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Sulphur Oxidizing Bacteria and Pulse Nutrition - A Review

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Abstract: Sulphur oxidizers are involved in oxidation of elemental sulphur to plant available sulphate. Sulphur is the key element for higher pulse production and plays an important role in the formation of proteins, vitamins and enzymes. Sulphur is a constituent of the essential amino acids cystine, cysteine and methionine. In the recent years the importance of sulphur in pulse nutrition has been well recognized because of the widespread occurrence of its deficiency in the soil world over. Majority of sulphur taken up by plant roots is in the form of sulphate (SO_4), which undergoes a series of transformation prior to its incorporation into the original compounds. The soil microbial biomass is the key driving force behind all sulphur transformations. The biomass acts as both a source and sink for inorganic sulphate. They make available sulphate from element or any reduced forms of sulphur, through oxidation process in the soil. The role of chemolithotrophic bacteria of the genus *Thiobacillus* in this process is usually emphasized. Use of sulphur oxidizers enhance the natural oxidation and speed up the production of sulphates.

Key words: Sulphur · Sulphur Oxidizing microorganisms · Pulse

INTRODUCTION

Sulphur is one of the essential plant nutrients and it contributes to yield and quality of crops. Sulphur occurs in a wide variety of organic and inorganic combinations. The transfer of sulphur between the inorganic and organic pool is entirely caused by the activity of the soil biota, particularly the soil microbial biomass, which has the greatest potential for both mineralization and also for subsequent transformation of the oxidation state of sulphur. *Thiobacilli* play an important role in sulphur oxidation in soil. Sulphur oxidation is the most important step of sulphur cycle, which improves soil fertility. It results in the formation of sulphate, which can be used by the plants, while the acidity produced by oxidation helps to solubilize plant nutrients and improves alkali soils [1].

Sulphur Oxidizing Microorganisms: The sulphur oxidizing microorganisms are primarily the gram negative bacteria currently classified as species of *Thiobacillus, Thiomicrospira* and *Thiosphaera*, but heterotrophs, such as some species of *Paracoccus, Xanthobacter, Alcaligens* and *Pseudomonas* can also exhibit chemolithotrophic growth on inorganic sulphur compounds [2].

Two clear metabolic types exist in this group: The obligate chemolithotrophs, which can only grow when supplied with oxidizable sulphur compounds (and CO_2 as the source of metabolic carbon) and heterotrophs that can also use the chemolithoautotrophic mode of growth. The obligate chemolithotrophs include *Thiobacillus thioparus, T. neapolitanus, T. denitrificans* (facultative denitrifier), *Thiobacillus thiooxidans* (extreme acidophile), *Thiobacillus ferrooxidans* (acidophilic ferrous iron-oxidizer), *Thiobacillus halophilus* (halophile) and some species of *Thiomicrospira*. The heterotrophs include *Thiobacillus novellus, T. acidophilus* (acidophile), *Thiobacillus aquaesulis* (moderate thermophile), *Thiobacillus intermedius, Paracoccus denitrificans, P. versutus, Xanthobacter tagetidis, Thiosphaera pantotroph* and *Thiomicrospira thyasirae.*

Several *Thiobacillus* species are able to utilize mixtures of inorganic and organic compounds simultaneously, often referred to as mixotrophic growth [2]. Depending on the ratio of inorganic and organic substrates, CO_2 may serve as an additional carbon source. Of the 13 species of the genus *Thiobacillus* recognized, occurring in diverse habitats, only five species are important in sulphur oxidation in soil [3]. Four of these *Thiobacillus thiooxidans, T. ferrooxidans, T. thioparus* and *T. denitrificans* are obligate chemoautotrophs while *T. novellus* is considered a facultative chemoautotroph [4].

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Also fungi are capable of oxidizing elemental sulphur and thiosulphate, which include, *Alternaria tenius, Aureobasidium pullulans, Epicoccum nigrum*, a range of *Penicillium* species [5], *Scolecobasidium constrictum, Myrothecium cinctum* and *Aspergillus* [6].

The Sulphur-Oxidizing Prokaryotes: Biological oxidation of hydrogen sulphide to sulphate is one of the major reactions of the global sulphur-cycle. Reduced inorganic sulphur compounds are exclusively oxidized by prokaryotes and sulphate is the major oxidation product. Sulphur oxidation in members of the *Eukarya* is mediated by lithoautotrophic bacterial endosymbionts [7]. In the domain *Archaea* aerobic sulphur oxidation is restricted to members of the *Sulfolobales* [8, 9] and in the domain *Bacteria* sulphur is oxidized by aerobic lithotrophs or by anaerobic phototrophs. The nonphototrophic obligate anaerobe *Wolinella succinogenes* oxidizes hydrogen sulphide to polysulphide during fumarate respiration [10].

