Fishery Technology 55 (2018) : 270 - 275

Isolation and Identification of Sulphur Oxidizing Bacteria from Freshwater Fish Farm Soil

N. Ranjit Kumar*, K. K. Archana, K. A. Basha, T. Muthulakshmi, T. C. Joseph and M. M. Prasad
ICAR-Central Institute of Fisheries Technology, Matsyapuri, P. O. Cochin - 682 029, India

Abstract

Sulphur oxidizing bacteria (SOB) are natural microflora in aquaculture farms that play a significant role in sulphur cycle. In this study isolation, characterization and sulphate ion production ability was carried out to assess the role of SOB in utilizing thiosulphate. Ninety six distinct morphological isolates of SOB were isolated from the freshwater fish farm soil employing six different isolation media. Subsequent dye reduction test revealed that 16 isolates on *Thiobacillus aquaesulis* medium, 12 isolates on *T. denitrificans* medium, 9 isolates on *T. thioparus* medium and 10 isolates on *Thiothrix* medium showed characteristic color change of the dye (bromo phenol blue). Eight sulphur oxidizing bacterial isolates (SOBA17, SOBA12, SOBA3, SOBA20, SOBD10, SOBD16, SOBD12 and SOBD14) were selected for further studies based on the pH reduction test. To screen the potential sulphur oxidising bacteria, medium was amended with sodium thiosulphate and the sulphate ion production ability of the isolates was measured spectrophotometrically. Bacterial isolate of SOBA17 (1.62 mg ml^{-1}) showed maximum sulphate ion production, followed by SOBA20 (1.50 mg ml^{-1}). The 16S rRNA sequencing revealed that the isolates belonged to *Thiobacillus aquaesulis*. The partially purified sulphur oxidase of SOBA17 in SDS-PAGE exhibited three consecutive protein bands of molecular weight 10, 17 and 30 kDa. The present study revealed that SOB has the capacity to utilize sulphur in its oxidized state to non-toxic sulphate. Further studies are needed to understand the diversity and role of these bacteria in sulphur dynamics in aquaculture farms.

Received 08 March 2018; Revised 11 October 2018; Accepted 12 October 2018

**E-mail: nranjeetkumar@gmail.com*

Keywords: Aquaculture, sulphur oxidizing bacteria, sulphur oxidation, *Thiobacillus aquaesulis*

Introduction

Aquaculture is expanding rapidly and making an important contribution to global food production. World aquaculture production of fish constituted 47% of total production in 2016 and has grown tremendously over the past 50 years to nearly 80 million tons worth 232 billion USD (FAO, 2018). Moreover, FAO estimates that the share of aquaculture in total fish production would surpass capture fisheries in 2021 and would occupy a share of 52% by 2025 (FAO, 2016). In order to improve the productivity, the present day farmers are going for intensive farming. As a result, different kinds of feeds are being used to enhance the growth of animals. Unutilized feed which is left over in the pond bottom is responsible for the generation of toxic gases such as ammonia and hydrogen sulphide as a result of bacterial degradation (Rojas et al., 2001). Sulphur oxidizing bacteria (SOB) can oxidize the inorganic sulphur compounds such as sulphide, sulphite, thiosulphate, and elemental sulphur in their metabolism to produce energy (Friedrich et al., 2001). SOB are divided into two groups based on photosynthesis *viz.,* green sulphur bacteria and purple sulphur bacteria (Jorgensen & Revsbech, 1983). They are heterotrophic, photoautotrophic and chemolithotrophic in metabolism. They play an important role in utilizing the hydrogen sulphide produced by the sulphur reducing bacteria (SRB) and also act as major driving force in sulphur transformations occurring in the pond bottom (Suzuki, 1999). These bacteria can be isolated almost in every life-supporting environment where reduced sulphur compounds are available (Kelly & Wood, 1998).

