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Delineation of Sulphur Bacteria Isolated from Rice Rhizosphere Soil

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ABSTRACT

Background: Crop yields are greatly reduced by sulfur-deficient conditions, as sulfur is an essential nutrient for plant growth. So, sulfur fertilizers are generally added to soils to alleviate this deficiency, usually in a reduced form, such as elemental sulfur. Despite this, microorganisms are required to oxidize reduced sulfur fertilizers into sulfate before they can be absorbed by plants. Recently, sulfur deficiency was reported widely in rice. Till date there are no suitable bio-inoculants for rice. Hence, the present study was aimed to develop a sulfur bio-inoculant for rice.

Methods: In this present investigation, totally 9 chemolithoautotropic sulfur oxidizing bacteria (3 from aerobic, 4 from wet land and 2 from SRI systems) were isolated from various rice ecosystems. Chemolithoautotrophic strain S1-3 isolated from SRI system and strain W3-1 isolated from wet land were found to efficient based on pH reduction and titrable acidity. Totally five facultative chemo lithoautotrophic sulfur oxidizing bacteria (two from wet land and 3 aerobic samples) were recovered from different rice ecosystems.

Result: Facultative chemolithoautotrophic strain S1Y1-bLL and S2Y2-b significantly reduced the medium pH (4.3) and consumed the maximum alkali 1.7 and 1.8 mL and found to be efficient. All the isolated facultative chemolithoautotrophic sulfur were autotrophic and chemoheterotrophic in nature. Molecular characterization study revealed that strains S1Y2-aS, S1Y1- BII, S2Y2-b, S2SA-Sa and S2Y2-an exhibited 99.5, 99.7, 99.5, 99.6 and 99.9% sequence similarities to *Pseudomonas beteli, Advenella kasmirensis, Pseudomonas beteli, Providencia stuartii* and *Bacillus tequilensis*, respectively. Further, developed lignite and compost based and clay pellet formulation for facultative chemolithoautotrophic sulfur oxidizing bacteria. Yet, plant inoculations studies should be carried out for isolated sulfur oxidizing bacteria from this study before recommended to the farmers.

Key words: Chemolithoautotrophic sulphur oxidation, Formulation, Sulfur-oxidizing bacteria, Thiosulfate.

INTRODUCTION

In addition to nitrogen, phosphorus and potassium, sulfur (S) is becoming increasingly recognized as a major plant nutrient. A compound containing reduced sulfur is produced from the amino acids cysteine and methionine, which act as precursors (Narayan *et al*., 2023). In addition to holding structural and functional roles within macrobiomolecules, sulfur modulates many physiological processes and makes plants more tolerant to abiotic stress (Haneklaus *et al*., 2003). Plants and animals require sulfur to survive. A sulfur deficiency in the field was first reported in 1933, despite plants' need for sulfur in comparable amounts to phosphorus. The first report of sulfur deficiency in wetland rice was made in 1938. Dry-land crops as well as wetland rice have been found to be affected by sulfur deficiency over the last 10 years. The tropics are generally characterized by sulfur deficiency in Andosols, Vertisols, Alfisols, Ultisols and Oxisols. Several Asian countries, including Bangladesh, Burma, India, Indonesia, Japan, Philippines and Sri Lanka, have reported sulfur deficiency of wetland rice (Roy *et al.,* 2017). Twenty-three crops have been reported to respond to sulfur in forty tropical countries (Jenni, 2020). A decrease in organic manures, intensive cropping and decreased atmospheric deposition have contributed to this increase in sulfur deficiencies (Dawar *et al*., 2023). The use of sulfur fertilizers is invariably used to alleviate sulfur deficiencies in soils, usually in reduced forms, such as elemental sulfur.

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Use of S oxidizers enhances the rate of natural oxidation of S and speed up the production of sulfates and makes them available to plants at their critical stages, consequently resulting in increased plant yield (Anandham *et al*., 2008a, b). Micororganisms contribute the biogeochemical cycling of sulfur (Friedrich *et al*., 2005). Various microorganisms (prokaryotes, green sulfur bacteria) have been described which utilize sulfur compounds obligately or facultatively as electron donors and oxidize them to sulfate. Sulfur oxidizing bacteria such as *Bosea thiooxidans, Paracoccus thiocyanatus, Pseudamino-bacter salicylatoxidans, Paracoccus pantotrophus, Paracoccus bengalensis, Tetrathiobacter kashmirensis* and *Mesorhizobium*

thiogangeticum were isolated from several ecological niches (Sowmya and Sivapriya, 2026).