Prokaryotes oxidize hydrogen sulphide, sulphur, sulphite, thiosulphate and various polythionates under alkaline [11] neutral or acidic conditions 12]. Aerobic sulphur oxidizing prokaryotes belong to genera like *Acidianus* 13], *Acidithiobacillus* [14], *Aquaspirillum* [15], *Aquifer* [16], *Bacillus* [17], *Beggiatoa* (Strohl, 1989)[18], *Methylobacterium* [19,20], *Paracoccus, Pseudomonas* [15], *Starkeya* [21], *Sulfolobus*, *Thermithiobacillus* [14], *Thiobacillus* and *Xanthobacter* [15] and are mainly mesophilic. Phototrophic anaerobic sulphur oxidizing bacteria are mainly neutrophilic and mesophilic [22,23] and belong to genera like *Allochromatium* [24], *Chlorobium, Rhodobacter, Rhodopseudomonas*, *Rhodovulum* and *Thiocapsa* [25]. Lithoautotrophic growth in the dark has been described for *Thiocapsa roseopersicina, Allochromatium vinosum* and other purple sulphur bacteria, as well as for purple non sulphur bacteria like *Rhodovulum sulfidohilum* [26], *Rhodocyclus genatinosus* and *Rhodopseudomonas acidopphila* [27, 28]. This capacity may be based on related biochemical mechanism of sulphur oxidation in lithotrophic bacteria.

Two groups of sulphur oxidizing lithotrophic bacteria have been distinguished previously; members of one group are able to utilize polythionates and members of the other group are not able to do this [29, 13]. On the basis of physiological and biochemical data, at least two major pathways have been proposed for sulphur oxidizing bacteria: (I) the sulphur oxidation pathway and (ii) the S_4 intermediate pathway involving polythionates [29]. Different metabolic reactions merge into common mechanism in lithotrophic and phototrophic sulphur oxidizing bacteria [30].

Other Sulphur Bacteria: Other bacteria may also oxidize sulphur compounds. The gliding sulphur oxidizers include those bacteria that have a gliding motion on the substrate; their cells are arranged in trichomes. The most important members of this group in relation to sulphur oxidation in soils are species of *Beggiatoa* bacteria that participate in sulphate oxidation in the root zone of rice. All strains of *Beggiatoa* deposit sulphur intracellularly in the presence of hydrogen sulphide. Phototrophic bacteria, such as *Chromatium* and *Chlorobium*, also play an important role in sulphide oxidation in rice soil, but not in aerobic agricultural soils [2]. A number of non-filamentous, chemolithotrophic sulphur oxidizing bacteria such as *Sulfolobus, Thiospira* or *Thiomicrospira* have also been isolated from special habitats.

Sulphur Transformation in Soil: The dominant form of sulphur taken up by plants and soil microbes is sulphate. The sulphur may originate from the weathering of soil minerals, from the atmosphere and from originally bound sulphur. Transformation of elemental sulphur to sulphate form was necessary for sulphur to be available for crop uptake [31]. The oxidation was faster in coarse textured soils and was completed in 3-4 weeks [32]. Sulphur transformations in soil are considered to result primarily from microbial activity which involves processes of mineralization, immobilization, oxidation and reduction.

Reduced sulphur compounds formed by assimilatory sulphate reduction are cell constituents and are therefore protected within the cells against reaction with oxygen. The reduction of SO_4 to H_2S is mediated by anaerobic sulphate-reducing bacteria. Sulphate reduction is a major component of the sulphur cycle in soils exposed to water logging conditions or periodic flooding, especially, where readily decomposable plant residues are present.

The biogeochemical transformations of sulphur have been formulated by Vernadskii [34].

In the magmatic crust of our planet, sulphur is always present in its reduced form as sulfide of metals. Oxidation of the magmatic metal sulphides reaching the crust and settling in ore deposits preceded with the participation of *Thiobacillus ferrooxidans* into the oceans. In arid regions, lagoons, or inland seas they may form gypsum deposits. In waterlogged soils, sulphates are reduced to

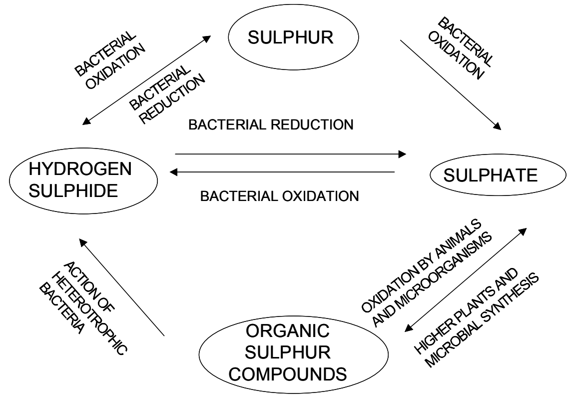

Fig. 1: The sulphur cycle [33]

hydrogen sulphide by *Desulfovibrio desulfuricans*. When the secondarily formed hydrogen sulphide enters the oxidative zone, it is oxidized by *Thiobacillus thioparus*. This is the pattern of formation of sedimentary deposits of native sulphur. The activity of *T. thioparus* and *T. thiooxidans* in the soil accounts for the oxidation of hydrogen sulphide to sulphur. The final oxidation stage of molecular sulphur to sulphuric acid is accounted for, by the activity of *T. thiooxidans*. The role of filamentous and pigmented sulphur bacteria in the metabolism of sulphur in water bodies and soil was of secondary importance [35].

Role of *Thiobacilli* in the Oxidation of Sulphur in Soil: Though sulphur traces may be oxidized in soil by various species of microorganisms and fungi, Waksman [36] pointed out *Thiobacilli* as the most characteristic group of microorganism performing the oxidative part of sulphur transformation in soil.

