INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 6, p. 110-121, 2019

RESEARCH PAPER OPEN ACCESS

Efficiency of sulphur oxidizing bacteria to solubilize phosphorus from rock phosphoate of Hazara, Pakistan

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Key words: *Enterobacter*; Sulphur oxidizing bacteria; Sulphur; Sulphur oxidation; Phosphorous solubilization.

http://dx.doi.org/10.12692/ijb/14.6.110-121 Article published on June 16, 2019

Abstract

The information about sulphur oxidising bacteria from rock phosphate mine area Hazara Pakistan is still unknown. This study was conducted to isolate sulphur oxidising bacteria from rock phosphate mine area Hazara Pakistan. Bacterial strains, specifically Sulphur oxidizing bacteria (SOB), have the ability to oxidize elemental sulphur (S^o) and overcome S compounds to release sulphuric acid. Sulphuric acid converts the insoluble phosphorous (P) compounds to simple plant available P compounds. Best strains were screened out on the basis of morphological and biochemical characteristics, pH reduction test, sulphate ion production test, indole acetic production test. Among 30 isolates 10 were capable of reducing the pH of the growth media below 4.0 from initial pH 8.0 and made the highest sulphate production in the growth media and concentration of produced sulfate ion ranged from 1.73 to 2.0063mg/mL. On the basis of pH reduction (in thiosulphate broth) and phosphorous solubilization index (PSI) best strains were screened out. A total of ten selected SOB were tested in 0.5% tricalcium phosphate (TCP) broth media for the efficiency Phosphorous solubilization. Results showed that the strain Endo2 released 2.0435 mg L-1 P (95.2%) during 45 days of incubation shows Capacity of Phosphorus dissolved had a significant correlation revise this line. The bacterial isolates were identified as *Bacillus* spp., *Serratia* spp. and *Enterobacter* spp *thiobacillus spp*. based on their 16Srna, morphological and biochemical characterization.

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Introduction

Sulphur is one of the most important plant nutrients after N, P, and K especially in pulses and oilseed crops as a component of essential amino acids cystine and methionine as well as cysteine (Reddy *et al.*, 2018). Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible (Rodríguez *et al.*, 2006). In biogeochemical cycle of sulphur compounds in the environment sulphur oxidizing bacteria (SOB) play an important role. The majority of SOB bacteria belong to the genera *Bacillus*, *Enterobacter*, *Serratia*, *Thiobacillus*. On the basis of morphological and bio-chemical characterization these are Gram-negative, facultative anaerobic, short rod-shaped prokaryotes (Rodríguez *et al.,* 2006). The genera within the family *Enterobacteriaceae* having plant growth promoting bacteria (PGPB) are *Enterobacter*, *Klebsiella*, and *Serratia* (Rodríguez *et al.,* 2006). For biological S oxidation, among the SOB in soil, the genus *Thiobacillus* is very important. Sulphur Oxidizing Bacteria (SOB) enhance the oxidation of 'S' and speed up the production of sulphates and make it available to plants. Soil phosphorous utilization in plants is directly linked with increase in world agriculture production and helps to meet the high demand of agriculture products. In crop yield, phosphorus supply in rhizosphere is essential to activate plant root growth for efficient soil nutrient uptake. Therefore, soil phosphorus is becoming an interesting subject to agriculturist. Phosphorus play a vital role in crop production as it is very important nutrients for plant growth. The uptake of Phosphorus (P) by plants is very low with estimated range of 10-25% and therefore results in P deficiency in crops. This P deficiency is mainly due to unavailability of P to plants as it becomes bound to calcium, especially in calcareous soil. Unfortunately much arable area of the Pakistani soil is calcareous and alkaline having pH less than 7.5 and $CaCO_3$ up to 3.0 % creating serious problem of P fixation (Sharif *et al.,* 2000). In Pakistan 90% soils have low available P status and are moderate to high P deficient (Rehman *et al.,* 2000; Solangi *et al.,* 2006).

Main contributors in the carbon, sulphur, nitrogen and phosphorous cycles are bacteria. For the degradation of organic compounds, sulphate reducing bacteria use sulphate as a terminal electron acceptor resulting in the production of sulphide. After this, the sulphide can be oxidized by sulphur oxidising bacteria to produce sulphate. As the novel source of reduced sulphur compounds is H_2S, hence it has the capacity to support plentiful populations of sulphur-oxidizing bacteria at the oxic-anoxic boundary (Behera *et al.*, 2014). For sulphur oxidizing bacteria (SOB) elemental S° is a fundamental substrate which oxidizes to sulphates during oxidation process (Pokorna *et al.,* 2007) and there exists a close bacteria-substrate relationship for S oxidation (Briand *et al.*, 1999). It is reported that genus *Bacillus*, sulphur oxidizing bacteria, oxidize S which results in phosphorus release from Rock Phasphate due to bacterially produced sulphuric acid during S oxidation phenomenon (Chi *et al.,* 2007). Currently, no information on the availability of phosphorous solubilizing, sulphur oxidizing bacteria has yet been reported from Hazara, Pakistan.

Therefore this study is the first attempt to isolate and characterize SOB from different locations of Hazara, especially rock phosphate mine area which are calcareous in nature. Moreover, the most efficient strains with P solubilisation abilities were screened out.

Material and methods

Soil sample collection

Soil samples were collected from ten different sources viz., rock phosphate mines (Mine), rhizosphere maize (RS), endo rhizospere (Endo), canal water (CW), sewage (S), broth (SOB).

