Characterization and evaluation of sulphur oxidizing ability of bacteria isolated from agricultural soil.

Naveen Joshi [*], Madhulika Singh,[**] Ragini Gothalwal[**]

[*&**]Department of Biotechnology and Bioinformatics Center, Barkatullah University, Bhopal, India 462026

[**]Department of Biotechnology Sadhu Vasvani College, Bairagarh, Bhopal, India 462030

Abstract

The diversity and habitat of sulphur-oxidizing bacteria recently gained the attention to consummate sulphur deficiency and to improve the fertility of soil. The present study strives to decrypt the abundance and diversity of sulphur-oxidizing bacteria in agricultural soil of Indore, Madhya Pradesh. A total of 40 different bacterial morphotypes were isolated from soil samples. Total of 14 isolates is screened on the basis of their efficacy to reduce pH of growth medium to 5 or less. The maximum pH reduction on starkey and thiosulphate broth is 4.37 and 4.41 by $IS_2$9GA, S12ND respectively. The maximum sulphate ion production was studied is $IS_2$10B (8.53mM), $IS_2$2B (7.25mM), $IS_2$9GA (7.14mM) and IS10NB (5.92mM), lowest by KA03B (0.782mM). Seven isolates showed mixotrophic growth utilize both elemental sulphur and thiosulphate as energy source; whereas 5 isolates utilized thiosulphate only and two utilize elemental sulphur only. These findings unveil the abundance of the SOB in agricultural soil and their role in the oxidation of sulphur.

Key Words: Oxidation, Sulphur-Oxidizing Bacteria (SOB), Sulphur, Sulphate ion, Thiosulphate.

Introduction

Sulphur is an essential nutritional element for plants and animals. It is one of the sixteen elements required for growth and development of plants. It is now considered as a fourth indispensable plant nutrient after N, P and K predominantly in legumes (Vidyalakshmi and Sridar, 2007). Sulphur is the chief constituent of three amino acids, viz. cysteine, cystine and methionine. It forms a basic framework for proteins; methionine is a basic constituent of seed protein in legumes. Sulphur is involved in various enzymatic and metabolic activities include photosynthesis, respiration, nitrogen fixation and production of large-sized granules (Srinivasrao *et al*., 2004).

In the environment, sulphur accumulation is the result of soil weathering, from the atmosphere and originally from bonded sulphur. Mostly sulphur is present in elemental form in the soil. Plants do not absorb elemental sulphur they uptake it in the form of sulphate. Sulphur prior to its incorporation in original compound undergoes a series of transformation.

Conversion of sulphur is necessary for crop uptake (Mahendra, 1988). Microbial biomass plays an important role in the transformation of sulphur; they mineralize, immobilize, oxidize and reduce sulphur. Microorganisms are the source and sink of sulphate ions. They oxidize the elemental sulphur and other forms of sulphur to produce sulphate and other reduced form of sulphur, available for uptake in soil (Sridar *et al.,* 2013).

Sulphur-oxidizing bacteria are an important group of microorganism involved in the oxidation of sulphur. Primarily they are gram-negative bacteria of Gammaproteobacteriaceae (Kelly *et al*., 2000). The diverse habitat of sulphur-oxidizing bacteria showed that they belong to Firmicutes, Betaproteobacteria and alphaproteobacteria (Marvi *et al.*, 2016). In soil oxidation of sulphur is majorly carried out by bacteria of genus *Thiobacillus*, importantly five species are predominant in soil (Starkey, 1966), four of them are *T. ferrooxidans, T. denitrificans*, *Acidithiobacillus thiooxidans* and *T. thioparus* obligate chemolithotrophs while *T.novellus* considered a facultative chemolithotrophs (Vidyalakshmi *et al.,* 2009). The sulphur oxidation in soil is not restricted to true sulphur-oxidizing bacteria; the sulphur oxidizing ability was also studied in the heterotrophs isolated from soil and marine environment (Das *et al.*, 1996). The number of *Thiobacillus* is not significant in most of the agricultural soil (Champman *et al.,* 1990; Lawrence *et al.*, 1991), the heterotrophic rhizospehric and soil bacteria are the driving source of oxidation. It is observed that species of *Paracoccus, Xanthobacter, Alcaligenes, Enterobacter, Pseudomonas* and *Bacillus* have the capability to oxidize sulphur in agricultural soil. They exhibit chemolithotrophic growth on inorganic sulphur.