Few studies have been carried out to elucidate the importance of sulphur oxidizing bacteria in the

© 2018 Society of Fisheries Technologists (India)

aquaculture systems. The levels of SOB counts in the soil and culture water were significantly higher in the *L. vannamei* culture ponds when treated with probiotics (Patil et al., 2015). Abraham et al. (2015) have concluded that H_2S was effectively converted to sulphur compounds that enhanced SOB counts in the shrimp farms. Patil et al. (2012) have reported the influence of water probiotics, soil probiotics and fermented juice on both SOB and SRB. Presumptive densities of SOB groups in water and sediment have been estimated by Devaraja et al. (2002). Mean SOB counts in tropical culture farms have been reported by Rao & Karunasagar (2000). Behera et al. (2014) have isolated and characterized heterotrophic SOB from mangrove soil of Mahanadi river, Odisha and studied their sulphur oxidizing capability. Behera et al. (2016) have carried out studies on partial purification and characterization of the enzyme sulphur oxidase produced by the heterotrophic SOB (*Micrococcus* sp. and *Klebsiella* sp.). Keeping in view of these earlier studies, the present investigation was carried out to isolate, identify the SOB from freshwater fish farms and characterize their sulphur oxidation capability.

Material and Methods

The soil samples were collected aseptically from the freshwater fish aquaculture farms located in Malayatoor of Kerala, India in which the fish were fed daily with chicken slaughter waste @ 3% of body weight and brought to the laboratory at Central Institute of Fisheries Technology, Cochin. Top soil (about 1 cm) was collected from all the four corners of the farm and pooled to represent soil sample from the whole farm. The soil temperature varied from 30-31 °C and the pH of the soil ranged from 6.4 to 6.7. Isolation of SOB was carried out by employing six different media *i.e., Thiobacillus aquaesulis* medium, *Thiobacillus thioparus* medium, *Paracoccus versutus* medium, *Thiobacillus neopolitanus* medium, *Thiobacillus denitrificans* medium and *Thiothrix* Medium (Kelly & Wood, 1998). The soil samples (10 g) were mixed with the above mentioned media in the ratio of 1:9 under aseptic conditions and the flasks were incubated at 30°C in the BOD incubator (Labline instruments, India) for 4 to 5 days. The same media were used for the preparation of the plates with the addition of 1.8 % agar (BD, Difco, India). One ml of enriched medium is taken and serially diluted using phosphate buffer saline[#] (1X) upto 10^{-3}. Hundred microliters of serially diluted samples were plated and the plates were kept for incubation under same conditions as mentioned above. The pH of the medium was monitored with pH meter (Eutech instruments, India) to assess the growth of bacteria.

The plates were observed after 4-5 days of incubation and well isolated colonies were picked on the same medium for purification by repeatedly transferring the isolates to the fresh media. The purified isolates were preserved at -80°C in 60% glycerol for characterization and further tests. Morphological and biochemical characterization were carried out as per the standard methods mentioned in Bergey's Manual of Systemic Bacteriology (Brenner et al., 2005).

The isolates were inoculated on respective media containing bromophenol blue dye (Behera et al., 2014).The isolates were screened on the basis of their ability to reduce the color of the dye from purple to yellow. The cultures which changed the colour of the broth from purple (red in case of *T. aquaesulis* medium) to yellow colour by reducing the pH after incubation for 4-5 days at 30°C were selected for further characterization.

The amount of sulphate ion (SO_4^{2-}) produced during growth of SOB on thiosulphate broth was determined spectrophotometrically (Cary 100, UV-VIS Spectrophotometer, Thermofisher Scientific, USA) (Behera et al., 2014). Amount of sulphate formed during growth of bacteria was measured by adding 1:1 barium chloride solution (10%) with bacterial culture supernatant and followed by mixing the suspensions vigorously (Cha et al., 1999). The resulting white turbidity due to barium sulphate formation was measured at 450 nm. Potassium sulphate (K_2SO_4) was used as standard to construct a sulphate calibration curve (Kolmert et al. 2000).