Studies on the distribution of *Thiobacillus thioxidans* and *T. thioparus* showed that these bacteria are found in an active state mainly in soils fertilized with sulphur. The distribution of *T. thioparus* in soils was studied by Starkey [37], who demonstrated the almost ubiquitous presence of bacteria in alkaline and neutral soils and their absence in strongly acid soils fertilized with sulphur. The widespread occurrence of *Thiobacilli* in soils fertilized with sulphur or in soils in which accumulation of sulphur compounds occurs under natural conditions (marshes and peats) indicates that these bacteria play an important role in the oxidation of sulphur and its compounds in soils.

Inoculation of *Thiobacilli* generally increases the rate of sulphur oxidation [38], although such amendment is unnecessary in many agricultural soils, as they possess a native population of sulphur oxidizers which is stimulated on sulphur addition. Kapoor and Mishra [38] observed that sulphur was rapidly oxidized in a field soil of pH 8.0 and the rate of oxidation could be enhanced by inoculation with *T. thiooxidans*.

Isolation, Characterization and Identification of Sulphur Oxidizing Bacteria

Isolation: In 1888, Winogradsky, on the basis of his studies on *Beggiatoa*, established that sulphur bacteria derive their energy from the oxidation of reduced sulphur compounds and utilize it for the uptake of CO_2. Similar organism was first isolated by Nathansohn [39], from the silts of the Bay of Naples. Their excellent growth in mineral media containing thiosulphate was accompanied by the production of a pellicle of elemental sulphur. According to Nathansohn [39], these organisms derive their energy from the exothermic oxidation of thiosulphate to tetrathionate and sulphuric acid.

Bacteria, closely resembling the organisms described by Nathanson [39], were isolated in pure culture by Beijernick [40], from seawater, silts of the Delft canals and freshwater. The new organism studied by Nathanson [39] and Beijerinck [40] was named *Thiobacillus thioparus*. The *T. thioparus* was isolated by various authors (Jacobsen, [41] Jofee, [42] Brown, [43]) from soil, ditchwater, sewage and sea water and its oxidation of sulphur to sulfate was also studied. Waksman [44] isolated *Thiobacillus thioparus* and *Thiobacillus novellus* from compost which were capable of sulphur oxidation under alkaline condition.

Waksman and Jofee [45] described another species of *Thiobacilli*, isolated from soil previously fertilized with sulphur and designated as *Thiobacillus thiooxidans.* Brown [43] obtained a similar organism from garden soil, activated sludge and soil-sulphur compost. Ayyar [46] and Ayyar *et al.* [47] obtained a culture from activated sludge which reacted much same as *Thiobacillus thiooxidans.*

Emoto [48, 49] isolated from hot springs several bacterial strains differentiated mainly by colonial morphology: *Thiobacillus thermitanus, T. umbonatus, T. lobatus* and *T. crenatus*. These are autotrophic, developing at low pH, morphologically resembling variants of *Thiobacillus thiooxidans.* Starkey [50] isolated sulphur oxidizing bacteria from soil. Parker [51], Parker and Prisk [52] isolated *Thiobacillus* X and *Thiobacillus concretivorus* from corroded concrete. The organism described as *Thiobacillus ferrooxidans* has been isolated from acid mine water by Colmer and Hinkle [53]. *Thiobacillus denitrificans* was first isolated by Beijerinck [40] and has been studied in detail by Lieske [54] and Baalsrud and Baalsrud [55].

Thiobacilli were isolated from only one out of six alkali soils in India [56]. Brock *et al.* [57] isolated members of a new genus of sulphur oxidizing acidophilic bacteria, *Sulfolobus,* the type species of which was *S. acidocaldarius*. It grew and oxidized elemental sulphur over a temperature range of 55-88°C. Khalid and Malik [58] isolated two acidophilic chemolithotrophic bacterial strains resembling *Thiobacillus thiooxidans* and *Thiobacillus* ferrooxidans from sewage waters.

Thiobacillus acidophilus and *Thiobacillus cuprinus* are mixotrophic acidophiles, isolated from acid strams [59]. Chapman [60] detected neutrophilic *Thiobacilli* in 84 per cent of the soils examined with a mode of 10^{-2} to 10^{-3} organisms per gram soil. Heterotrophic sulphur oxidizing isolates, colonizing elemental sulphur beads were obtained by Lawrence and Germida [61]. *Sulfolobus* and *Thermus* like strains and thiosulphate oxidizing organism were isolated from the thermal spring waters of Surat by Dave and Upadhyay [62]. Shooner and Tyagi [63] isolated moderate thermophilic organism *Thiobacillus thermosulfatus* from sewage sludge. Two novel chemolithotrophic sulphur bacteria were isolated from oil field brine by Gevertz *et al.*, [64].

Sulphur oxidizing bacteria were isolated from paddy rhizosphere, pulse rhizosphere, sewage, biogas slurry, tannery effluent and mine soil by Vidyalakshmi and Sridar, [65]. Out of the 28 *Thiobacillus* isolates obtained, 14 were screened based on their efficacy to reduce the pH of the growth mediu m from 8.0 to 5.0.

Characterization and Identification: Winogradsky's [66] observation, that hydrogen sulphide could serve as electron donor in the respiration of *Beggiatoa* species was the first in his series of studies which culminated in the discovery of the chemoautotrophic mode of life. The experiments of Cataldi [67] suggested that at least some strains of *Beggiatoa* are heterotrophic.

