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ISOLATION AND CHARACTERIZATION OF SULPHUR OXIDIZING BACTERIA

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Summary

Sulphur oxidizing bacteria were isolated from different samples viz., paddy rhizosphere, pulse rhizosphere, sewage, biogas slurry, tannery effluent and mine soil. Out of the 28 isolates obtained, 14 were screened based on their efficacy to reduce the pH of the growth medium from 8.0 to ≤5.0. The selected isolates were characterized and related to the genus Thiobacillus.

Key words*: sulphur oxidizing bacteria, Thiobacillus, sulphate.*

Introduction

Sulphur is now considered the fourth major plant nutrient after N, P and K, and is one of the sixteen nutrient elements which are essential for the growth and development of plants, especially in the agricultural crop production. This is mainly because of its widespread deficiency in the soil world over. The importance of sulphur is equal to that of nitrogen in terms of protein synthesis, and in terms of crop uptake it exceeds even that of phosphorus. The majority of sulphur taken up by plant roots is in the form of sulphate (SO_4), which undergoes a series of transformations prior to its incorporation into the original compounds [10].

The soil microbial biomass is the key driving force behind all sulphur transformation. The biomass acts as both a source and sink for inorganic sulphate. The latter make sulphate available from element sulphur or any reduced forms of sulphur through its oxidation process in the soil. The role of chemolithotrophic bacteria of the genus *Thiobacillus* in this process is essential. The objective of this study was to isolate and characterize the sulphur oxidizing bacteria from various sources.

Materials and Methods

Isolation of sulphur oxidizing bacteria. Isolation was performed using samples collected from seven different sources viz., paddy rhizosphere soil (Wetland, Tamil Nadu Agricultural University, Coimbatore), lab rhizosphere soil (Millet Breeding Station, Tamil Nadu Agricultural University, Coimbatore), black gram rhizosphere soil (Farmers field, Pondicherry), sewage water (Department of Bioenergy, Tamil Nadu Agricultural University, Coimbatore), tannery effluent (Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore) and mine soil (Neyveli Lignite Corporation, Neyveli).

Microbiological media. The media employed for the isolation of sulphur oxidizing bacteria include Starkey broth [13] composed of 3.0 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.2 g $CaCl_2 \cdot 2H_2O$,

0.5 g $(NH_4)_2SO_4$, traces of $FeSO_4$ in 1000 ml distilled water with pH 8.0; NCL broth [15] consisted of 0.2 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.25 g $CaCl_2$ $2H_2O$, traces of $FeSO_4$, in 1000 ml of distilled water with pH 3-5; and the thiosulphate broth [2] contained 5.0 g $Na_2S_2O_3$, 0.1 g K_2HPO_4, 0.2 g $NaHCO_3$, 0.1 g NH_4Cl in 1000 ml distilled water, with pH 8.0. Bromo cresol purple was the indicator used. For isolation of heterotrophic oxidizers 5.0 g of glucose per litre of Starkey, NCL and Thiosulphate broth was added. Elemental sulphur at 10 g per litre was added to Starkey and NCL broths and half-an-hour steam sterilized for three consecutive days. Thiosulphate broth was devoid of S^0. One gram or one ml of the sample was added to 20 ml of the broth dispensed in tubes, under aseptic conditions. The tubes were incubated in BOD incubator at 32 ^oC for 25 days. The isolates obtained were purified by transferring to fresh broth thrice 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.

Screening of isolates by pH reduction test. The obtained isolates were inoculated in the growth media with initial pH adjusted to 8.0 and incubated at 32 ^oC for 15 days. The final pH of the growth media was measured using a 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 morphology, Gram reaction, colony characters and nutritional type.

Cell and colony morphology. Negative staining was done and the morphology of the isolates was studied under a microscope. Gram staining [7] of the isolates was performed. For colony characterization Starkey agar and thiosulphate agar media were prepared with pH adjusted to 8.0. The isolates were plated in sterile Petri dishes by the pour plate method and the plates incubated at 32 ^oC for 15 days. Colony characters were observed after the incubation period.

