

Original Research Article

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Isolation, Characterization and Screening of Sulphur Oxidizing Bacteria from Rhizosphere Soils of Groundnut

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ABSTRACT

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This study was conducted to isolate sulphur oxidising bacteria from saline soils of groundnut growing places in Andhra Pradesh, India and they were characterized based upon their morphological and biochemical characteristics. Among 10 isolates five were capable of reducing the pH of the growth media below 5.0 from initial pH 8.0 and made the highest sulphate production in the growth media and sulphate production of isolates ranges from 1.264 mM to 0.390 mM.

Introduction

Sulphur Oxidizing bacteria (SOB) are the group of bacteria which play an important role in the biogeochemical cycle of sulphur compounds in the environment. Members of this group include free living rods or ovoids of the genera *Thiobacillus*, *Thiomonas*, *Acidiphilium* and *Thiomicrospira* as well as the morphologically conspicuous gliding and non-gliding filamentous forms of the genera *Beggiatoa* and *Thiothrix* (Wirsen *et al.*, 2002).

Sulphur is one of the major plant nutrients after N, P, and K especially in pulses and oilseed crops as a constituent of essential

amino acids cystine, cysteine and methionine. Sulphur Oxidizing Bacteria (SOB) enhance the oxidation of 'S' and speed up the production of sulphates and make it available to plants. In the present study attempt was made to isolate SOB from rhizosphere soils of groundnut which are saline in nature.

Materials and Methods

Sample collection

Isolation of Sulphur Oxidizing Bacteria was done by using rhizosphere soil samples collected from various groundnut growing places of Andhra Pradesh, India.

Culture media, growth conditions, and maintenance

The culture media used for isolation of sulphur oxidizing bacteria include modified thiosulphate broth (Parker, 1957) contained 5.0 g Na₂S₂O₃, 0.1 g K₂HPO₄, 0.2 g NaHCO₃, 0.1 g NH₄Cl, 5.0g Glucose in 1000 ml of distilled water, with pH 8.0. Bromocresol purple was the indicator used (Vidyalakshmi and Sridar, 2006). Five grams of sample was added to 50ml of broth dispensed in conical flasks under aseptic conditions. The flasks were incubated at room temperature for three weeks.

The isolates obtained were purified by transferring to fresh broth twice at fortnightly intervals. The isolates were then streaked on thiosulphate agar medium and individual colonies were obtained. The single colonies were picked and preserved on thiosulphate slants. The pure cultures were labelled and used for characterization and further studies.

The pH reduction test

The pure cultures were inoculated on the growth media with initial pH adjusted to 8.0 and incubated at room temperature for 7 days. The final pH of the growth media was measured using pH meter. The isolates were screened for their efficacy to reduce the pH from 8.0 to 5.0 or less than 5.0. The selected isolates were further studied for their morphological and biochemical characterization.

Morphological characterization

Obtained isolates were plated over thiosulphate agar media with pH 8.0 to study the colony morphology. Single colony was picked and prepared slide for studying cell morphology (Bergey and Boone, 2009), Gram staining and shape.

Biochemical characterization

Indole test, starch hydrolysis, citrate utilization, phosphate solubilization and sulphate production were done to study the biochemical characters of the isolates.

Starch Hydrolysis: Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ±2° C for 48-72 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive reaction for the test.

Indole production: Sterilized SIM agar slants were inoculated with the overnight cultures of the isolates and incubated for 72h at 28 ±2° C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

Gelatin liquefaction: The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin tubes and incubated for 48h at 28 ±2° C. Then the tubes were kept in the refrigerator for 30 minutes at 4°C. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative.

Citrate utilization: Isolates were streaked on Simmon's citrate agar slants and incubated at 28±2°C for 24h. Change in colour from green to blue indicates the positive reaction for citrate utilization.

Catalase test: This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of Pure isolates were taken on glass slides and one drop of H₂O₂ (30 %) was added. Appearance

of gas bubble indicated the presence of catalase enzyme.

Sulphate estimation: The isolates were further screened on the basis of production of sulphate ion (SO_4^{2-}). The amount of sulphate ions (SO_4^{2-}) produced during growth of sulphur-oxidizing bacteria on Thiosulphate broth medium was determined spectrophotometrically. A loopful of 48 hrs old culture of each isolate was inoculated into 10 ml of Thiosulphate broth. All the inoculated tubes were incubated at 30°C for 7 days. After 7 days of incubation, the broths were centrifuged at 15000 rpm for 10 minutes to separate the supernatant from the cell growth. Sulphate production was measured by adding 1:1 barium chloride solution (10%, w/v) with bacterial culture supernatant followed by mixing the suspensions vigorously (Cha *et al.*, 1999). A resulting white turbidity due to barium sulphate formation was measured at 450 nm. The values obtained were compared with the sulphate standard curve.

Results and Discussion

Isolation and screening of isolates: A total of 10 isolates were obtained from rhizosphere soils of groundnut based upon the pH

reduction test (Fig. 1). They were named as BSS2, AKS2, NNS1, VKS3, NJS2, NDS1, NGS2, NUS2, KKS1 and KAS1. Among the 10 isolates, AKS2, NNS1, NUS2, NGS2 and NJS2 reduced the pH of thiosulphate broth to 5.0, 4.5 and 4.0 respectively after seven days of incubation. VKS3, NDS1, BSS2 and KKS1 reduced the pH to 5.5, 6.0 and 6.5 on thiosulphate broth respectively after 7 days. A control for was maintained with pH 8.0. The results are shown in Table. 1.