Beijerinck [40] characterized *Thiobacillus thioparus* as a strictly aerobic motile, minute thin rods, 0.5×3µ bacterium which grew rapidly in a mineral medium containing thiosulphate and frequently deposited large amounts of molecular sulphur and gave the colonies a milky white to canary yellow appearance. The organisms grew rapidly near pH 7.0 at 30°C, whereas Starkey [37] described it as non-motile coccoid cells, 0.5 × 0.7µ; while Parker and Prisk (1953) [52] described them as medium-sized rods, single or in pairs measuring 0.5× 1-1.5µ.

Thiobacillus thiooxidans are described as short rods with round ends, 1µ long and 0.5µ wide, gram positive, motile [45] and bore one polar flagellum [68], revealed that the protoplasm of *Thiobacillus thiooxidans* is gram negative, while the vacuoles are gram positive. Young cells devoid of reserve substances are gram negative. Sokolova and Karavaiko [35] described them as rod-shaped cells of 0.5-0.7 × 1-1.2 µ, gram negative cells which occurred singly and occasionally in pairs and short chains.

Guay and Silver [69] characterized *Thiobacillus acidophilus* as short rods, 0.5-0.8µm × 1-1.5µm with round ends, gram negative and motile non-sporulating. The cells occured singly, mainly in pairs and rarely in chains.

Wood and Kelly [70] isolated and characterized *Thiobacillus halophilus* sp. nov. a sulphur oxidizing eubacterium from a Western Australian hypersensitive lake. The organism was obligately halophilic, grew best with 0.8-1.0µ NaCl and tolerated upto 4M NaCl. Optimum growth was obtained at about 30°C and pH 7.0-7.3.

Microbial Production of Sulphate: Starkey [37] reported that sulphate production from elemental sulphur in sulphur media ranged from 31.7 mg per 100 cc. to 118.9 mg per 100 cc. Sulphate-sulphur over the range of 100-200 µg per ml with a precision of ± 10 µg was obtained on the oxidation of elemental sulphur by *Thiobacillus thiooxidans* [71].

In a experiment involving the biological oxidation of pyrite conducted in mine spoil, Pichtel and Dick [72] noticed concentration of sulphate was found to be 81 m. mol kg^{-1} (at controlled atmospheric conditions) spoil after 28 days of incubation.

Sulphur Deficiency Symptoms in Pulses: Plants suffering from severe sulphur deficiency show characteristic symptoms. Natesan *et al.* [73] reported that sulphur deficiency leads to reduction in growth rate of the plant and generally the growth of shoots were more affected than that of roots. Sulphur deficient plants were yellow, small and spindle with short slender stalks [74]. Randall [75] opined that the mobility of sulphur in the plants is relatively lesser than nitrogen, leading to chlorosis of younger leaves. The leaves at later stages show necrotic symptoms and die [76].

The classical symptom was the yellowing of younger leaves, while old leaves remained green [77]. Joseph and Verma [78] reported that nodulation in legumes was affected and nitrogen fixation was reduced.

Role of Sulphur in Pulse Crops: Sulphur is one of the essential plant nutrients classified as secondary nutrient. It is essential for all plants and is indispensable for the growth and metabolism. The concentration was found to be the highest in oilseeds (1.1-1.7 per cent), intermediate in pulses (0.24-0.32 per cent) and the lowest (0.12-0.20 per cent) in cereals [79]. Sulphur has a number of oxidizing function in plant nutrition and is best known for its role in protein synthesis [75]. Sulphur is a constituent of certain aminoacids like methionine, cystine and cysteine and also a constituent of Fe-S proteins called ferrodoxins [80].

Khandkar *et al.* [81] observed that the nodule in blackgram was increased due to sulphur application. Saraf [82] reported that sulphur is involved in the formation of nitrogenase enzyme and is known to promote nitrogen fixation in legumes. Sulphur is involved in the formation of chlorophyll [83]. Hanesklaus and Schnug [84] reported that sulphur is associated with production of crops of superior nutritional and market quality. Poorani [85] reported that sulphur application increased the total chlorophyll content of greengram. Sulphur plays a dominant role in improving the quality of pulses [86].

Sharma and Room Singh [87] found that application of sulphur at the rate of 40 kg ha^{-1} enhanced plant height, branches, pods $plant^{-1}$ and the 1000 gram weight in green gram. Singh and Aggarwal [88] found that application of gypsum at the rate of 60 kg ha^{-1} produced significantly higher pod length, seeds pod^{-1} to 1000 weight in blackgram. They also reported that application of 30 kg sulphur ha^{-1} significantly increased the yield contributing characters and yield of blackgram.

Interaction of Sulphur with Other Nutrients: Tiwari [89] studied the interaction of sulphur with other nutrients. He reported that sulphur had synergistic relationship with N, P, K, Mg and Zn. Acidity produced on oxidation of reduced inorganic sulphur compounds in soil was known to increase the solubility of micronutrients, like iron, zinc and manganese.

Effect of Sulphur Application and *Rhizobium* Interaction: Studies in pigeon pea by Umarani [90] indicated increased uptake of N, P and K due to sulphur nutrition. Sulphur application (40 kg ha^{-1}) with *Rhizobium* inoculation in greengram, increased the bacterial population significantly [91,92].