Sulphur-oxidizing bacteria are isolated from different habitats includes paddy rhizosphere soil, black gram rhizosphere soil, sewage water, tannery effluent and mine soil (Vidyalakshmi and Sridar, 2007; Marvi *et al.,* 2016; Sultan and Faisal, 2016). Their presence is also studied in rhizosphere of mustard, decomposing sites, pit house and mangrove soil of Mahanadi river delta (Chaudhary *et al.,* 2017; Behera *et al.,* 2014). During the transformation of elemental and other forms of sulphur bacteria reduce the pH which helps in the uptake of other nutrients like phosphorous, nitrogen and potassium. This property of sulphur-oxidizing bacteria helps in the reclamation of soil and reduces the alkalinity of soil, they are the source of sulphate which full fills the sulphur requirement of crops. The present study was to isolate, characterize and evaluate the sulphur oxidizing ability of bacteria in agricultural soil.

Materials and Method

Collection of soil sample

Ten soil samples were collected from agricultural fields of Indore, Madhya Pradesh. The samples were collected randomly from the 10-15cm depth and mixed in sterile polyethylene bags and shifted to the laboratory for studies.

Isolation of Sulphur Oxidizing Bacteria

The media used for isolation includes Starkey broth (Starkey and Collins 1923) comprises 3.0g KH_2PO_4, 0.2g $MgSO_4.7H_2O$, 0.2g $CaCl_2.2H_2O$, 0.5g (NH_4) SO_4, trace of $FeSO_4.7H_20$ per liter of deionized water and pH is adjusted to 8 and Thiosulphate broth contained 5.0g $Na_2S_2O_3$, 0.1g K_2HPO_4, 0.2g $NaHCO_3$, 0.1g NH_4Cl per liter of deionized water and final pH adjusted to 8.0. 0025g per liter bromocresol purple is added as an indicator (Vidyalakshmi and Sridar, 2007). 5.0g of glucose per liter of media was added for isolation of heterotrophs. Elemental sulphur 10.0g per liter was added to Starkey broth and sterilized for three successive days. Thiosulphate broth was deprived of elemental sulphur. 1.5g of soil was added to 25ml of broth and incubated at 32°C with initial shaking condition for 25days. The isolates were purified by transferring thrice in fresh medium every biweekly. The isolates streaked on corresponding media and pure individual colony was obtained. The pure isolates were streaked on thiosulphate agar and stored at 4°C for further studies.

Screening of Isolates by pH reduction test

Pure isolated bacteria were inoculated in thiosulphate broth with initial pH adjusted to 8 and incubated at 32°C for 15 days; the experiment was performed in triplicate. The pH reduction was recorded using pH meter and the average was taken. The isolates were screened based on their efficacy to reduce pH from 8 to 5 or less.

Quantification of sulphate ion

The amount of sulphate ion ($SO4^{2-}$) produced by isolates in thiosulphate broth was determined spectrophotometrically by method explained by Cha *et al.,* (1999). The fully grown culture media was centrifuged at 6000 rpm for 5 min. to obtain the supernatant. Equal volume of barium chloride (10%w/v) was added in supernatant in ratio of 1:1 and mixed strenuously to find out sulphate ion. Tubes showed formation of white turbid solution due to formation of barium sulphate. Absorbance was measured at 450 nm using UV-Visible spectrophotometer. Each sample was tested in triplicate and mean absorbance was considered to calculate sulphate ion ($SO4^{2-}$) concentration. The value obtained was compared with standard curve, K_2SO_4 was used to prepare standard curve according to Kolmert *et al.,* (2000). The standard K_2SO_4 solution was prepared in a known concentration of 0 to5 mM. The turbidity produced is directly proportional to sulphate ion concentration.

