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DIVERSITY OF PURPLE NON SULFUR BACTERIA (PNSB) FROM SHRIMP PONDS IN NAGAI COASTAL REGION, SOUTH EAST COAST OF INDIA

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ABSTRACT

Shrimps ponds are suitable for the growth and development of purple non sulfur bacteria (PNSB) as there is excess accumulation of nutrients in the shrimp pond bottom due to feed, detritus and the excreta from the shrimps, leads to the establishment of PNSB members in shrimp ponds. Hence the present study focuses its attention on the diversity of PNSB from shrimp ponds (brackish) from the Nagai Coastal Region, South east coast of India, as no earlier reports exists on PNSB diversity in the Nagai coastal area, this systematic survey gives a clear picture of existing PNSB Diversity.

Key Words: PNSB, Alpha- proteobacteria, Pfennig s medium, shrimp ponds.

INTRODUCTION:

The phototrophic purple Alpha- proteobacteria are purple non sulfur bacteria (PNSB), able to perform anoxygenic photosynthesis. Phototrophic purple non sulfur bacteria are a highly diverse and heterogenous group of bacteria, both phenotypically and genetically. On the basis of 16s rDNA sequence similarities, phototrophic purple bacteria belong to the α, ß, and γ-proteobacteria (Woese *et al.,* 1984a, b, 1985; Stackebrandt *et al.,* 1988; Woese, 1987).

While purple sulfur bacteria are γ-proteobacteria, purple non sulfur bacteria are found in the ß and α –proteobacteria. Ecological niches of phototrophic α-proteobacteria are the anoxic parts of water and sediments that receive light of sufficient quantity and quality to allow phototrophic development. Representatives of the PNSB are widely distributed in nature and are found not only in all kinds of stagnant water bodies including lakes, waste water ponds, coastal lagoons and in other aquatic habitats, but also in sediments, moist soils and paddy fields. They have been reported from a wide range of environments such as fresh water, marine and hypersaline environments, habitats of moderate temperatures, thermal springs, cold polar habitats etc. They have been isolated from all kinds of aquatic habitats (lakes, sewage and brackish waters) even from wet decaying leaves and from soil (Biebl and Pfennig, 1981; Imhoff and Truper, 1992). PNSB are known to live in aquatic habitats with significant amounts of soluble organic matter and low oxygen tension and also utilize various nitrogenous substances for their growth (Gobel, 1978 and Imhoff, 2006). This makes the shrimp pond an ideal habitat for PNSB members to thrive.

In shrimp ponds there is excessive accumulation of organic matter leading to intensive organic matter degradation at the pond bottom and higher sediment oxygen demand exceeds the oxygen renewal rate. This leads to the development of anoxic conditions in the sediments and at the sediment water interfaces (Avnimelech and Ritvo, 2003).

The present survey aims to isolate and characterize purple non sulfur bacterial members from marine environments. Even though it is a well established fact that PNSB members are prevalent in marine shrimp ponds, a systematic survey of PNSB members prevalent in those artificial man made marine environments, remains uncharted and no work to the best of our knowledge, is available on PNSB population from shrimp ponds rearing Tiger prawn (*Penaeus monodon*), Nagai Coastal Region, south east coast of Tamilnadu.

Materials and Methods:

Collection of samples:

The sub surface water samples from the shrimp farms were taken from locations like, water source /inlet (brackish canals/pump house), culturing ponds and draining channel in the study area using sterilized 1000ml plastic bottles and 300 ml glass stoppered bottles. Shrimp pond soil sediment samples were collected in fresh zip-lock polythene covers, from the above mentioned locations.

Enumeration of purple non sulfur bacteria (PNSB) from shrimp ponds:

Water and soil sediment samples from locations of the shrimp pond like inlet, culture pond and draining channel were used for the enumeration of PNSB. The enumeration of PNSB was done by Paraffin wax overlay of pour plate method (Archana et al., 2004). The water and sediment samples were serially diluted by taking, one ml of the water sample mixed in 9 ml saline (0.7% NaCl w/v) and one gram of soil sample suspended in 9 ml saline (0.7% NaCl w/v) mixed by vortexing and tenfold dilutions in the saline were made to give dilutions upto 10-6. 0.5 ml from each of the dilution were used as inoculum, and pour plated with 20 ml of modified Biebl and Pfennig"s (1981) agar medium at 40–45°C). The medium was allowed to solidify. The plates were then over layed with molten paraffin wax (55–60 °C), which solidified upon layering. The plates were rotated gently in a circular motion while pouring the wax in order to spread it evenly over the agar surface. The plates were kept open fora period of 10 min after pouring the paraffin wax in order to radiate the heat of the wax before closing the lid and incubated at a temperature of 30±2 °C with the agar side of the plate exposed to a light intensity of 2400 lux, for 12- 15 days. At the end of the incubation period the brownish red- pink coloured colonies were counted.