Effect of Sulphur and Phosphorus Application: Legumes usually require almost equal amount of phosphorus and sulphur. When phosphorus and sulphur are present below the critical level in the soil, plant growth and quality of produce are affected adversely [93]. Sulphur oxidized by *Thiobacilli* in soil leads to production of H_2SO_4, which would then solubilize phosphorus. The acidity generated on oxidation of pyrite can be coupled to solubilization of rock phosphate or to reclaim alkali soil [38]. The solubilization of Mussoorie rock phosphate on addition of elemental sulphur and pyrite and on inoculation with sulphur and iron-oxidizing bacteria in soil was studied by Kapoor *et al.* [94]. Inoculation with *Thiobacillus thioxidans* and *Thiobacillus ferrooxidans* increased oxidation of sulphur and pyrite and consequently the solubilization of rock phosphate. A similar finding was also reported by Costa *et al.* [95].

Also, the grain yield of green gram was maximum when 50 kg P_2O_5 + 40 kg sulphur ha^{-1} was applied [86]. Application of 40 kg sulphur along with 40 kg P_2O_5 ha^{-1} resulted in higher grain yield than sulphur alone, in chickpea [96]. Thus phosphorus and sulphur are reported to have synergistic effect on the productivity of crops.

REFERENCES

1. Wainwright, M., 1984. Sulphur oxidation in soils. Adv. Agron., 37: 350-392.
2. Kuenen, J.G. and R.F. Beudeker., 1982. Microbiology of Thiobacilli and other sulphur oxidising autotrophs mixotrophs and heterotrophs. In: Sulphur bacteria. J.P. Post Gate and D.P. Kelly (Eds.). University Press, Cambridge. pp: 473-497.
3. Starkey, R.L., 1966. Oxidation and reduction of sulfur compounds in soils. Soil Sci., 101: 297-306.
4. Taylor, B.F. and D.S. Hoare, 1969. J. Bacteriol., 100: 487-497.
5. Wainwright, M., 1978. A modified sulphur medium for the isolation of sulphur oxidising fungi. Plant and Soil, 49: 191-193.
6. Shinde, D.B., P.L. Patil and B.R. Patil., 1996. Potential use of sulphur oxidizing microorganism as soil inoculant. Crop Res., 11: 291-295.
7. Nelson, D.C. and C.R. Fisher., 1995. Chemoautrophic and methanotrophic endosymbiotic bacteria at deep–sea vents and seeps. In: The microbiology of deep-sea hydrothermal vents. D.M. Karl (Ed.). CRC Press, Boca Raton. pp: 125-167.
8. Setter, K.O., G. Fiala, G. Huber, H. Huber and A. Segerer., 1990. Hyperthermophilic microorganisms. FEMS Microbiol. Rev., 75: 117-124.