Utilization of sulphur source

The selected isolates were tested for utilization of sulphur source i.e. elemental sulphur and thiosulphate. Isolates were inoculated in Starkey and Thiosulphate broth with initial pH 8. Bromocresol purple was added as indicator.

The tubes were incubated in a BOD incubator at 32°C for 15 days. The sulphur utilization was measured with reduction in

pH and change in colour due to production of sulphate ions.

Characterization of Isolates

The isolates were characterized primarily by studying morphological and biochemical characters. The morphology of cell was confirmed microscopically using monochrome staining, gram staining was performed. Single colony was obtained on thiosulphate agar and colonial characters, viz. size, shape, elevation, opacity, margin, texture and pigmentation were recorded. Biochemical tests including Catalase test, Oxidase test, Citrate utilization, Urease test, Indole, MR-VP test, Sugar utilization (TSI) test, H_2S production, Gelatin liquefaction test and Starch hydrolysis was performed according to standard protocol.

Results

Isolation and screening

In the present study total 40 isolates have been isolated from agricultural soil samples. Among them 14 isolates are selected on the basis of pH reduction on thiosulphate and Starkey media (table:1, Fig.:1) and coded as $IS_2$2B, IS9NA, IKI09A, S12ND, $IS_2$10B, KA03B, IS10NB, KA01GA ,IK01GB, $IS_2$9GA, IK02GA, KA01GC, IS1NGI and KA01GF. The isolate $IS_2$9GA showed maximum pH reduction to 4.37 and minimum reduction was observed in KA03B 5.99 results are presented in (Table: 1). the resulting change in colour of media due to pH reduction is shown in Fig1. Seven isolates are chemolithoautotrophic and seven are chemolithohetrotrophic as they utilize glucose.

Quantification of sulphate ion

Extracellular sulphate ion production by screened isolates in thiosulphate medium was found maximum in chemolithoautotrophic isolates $IS_2$10B (8.53 mM) followed by $IS_2$2B (7.25mM), IS10NB (5.92mM), IS9NA (4.96mM) and S12ND (4.16mM). Whereas in chemolithohetrotrophic isolates it was $IS_2$9GA (7.14 mM), IK02GA (4.51 mM), IS1NGI (3.90 mM) and IK01GB (3.47 mM) respectively (Table: 1). Values obtained indicate that isolates have potential to produce high amount of sulphate ions. The comparative account of amount of sulphate ion produced is shown in fig.2.

Utilization of sulphur source

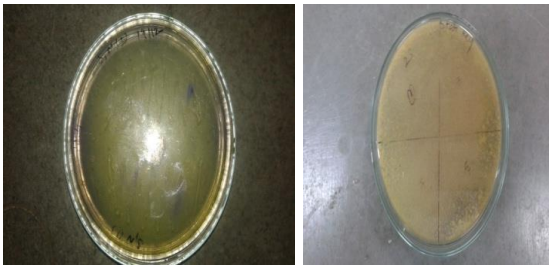
The potential isolates were studied for utilization of sulphur source, viz. elemental sulphur and thiosulphate. The isolates showed mix response on the sources. Isolate $IS_2$2B, IS9NA, IKI09A, S12ND, KA01GA, IK01GB and $IS_2$9GA are utilizing both elemental sulphur and thiosulphate; Isolate $IS_2$10B, IS10NB, IK02GA, KA01GC and IS1NGI utilizes only thiosulphate while only elemental sulphur is utilized by KA01GF and KA03B respectively. The details are shown in table 1.

Table 1. pH reduction, sulphate ion production and sulphur utilization by

potential isolates, + Utilizing and - Not Utilizing.