MATERIALS AND METHODS

Rhizosphere sample collection

Rhizosphere soil samples were collected from rice cultivated land at various ecosystems (Wetland, SRI and aerobic) (Table 1). Three plants at flowering stages were selected and removed from the soil, placed in a polythene bag, immediately transported to the laboratory and processed. The plants were shaken gently to separate the soil that was not tightly adhering to the roots. The rhizosphere soil (soil attached to the roots after gentle shaking) and fine roots (approximately 1 cm length) were collected from the plants and used for enrichment isolation (Anandham *et al*., 2008).

Enrichment Isolation

Enrichment isolation of chemolithoautotrophic sulfur-oxidising bacteria

For isolation of chemolithoautotrophic sulfur oxidizing bacteria, three 1 g replicates of each rhizosphere soil samples were added into 10 mL of liquid mineral salts thiosulfate (MST) medium containing (g L^{-1}) NH_4Cl, 1.0; K_2HPO_4, 4.0; KH_2PO_4, 1.5; $MgSO_4.7H_20$, 0.5; $Na_2S_2O_3.5H_20$, 5.0; yeast extract, 0.05; bromocresol purple, 0.002; trace element solution 5 ml; pH 7.5). The medium was then incubated at 30°C in the dark to avoid growth of phototrophic bacteria on a rotary shaker (120 rpm) until the color of the bromocresol purple changed to yellow.

Enrichment isolation of facultative chemolithoautotrophic sulfur-oxidizing bacteria

For isolation of chemolithoautotrophic sulfur-oxidizing bacteria, three I g replicates of each rhizosphere soil sample were added into 10 mL of liquid mineral salts thiosulfate yeast extract (MSTYE) medium containing (g L-l) NH_4Cl, 1.0; K_2HPO_4, 4.0; KH_2PO_4, 1.5; $MgSO_4.7H_20$, 0.5; $Na_2S_2O_3.5H_20$, 5.0; yeast extract, 5.0; bromocresol purple, 0.002; trace element solution 5 ml; pH 7.5). The medium was then incubated at 30°C in the dark to avoid growth of phototrophic bacteria on a rotary shaker (120 rpm) until the colour of the bromocresol purple changed to yellow.

Purification of sulfur-oxidising bacteria

For isolation of pure cultures, streak plate method is followed a Loop full of culture is streaked on the surface of MST/MSTYE agar plates (Fig 6). The colonies that developed yellow halo against purple background, indicative of the production of sulfuric acid resulting from the oxidation of thiosulfate were picked and streaked on solid MST medium until uniform colony morphology was observed. Colonies were transferred 3 times to be considered as pure, in addition, the purity of the strains was checked microscopically. The pure bacterial strains were maintained on MST agar plates, sub cultured every week and subjected to further studies.

Screening of efficient sulfur-oxidizing bacteria

pH reduction test

Isolated chemolithoautotrophic and facultative chemolitho-autotrophic sulfur-oxidising bacteria were inoculated in MST and MSTYE broth and incubated for 5 days at 30°C. The End of sulfur oxidation is the production of sulfuric acid; hence, the pH of the medium would be reduced. The reduction in pH was recorded using the pH meter (Medox, MX-1292-01, India) at 25°C.

Titrable acidity

One mL of stationary phase cultures of chemolitho-autotrophic and facultative chemolithoautotrophic sulfur-oxidising bacteria

Table 1: Plant source for isolation of sulfur oxidizing bacteria.

Plant variety	Ecosystem	Stage
Anna R4	Aerobic rice	Flowering stage
ADT45	Wet land	Flowering stage
ADT43	SRI*	Flowering stage

*SRI-System of rice Intensification.

Table 2: Isolation of chemolithoautotropic sulfur oxidizing bacteria from different ecosystems.