9. Fuchs, T., H. Huber, S. Burggraf and K.O. Stetter, 1996. 16S rDNA –based phylogeny of the archaeal order *Sulfolobales* and reclassification of *Desulfurolobus ambivalens* as *Actdianus ambivalens* comb. nov. Syst. Appl. Microbiol., 19: 56-60.
10. Macy, J.M., I. Schroder, R.K. Thauer and A. Kroger., 1986. Growth of *Wolinella succinogenes* on H_2S plus fumarate and on formate plus sulfur as energy sources. Arch. Microbiol., 144: 147-150.
11. Sorokin, D.Y., A.M. Lysenko, L.I. Mityushina, T.P. Tourova, B.F. Jones, F.A. Rainey, L.A. Robertson and J.G. Kuenen, 2001. *Thioalkalimicrobium aerophulum* gen. nov., sp. nov. and *Thioalkalimicrobium sibericum* sp. nov. and *Thioalkalivibrio versutus* gen. nov., sp. nov. and *Thioalkalivibrio nitratis* sp. nov. and *Thioalkalivibrio denitrificans* sp. nov., novel obligately alkalophilic and obligately chemolithoautotrophic sulfur oxidizing bacteria from soda lakes. Int. J. Syst. Evol. Microbiol., 51: 565-580.
12. Harrison, A.P., 1984. The acidophilic Thiobacilli and other acidophilic bacteria that share their habitat. Annu. Rev. Microbiol., 38: 265-292.
13. Friedrich, C.G., 1998. Physiology and genetics of sulfur–oxidizing bacteria. Adv. Microb. Physiol., 39: 235-289.
14. Kelly, D.P. and A.P. Wood., 2000. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halobacillus* gen. nov. and *Themithiobacillus* gen. nov. Int. J. Syst. Evol. Microbial., 50: 511-516.
15. Friedrich, C.G. and G. Mitrenga., 1981. Oxidation of thiosulfate by *Paracoccus denitrificans* and other hydrogen bacteria. FEMS Microbiol. Lett., 10: 209-212.
16. Huber, R. and K.O. Stetter., 1999. Aquificales. In: Embryonic ELS, No. 785998. Macmillan, Houndmills, England. pp: 1-7.
17. Aragno, M., 1991. Aerobic chemolithoautotrophic bacteria, In: Thermophilic bacteria. J.K. Kristjansson (Ed.) CRC Press, Boca Raton, Fla pp: 7-103.
18. Strohl, W.R., 1989. Genus I. *Beggiatoa.* In: Bergey's manual of systematic bacteriology, vol 3. J. T. Staley, M.P. Bryant, N. Pfennig and J.G. Holt (Eds.). Williams and Wilkinson, Baltimore, Md., pp: 2091-2097.
19. Kelly, D.P. and N.A. Smith., 1990. Organic sulfur compounds in the environment. Adv. Microb. Ecol., 11: 345-385.
20. De Zwart, J.M.M., P.N. Nelisse and J.G. Kuenen., 1996. Isolation and characterization of *Methylophaga sulfidovorans,* sp. nov. an obligately methylotrophic, aerobic, dimethyl sulfide oxidizing bacterium from a microbial mat. FEMS Microbiol. Ecol., 20: 261-270.
21. Kelly, D.P., I.R. McDonald and A.O. Wood, 2000. Proposal for the reclassification of *Thiobacillus novellus* as *Starkeya novella* gen. nov., comb. nov., in the ∝-subclass of the Proteobacteria. Int. J. Syst. Evol. Microbiol., 50: 1797-1802.
22. Truper, H.G. and U. Fischer. 1982. Anaerobic oxidation of sulfur compounds as electron donors for bacterial photosynthesis. Phil. Trans. R. Soc. Lond., 298: 529-542.
23. Brune, D.C., 1995. Sulfur compounds as photosynthetic electron donors. In: Anoxygenic photosynthetic bacteria. R.E. Blankenship, M.T. Madigan and C.E. Bauer (Eds.). Kluwer, Dordrecht, The Netherlands. pp: 847-870.
24. Imhoff, J.F., J. Siiling and R. Petri., 1998. Phylogenetic relationship and taxonomic reclassificiation of *Chromatium* species and related purple sulfur bacteria. Int. J. Syst. Bacteriol., 48: 1029-1043.
25. Brune, D.C., 1989. Sulfur oxidation by phototrophic bacteria. Biochem. Biophys. Acta, 975: 189-221.
26. Hiraishi, A. and Y. Umeda., 1994. Intragenic structure of the genus *Rhodobacter*: transfer of *Rhodobacter sulfidophilas* and related marine species to the genus *Rhodovulum* gen. nov. Int. J. Syst. Bacteriol., 44: 15-23.
27. Siefert, E. and N. Pfennig, 1979. Chemoautotrophic growth of *Rhodopsea domonus* species with hydrogen and chemotrophic utilization of methanol and formate. Arch. Microbiol., 122: 177-182.
28. Kondratieva, E.N., 1989. Chemolithotrophy of phototrophic bacteria. In: Autotrophic bacteria. H.G. Schlegel and B. Bowien (Eds.). Springer, Germany, pp: 283-287.
29. Kelly, D.P., J.K. Shergill, W.P. Lu and A.P. Wood, 1997. Oxidative metabolism of inorganic sulphur compounds by bacteria. Antonie Leeuwenhoek, 71: 95-107.
30. Friedrich, C.G., D. Rother, F. Bardischewsky, A. Quentmeier and J. Fischer., 2001. Oxidation of reduced inorganic sulphur compounds by bacteria: Emergence of a common mechanism? Appl. Environ. Microbiol., 67: 2873-2882.
31. Mahendra Singh., 1988. Sulphur management in coarse textured alluvial soils. Proc. of TSI-FAI symp. Sulphur in agriculture, Mar. 9-11. New Delhi. pp: S III/2(1-9)