Freshly grown culture (10 ml) from the *Thiobacillus* medium was taken in Eppendorf tube and centrifuged at 4000 g for 10 min. The supernatant was discarded and 1 ml phosphate buffer saline (1X) was added, mixed well and centrifuged again at 4000 g for 10 min. Further the supernatant was discarded and 0.5 ml 1X tris EDTA (TE) buffer (pH 8.0) was added. The tube was kept in dry bath at 95°C for 10 min and transferred immediately to -20°C for crude DNA isolation.

PCR was carried out to amplify the 16S rRNA gene from bacterial isolates (Joseph et al., 2015) in three steps *i.e.*, initial denaturation at 94°C for 2 min

[#]Composition of phosphate buffer saline (g lit-1): NaCl- 8; KCl-0.2; Na2HPO4-1.44; KH2PO4-0.24

followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 60 sec and extension at 68°C for 90 sec. The reaction was performed as 25 µL reaction mixture composed of 10X PCR Buffer (2.5 µl), dNTP (0.5 µl), $MgCl_2$ (1.5 µl), forward primer (1 µl), reverse primer (1 µl), 5U Taq Polymerase (0.2 µl), distilled water (16.8 µl) and template DNA (2.5 µl). The primers used for amplification of gene are universal primers which are specific to 16S rRNAa) 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and b) 1544R (5′-AGAAAGGAGGTGATCCAGCC-3′). After adding all the components, the tubes were vortexed for proper mixing. Then the tubes were placed in the thermo cycler (Veriti 96-Well Thermal Cycler, India) for completing 35 cycles of the above mentioned steps with final extension time of 10 min. for completing the PCR cycle.

Agarose gel electrophoresis was carried out for the effective separation of the isolated DNA. The gel (1.5%) was prepared in 1X tris acetate EDTA (TAE) buffer. Ethidium bromide (0.5 µg ml^{-1}) was added and poured after placing the comb and was allowed to set after which the comb was removed and the gel was placed in electrophoresis tank. The PCR products were analyzed by loading into the well after mixing with 6X loading dye (Thermo Fisher Scientific, USA) and ran at 25 V until the dye reached three-fourth of the gel. Later the gel was observed under UV transilluminator (Biorad, Germany) and the image was captured.

Partial purification of sulphur oxidase enzyme from the culture broth was carried out by gradient ammonium sulphate precipitation (70%) followed by dialysis (Behera et al., 2016). The supernatant was collected by centrifugation at 8000 rpm for 10 min and was precipitated using ammonium sulphate for 2 h at 4°C. Later, the precipitate was collected and dissolved in 0.2 M phosphate buffer (pH 8). The enzyme extract was dialyzed over night against the same buffer at 4°C. To estimate the molecular weight of the partially purified enzyme, SDS-PAGE was carried using 5% stacking gel and 10% resolving gel at 15 mA fixed current.

Results and Discussion

A total of 96 sulphur oxidising bacteria were isolated from freshwater fish aquaculture farms using different sulphur oxidizing media. Distinct growth of bacteria was observed on the plates of *T. aquaesulis* medium, *T. denitrificans* medium, *T. thioparus* medium and *Thiothrix* medium. However, no bacterial growth is observed on *Paracoccus versutus* medium and *Thiobacillus neopolitanus* medium.

Table 1. Characteristics of sulphur oxidizing bacterial isolates

Sl. No.	SOBA17	SOBA20	SOBA3	SOBA20	SOBD10	SOBD16	SOBD12	SOBD14
Shape	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod
Shape	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod	Thin rod
Spore	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Motile	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Aerobic growth	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Gram stain	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Catalase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Oxidase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
MR test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Indole production	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
D-glucose fermentation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Nitrate reduction	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Nitrite reduction	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Growth at pH 5	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Growth at 35°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

The plates having bacterial growth showed change of color due to the reduction in pH. From these plates, colonies showing distinct morphological differences were selected and streaked onto the respective fresh media. Twenty four isolates each from *T. aquaesulis, T. thioparus, T. denitrificans* and *Thiothrix* media were picked and purified. Based on the reduction in colour from purple to yellow (red to yellow in case of *T. aquaesulis medium*), 16 isolates from *T. aquaesulis* medium, 12 isolates from *T. denitrificans* medium, 9 isolates from *T. thioparus* medium and 10 isolates from *Thiothrix* medium were selected for subsequent dye reduction test.