Isolates	Growth	Color change
A1-1	+	Purple to light yellow
A1-2	+++	Intense yellow
A1-3	++	Medium yellow
A2-1	+	Light yellow
A2-2	++	Medium yellow
A2-3	++	Medium yellow
A3-1	++	Medium yellow
A3-2	+++	Intense yellow
A3-3	-	Purple
W1-1	++	Medium yellow
W1-2	++	Medium yellow
W1-3	+++	Intense yellow
W2-1	+++	Intense yellow
W2-2	+++	Intense yellow
W2-3	+++	Intense yellow
W3-1	+++	Intense yellow
W3-2	+++	Intense yellow
W3-3	+++	Intense yellow
S1-1	+	Light yellow
S1-2	++	Medium yellow
S1-3	++	Medium yellow
S2-1	+++	Intense yellow
S2-2	+++	Intense yellow
S2-3	+	Light yellow
S3-1	+	Light yellow
S3-2	++	Medium yellow
S3-3	-	Purple

+ Positive growth/color changes from purple to yellow; -No growth/ no color change.

A1, A2, A3- Aerobic rice(Anna R4); W1,W2,W3- Wetland rice (ADT45); S1, S2, S3- SRI (ADT43).

cultivated in liquid MST and MSTYE were collected in a beaker and drops of 0.1% phenolphthalein were added. At this moment, it is colourless. It was titrated against 0.1N NaOH until the appearance of pink colour, which indicates the end of the titration. Titrable acidity indicates the indirect production of acid.

Morphological characterization

Phenotypic characters such as colony colour, colony diameter, cell shape and Gram staining were performed as per the standard procedure (Smibert and Krieg, 1994).

Nutritional characterization of facultative chemoltiho-autotrophic sulfur oxidizing bacteria

Facultative cheomolithoautotrophic sulfur oxidizing bacteria were streaked on MSTA (Mineral salts thiosulfate agar), NA (Nutrient agar), TSA (tryptic soy agar) and LBA (Luria Bertani agar) and incubated at 30°C for 5 d then growth was checked.

Molecular characterization and phylogenetic analysis

The purified bacteria were grown in MST broth and harvested in the log to initial stationary phase. The genomic DNA was extracted using standard methods described (Wright *et al*., 2017). The genomic DNA was amplified using 16S rRNA universal primer (27f: 5′ -AGA GTT TGA TCC TGG CTC AG-3′ and 1492r: 5′ GGT TAC CTT GTT ACG ACT T-3′) in a thermocycler (Veriti™ 96-Well Fast Thermal Cycler). The authentication potential of SOB was verified by 16S rRNA amplification followed by sequencing for identification. The obtained sequences were performed with a similarity search using BLAST. A phylogenetic tree was constructed with existing 16S rRNA sequences of SOB from different eubacteria obtained from NCBI GenBank database.

Table 3: Selection of chemolithoautotrophic sulfur oxidizing bacteria for purification.

Samples	Growth rate	
	Initial	Final
A1-2	+++	+++
A2-3	++	+++
A3-2	+++	+++
W1-3	+++	+++
W2-2	+++	+++
W3-1	+++	+++
S1-3	++	+++
S2-1	+++	+++

A1, A2, A3- Aerobic rice(Anna R4); W1,W2,W3- Wetland rice (ADT45); S1, S2, S3- SRI (ADT43).

Table 4: Screening of chemolithoautotrophic sulfur oxidizing bacteria based on pH reduction and titrable acidity.

Samples	Name of the isolates recovered	pH reduction (Initial pH 7.5)	Titrable acidity (mL)
Aerobic rice	A2-3	5.9	0.6
	A3-2	6.5	1.0
	A1-2	6.1	1.1
Wetland rice	W2-2	5.9	2.0
	W3-1	5.6	1.3
	W1-3	6.1	0.9
SRI	S1-3	5.4	1.2

A1, A2, A3- Aerobic rice(Anna R4); W1,W2,W3- Wetland rice (ADT45); S1, S2, S3- SRI (ADT43).

pH reduction test and Titrable acidity were performed in the minimal salts thiosulfate medium without bromocresol purple indicator. Initial pH of the medium was 7.5.

Development of formulation

Development of powdered formulation

Facultative chemolithoautotrophic sulfur oxidizing bacteria grown in nutrient broth were used for formulation development. One kg of powdered, neutralized and/or sterilized lignite and compost were mixed with stationary phase culture (1 $\times 10^9$ cfu mL^{-1}) under aseptic condition, allowed for 2d and packed.

Development of powdered formulation

One Kg of clay soil was powdered and sterilized and cooled. Then clay soil was mixed with 400 mL of stationary phase Facultative chemolithoautotrophic sulfur oxidizing bacterial culture (1 $\times$ 10^9 cfu mL^{-1}) and pelletized with hand pelletizer and air dried further cut into approximately 1 cm size and stored.