Isolation and purification of sulphur oxidizing bacteria

Sulphur-oxidizer medium was prepared for isolation of SOB which contained (1 L) 10g of Bacto-Peptone, 1.5g of K_2HPO_4, 0.75g of ferric ammonium citrate and 1.0g of $Na_2S_2O_3.5H_2O$, 15g of agar, and pH adjusted to 7.0 using 1M HCl before sterilization (Behera *et al.*,

2014). Isolation of sulphur-oxidizing bacteria was performed by using direct spread plating method. The samples were serially diluted up to 10 times and 1mL of final dilution was spread onto prepared SOB medium plates and incubated for 24 hrs at 30°C. The observed well-defined colonies were loop transferred to fresh sulphur-oxidizer medium agar plate for purification. Qualitative screening of recovered SOBs was done by growing the isolates in thiosulphate broth medium (Beijerinck, 1904), containing (per 1L) $Na_2S_2O_3$, 5.0 g; K_2HPO_4, 0.1 g; $NaHCO_3$, 0.2 g; NH_4Cl, 0.1 g and pH of final medium adjusted to 8.0. Bromo cresol purple was used as indicator (Vidyalakshmi and Sridar, 2007). The cultures were incubated for 11 days at 30°C. The pH of the control and culture inoculated broth medium was measured at 24 hrs regular intervals. The strains showing the ability to reduce the pH of broth medium with time and change its colour from purple to yellow will be taken as an indication of SOB growth. These selected SOB strains will be purified by transferring the culture to a new freshly prepared broth medium for three consecutive days after 24 hrs interval. The purified cultures will be used characterization and further screening tests (Smibert and Kreig, 1994).

Morphological characterization

Characterization was carried out by culturing the recovered isolates over thiosulphate agar (pH 8.0) plates. Cell morphology and Gram staining was recorded for each isolate (Bergey and Boone, 2009). Screening of efficient sulphur-oxidizing bacteria.

To screen out the most efficient SOBs, pH reduction test, phosphorous solubilization index, sulphate ion production test, and indole actic acid test were performed.

The pH reduction test

Thiosulphate broth medium (pH 8.0) 20 mL was inoculated with 1ml (10^6 cells mL^{-1}) fresh culture of bacterial strains and incubated at 30°C for 16 days. Completely randomized design (CRD) with three replications per strain and respective control was arranged. Sob efficacy of the strains was measured on the basis of its ability to reduce pH of medium. Metrohm Highprecision 780 pH meter was used to estimate the pH of treatments (Behera *et al.*, 2014).

Phosphorous solubilization index

Preserved culture of each SOB (0.1 mL) was placed on thiosulphate tricalcium phosphate (TCP) 0.5% agar plates and incubated for 8 days at 30°C. The TCP agar plates were arranged in completely randomized design (CRD) having three replications. Phosphorous solubilization zones were formed on the thiosulphate TCP agar plates. Phosphorous solubilization index (PSI) was measured by using the following formula (Edi-Premono *et al.*, 1996).

$$PSI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

Quantification of phosphorous solubilization through bioleaching test

The most efficient strains screened via PSI measurement were quantified for phosphorus solubilisation using Tricalcium phosphate (TCP) bioleaching test. Fresh culture (1.0ml) of each SOB strain was used to inoculate thiosulphate broth medium (100 mL) amended with 0.5% tricalcium phosphate. The pH of medium was adjusted to 8.0. The experimental setup was arranged in completely randomized design (CRD) with 3 replications per SOB strain treatment and 3 broth flasks were kept as un-inoculated control. The flasks were kept in shaker incubator (100 rev. min^{-1}) at 30°C for 45 days. After 7, 10, 16, 21, 30 and 45 days of incubation aliquot samples (5 mL) were taken and centrifuged. Bacterial supernatants were tested for pH, sulphate contents and P solubilization. P ratio was detected (Watanabe and Olsen, 1965) with the help of two reagents viz., reagent a (ammonium heptamolybdate 12 g in 250 mL distilled water + antimony potassium tartrate 0.2908 g in 100 mL distilled water. Both were added in 1 L 5 N H_2SO_4 in a 2 L volumetric flask and made the volume with distilled water) and reagent B (L-ascorbic acid ($C_6H_8O_6$) 1.056 g + 200 mL of reagent A). Liquid sample of 5.0 mL in 50 mL volumetric flask was taken then 8 mL of reagent B was added.

The volume of the reaction was then raised to 50 mL with distilled water. The absorbance of blank, standards and samples were read after 10 min at 882 nm wavelength in spectrophotometer and P concentration was read from the calibration curve.

Sulphate production

By using spectrophotometer sulphate ion (SO_4^{-2}) production was observed on thiosulphtae broth medium during growth of sulphur oxidising bacteria in it. Sulphate contents were measured by using barium chloride solution with 1:1 (10% w/v) with addition of bacterial supernatant culture followed by mixing the suspensions thoroughly (Behera *et al.*, 2014).

The resulting white precipitate due to barium sulfate formation was measured at 450 nm with a systronics-119 spectrophotometer. The results obtained were compared with the sulphate standard curve. To construct a sulfate calibration curve Potassium sulfate (K_2SO_4) was used as standard according to Behera *et al.*, (2014).

Sulphate Standard solution was prepared by dissolving K_2SO_4 in distilled water of known concentrations between the ranges 0 to 3 mM. The amount of precipitates formed is directly proportional to the sulphate concentration.

Indole production

Broth culture medium (500 mL) was prepared containing, 5 g tryptone, .5 g L-Tryptophan and 2.5 g NaCl mixed in distilled water. Fresh SOB cultures strains were transferred into test tubes containing broth solution. Broth cultural tubes were kept in incubator for 7 days at 28°C. Five drops of Kovac's reagent was added into each test tube along with control. Development of cherry red rings was taken as positive and green colour rings were taken as negative.

Results

Bacterial Isolates

A total of 50 isolates recovered from sampling locations were tested for sulphur oxidizing ability. Only 30 of the isolates were found to be SOB positive. It was depicted from data that canal water, sewage water mine, rhizosphere and endo rhizosphere had the highest frequency (60%) of SOB (Figure 1).