Utilization of sulphur sources. The isolates were studied for the utilization of elemental sulphur and thiosulphate broth. They were inoculated in both the sterilized broths (initial pH 6.0) and incubated in the BOD incubator for 15 days. The growth was assessed by pH reduction and microscopic observation.

Selection of suitable media for autotrophic and heterotrophic sulphur oxidizing bacteria. Starkey broth with initial pH of 8.0 was prepared with and without glucose. The isolates were inoculated and incubated at 32 ^oC for 15 days. Based on the pH reduction of the broth with and without glucose, the isolates were classified as chemoheterotrophs and chemoautotrophs. Heterotrophic isolates were also inoculated in NCL and thiosulphate broth prepared with glucose. Autotrophic isolates were inoculated in the same broths prepared without glucose. Inoculated tubes were incubated at 32 ^oC for 15 days. Control tubes and three replications were maintained. The pH of the broth was recorded after the incubation period.

Influence of biotin on the elemental sulphur oxidation. Filter sterilized biotin solution (0.1 per cent) was added to the Starkey broth. The pH reduction was measured at 24 hour intervals from the tenth up to the 15th day of incubation using a pH meter. Simultaneously, a control was maintained.

Growth of type culture. The strain *Thiobacillus thiooxidans* MTCC 468 was obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, Haryana and was grown in the medium as recommended by the MTCC and used as a reference culture.

Results

Isolation and screening of sulphur oxidizing bacteria

A total of twenty eight isolates were obtained from seven different samples. Among them, 14 isolates were selected based on the pH reduction test (Fig. 1) and named as follows: SR, AP2, AN, NP2, NS, TP2, TB, TR, ASE, ATE, ATR, ASS and ATB. Among the 14 isolates obtained, the isolate from the black gram rhizosphere soil (TP2) reduced the pH to 4.5, 5.5 and 4.0 (control 8.0) of the Starkey, NCL and thiosulphate broth respectively, within 12 days. The isolate obtained from paddy rhizosphere soil (SR) caused a pH reduction of 4.5 and 6.5 (control 8.0) in Starkey and thiosulphate broth respectively, within 10-15 days and no reduction was found in NCL broth. The results are furnished in Table1. The isolate NP2 reduced the pH to 5.0 of Starkey, NCL and thiosulphate broths. The isolates SN, NS, TR, ATE and ATB did not show a remarkable reduction in pH of the growth media.

Starkey broth NCL broth Thiosulphate broth

Fig.1. Reduction of pH in the growth media by sulphur oxidizing bacteria.

Table 1. Reduction of pH in the growth media by sulphur oxidizing bacteria (SOB).

Name of SOB isolate	pH Reduction in growth media		
	Starkey broth	NCL broth	Thiosulphate broth
SR	4.5	8.0	6.5
SP2	4.5	8.0	5.0
SN	6.5	8.0	8.0
SS	5.5	5.5	8.0
NP2	5.0	5.0	5.0
NS	8.0	5.5	6.0
TP2	4.5	5.5	4.0
TB	5.5	5.5	8.0
TR	8.0	8.0	6.0
ASE	8.0	6.0	4.5
ATE	8.0	6.5	8.0
ATR	8.0	6.0	5.5
ASS	6.0	5.5	6.0
ATB	6.0	6.0	8.0

Characterization of the selected isolates

The results of the isolates characterization are presented in Table 2 and Fig. 2. Invariably, all the organisms were short rods and gram negative, but differed in the utilization of sulphur sources.

The isolates SN and SS utilized only S^0, whereas TR, ATE and ATR utilized thiosulphate only. The isolates SR, SP2, NP2, NS, TP2, TB, ASE, ASS and ATB utilized both S^0 and thiosulphate. The heterotrophic isolates utilized glucose, whereas the autotrophic isolates did not utilize glucose. All the isolates were positively influenced by biotin. The heterotrophic colonies were smooth, round straw yellow coloured, and autotrophic colonies were smooth, raised, pink coloured.