Isolate	pH reduction		Sulphate ion concentration in mM	Substrate utilization	
	Starkey Broth	Thiosulphate Broth		Elemental Sulphur	Thiosulphate
$IS_2$2B	4.46	4.72	7.25	+	+
IS9NA	4.72	4.85	4.96	+	+
IKI09A	5.05	5.25	2.65	+	+
S12ND	4.56	4.41	4.16	+	+
$IS_2$10B	4.71	4.65	8.53	-	+
KA03B	5.25	6.99	0.782	+	-
IS10NB	5.51	5.14	5.92	-	+
KA01GA	4.44	4.54	2.30	+	+
IK01GB	4.84	4.56	3.47	+	+
$IS_2$9GA	4.37	4.75	7.14	+	+
IK02GA	4.90	4.5	4.51	-	+
KA01GC	4.80	4.6	3.22	-	+
IS1NGI	5.01	4.9	3.90	-	+
KA01GF	5.02	7.02	2.08	+	-

(A) **(B)**



(C) **(D)**

Fig. 1. (A) Change in colour due to pH reduction after 15 days in thiosulphate broth. (B) Change in colour due to pH reduction after 15 days in Starkey broth. (C) Change in colour due to pH reduction after 15 days on thiosulphate agar. (D) Change in colour due to pH reduction after 15 days in Starkey Agar.

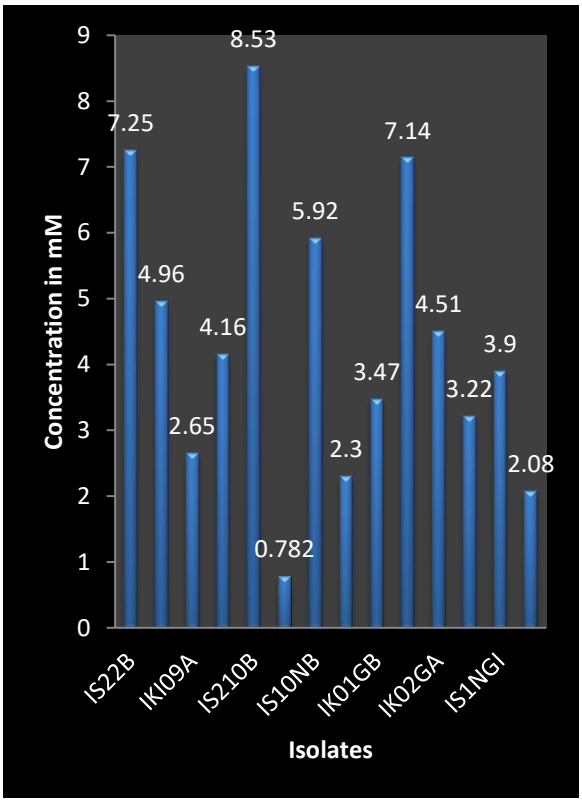


Fig.2 Amount of sulphate ion produced by Isolates.

Characterization of potential Isolates

The bacterial isolates were studied for morphological and biochemical characters (table 2). Microscopic studies of isolates shows that all morphotypes are rod shaped motile except IS9NA which is a cocci. They have variability in gram staining. All the bacterial isolates are catalase producer. In conformity with morphological and biochemical studies bacterial isolates identified as $IS_2$2B and KA01GA *Pseudomonas Spp.*, S12ND, $IS_2$10B, IK01GB and IK02GA *Bacillus Spp.*, KA01GC and KA01GF *Kosakonia Spp.*, IS9NA and IS1NGI *Paracoccus* and *Enterobacter* respectively.

Table 2. Morphology and Biochemical Characters of Potential Isolates, P: Positive and N: Negative.