Only 30 of the isolates were found to be SOB positive. Among them, 10 isolates were selected based on the better pH reduction ability on the bromophenol blue containing sulphur oxidising broth and agar medium by changing the color of the media purple to colourless (Figure2). It was depicted from data that canal water, sewage water mine, rhizosphere and endow rhizosphere had the highest frequency (60%) of SOB (Figure 3) and Table 2.

Table 1. Ecology-wise description of sulphur oxidizing bacteria**.**

Ecology	Total	SOB + ve	No. of samples	SOB -ve
Sewage (S)	6	02	(s3, s2)	4
Cow dung (SOB)	5	20	(SOB11, SOB4,)	3
Rock phosphate mines (mine)	8	05	(mine, mine1, mine5, mine2, mine4)	3
Rock phosphate area(RP)	4	2	RP1,RP3	2
Rhizosphere (rs)	8	04	(rs8, rs6 , rs4, rs7,	4
Canal water (cw)	5	02	(cw2,cw3)cw4	3
Endo rhizosphere (Endo)	6	06	(Endo4, Endo2, Endo3, Endo9, Endo5, Endo11)	0
Endo rhizosphere maize (R.E)	7	7	R.E9, R.E 3,R.E 1,R.E12,, R.E4, R.E7, R.E2,	0
Total :	50	30		20

SR: short rod, SRY: smooth, round, yellow: SRP: smooth, round, pink: SRW: smooth, round, white, AT: Autotrophic, HT: Heterotrophic, M: motile, NM non motile

The effect of pH and holozones

After 45 days of incubation all the 30 SOB isolates successfully reduced pH to an average 5.65 with significant reduction in pH observed only with strain RS4 (pH reduction 2.3), while minimum decrease in pH was observed in isolate R.endo7 with final pH value of 5.9617.

It had been observed that at minimum drop in isolated that as a whole dropped to 2.0383 values. Isolating R.E3 (2.348), Mine4 (3.519), (4.392), C.way4 (3.547), S2 (3.74) defined PH values in the range of 2.345 to 4.392. The growth media of 30 isolated after 16 days ranged pH value of 5.50 to 7.00 with a total decline of 1.5 to 1. Where there was no inoculation carried out there was no conspicuous change in pH values was observed. Out of 50 isolated, 30 SOB (Mine1, endo1, rp1, R.E1, Mine, RS1, RP2, S3, RP1, RS6, C.way1, RS41, RS4, Endo3, Endo2, Mine5, Mine4, Broth, RP41, RS8, C.way4, RS6, R.E9, R.E3, RS8, Endo3, P.mud3, RS4, Endo5, Mine2, R.Endo6, R.Endo7, S4,RP3 and S1) had been selected on the PH values reduction standard. Later, this Isolated were tested for phosphorous solubilization index PSI as per that test 7 SOB (R.E3, C.way4, RS6, Mine4, Endo2, Rs4, Mine1 and Endo3) were able to make holozones within 1day and 12 holozones on the second day and 8 made within 4 days. When C.W was isolated the highest PSI had been observed with PSI 8.42 (Figure 6) of Endo2, the lowest PSI 0.86 had been observed when Mine1 was isolated.

Table 2. Morphological, physiological and biochemical characterization of sulphur oxidizing bacteria.

Characteristics	R.E3	RS41	Mine1	Mine 4	RS6	Endo3	cw 4	Endo2
Morphology	SR	SR	SR	SR	SR	SR	SR	SR
Gram reaction	-	-	-	-	-	-	-	-
Elemental S° utilization	+	+	-	-	+	+	+	+
Thiosulphate utilization	+	+	+	+	+	+	+	+
Colony character	SRY	SRY	SRP	SRP	SRW	SRY	SRW	SRW
Cell	0.7-0.9	0.9-1.3	0.7-0.8	0.8-1.1	1.1-1.3	1.5-1.9	0.7-2.3	1.1-2.1
pH reduction	+++	+++	++	++	+++	+++	++	+++
Sulfates production	++	++	+	++	++	+++	+++	+++
Nutritional type	AT	AT	HT	HT	AT	AT	HT	HT
Pectinase	+	+	+	+	+	+	+	+
Motility	M	M	NM	M	M	M	M	M
Catalase	-	+	-	+	-	-	+	+
Oxidase	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	-	+	+	+	+	+
NH3 production	-	-	+	+	+	-	+	+
H_2S production	-	-	-	-	-	-	-	-
IAA	+++	+++	++	++	+++	+++	+++	+++
Amylase	-	-	-	-	-	-	-	-
Citrate	-	+	-	-	+	-	-	-
HCN	-	-	-	-	-	-	-	-

There was no existence of holozone in thiosulphate agar plates as no inoculation was carried out. Then 10 other SOB R.E3, R.E9, Endo2, Endo3, RS6, Mine4, Mine1, C.w 4, Rp1,Rs4 had been isolated at the criterion of PH reduction and were further tested for quantitative estimation of phosphorous solubilization in thiosulphate tricalcium phosphate TCP 0.5% media containing 1000 mg L-1 insoluble P. Obtaining data by change of SOB by isolation in TCP both Media are given in Figure 4.

There was significant drop in PH by treatment

compared from 7 to 45 days. It had been observed the maximum drop was in first 7 days that interprets that maximum S oxidation had taken place in those 7 days. The Highest values of pH were recorded as 2.3449, 2.3488, 3.547 and 3.859 (net reduction of 5.6551, 5.65, 4.453, 4.141 and 5.54 points) in RS4, R. E3, C.W, and mine4 whereas the lowest values were 5.9617, 4.443, 4.336 and 4.392 (net reduction of 2.033, 3.557, 3.664 and 3.608 points) in treatment MINE5, SOB4, C.W4 AND ENDO3 after 7, 10, 16, 21 and 45days, respectively.