The bacterial isolates inoculated on thiosulphate broth medium containing bromophenol blue (BPB) as an indicator. Four isolates from *T. aquaesulis* medium and four isolates from *T. denitrificans* medium were able to change the colour of the BPB in thiosulphate broth medium by reducing the pH of the medium from initial pH 7.0 to 5 in 4-6 days incubation at 30°C. Based on the dye reduction capability, eight isolates were selected for further studies.

Morphologically the bacterial isolates appeared as small, white colour colonies having smooth surface on the growth media. Biochemically they were identified as Gram negative non-spore forming thin short rod shaped bacteria with scattered arrangement. All the isolates were negative for catalase, oxidase and Methyl red. They produced indole and acid from D-glucose, reduced nitrate but not nitrite. Growth was found to be heterotrophic at pH 5 and temperature 35°C (Table 1). The isolates were obligate aerobic bacteria and metabolically chemolithoautotrophic.

Selected bacterial isolates that were screened by dye reduction test were further tested using barium chloride test. The amount of sulphate produced by different bacterial isolates ranged from 1.42 to1.62 mg ml^{-1} concentration (Table 2). Bacterial isolate of SOBA17 showed maximum sulphate production of 1.62 mg ml^{-1} followed by SOBA20. Amplicons of 1500 bp size (Fig. 1) were purified and sequenced. Blast analysis revealed that both the sequences showed similarity to *T. aquaesulis*. Partial purification of sulphur oxidase (Fig. 2) enzyme was performed by 70% of ammonium sulphate precipitation followed by dialysis and SDS-PAGE showed specific bands of approximately 10 kDa, 17 kDa and 30 kDa.

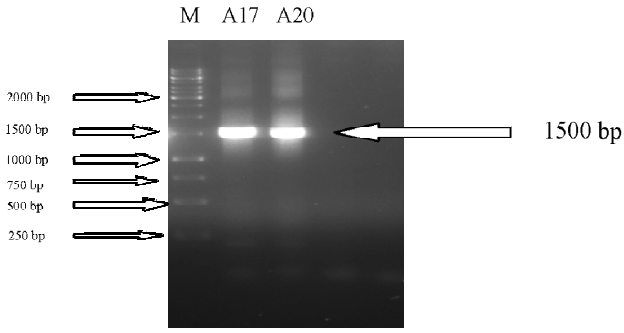

Fig. 1. Amplification of 16S rRNA gene of sulphur oxidizing bacteria by PCR. Lane 1- Molecular marker (1 kb), Lane 2 and 3- sulphur oxidizing bacterial isolates

Table 2. Sulphate ion production by sulphur oxidizing bacterial isolates

Bacterial isolate	Sulphate ion produced (mg ml^{-1})
SOBA17	1.62
SOBA12	1.50
SOBA3	1.46
SOBA20	1.58
SOBD10	1.42
SOBD16	1.51
SOBD12	1.53
SOBD14	1.47