Control 2^{nd} day of incubation 4^{th} day of incubation 7^{th} day of incubation

Fig. 2. Growth of sulphur oxidizing bacteria on thiosulphate agar medium.

Table 2. Characterization of the screened isolates.

Isolates	Morphology	Gram reaction	Utilization			Influence of biotin	Colony character	Nutritional type
			Elemental sulphur	Thio-sulphate	Glucose			
SR	Short rods	Negative	+	+	+	+	Smooth, round straw yellow colour colonies	Hetero-troph
SP2	Short rods	Negative	+	+	+	+		
SN	Short rods	Negative	+	-	+	+		
SS	Short rods	Negative	+	-	+	+		
NP2	Short rods	Negative	+	+	+	+		
NS	Short rods	Negative	+	+	+	+		
TP2	Short rods	Negative	+	+	+	+		
TB	Short rods	Negative	+	+	+	+		
TR	Short rods	Negative	-	+	+	+		
ASE	Short rods	Negative	+	+	-	+	Smooth, raised pink colour colonies	Auto-troph
ATE	Short rods	Negative	-	+	-	+		
ATR	Short rods	Negative	-	+	-	+		
ASS	Short rods	Negative	+	+	-	+		
ATB	Short rods	Negative	+	+	-	+		

Legend: utilized (+), unutilized (-).

Discussion

Twenty-eight isolates were obtained from different sources *viz*., soil, sewage, biogas slurry, mine soil, tannery effluent etc. Earlier studies on isolation of sulphur oxidizing bacteria by various researchers reveal their existence in mud soil, canal water, other fresh water sources [11] and acid streams [9]. *T. ferrooxidans* and other *Thiobacillus* sp. were isolated from uranium mines by Deborah Berthelot [3]. A similar organism was also reported from garden soil, activated sludge [1] and soil sulphur compost [4]. Dave and Upadhyay isolated thiosulphate oxidizing organisms from thermal springs [5]. Heterotrophic isolates of thiosulphate producing bacteria, which acidified the medium moderately by ca.0.5 to 1.0 pH units, were obtained from marine sediments and hydrothermal vents [10].

Out of the 28 isolates 14 were selected based on pH reduction (initial 8.0, final $\leq$ 5.0 in 15 days). The pH reduction of the medium was due to the production of sulphuric acid. Reduction in pH of the growth medium by sulphur oxidizing bacteria was reported by Donati [6]. The selected isolates were characterized to be short rods, Gram negative, utilizing biotin and one or more of the sulphur sources. The heterotrophic colonies were smooth, round and straw yellow probably due to the sulphur deposition. Similar results were reported by several workers [8, 11, 16] who isolated from soil compost sulphur oxidizing bacteria which were short rods and morphologically similar to *Thiobacillus*. The organisms reduced the pH from 5.6-6.2 to 2.6-2.8 and utilized both elemental sulphur and thiosulphate [16]. All the autotrophic and heterotrophic isolates were positively influenced by biotin, which was in conformity with the findings of Kelly and Harrison [11]. Based on the morphological and physiological studies and on comparison with the reference culture *T. thiooxidans* MTCC 468, all the 14 isolates were confirmed to belong to the genus *Thiobacillus*.

The present study emphasizes the importance and the role of sulphur oxidizing bacteria in the oxidation of sulphur in soil. These *Thiobacillus* isolates can be incorporated to enhance sulphur oxidation in soil and to increase soil available sulphate. Also, the pH reducing property of sulphur oxidizing bacteria by the production of sulphuric acid can be utilized for reclamation of alkali soils.

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ИЗОЛИРАНЕ И ХАРАКТЕРИЗИРАНЕ НА СЯРА-ОКИСЛЯВАЩИ БАКТЕРИИ

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Резюме

Изолирани са сяра-окисляващи бактерии от различни проби – ризосфера на оризище и бобови култури, канализационни води, биогазна тиня, отпадни води при обработка на кожи и минна почва. От получените 28 изолата 14 са скринирани на база на ефикасността им да понижават pH на растежната среда от 8.0 до ≤5.0. Избраните изолати са характеризирани и отнесени към род Thiobacillus.