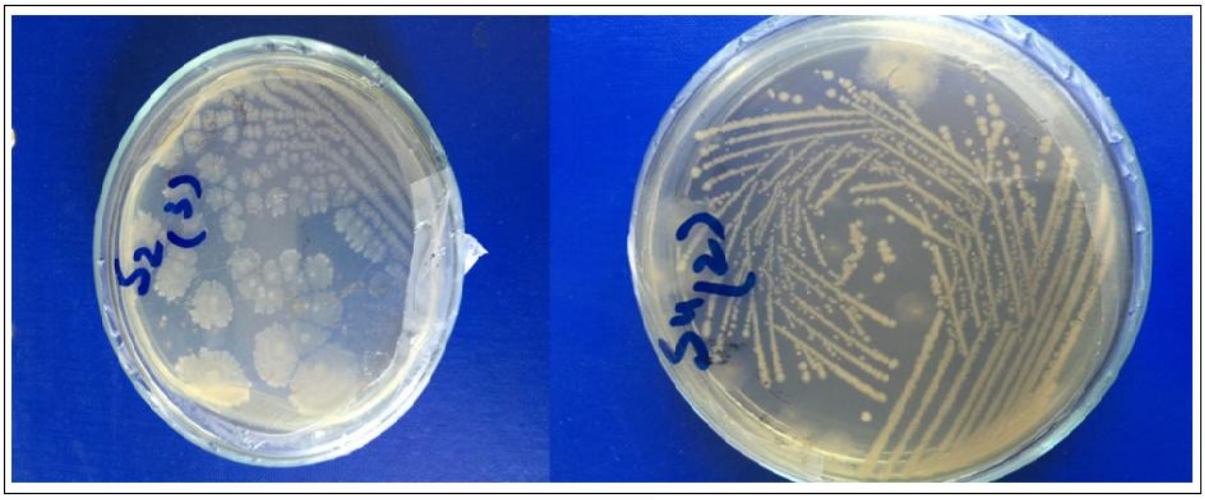

Fig. 1. SOB bacterial isolates recovered from Hazara region.

The value of PH without inoculation had remained the same during those 7 days. The sulphate free contents which stayed neutral in reaction with TCP gradually increased 7days to 45 days of leaching time in all treatments and decreased pH except in control where no changes had been noticed Figure 5.

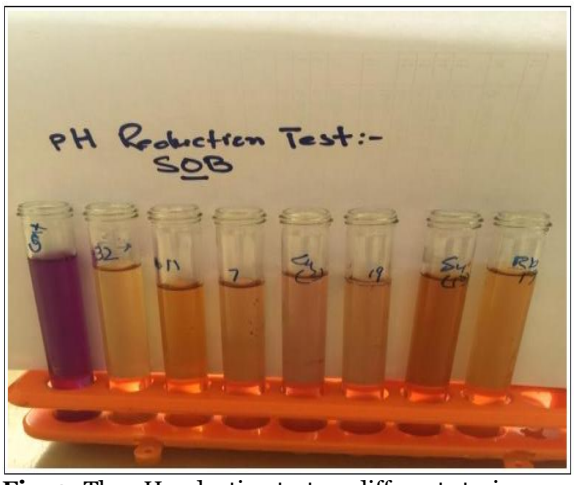

Fig. 2. The pH reduction test on different strains.

Sulphate contents

Amongst the ten SOB isolates RS4 produced the highest amount of sulphate contents 2.6539mg L-1 in 45 incubation days respectively, Whereas the lowest sulphate e contents (1.32mg L-1 after 7, 10, 21, and 45 days respectively had been found in SOB4 Figure 7. The quantities of P solubilized by 10 selected SOB isolates in TCP 0.5% media are presented Figure 9. The Strain RS4 dissolved 2.0435 mg L-1 of P in 45 days of leaching time respectively and remained the highest amongst the 10 SOB isolated and the lowest performance was observed Figure 9 in case of SOB4 that solubilized 1.6101 mg L^{-1} of P IN 45days respectively. Table1 showed that morphological and biochemical characterization of all the isolates was conducted. SR: short rod, SRY: smooth, round, yellow: SRP: smooth, round, pink: SRW: smooth, round, white, AT: Autotrophic, HT: Heterotrophic, M: motile, NM non motile All characteristics of Morphological, physiological biochemical of the 10 SOB isolates indicated that they were Gram negative and short rods.

Morphological, physiological and biochemical characteristics

The SR: short rod, SRY: smooth, round, yellow: SRP: smooth, round, pink: SRW: smooth, round, white, AT: Autotrophic, HT: Heterotrophic, M: motile, NM non motile were recorded.