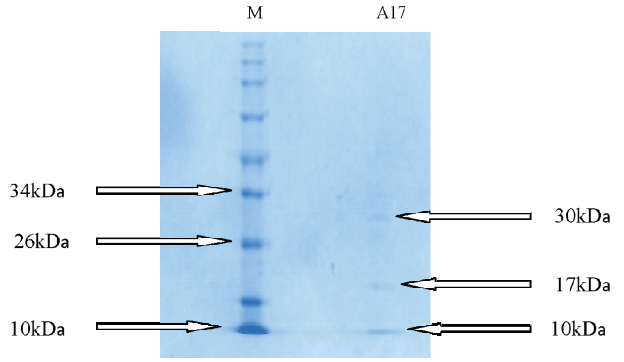

Fig. 2. SDS PAGE of partially purified sulphur oxidase enzyme. M- Marker A17- SOB isolate

Preliminary studies showed eight among 47 bacterial isolates changed the color of the dye (evidence of pH reduction from 7 to 5) in one week of incubation and therefore were selected for further studies. The genus *Thiobacillus* spp. can oxidize

sulphur at pH ranged from 1 to 9, but show optimum growth only under acidic conditions of pH 1 to 5 (Suzuki et al., 1999). In the earlier studies, Lee et al. (2000) have isolated SOB from the activated sludge. Similarly, Vidyalakshmi & Sridar (2006) have isolated SOB from pulses rhizosphere, biogas slurry, paddy rhizosphere, mine soil, sewage and tannery effluent. They have reported 14 out of 28 isolates reduced the pH from 8.0 to less than 5.0 and maximum dye (bromo cresol purple) reduction by changing the colour of the media from purple to yellow. Another study conducted by Behera et al. (2014) from mangrove soil of Mahanadi river have also reported screening of SOB isolates on the basis of pH reduction due to sulphuric acid production and bromo phenol dye reduction test. The pH reduction of the medium was due to the production of sulphuric acid (Friedrich et al., 2001). Sulphate, sulphur, polythionatesare produced transiently by major species of SOB before the end product sulphate. Decrease in pH from neutral to 3 during growth of SOB varies within species. In majority, optimum growth was noticed at pH 6.5 to 8.0 and temperatures from 30 to 42°C (Kelly & Wood, 2003). However, in the present study, heterotrophic growth for all the eight isolates was observed at 35°C.

Barium chloride test was employed to measure the amount of sulphate ion produced during the oxidation of sulphur into sulphate by SOB. Out of ninety six bacterial isolates, eight isolates were selected for the barium chloride test. Maximum sulphate ion (1.62 mg ml^{-1}) was produced by bacterial isolate SOBA17 followed by SOBA20. In previous studies, Teske et al. (2000) have reported sulphate production of 2 to 4.6 mM from thiosulphate by SOB isolates (isolated from hydrothermal vents). Vidhyasri & Sridar (2011) have also showed the sulphate production of 79.20 and 68.80 mg per 100 ml^{-1} broth by the isolates BGS2 and TRY2 respectively. In another study, sulphate production of 13.4 to 571.6 mg l^{-1} after 8 days of incubation by ten bacterial strains isolated from industrial wastewater, sewerage water and sulphur mud has been reported by Ullah et al. (2014).

Conventional microbiological identification which includes morphological, physiological and characterization study confirmed the characteristic of the *Thiobacillus*. Further the analysis of 16SrRNA gene revealed that these isolates were closely related to *T. aquaesulis*. 16S rDNA sequencing analysis revealed that the SOB bacteria isolated in the present study belongs to the genus *Thiobacillus*of *ã-Proteobacteria* which are frequently isolated from the soil, freshwater, and marine environments (Kelly & Wood, 2003).

The partially purified sulphur oxidase of SOBA17 in SDS-PAGE exhibited three consecutive bands of 10, 17 and 30 kDa. According to Mohapatra et al. (2006), the purified sulphur oxidase was found to be monomer with a molecular weight of 43 kDa. Similarly, the purified sulphur oxidase from *Bacillus* sp. BN53-1 showed a band of 37 kDa (Nakada & Ohta, 1999). Also, Behera et al. (2016) reported that SDS-PAGE of partially purified sulphur oxidase enzyme of SOB-7 shows specific bands of approximately 72 kDa, 55 kDa, 50 kDa and 43 kDa.