Isolate	Morphology	Gram Reaction	Catalase test	Oxidase test	Citrate Utilization Test	Urease Test	Indole Test	MR	VP	H_2S Production	Gelatin Hydrolysis	TSI	Starch Hydrolysis
$IS_2$2B	Rods	Negative	P	N	N	P	N	N	P	N	P	P	N
IS9NA	Cocci	Negative	P	N	N	N	N	N	P	N	P	P	N
IKI09A	Rods	Negative	P	N	P	N	N	P	P	P	N	N	P
S12ND	Rods	Positive	P	N	P	N	N	N	P	N	P	P	N
$IS_2$10B	Short Rods	Positive	P	N	N	N	N	N	P	N	P	P	N
KA03B	Rods	Negative	P	N	P	N	N	N	P	N	N	P	N
IS10NB	Rods	Negative	P	N	P	N	N	N	P	N	N	P	N
KA01GA	Short Rods	Negative	P	N	P	N	N		P	N	P	P	N
IK01GB	Rods	Positive	P	N	P	N	N	N	P	N	P	P	P
IS29GA	Rods	Negative	P	N	P	N	N	P	P	N	N	P	N
IK02GA	Rods	Positive	P	N	N	N	N		P	N	P	N	N
KA01GC	Rods	Negative	P	N	P	N	N	P	P	N	N	P	N
IS1NGI	Rods	Negative	P	N	P	N	N	P	P	N	N	P	N
KA01GF	Rods	Negative	P	N	P	N	N	P	P	N	N	P	N

Discussion

Sulphur-oxidizing bacteria have an important role in transformation of sulphur in soil. They undergo a series of steps to convert sulphur in its original compound. In present investigation 40 bacterial isolates were obtained from soil. The finding of investigation reveals the role of sulphur-oxidizing bacteria in soil transformation. As they are present in soil, SOB becomes an important microbial biomass of rhizospheric soil. Their existence in rhiosphere is confirmed by various researchers, they isolated SOB from rhizosphere of mustard, paddy rhizosphere soil, black gram rhizosphere soil and sesame rhizosphere soil (Chaudhary *et al.,* 2017; Vidyalakshmi and Sridar, 2007; Anandham *et al.,* 2010). Studies showed their presence in different habitats; acid mine drainage and Black Shale soil; copper mines and surrounding agricultural soil; mangroove soil (Sajjad *et al.,* 2016, Marvi *et al.,* 2006, Behera *et al.,* 2014). Their abundance and diversity in cropping soil; landfill cover soil is confirmed by *Sox*B gene analysis (Zhao *et al.,* 2017, Xia *et al.,* 2014).

The potential sulphur oxidizing bacteria has characteristics to reduce the pH of medium. Bacteria oxidize the sulphur, thiosulphate and other reduced forms of sulphur finally in to sulphuric acid. This drastically reduces pH of the medium to 3 or less. The bacterial isolates are subjected to pH reduction test and 14 potential isolates were screened out on the basis of pH reduction. The test was the basis of screening in various studies (Ullah *et al.,* 2014). This property of potential isolate may help in reclamation of soil alkalinity. Reports showed decrease in pH is due to oxidation of sulphur and due to formation of sulphate to sulphuric acid (Donati *et al.,* 1996, Vidyalakshmi and Sridar, 2007). Acidophilic microbes oxidize environmental pyrite and other sulphide quickly in presence of oxygen and ferric iron. The pH reduction by bacterial isolate is clearly seen in Fig.1.

The screened potential isolates are tested for quantification of sulphate ion, using barium chloride precipitation. The bacterial isolates oxidize sulphur to produce sulphate ion. In test they oxidize thiosulphate and produce sulphate ion. The maximum sulphate ion production was 8.5 mM by $IS_2$10B, followed by $IS_2$9GA 7.14 mM. The lowest sulphate ion was produced by KA03B 0.78mM. other studies also reported sulphate ion production to 3.012 mM, 2.268 mM, and 2.785 mM (Chaudhary *et al.,* 2017), 1.51 mM and 1.60 mM (Ohba and Owa, 2005). The ability to produce sulphate ion reveals that $IS_2$10B and $IS_2$9GA are the potential sulphur oxidizer amongst all other. The form of sulphur oxidized by bacterial isolate provides information and helps in characterization of bacteria. Sulphur-oxidizing bacteria can utilize a variety of organic and inorganic forms of sulphur. The potential isolate was checked for the sulphur source utilization. It was observed that 7 bacterial isolates showed mixotrophic growth they utilize both elemental sulphur and thiosulphate, 5 isolates utilize strictly thiosulphate only and remaining 2 utilize elemental sulphur only. Authors reported properties of sulphur-oxidizing bacteria in their studies (Vidyalakshami and Sridar, 2007).