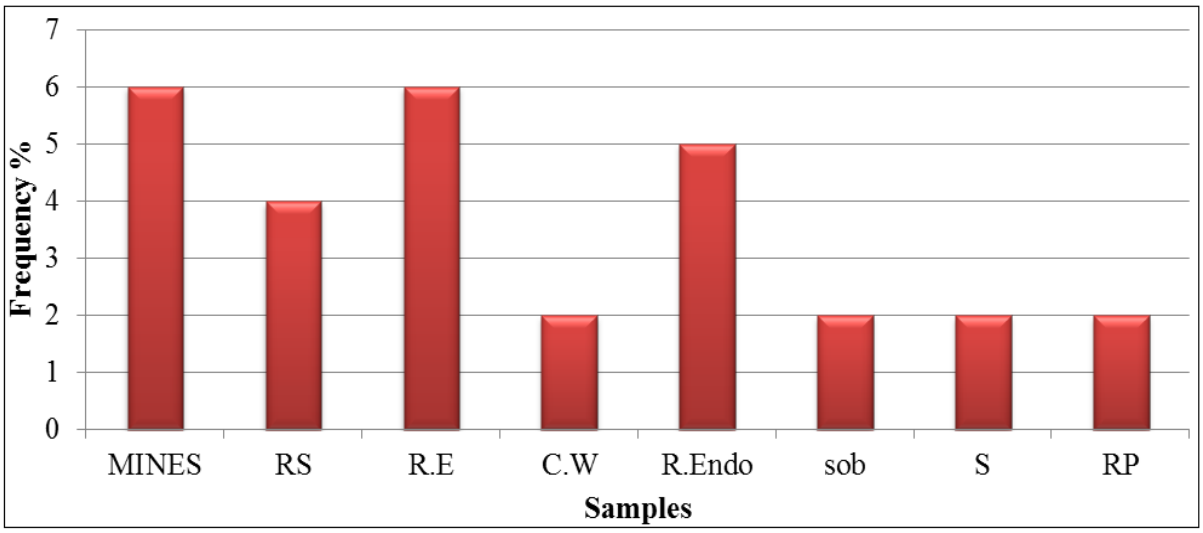

Fig. 3. Frequency of sulfur oxidizing bacteria in the sampling ecologies indicating the highest number different SOB in industrial wastewater. Mine (rock phosphate mine area), RS (maize rhizosphere), CW (canal water), R.Endo (endo rhizosphere), CW (canal water), S (sewage water) and RP(Rock Phasphate area).

All characteristics of Morphological, physiological and biochemical of the 10 SOB isolates indicated that they were Gram negative and short rods. Out of 10 isolates 7 isolates ENDO2, RS4, R.E3, C.W4, MINE4, MINE5 and C.W utilized both elemental S and Thiosulphate, while 3 isolates sob4, endo7 and s2 utilized only thiosulphate. Three SOB isolates endo2, endo3 and R.E3 had smooth, round and yellow coloured colonies, 2 SOB isolates RS4 and S2 had smooth, short rod and dark purple coloured colonies, while 2 SOB isolates C.W and C.W4 had Smooth, round and dark yellow colonies.

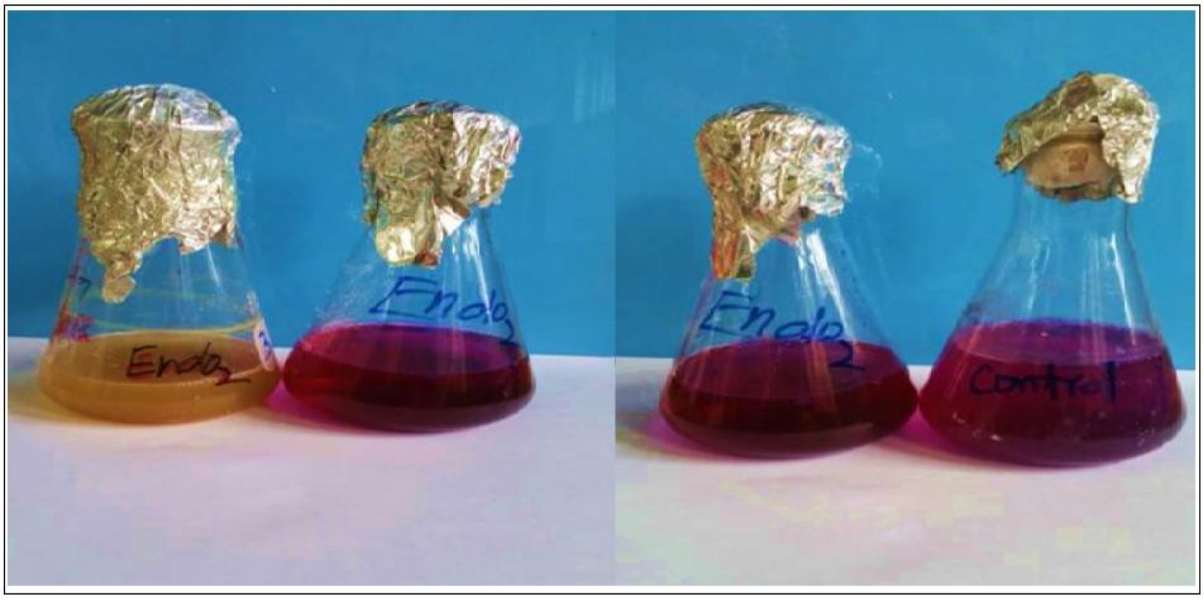

Fig. 4. Growth of sulphur oxidising bacteria in thiosulphate broth medium supplied with bromophenol blue use as an indicator.

Discussion

In this study Phosphorous solubilizing of sulphur oxidizing bacteria through bacterial sulphur oxidation mechanism has been given in this observation. The data of isolation of SOB indicated that maximum percentage of SOB were found in sulphur rich ecologies such as industrial water and sulphur mud and sewer water, it is due to sulphur or reduced

sulphur compounds are crucial for the existing of SOB as its dependent on S oxidation for their energy requirements (Pakoma et, 2007). Presence of SOB in rock phosphate mines, canal water and maize rhizophere, Endo rhizosphere and sewage depicted the occurrence of reduced S compounds in the soil. Biological sulphur oxidation is an exclusive characteristic of SOB through which they oxidize S or compounds and produce sulphuric acid. The Sulfuric oxidation is sulphuric acid generation process given below in the chemical equation: SOB S° + 1.5 O2 + H2O SO4 2– + 2H (_ Go= –587.1kJ/reaction).