RESULTS AND DISCUSSION

Isolation of chemolithoautotrophic sulfur-oxidising bacteria

In total, 27 rice rhizosphere soil samples were collected from various rice ecosystems, such as aerobic, wetland and SRI rice, of which 25 samples contained the chemolitho-

Table 5: Isolation of facultative chemolithoautotrophic sulfur oxidizing bacteria.

Source	Change in colour		
	Initial	Final	Isolates recovered
A1-2	Purple	Intense yellow	S1Y2 - aS
W2-2	Purple	Intense yellow	S1Y1 - bLL
A2-3	Purple	Pale yellow	S2Y2 - b
W3-1	Purple	Medium yellow	S2SA - Sa
A3-2	Purple	No change	S2Y2 - a

A1, A2, A3- Aerobic rice (Anna R4); W1,W2,W3- Wetland rice (ADT45); S1, S2, S3- SRI (ADT43).



Fig 1: Purification of facultative chemolithoautotophic sulfur oxidizing bacteria.

autotrophic sulfur-oxidising bacteria. It was inferred that the change of colour from purple to yellow, which indicated the end of sulfur oxidation, is the production of sulfuric acid, ultimately reducing the pH of the medium, in turn changing the medium colour from purple to yellow (Table 2). Above 25 positive samples, the isolates that showed good growth (3 from aerobic, 4 from wetland and 2 from SRI systems) were taken for purification (Table 3).

Screening of chemolithoautotrophic sulfur-oxidising bacteria

Efficient chemolithoautotrophic sulfur-oxidising bacteria were screened based on pH reduction and Titrable acidity, indirectly indicating sulfur oxidation. Among isolates, strain S1-3 isolated from the SRI system showed the maximum reduction of pH (5.4), followed by the wetland strain W3-1 (5.6). Strains W3-1 and S1-3 consumed the maximum alkali 2 and 1.2 mL, respectively (Table 4).

Isolation of facultative chemolithoautotrophic sulfur-oxidising bacteria

In total, 27 rice rhizosphere soil samples were collected from various rice ecosystems, such as aerobic, wetland and SRI rice. Of which 5 samples (two wetlands and 3 aerobic samples) contained the efficient facultative chemolithoautotrophic sulfur-oxidising bacteria (Table 5). Above 5 positive samples, 5 facultative chemolithoautotrophic sulfur-oxidising bacteria were recovered (Table 5; Fig 1).

Screening of facultative chemolithoautotrophic sulfur-oxidising bacteria

Efficient facultative chemolithoautotrophic sulfur-oxidising bacteria were screened based on pH reduction and titrable acidity, indirectly indicating sulfur oxidation. Strain S1Y1-bLL and S2Y2-b significantly reduced the medium pH (4.3) and consumed the maximum alkali 1.7 and 1.8 mL (Table 6).

Morphological characterisation of facultative chemolitho-autotrophic sulfur-oxidising bacteria

Among the screened facultative chemolithoautotrophic sulfur-oxidising bacteria, all were Gram-negative rods except strain S2Y2-a, which was Gram-positive (Table 7).

Nutritional characterisation of facultative chemolitho-autotrophic sulfur- oxidising bacteria

All the isolated facultative chemolithoautotrophic sulfur-oxidising bacteria strains were able to grow on autotrophic medium (MSTA) as well as on NA, TSA and LBA. This indicated that all the facultative chemolithoautotrophic sulfur oxidizing bacteria isolated from this study were autotrophic as well as chemoheterotrophic in nature (Table 8; Fig 2).

Molecular characterization of facultative chemolitho-autotrophic sulfur oxidizing bacteria

Molecular characterization of sulfur oxidizing bacteria was performed through 16S rDNA sequencing. Sequence results revealed that strains SlY2- as, S1Y1-bLL, S2Y2-b. S2SASa and S2Y2- a cxhibited 99.5, 99.7, 99.5, 99.6 and 99.9% sequence similarties to Pseudomonas beteli, Advenella kashmirensis, Pseudomonas beteli Providencia stuartii and Bacillus tequilensis, respectively. In phylognelic analyses revealed that clustering patern were similar in both neighbor-joining and maximum likely hood trees. Irrespective of phylogenetic methods used, strain S1Y 1- bLL formed cluster with Advenella kashmirensis which was supported by bootstrap value of 100, 92 and 9 in neighbor -joining (NI), maxmimum-likely hood (ML) and maximum-parsimony (MP) trees. Similarly, strains S1Y2- aS and S2Y2-b clustered with Pseudomonas beteli. Strains S2SA -Sa and S2Y2 formed separate clade with Providencia stuartiiand Bacillus tequilensis, respectively (Table 9; Fig 3, 4, 5).

