] and Anandham *et al.* [1], respectively. The least growth was observed for *M. fujisawaense* CBMB37 in mineral medium containing methanol devoid of thiosulfate (Fig. 1B). Previously, low yield on methanol was reported for *M. sulfidovorans*, *Hyphomicrobium*, and *Arthrobacter* [3, 7]. $NH_4Cl$ (50 mM) was required for methanol dehydrogenase activity, indicating that the enzyme is PQQ dependant [8]. In this experiment, 18.7 mM of $NH_4Cl$ (equal to 1.0 g in 1,000 ml of distilled water) was used, this may be attributed for low yield on methanol; however, this postulation has to be confirmed with further experiments.

In all growth experiments presented here, thiosulfate was oxidized to sulfate, and intermediates such as tetrathionate, trithionate, polythionate, sulfite, and sulfur were not detected in spent medium. It has been postulated that the *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 are likely to oxidize thiosulfate *via* a non-$S_4$ intermediate sulfur oxidation pathway [1]. In this study, mixotrophically grown cells showed the highest rate of oxygen consumption with thiosulfate (Table 3). A similar trend has been noticed in *M. oryzae* [1]. In the present study, the enzymes of thiosulfate oxidation were quantified in cell-free extracts of *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 (Table 3). Similar to *M. oryzae,* both of the methylotrophic bacteria tested in this study did not exhibit thiosulfate oxidase activity [1]. Rhodanese and sulfite oxidase activities were noted in *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37. Similar results of enzyme activity have been reported in *Bosea thiooxidans* and *M. oryzae* when grown in mixotrophic medium containing succinate or glutamate amended with thiosulfate [1, 6].

The *soxB* amplification was not found in *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37. In a previous study, Anandham *et al.* [1] successfully amplified the *soxB* gene from *M. extorquens* but not from *M. oryzae,* and both organisms possessed the PSO and non-$S_4$I pathways of thiosulfate oxidation, respectively. In the present study, failure to obtain positive *soxB* amplification implies that the existence of a distinct *soxB* gene or distinct thiosulfate oxidation pathway in *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37. As *soxB* is generally part of the *sox* gene cluster, its PCR-based detection in these strains might be used as a first indication for the putative presence of components of the Sox enzyme system [21]. Friedrich *et al.* [9] reported that the sulfur oxidation (*sox*) gene cluster (*sox* SRT-VW-XYZABCDEFGH) encodes TOMES in PSO pathway organisms. The highly degenerated primers used in this study are complementary to the target sites of *Chlorobiaceae*, *Betaproteobacteria*, and most *Gamma*- and *Alphaproteobacteria soxB* sequences [21]. Therefore, negative amplification with these primer sets was most probably not caused by inhibited primers annealing but is indicative of the absence of this gene in the respective strain. However, this postulation has to be confirmed with southern blot analysis [21]. Moreover, both the organisms (*M. goesingense* CBMB5 and *M. fujisawaense* CBMB37) oxidized thiosulfate directly to sulfate and did not show positive amplification for *soxB* primers and hence presumed that they possessed a non-$S_4$I pathway for thiosulfate oxidation. Overall, it is concluded from this study that *M. goesingense* CBMB5 and *M. fujisawaense* CBMB37 oxidized thiosulfate and exhibited mixotrophic growth. The mixotrophic growth might be the preferred metabolic trait of these organisms, since co-oxidation of sulfur compounds together with organic substrates might ensure better survival. Furthermore thiosulfate oxidation and the existence of a PSO pathway in pink-pigmented facultative methylotrophic bacteria seem to be species dependant.

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