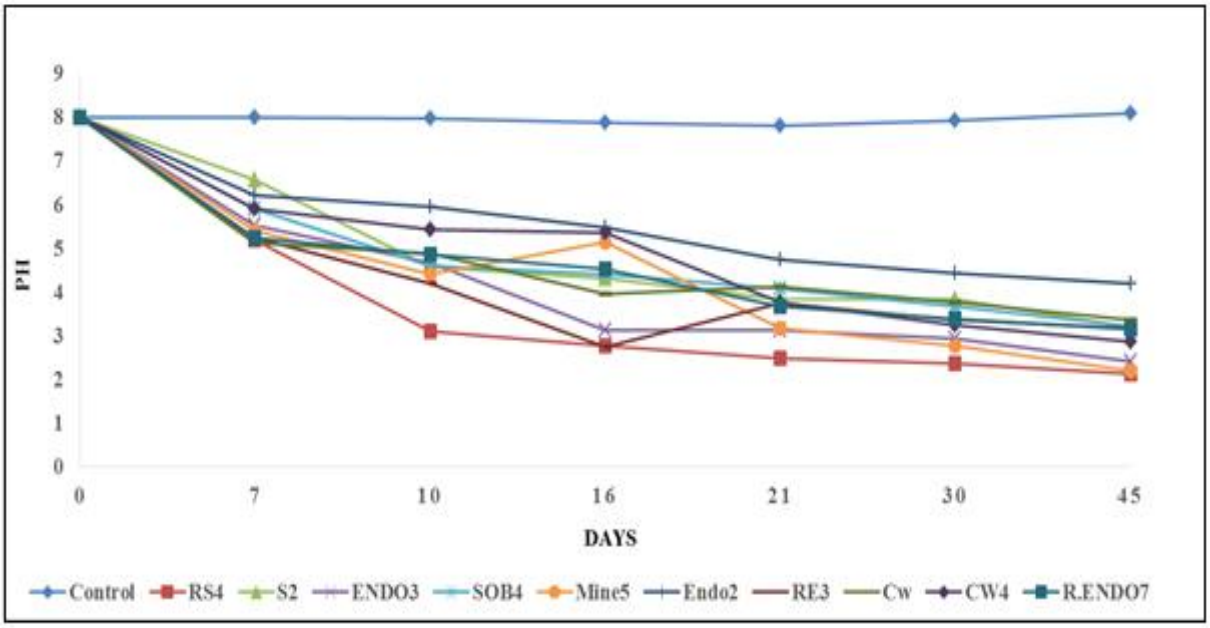

Fig. 5. Effect of pH values in sulfate ion production and phasphorus soloblization of SOB.

The most efficient SOB isolates oxidize S compounds quickly and produce sulphuric acid in huge quantity and drop pH sharply like strain IW16 (Hassan and Van Ginkel, 2011). In the same way highly efficient SOB strains (IW16 and SW2) produced sulphuric acid rapidly and started making holozones from the days 1st of inoculation and consequently their PSI (9.83 and 8.42, respectively) was very high (Islam *et al.,* 2007).

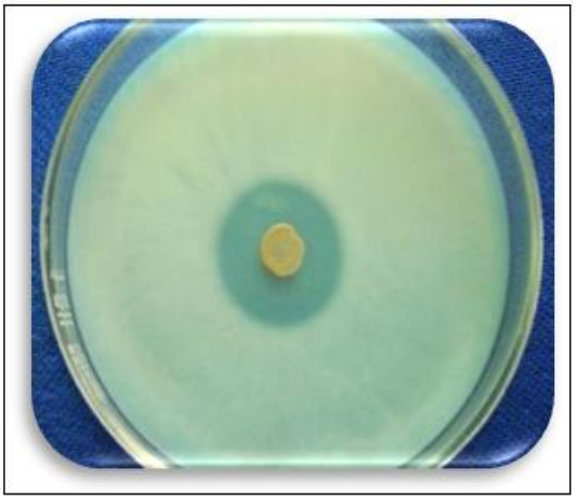

Fig. 6. The C.W SOB shows holozone.

The strains having high PSI are reported to be the most efficient in solubilizing and enhancing P in different media (Hariprasad and Niranjana, 2009; Ahemad and Khan, 2010).

During tricalcium phosphate bioleaching (TCP) test one part of the bacterially produced sulphuric acid was used in solubilizing P from TCP, while the other part decreased pH of the media.

The most efficient SOB (IW16 and SW2) produced rich amount of sulphuric acid and dropped pH drastically. Therefore, pH reduction in the media predicted the efficiency and capability of SOB isolates in P solubilization (Aria *et al.,* 2010; Ullah *et al.,* 2014). Likewise, the concentration of sulphates in the leach solutions depicted the efficiency of SOB isolates to oxidize S or S compounds. The most efficient SOB isolates rapidly oxidized S compounds into sulphates whereas less efficient SOB isolates did this slowly and consequently low quantity of sulphates were present in their leach solutions.