Formulation development

Powder and granular based formulations for facultative chemolithoautotrophic sulfur oxidizing bacteria were developed using lignite, compost and clay soil (Fig 6).

Table 6: Screening of facultative chemolithoautotrophic sulfur oxidizing bacteria based on pH reduction and titrable acidity.

Sample	pH reduction	Titrable acidity (mL)
S1Y2 - aS	4.6	1.6
S1Y1 - bLL	4.3	1.7
S2Y2 - b	4.3	1.8
S2SA - Sa	5.3	1.7
S2Y2 - a	4.4	1.6

pH reduction test and Titrable acidity were performed in the minimal salts thiosulfate medium without bromocresol purple indicator. Initial pH of the medium was 7.5.

Table 7: Morphological characterization of facultative chemo lithoautotrophic sulfur oxidizing bacteria.

Isolates	Gram staining	Colony morphology	Colony diameter
S1Y2 - aS	Gram negative	Rod	0.8 mm
S1Y1 - bLL	Gram negative	Rod	0.1 mm
S2Y2 - b	Gram negative	Short rod	0.8 mm
S2SA - Sa	Gram negative	Short rod	0.2 mm
S2Y2 - a	Gram positive	Short rod	7 mm

Table 8: Nutritional characterization of facultative chemo lithoautotrophic sulfur oxidizing bacteria.

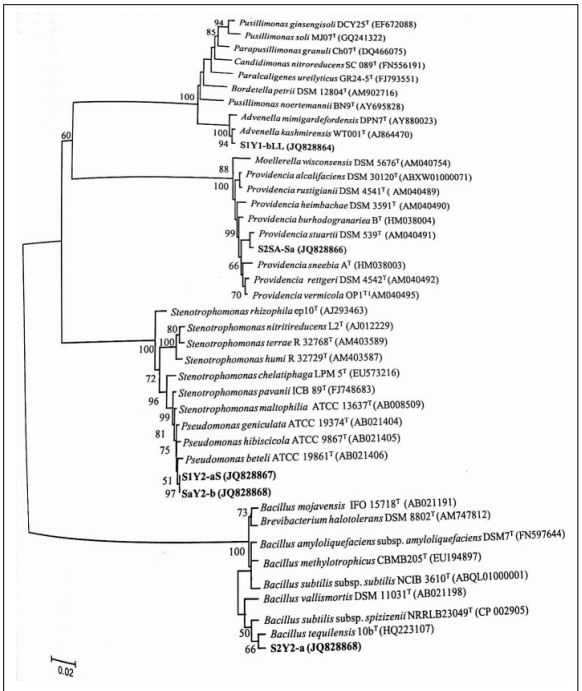
Isolates	Growth on media				
	MSTA	MSTYEA	NA	TSA	LBA
S1Y2 - aS	+	+	+	+	+
S1Y1 - bLL	+	+	+	+	+
S2Y2 - b	+	+	+	+	+
S2SA - Sa	+	+	+	+	+
S2Y2 - a	+	+	+	+	+

MSTA- Mineral salts thiosulfate agar medium; MSTYEA- Mineral salts thiosulfate yeast extract agar medium; NA- Nutrient agar medium; TSA- Tryptic soy agar medium: LB- Luria bertani agar medium.

Sulfur is cycled biogeochemically by microorganisms (Friedrich *et al*., 2005). Sulfur-oxidizing bacteria, including chemolithotrophs and chemoheterotrophs, utilize thiosulfate. It is most suitable for the investigation of sulfur lithotrophic process (Mukhopadhayaya *et al*., 2000). In the Present study, 7 chemolithoautotrophic and 5 facultative chemolithoautotrophic sulfur oxidising bacteria were isolated from different rice ecosystems such as wetland, aerobic and SRI. In an earlier study, Starkey (1935) isolated sulfur-oxidising bacteria from black clay loams. He also reported that the characteristics of growth were diverse; in some cases, the medium was turbid. A low level of sulfur oxidizing bacteria is found in alkali soils. An alkali soil enriched with appropriate bacteria resembles the