In conclusion, SOB are of great biological importance in the environment. Among SOB, the genus *Thiobacillus* are very important in biological sulphur oxidation in aquaculture soils. These bacteria enhance sulphur oxidation rate and can utilize the products derived from the sulphur metabolism such as sulphide, sulphate, polythionate. Their presence in aquaculture systems signifies an important role in maintaining the levels of toxic gases such as hydrogen sulphide. Sulphur oxidase enzyme can be used in various environmental monitoring applications such as mitigation of sulphides in effluent waters, deodorizing animal feces, for treating waste waters, and in oil-field brine. Further studies on developing the SOB consortium and their application in bioremediation of the culture environment in aquaculture systems need to be carried out.

Acknowledgments

Authors are grateful to the Indian Council of Agricultural Research, New Delhi, India, for the financial support and to the Director, ICAR-Central Institute of Fisheries Technology, Cochin, India, for providing all the facilities required for the present study.

References

Abraham, T. J. S., Ghosh, S., Nagesh, T. S. and Sasmal, D. (2015) Assessment of nitrogen and sulphur cycle bacteria and shrimp production in ponds treated with biological products. J. Coast. Life Med. 3(6): 466-470

Behera, B. C., Patra, M., Dutta, S. K. and Thatoi, H. N. (2014) Isolation and characterization of sulphur oxidising bacteria from mangrove soil of Mahanadi River Delta and their sulphur oxidizing ability. J. Appl. Environ. Microbiol. 2(1): 1-5

Behera, B. C., Singh, S. K., Patra, M., Mishra, R. R., Sethi, B. K., Dutta, S. K. and Thatoi, H. N. (2016) Partial purification and characterisation of sulphur oxidase from *Micrococcus* sp and *Klebsiella* sp isolated from mangrove soils of Mahanadi river delta, Odisha, India. Univ. J. Microbiol. Res. 4(3): 66-78

Brenner, D. J., Krieg, N. R. and Staley, J. T. (2005) Bergey's manual of systemic bacteriology. Volume 2. The proteobacteria, Part B: The Gammaproteobacteria. Editor-in-chief: Garrity, G., Brenner, D.J., Krieg, N.R. and Staley, J.R. 1106 p

Cha, J. M., Cha, W. S. and Lee, J. H. (1999) Removal of organo-sulphur odour compounds by *Thiobacillus novellus* SRM, Sulphur-Oxidizing Bacteria. Process Biochem. 34: 659-665

Devaraja, T. N., Yusoff, F. M. and Shariff, M. Y. (2002) Changes in bacterial populations and shrimp production in ponds treated with commercial microbial products. Aquaculture. 206(3-4): 245-256

FAO (2018) The State of World Fisheries and Aquaculture 2018, Meeting the Sustainable Development Goals, Rome. 227 p

Friedrich, C. G., Rother, D., Bardischewsky, F., Quentmeier, A. and Fischer, J. (2001) Oxidation of reduced inorganic sulphur compounds by bacteria: Emergence of a common mechanism. Appl. Environ. Microbiol. 67: 2873-2882

Jorgensen, B. B., and Revsbech, N. P. (1983) Colorless sulfur bacteria, *Beggiatoa* spp. and *Thiovulum* spp, in O_2 and H_2S Microgradients. Appl Environ Microbiol. 45(4): 1261-1270

Joseph, T. C., Murugadas, V., Reghunathan, D., Shaheer, P., Akhilnath, P. G. and Lalitha, K. V. (2015) Isolation and characterization of *Vibrio cholerae* O139 associated with mass mortality in *Penaeus monodon* and experimental challenge in postlarvae of three species of shrimp. Aquaculture. 442: 44-47

Kelly, D. P. and Wood, A. P. (1998) Microbes of the sulfur cycle. Techniques in Microbial Ecology (Burlage, R. S., Atlas, R., Stahl, D., Geesey, G. and Sayler, G., Eds), Oxford University Press, New York. pp 31-57