32. Tandon, H.L.S., 1989. Sulphur fertilizers for the tropics. Proc. TSI-FAI symp. Sulphur in Indian agriculture, Mar. 9-11, New Delhi. pp: S IV / 2(1-11).
33. Burns, G.R., 1967. The oxidation of sulphur in soils. In: Sulphur, energy and environment. B. Meyer (Ed.). Sulphur Institute Tech. Bull. 13. Elsevier. New York. pp: 261.
34. Vernadskii, V.I., 1927. Istoriya mineralov zemnoi kory (Hisotry of the minerals of the earth crust), Vol.1, No: 2 (see Izbrannye Trudy, Vol. 3, Izd. An SSSR. 1955).
35. Sokolova, G.A. and G.I. Karavaiko, 1968. Physiology and geochemical activity of black gram*Thiobacilli*. In: Sulphur bacteria J.R. Postgate and D.P. Kelley (Eds). University Press. Cambridge. pp: 133-152.
36. Waksman, S.A., 1932. Principles of soil microbiology. 2nd edition. Williams and Williams Co., Baltimore.
37. Starkey, R.L., 1934. Cultivation of organisms concerned in the oxidation of thiosulphate. J. Bacteriol., 28: 365-386.
38. Kapoor, K.K. and M.M. Mishra., 1989. Microbial transformation of sulphur and plant nutrition. In: Soil microorganisms and crop growth. L.L. Somani and S.L. Bhandari. (Eds.). Diyajyoti Prakasam, India, pp: 1-30.
39. Nathansohn, A., 1902. Ober eine neue GTruppe Von schwefelbacterien undihren stoffwechsel. Mitt. Zool. Stn. Neapel., 15: 655-680.
40. Beijerinck, M.W., 1904. Phenomenes de reduction proguits parles microbes. Arch. Sci. Exactes et Nat. Haarlem. Ser., 2: 9131-9157.
41. Jacobsen, H.C., 1912. Die oxidation von elementarem schwetel dutch Bakterien. Folia Microbiol., 1: 487-496.
42. Jofee, J.S., 1922. Biochemical oxidation of sulphur and its significance to agriculture. N. J. Agr. Exp. Sta. Bull., 374.
43. Brown, H.D., 1923. Sulfofication in pure and wined cultures with special reference to sulphate production, hydrogen ion-concentration and nitrification. J. Amer. Soc. Agron., 15: 350-382.
44. Waksman, S.A., 1922. Microorganisms concerned in the oxidation of sulphur in the soil: III. Media used for the isolation of sulphur bacteria from the soil. J. Bacteriol., 7: 605-608.
45. Waksman, S.A. and J.S. Jofee., 1922. Microorganisms concerned in the oxidation of sulphur in the soil: II. *Thiobacillus thiooxidans*, a new sulphur-oxidizing organism isolated from the soil. J. Bacteriol., 7: 239-256.
46. Ayyar, C.V.R., 1928. Biological oxidation of sulphur. Part II. Effect on the microflora of activated sludge. J. Indian Inst. Sci., 12: 275-277.
47. Ayyar, C.V.R., T.S.S. Perumal and R.V. Norris, 1928. The oxidation of sulphur in suspensions of activated sludge and its influence on the solubilization of mineral phosphate. J. Indian.lnst. Sci., 11: 85-90.
48. Emoto, Y., 1928. uber'einneue Schwefelonidievende Bakterie. Botan. Mag. (Tokyo), 42: 421-425.
49. Emoto, Y., 1933. Studien uber die Physiologie der schwefeloxydierenden Bakterien. Botan. Mag. (Tokyo), 47: 405-588.
50. Parker, C.D., 1947. Species of sulphur bacteria associated with the corrosion and concrete. Nature, 159.
51. Starkey, R.L., 1935. Isolation of some bacteria which oxidize thiosulphate. Soil Sci., 39: 197-219.
52. Parker, C.D. and J. Prisk., 1953. The oxidation of inorganic compounds of sulphur by various sulphur bacteria. J. Gen. Microbiol., 8: 344-364.
53. Colmer, A.R. and M.E. Hinkle., 1947. The role of microorganism in acid mine drainage. A preliminary report. Science, 106.
54. Lieske, R., 1912. Untersuchungen uber die physiologie der denitrifizierenden Schwe-felbakterien. Ber. deut. botan. Ges., 30: 12-22.
55. Baalsurd, K. and K.S. Baalsrud., 1954. Studies on *Thiobacillus denitrificans.* Arch. Mikrobiol., 20: 34-62.
56. Rupela, O.P. and P. Tauro., 1973. Isolation and characterization of *Thiobacillus* from alkali soils. Soil Biol. Biochem., 5: 891-897.
57. Brock, T.D., K.M. Brock, R.T. Belly and R.L. Weiss, 1972. *Sulfolobus*: a new genus of sulphur-oxidising bacteria living at low pH and high temperature. Arch. Microbiol., 134: 42-50.
58. Khalid, J.M. and K.A. Malik, 1987. Isolation and characterization of some acidophilic *Thiobacilli* from sewage waters. Pakistan J. Scientific and Industrial Res., 30: 905-908.
59. Barrie Jhonshon, D., M.A. Pal Ghauri and Mazinf Said, 1992. Isolation and characterisation of an acidophilic, heterotrophic bacterium capable of oxidizing ferrous ion. Appl. Environ. Microbiol., 58: 1423-1428.
60. Chapman, S.J., 1990. *Thiobacillus* population in some agricultural soils. Soil Biol. Biochem., 22: 479-482.
61. Lawerence, J.R. and J.J. Germida., 1991. Microbial and chemical characteristics of elemental sulphur beads in agricultural soils. Soil Biol. Biochem, 23: 617-622.
62. Dave, S.R. and N.M. Upadhyay., 1993. Thiosulphate oxidizing organisms from thermal spring. Indian J. Micorbiol., 33: 241-244.