Isolates were identified as $IS_2$2B and KA01GA *Pseudomonas Spp.*, S12ND, $IS_2$10B, IK01GB and IK02GA *Bacillus Spp.*, KA01GC and KA01GF *Kosakonia Spp.*, IS9NA and IS1NGI *Paracoccus* and *Enterobacter* respectively. All are present abundantly in soil. Behera *et al.*, 2014 isolated *Pseudomonas Spp, Bacillus pumilus, Bacillus subtilis, Micrococcus spp. and Bacillus megaterium* from soil. Sulphur oxidizing *Pseudomonas Spp.* was studied in Odisha (Thatoi *et al.*, 2012). Earlier sulphur oxidizing ability of *Micrococcus* and *Bacillus spp.* was reported in soil (Chattopadhyaya *et al.*, 1993). Chaudhary *et al.*, 2017 isolated sulphur oxidizing bacteria from rhizospheric soil of mustard and characterized them as *Pseudomonas Spp.* and *Xanthobacter*. Findings revealed the abundance of heterotrophic sulphur-oxidizing bacteria in soil. These bacteria serve as source and sink of sulphur in soil.

Conclusion

The present study emphasizes on abundance and significance of sulphur-oxidizing bacteria in oxidation of sulphur in soil. All the isolates were using sulphur containing chemicals as their energy source. The level of sulphate production is significant and potential bacteria isolated can be used as bioinoculants in soil to increase sulphur oxidation in turn increase available sulphate. The pH reduction and sulphate ion production ability can help in uptake of other nutrients from soil and increase soil fertility. They can be used in reclamation of alkaline soil. They increase the protein content and oil yield in oil seed crops; leading to sustainable agriculture with increased crop yield.

References

Anandham R, Indiragandhi P, Kwon SW, Sa TM, Jeon CO, Kim YK and Jee HJ (2010) *Pandoraeathyooxydans*sp. Nov., A facultativelychemolithotrophic, thiosulfate-oxidizing bacterium isolated from rhizosphere soils of sesame (*Sesamumindicum* L.). International Journal of Systematic and Evolutionary Microbiology, 60:21-26.

Behera BC, Patra M, Dutta SK and Thatoi HN (2014) Isolation and characterization of sulphur oxidizing bacteria from mangrove soil of mahanadi river delta and their sulphur oxidizing ability. Journal of Applied and Enviornmental Microbiology, 2(1): 1-5.

Cha JM, Cha WS and Lee JH (1999) Removal of organo sulphur odour compounds by *Thiobacillus novellus* SRM, sulphur-oxidizing bacteria. Process Biochemistry, 34: 659-665.

Chapman SJ (1990) *Thiobacillus* population in some agricultural soils. Soil Biol. Biochem., 22: 479-482.

Chattopadhyaya N and Dey BK (1993) Chemoheterotrophic sulphur oxidising microorganisms of a Terai soil I. Oxidation of inorganic and organic sulphur by microorganisms, isolated in sucrose-sodiumthiosulphate agar. Zentralblatt fur Mikrobiologie, 148(7):517-522.

Chaudhary S, Dhanker R, Tanvi and Goyal S (2017) Characterization and optimization of culture condition for sulphur oxidizing bacteria after isolation from thizospheric mustard soil, decomposing sites and pit house. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 11(6): 379-383.

Das SK, Mishra AK, Tindall BJ, Rainey FA and Stackerbrandt E (1996) Oxidation of thiosulphate by a new bacterium, *Bosea thiooxidans* (strain BI-42) gen. nov., sp. nov.: Analysis of phylogeny based on chemotaxonomy and 16S Ribosomal DNA sequencing. International Journal of Systematic Bacteriology, 46(4): 981-987.

Donati E, Curutchet G, Pogliani C and Tedesco P (1996) Process Biochem., **31**:129-134.