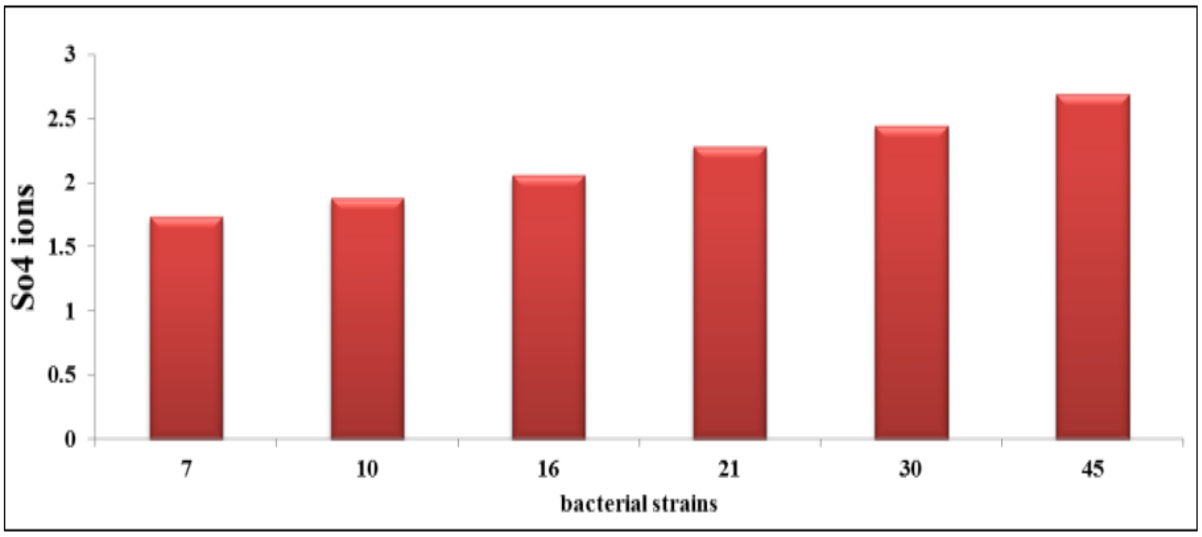

Fig. 7. The sulphate ion productivity on different days.

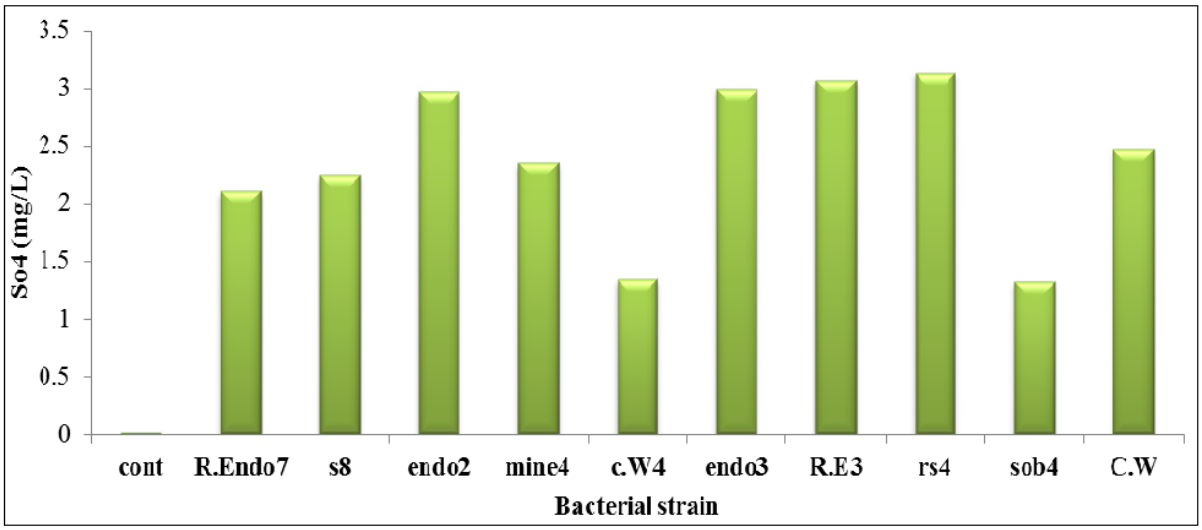

Fig. 8. Sulphate ion production by ten sulphur oxidizing bacterial isolates.

Therefore, SOB isolates could be scrutinized on the basis of sulphate concentration detected from their leach solutions (Lee *et al.,* 2010). Phosphorous solubilization data revealed that the isolates, which have the highest potential to produce sulphates (RS4 and ENDO2) solubilized maximum quantity of P from tricalcium phosphate, while the isolates possessing less efficiency to oxidize S compounds (SOB4) dissolved minimum amount of P in the leaching media.

The chemical reaction for P solubilization is as under: $2Ca_3(PO_4)_2 + 4H_2SO_4$ $2Ca(H_2PO_4)_2 + 4CaSO_4$ (Insoluble tricalcium phosphate) (Soluble calcium dihydrogen phosphate).

The above chemical reaction shows that bacterially produced sulphuric acid attacked on insoluble tricalcium phosphate and converted it into soluble and bio-available dihydrogen phosphate (NareshKumar and Nagendran, 2008; Bhatti and Yawar, 2010). It was noted that the soluble P contents were maximum in the first 16 days in all treatments which indicated that maximum S oxidation was up to the first 16 days of incubation (Aria *et al.,* 2010). The correlation coefficient (*r*) values between pH and solubilized P contents (-0.95, -0.91, -0.89 and -0.86 after 7, 10,16, 21 and 45 days, respectively) and between sulphate concentration and solubilized P contents (0.80, 0.89, 0.91 and 0.92 after 7, 10,16, 21 and 45 days of leaching time, respectively) indicated

that pH had a huge negative significant correlation with the quantity of P solubilized and the concentration of sulphates predicted massive positive correlation with the amount of P solubilized. It showed that with the decrease in pH, sulphate contents increased and subsequently the amount of P increased in the leach suspensions due to enhancement in P dissolution phenomenon (Bhatti and Yawar, 2010). Moreover, figure 8 presented simple regression analysis of pH with the amount of P solubilized.