63. Shooner, R. and R.D. Tyagi., 1995. Microbial ecology of simultaneous thermophilic microbial leaching and digestion of sewage sludge. Can. J. Microbiol., 41: 1071-1080.
64. Gevertz, D., A.J. Telang, G. Veerdouw and G.E. Jenneman., 2000. Isolation and characterization of strains CVO and FWKO B, two novel nitrate-reducing, sulfide-oxidizing bacteria isolated from oil field brine. Appl. Environ. Microbiol., 66: 2491-2501.
65. Vidyalakshmi, R. and R. Sridar., 2007. Isolation and characterization of sulphur oxidizing bacteria. J. of Culture Collections, 5: 73-77.
66. Winogradsky, S., 1888. Uber Eisenbak-terien. Botan. Zeitung, 46: 261-270.
67. Cataldi, M.S., 1940. Aislamiento de *Beggiatoa* alba en cultivo puro. Rev. inst. bacteriol. dep. nac. hig. (Buenos Aires), 9: 393-423.
68. Knaysi, G., 1943. A cytological and micro chemical study of *Thiobacillus thiooxidans.* J. Bacteriol., 46: 451-461.
69. Guay, R. and M. Silver., 1975. *Thiobacillus acidophilus* sp. nov; isolation and some physiological characteristics. Can. J. Microbiol., 21: 281-288.
70. Wood, A.P. and D.P. Kelly., 1991. Isolation and characterization of *Thiobacillus halophilus* sp. nov., a sulphur oxidizing autotrophic eubacterium from a Western Australian hypersaline lake. Arch. Microbiol., 156: 277-280.
71. Vogler, K.G. and W.W. Umbreit., 1941. The necessity for direct contact in sulfur oxidation by *Thiobacillus thiooxidans.* Soil Sci., 51: 331-337.
72. Pitchel, J.R. and W.A. Dick., 1991. Sulphur, iron and solid phase transformations during the biological oxidation of pyretic mine spoil. Soil Biol. Biochem., 23: 101-107.
73. Natesan, S., N. Palani and V. Ranganathan, 1985. Sulphur for tea. Proc. of TNAU-FACT National seminar on Sulphur in agriculture, Oct. 18-19, 1985, TNAU, Coimbatore. pp: 129.
74. Ramaswamy, P.P. and T.S. Manickam., 1985. Sulphur nutrition in agricultural crops. Proc of TNAU-FACT National seminar on Sulphur in agriculture, Oct. 18-19, 1985, Tamil Nadu Agricultural University, Coimbatore. pp: 69.
75. Randall, P.J., 1988. Evaluation of the sulphur status of soils and plants. Techniques and interpretation. Proc. of TSI-FAI symp. Sulphur in Indian agriculture. Mar. 9-11, 1988, New Delhi, pp: Sl/3 (1-15).
76. Singh, S.K., R. Singh and H.P. Singh., 1994. Effect of sulphur on growth and yield of summer moong. Leg. Res., 17: 53-56.
77. Tandon, H.L.S., 1991. In: Sulphur research and agricultural production in India. 3rd edition, TSI, Washington. pp: 140.
78. Joseph, B. and S.C. Verma., 1994. Response of rainfed chickpea to jalsakthi incorporation and P and S fertilization. Indian J. Agron., 39: 312-314.
79. Singh, M.V., 2001. Importance of sulphur balanced fertilizer use in India. Fert. News, 46: 13-18, 21-28, 31-35.
80. Goswami, N.N., 1988. Sulphur in Indian agriculture. Proc. T'SI-FAI sym. On sulphur in Indian agriculture, Mar, 9-1. New Delhi, pp: 1-90.
81. Khandkar, M.R., D.A. Shinde, V.S. Kanekar, N.R. Jambley and N.K. Jain, 1985. Growth, nodulation and yield of rainfed black gram as influenced by phosphate and sulphate fertilization in vertisol. Indian J. Pl. Physiol., 28: 318-322.
82. Saraf, C.S., 1988. Sulphur fertilization to pulses in yield and quality. Proc.TSI-FAI symp. Sulphur in Indian agriculture, Mar 9-11, New Delhi. pp: S II/2-1.
83. Mehta, U.R. and H.G. Singh., 1979. Response of greengram to sulphur in calcareous soils. Indian J. Agric. Sci., 49: 703-706.
84. Hanesklaus, S. and E. Schnug, 1992. Baking quality and sulphur content of wheat. Sulphur in Agriculture, 16: 35-36.
85. Poorani, B., 1992. Radiotracer studies on the interaction effects of application of P and S on available nutrients, yield and quality of greengram. M.Sc.(Ag.) Thesis Tamil Nadu Agricultural University, Coimbatore.
86. Pasricha, N.S. and R.L. Fox., 1993. Plant nutrient sulphur in the tropics and subtropics. Adv. Agron., 50: 209-255.
87. Sharma, M.P. and Room Singh, 1997. Effect of phosphorus and sulphur on greengram *(Phaseolus radiatus).* Indian J. Agron., 42: 650-652.
88. Singh, Y.P. and R.L. Aggarwal., 1998. Effect of sulphur and levels on yield, nutrient uptake and quality of black gram *(Phaseolus mungo).* Indian J. Agron., 43: 448-452.
89. Tiwari, K.M., 1997. Sulphur in balanced fertilisation in Northern India. Proc. TSI/PM/IFA symposium on sulphur in balanced fertilisation. Feb 13-14. at New Delhi. pp: SI-I/I-SI-1/15.

90. Umarani, N., 1994. Response of red gram genotypes to varying plant densities, sulphur application and ratooning. MSc (Ag.) Thesis. Tamil Nadu Agricultural University, Coimbatore.
91. Naidu, M.V. and H. Ram, 1995. Effect of sulphur and *Rhizobium* inoculation on rhizosphere and non rhizosphere microflora. Agropedology, 5: 53-58.
92. Naidu, M.V.S., H. Ram and J. Prasad, 1998. Effect of sulphur application and *Rhizobium* inoculation to green gram on nitrifying capacity of rhizosphere and non rhizosphere soils. J. Indian Soc. Soil Sci., 46: 313-314.
93. Dube, S.D. and P.H. Mishra., 1970. Effect of S deficiency on growth, yield and quality of some important leguminous crops. J. Indian Soc. Soil Sci., 18: 375-378.
94. Kapoor, K.K., M.M. Mishra, R.S. Malik and K.C. Banger., 1991. Solubilization of Mussoorie rock phosphate by use of pyrite and *Thiobacilli*. Environ. Ecol., 9: 635-637.
95. Costa, A.C.A., R.A. Medronhe and R.P. Pecanha, 1992. Phosphate rock bioleaching. Biotechnol. Lett., 14: 233-238.
96. Shivakumar, B.G., 2001. Performance of chickpea (*Cicer arietinum*) varieties as influenced by sulphur with and without phosphorus. Indian J. Agron., 46: 273-376.