Kelly DP, Wood IR and McDonald AP (2000) Proposal for the reclassification of *Thiobacillus novellus* as Starkeya *Novella* gen. nov., comb. nov., in the alpha subclass of the proteobacteria. Int. J. syst. Evol. Microbial, 50:1797-1802.

Kolmert A, Wikstrom P and Hallberg KB (2000) A fast and simple turbidimetric method for the determination of sulfate in sulfate-reducing bacterial cultures. Journal of Microbiological Method, 41: 179-184.

Lawerence JR and Germida JJ (1991) Microbial and chemical characteristics of elemental sulphur beads in agricultural soils. Soil Biol. Biochem, 23: 617-622.

Mahendra S (1988) Sulphur management in coarse textured alluvial soils. Proceeding of TSI-FAI Symposium, Sulphur in Agriculture, Mar. 9-11. New Delhi, pp:SIII/2(1-9).

Marvi SPM, Pourbabaee AA, Alikhani HA, Haidari A and Manafi Z (2016) The diversity of sulphur oxidizing bacterial population at an Iranian copper mine and the surrounding agricultural soil. Applied Ecology and Environmental Research, 14(3):509-533.

Ohba H and Owa N (2005) Isolation and Identification of sulphur-oxidizing bacteria from the buried layer containing reduced sulphur compounds of a paddy field on sado Island in Niigata Prefecture. Bull. Facul. Agric. Niigata Univ., 58(1): 55-61.

Sajjad W, Tariq MB, Fariha H, Samiullah K, Malik B, Abbas AN and Aamer AS (2016) Characterization of sulfur oxidizing bacteria isolated from acid mine drainage and black shale sample. Pakistan Journal of Botany, 48(3):1253-1232.

Sridar R, Raveendran M, Sivaji M and Gayathri R (2013) Isolation of autotrophic sulphur oxidizer from different ecological niches. J.Soil Biol. Ecol., 33(1&2):66-76.

Srinivasarao CH, Ganeshamurthy AN, Ali M, Singh RN and Singh KK (2004) Sulphur fractions, distribution and their relationship with soil properties in different soil types of major pulse growing regions of India. Commun.Soil Sci. Plant Anal., 35:2757-2769.

Starkey RL (1966) Oxidation and reduction of sulphur compound in soil. Soil Science. 101:297-306.

Starkey RL, Collins VG (1923) Autotrophs. In: Methods in Microbiology, J. R. Norris, D. W.Ribbons (Eds), New York: Academic Press, 38, 55-73.

Sultan S and Faisal M (2016) Isolation and characterization of Iron and Sulphur Oxidizing bacteria from coal mines. Journal of Environment and Earth Science, 6(3): 153-157.

Thatoi HN, Behera BC, Dangar TK and Mishra RR (2012) Microbial biodiversity in mangrove soil of Bhitarakanika, Odisha, India. International Journal of Environmental Biology, 2(2):50-58.

Ullah I, Jilani G, Khan KS, Akhtar MS and Rasheed M (2014) sulphur oxidizing bacteria from sulphur rich ecologies exhibit high capability of phosphorous solubilization. Int. J. Agric. Biol., 16:550-556.

Vidyalakshmi R and Sridar R (2007) Isolation and characterization of sulphur oxidizing bacteria. J. of Culture Collections, 5: 73-77.

Vidyalakshmi R, Paranthaman R and Bhakyaraj R (2009) Sulphur oxidizing bacteria and pulse nutrition- A Review. World J. of Agri. Science, 5(3): 270-278.

Xia FF, Su Y, Wei XM, He YH, Wu ZC, Ghulam A and He R (2014) Diversity and activity of sulphur oxidizing bacteria and sulphate reducing bacteria in landfill cover soils. Letters in Applied Microbiology, 59:26-34.

Zhao C, Gupta VVS, Degryse F and McLaughlin MJ (2017) Abundance and diversity of sulphur-oxidizing bacteria and their role in oxidizing elemental sulphurin cropping soil. Biol Fertil Soils, 53:159-169.