Characterization of isolates: Morphological and Biochemical characterization of all the isolates were done. Colony appearance ranges from milky white to dull white in colour and margin is regular to irregular in nature; colony surface of most of the isolates was raised and flattened. Cells were Rod shaped. All the isolates were Gram negative in nature. The characters of isolates were presented in Table 2. Ten isolates were obtained from rhizosphere soil samples of groundnut. In earlier studies various researchers isolated Sulphur oxidizing bacteria isolates from diverse habitats, including mangroves soils, acid mines drainage, paddy rhizosphere, treated tannery effluent, untreated tannery effluent, rhizosphere soil, acidic soil and treated beverage waste.

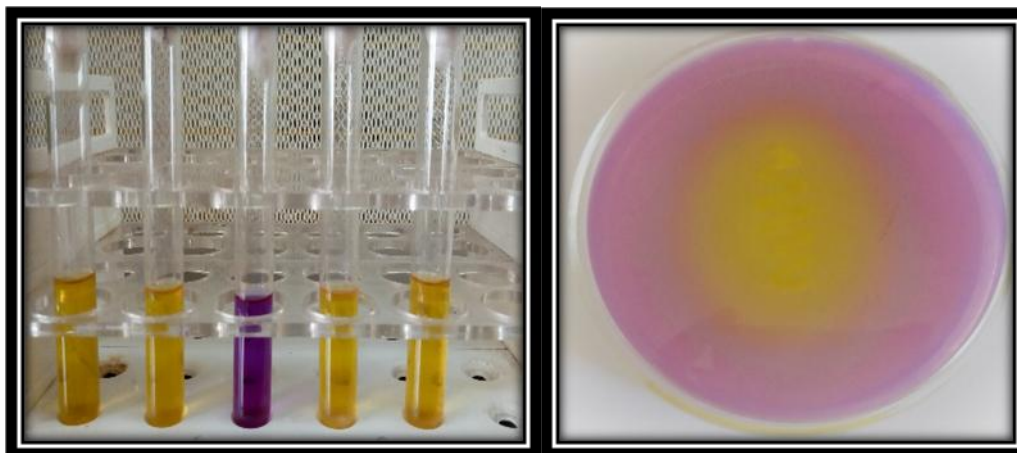
Table.1 pH reduction in growth media by sulphur oxidizing bacteria

| Name of the SOB isolate | Initial pH of the growth media | Reduction of pH in growth media of Thiosulphate broth |
|-------------------------|--------------------------------|---|
| BSS2 | 8.0 | 6.5 |
| AKS2 | 8.0 | 5.0 |
| NNS1 | 8.0 | 5.0 |
| VKS3 | 8.0 | 5.5 |
| NJS2 | 8.0 | 4.0 |
| NDS1 | 8.0 | 6.0 |
| NGS2 | 8.0 | 4.5 |
| NUS2 | 8.0 | 5.0 |
| KKS1 | 8.0 | 6.5 |
| KAS1 | 8.0 | 7.0 |

Table.2 Morphological and biochemical characteristics of SOB isolates

| Characters | | Isolates | | | | | | | | | |
|--------------------------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-------------------|-----------------|----------------|-------------------|-------------------|
| | | BSS2 | AKS2 | NNS1 | VKS3 | NJS2 | NDS1 | NGS2 | NUS2 | KKS1 | KAS1 |
| Morphology | | Rod shape | Rod shape | Rod shape | Rod shape | Rod shape | Rod shape | Rod shape | Rod shape | Rod shape | Rod shape |
| Gram reaction | | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Colony characters | | Smooth, Regular | Smooth, Regular | Smooth, Regular | Smooth, Regular | Mucoid, Regular | Smooth, Irregular | Smooth, Regular | Slimy, Regular | Smooth, Irregular | Smooth, Irregular |
| Optimum pH | 4 | + | + | + | + | + | + | + | + | + | + |
| | 7 | + | + | + | + | + | + | + | + | + | + |
| | 9 | + | + | + | + | + | + | + | + | + | + |
| Indole production | | - | + | + | + | + | - | - | - | + | + |
| Citrate utilization | | + | + | + | - | + | + | - | - | - | + |
| Gelatin liquefaction | | - | - | - | - | - | - | - | - | - | - |
| Starch hydrolysis | | - | + | - | + | + | - | + | - | - | - |
| Methyl red test | | - | - | - | - | + | - | + | - | - | + |
| Vogues-Prausker's test | | - | - | - | - | - | - | - | - | - | + |
| Sulphate production (mM) | | 0.643 | 0.626 | 0.953 | 0.441 | 1.264 | 0.854 | 1.103 | 0.972 | 0.575 | 0.390 |

Fig.1 Reduction of pH in growth media by SOB



Thiosulphate broth Thiosulphate agar (isolate NJS2)

The isolates were morphologically and physiologically best described as *Pseudomonas*, *Stenotrophomonas*, *Alcaligenes*, *Bordetella spp* and *Thiobacillus spp*. Sulfur oxidizing *Pseudomonas spp*. have also been isolated from soils of Bhitarkanika, Odisha, India (Thatoi *et al.*, 2012) and mangrove soils of Mahanadi River Delta (Behera *et al.*, 2014) that produce sulfate ion. In the present investigation, 10 isolates were obtained from rhizosphere soil samples of groundnut and among them five isolates showed good reduction of pH in growth media (initial 8.0, final below 5.0 in 7 days). The pH reduction is due to production of sulphuric acid by the oxidation of elemental sulphur by the sulphur oxidizers (Anandham *et al.*, 2005). And sulphate production is highest in the isolate NJS21.264 mM and further the isolates were characterized as rod shaped; all the isolates were Gram negative. The colonies of isolates were circular or irregular in shape.

In conclusion, the sulphate production property of these isolates from rhizosphere soils of groundnut can be used for developing inoculants for oil seed crops and pulses needing more sulphate for improving their productivity and quality.

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