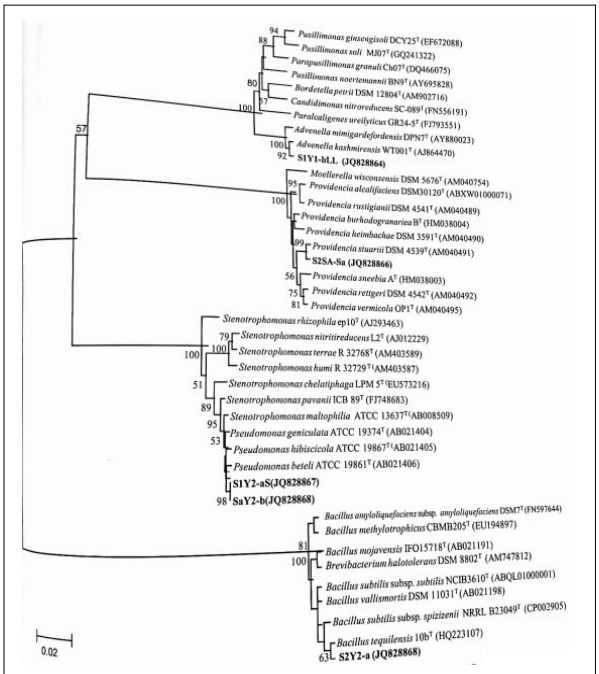


Fig 2: Growth of facultative chemolithoautotrophic sulphur oxidizing bacteria in NA medium.



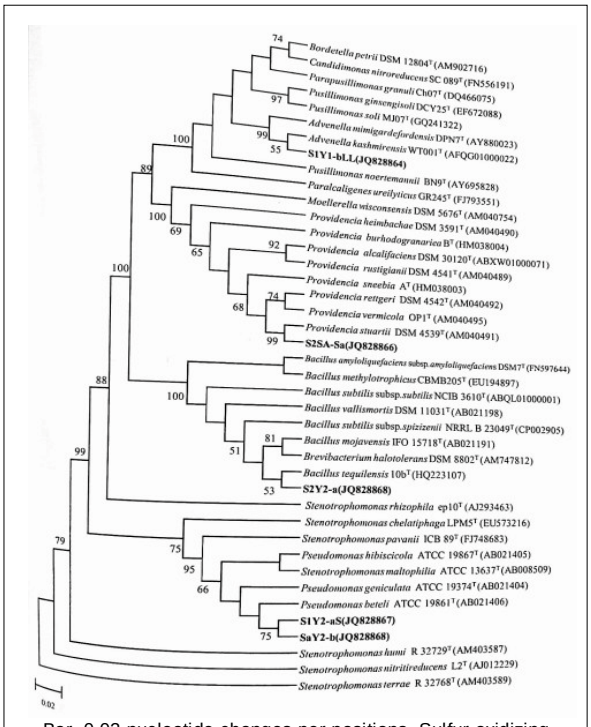
Bar, 0.02 nucleotide changes per positions. Sulfur oxidizing bacterial strains obtained in this study are shown in bold. Bootstrap value ≥ 50 are shown.

Fig 3: Neighbour-joining phylogenetic tree based on complete 16S rRNA sequences.



Bar, 0.02 nucleotide changes per positions. Sulfur oxidizing bacterial strains obtained in this study are shown in bold. Bootstrap value ≥ 50 are shown.

Fig 4: Maximum likely hood phylogenetic tree based on complete 16S rRNA sequences.



Bar, 0.02 nucleotide changes per positions. Sulfur oxidizing bacterial strains obtained in this study are shown in bold. Bootstrap value ≥ 50 are shown.

Fig 5: Maximum parsimony phylogenetic tree based on complete 16S rRNA sequences.



Fig 6: Various formulations of facultative chemolithoautotrophic sulphur oxidizing bacteria.

Table 9: Molecular characterization of facultative chemolithoautotrophic sulfur oxidizing bacteria isolated from different ecosystems.