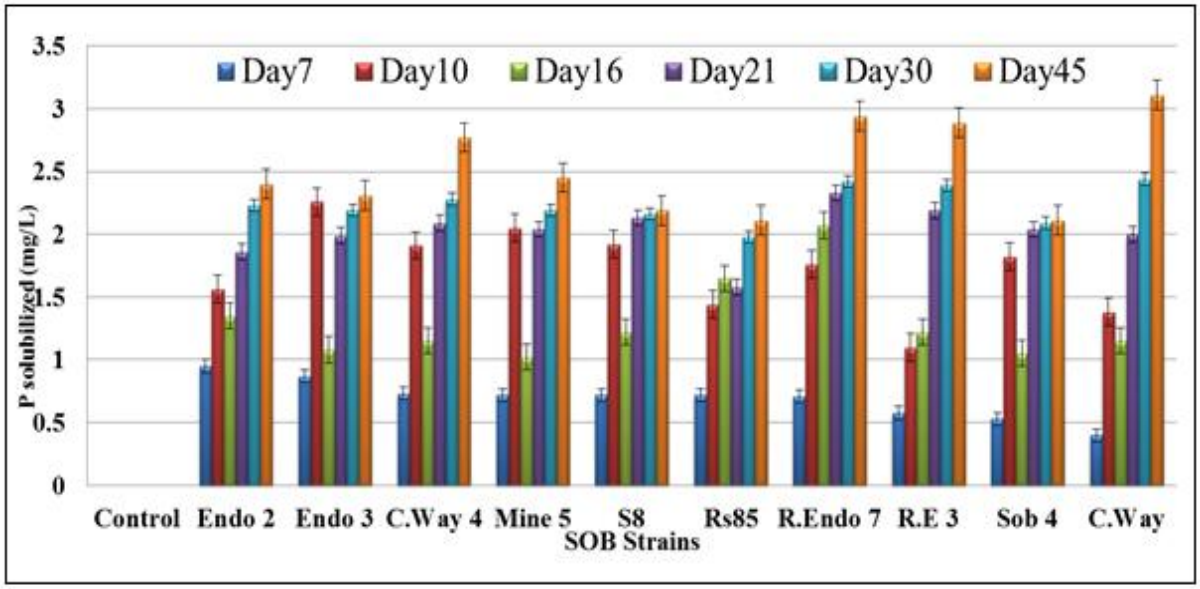

Fig. 9. Sulphate contents of P increased linearly from 7 to 45 days of incubation in all treatments except in control.

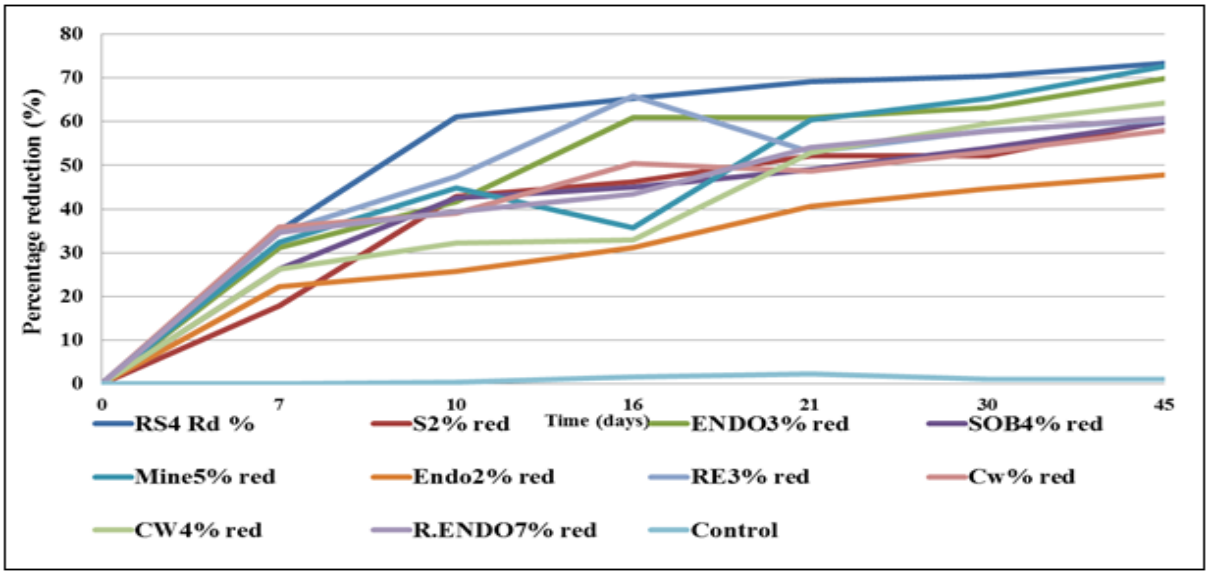

Fig. 10. The percentage reduction of pH by isolates of SOB over six weeks duration (n=3).

It showed that the relationship was linear and significant with the values of coefficient of determination (R2) 0.91, 0.83, 0.79 and 0.73 after 8, 16, 24 and 32 days, respectively (Stamford *et al.,* 2003). The selected SOB isolates were recognized as *Thiobacillus* spp, *Bacillus* spp., *Serratia* spp. and *Enterobacter* spp. because they were Gram negative, short rods and possessed high ability to utilize S or thiosulphate as the only source of energy and carbon dioxide as a sole source of carbon. Furthermore, they

showed great efficiency to produce sulfates and they also reduced pH of the growth media intensely. These characters showed that all these seven SOB isolates belonged to the genus *bacillus, Enterobacter* (Behera *et al.*, 2014; Vidyalakshmi and Sridar, 2007; Babana *et al.,* 2011; Jha *et al.*, 2011). The average percentage reduction of the total recovered bacterial isolates which exhibited a change in pH was calculated and expressed as the figure 10.

Conclusion

In the present study most promising ten bacterial isolates decrease the pH of the culture medium and resourcefully produce sulphate ion in thiosulphate brot and. thiosulphate tricalcium phosphate media. Moreover, these bacteria displayed high P dissolution capability and P solubilization rate was positively correlated with the rate of bacterially produced sulphates. Therefore, the sulphur oxidizing bacteria can be effectively utilized for solubilizing already present huge quantity of fixed P in alkaline and calcareous soils. By Using of these SOB as bio-inoculants can be integrated to enhance sulphur oxidation in soil and to increase availability of sulphate to minimize S-fertilizers application and reduce environmental pollution because using inorganic fertilizer and promotes sustainable and healthy crops, vegetables and cereals agriculture world.

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