Kelly, D. P. and Wood, A. P. (2003) Genus III Halothiobacillacae. In: Bergey's Manual of Systematic Bacteriology (Brenner, D. J., Krieg, N. R., Staley, J. T. and Garrity, G. M., Eds)., 2nd edn., Springer, New York. 2(B) 58-59

Kolmert, Å. P., Wikström, P. and Hallberg, K. B. (2000) A fast and simple turbidimetric method for the determination of sulfate in sulfate-reducing bacterial cultures. J. Microbiol. Methods. 41: 179-184

Lee, E. Y., Cho, K. S. and Ryu, H. W. (2000) Characterization of sulphur oxidation by an autotrophic sulphur oxidizer, *Thiobacillus* sp. ASWW-2. Biopro. Eng. 5: 48-52

Mohapatra, B. R., Gould, W. D., Dinardo, O., Papavinasam, S. and Revie, R. W. (2006) Optimization of culture conditions and properties of immobilized sulfide oxidase from *Arthrobacter* species. J. Biotechnol. 124: 523-531

Nakada, Y. and Ohta, Y. (1999) Purification and properties of hydrogen sulfide oxidase from *Bacillus* sp. BN53-1. J. Biosci. Bioeng. 87(4): 452-455

Patil, P. K., Muralidhar, M., Solanki, H. G., Patel, K., Gopal, C. and Pillai, S. M. (2012) Trends in beneficial and pathogenic bacterial populations and their relation with environmental parameters in tiger shrimp, *Penaeus monodon* culture ponds. Int. J. Bio-Res. Stress Manag. 4(2): 107-113

Patil, P. K., Muralidhar, M., Solanki, H. G., Patel, K., Patel, P. P. and Gopal, C. (2015) Effect of culture intensity and probiotics application on microbiological and environmental parameters in *Litopenaeus vannamei* culture ponds. J. Environ. Biol. 37: 21-29

Rao, P. S. S. and Karunasagar, I. (2000) Incidence of bacteria involved in nitrogen and sulphur cycles in tropical shrimp culture ponds. Aquacul. Int. 8: 463-472

Rojas, A., Holguin, G., Glick, B. R. and Bashan, Y. (2001) Synergism between *Phyllobacterium* sp. (N2-Fixer) and *Bacillus licheniformis* (P-Solubilizer), both from a semiarid mangrove rhizosphere", FEMS Microbiol. Ecol. 35: 181-187

Suzuki, I., Lee, D., Mackay, B., Harahuc, L. and Oh, J. K. (1999) "Effect of various ions, pH and osmotic pressure on oxidation of elemental sulfur by *Thiobacillus thiooxidans*", Appl. Environ. Microbiol. 65: 5163-5168

Suzuki, I. (1999) Oxidation of inorganic sulphur compounds: Chemical and enzymatic reactions. Can. J. Microbiol. 45: 97-105

Teske, A., Brinkhoff, G., Muyzer, D., Moser, P., Rethmeier, J. and Jannasch, H.W. (2000) Diversity of thiosulphate oxidizing bacteria from marine sediments and hydrothermal vents. Appl. Environ. Microbiol. 66: 3125-3133

Ullah, I. G., Jilani, K. S., Khan, M., Akhtar, S. and Rasheed, M. (2014) Sulphur oxidizing bacteria from sulphur rich ecologies exhibit high capability of phosphorous solubilization. Int. J. Agricult. Biol. 16(3): 550-556

Vidhyasri, S. and Sridar, R. (2011) Development of a carrier based formulation of sulphur oxidizing bacterium for enhancing the productivity of crops requiring sulphur nutrition. J. Basic Appl. Sci. Res. 1(5): 345-353

Vidyalakshmi, R. and Sridar, R. (2006) Isolation and characterization of sulphur oxidizing bacteria. J. Cult. Collec. 5: 73-77