Strain no.	Closest relative from eztaxon and accession no.	Similarity (%)	Nucleotide length (bp)	NCBI accession no.
S1Y2 - aS	*Pseudomonas beteli* ATCC 19861^{T}AB021406	99.51	1478	JQ828867
S1Y1 - bLL	*Advenella kashmirensis* WT 001^{T}AJ864470	99.66	1477	JQ828864
S2Y2 - b	*Pseudomonas beteli* ATCC 19861^{T}AB021406	99.51	1472	JQ828868
S2SA - Sa	*Providencia stuartii* DSM 4539^{T}AM040491	99.59	1485	JQ828866
S2Y2 - a	*Bacillus tequilensis* 10b^{T} HQ223107	99.93	1485	JQ828865

A. thiooxidans and *S. novella* bacterial species. From Galapagos hydrothermal vent, obligate heterotrophic sulfur oxidizers were repeatedly isolated that presumably oxidized thiosulfate either to sulfate (acid producing *Thiobacillus* like) or to polythionates (base producing *Pseudomonas*) (Vidyalakshmi and Sridar, 2013). Ito *et al*. (2005) isolated bacteria from wastewater biofilms that oxidize sulfur aerobically.

Sulfur oxidizing bacteria such as *Bosea thiooxidans, Paracoccus thiocyanatus*, *Pseudaminobacter salicylatoxidans, Paracoccus pantotrophus, Paracoccus bengalensis, Tetrathiobacter kashmirensis and Mesorhizobium thiogangeticum,* were isolated from rhizosphere and bulk soils of agricultural fields of India (Ghosh *et al*., 2005; 2006; Das *et al*., 1996; Ghosh and Roy, 2006a, b, 2007; Deb *et al*., 2004). Recently, Anandham *et al*. (2005; 2007; 2008a; 2009; 2010) have been documented that the rhizosphere soils of crop plants in Korea are dominated by both obligate and facultative chemolithotrophic thiosulfate oxidizing bacteria (Yim *et al*., 2008). Isolated sulfur oxidizing bacteria were screened based on pH reduction and titrable acidity. Similarly, earlier study, Anandham *et al*. (2005; 2007) screened sulfur oxidizing bacteria based on pH reduction test. Vassilev *et al*. (2001) adopted titrable acidity as one of the criteria to screen phosphate solubilizing bacteria. pH reduction and titrable acidity are indication of indirect oxidation of sulfur oxidation. Sulfur oxidizing bacteria oxidize thiosulfate into sulfuric acid which was responsible for reduction of pH of the medium (Anandham *et al*., 2007; Chaudhary *et al.,* 2022). All the isolated sulfur oxidizing bacteria in the present investigation are Gram negative rods except *Bacillus tequilensis* S2Y2- a is Gram positive. In a previous study, Anandham *et al*. (2008b) reported the presence of sulfur oxidation traits in Gram positive *Microbacterium phyllosphaerae* and *Leifsonia shinshuensis*. In the current investigation, isolated facultative chemo lithoautotrophic sulfur oxidizing bacteria exhibited different nutritional ability. In a previous study, facultative chemo lithoautotrophic *Pandoraea thiooxydans, Burkholderia kururiensis subsp. thiooxydans, Dyella thiooxydans* exhibited autotrophic, heterotrophic abilities (Anandham *et al*., 2009; 2010; 2011).

To best of our knowledge this is the first study to report the presence of sulfur oxidation trait in *Pseudomonas beteli, Providencia stuartii* and *Bacillus tequilensis. Tetrathiobacter kashmirensis* reclassified as *Advenella kashmirensis* was originally recovered in in garden soils of Kashmir, the same strain also isolated from paddy soil of present study (Ghosh *et al*., 2005). It was attributed that selective pressure (sulfur) used by Ghsoh *et al*. (2005) same selective pressure was also used in this study.

CONCLUSION

Totally five facultative chemolithoautotrophic sulfur oxidizing bacteria were autotrophic and chemo heterotrophic in nature recovered from different rice ecosystems which efficiently utilizes inorganic sulfur compounds as electron donors to generate energy. This investigation is widely distributed in isolated genera namely, *Pseudomonas beteli, Advenella kasmirensis, Pseudomonas beteli, Providencia stuartii* and *Bacillus tequilensis*. The current research explores the presence of facultative chemolithoautotrophs in rice ecosystems, which have a great ability to lower the pH of the culture medium and effectively create sulfate ion. The pH lowering ability of SOB allows them to be used for alkali soil restoration and phosphate solubilisation in the future.

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Disclaimers

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Conflict of interest

The authors declare no competing interests.

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