Enrichment of water and sediment soil samples:

The soil and water samples were enriched separately in 100 ml screw capped bottles, using modified Biebl and Pfennig s medium (1981) where 1 gm of soil sample and 1mL of water sample were introduced aseptically into 100 ml screw capped bottles and the medium was poured up to the brim , screw capped tightly and sealed with parafilm. Initially the tubes were incubated in dark for 24 hours and later the tubes were kept under constant illumination (2,400 lux) at 30 + 2C for 7 to 12 days and observed for brown / brownish-red / purple colour, which indicates the presence of PNSB. Enrichment of the water and soil samples from each station was done during different periods of the shrimp culture cycle (i.e. from seed stocking to harvest).

Purification of PNSB (Archana *et al.*, 2004)

Enrichment cultures were purified by repeatedly streaking on Biebl and Pfennig"s agar slants (1981) prepared in 25 x 150 mm. rim-less test tubes and sealed with polybutyrate rubber stoppers (suba seals). The gas phase in the test tube was replaced by flushing with argon for 2 min by using a pair of hypodermic needles and incubated under constant illumination (2400 lux) at 32ºC. The purification was performed till the colonies appearing on two successive slants were all identical. Purity of the culture was checked by streaking on Nutrient agar (Ap-

pendix) plates and incubated anaerobically under illumination at 2400 lux. Contamination from other phototrophic bacteria was checked by monitoring the cultural characters like color of the culture, colony morphology and by microscopic observation.

Results and Discussion

Enumeration of purple non sulfur bacteria (PNSB) from shrimp ponds

Appearance of brownish red-pink coloured colonies in the Paraffin wax overlay of pour plates containing water and sediment samples from various locations of shrimp ponds in the study area revealed the occurrence of PNSB.

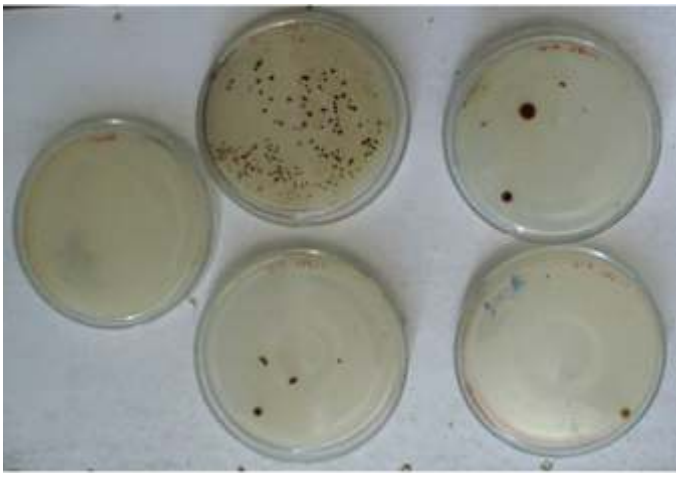

Fig-1

Table- 1 Enumeration of PNSB from different areas of Nagai Coastal Region. (CFU/ml of water and soil sample).

S No.	water and soil samples (CFU/ml) from various locations in shrimp ponds					
	Water	soil	Water 1	Soil 1	Water 2	Soil 2
1	4.8×10^3	4.9×10^3	5.2×10^3	2.9×10^5	3.1×10^4	$3.4 \times 10^6$4
2	1.3×10^4	3.9×10^4	6.0×10^4	1.2×10^5	3.8×10^5	$6.2 \times 10^6$5
3	1.3×10^4	2.8×10^4	4.1×10^4	7.2×10^4	1.2×10^5	4.3×10^5
4	1.3×10^4	6.2×10^4	8.7×10^4	0.7×10^6	1.9×10^5	3.3×10^6
5	1.3×10^4	4.6×10^4	7.1×10^4	1.8×10^5	1.0×10^6	2.9×10^6
6	1.3×10^4	0.9×10^5	1.4×10^5	0.9×10^6	2.0×10^6	3.9×10^6
7	1.3×10^4	1.2×10^5	1.4×10^5	1.0×106	2.3×10^6	4.4×10^6
8	1.4×10^4	4.2×10^4	6.3×10^4	1.3×105	4.8×10^5	7.2×10^5
9	1.2×10^4	3.6×10^4	5.0×10^4	0.9×10^5	1.3×10^5	5.7×10^5
10	1.3×10^4	5.7×10^4	7.8×10^4	2.7×10^5	1.2×10^6	3.0×10^6
11	1.3×10^4	3.1×10^4	4.8×10^4	0.8×105	1.2×10^5	5.1×10^5
12	1.3×10^4	3.7×10^4	5.2×10^4	0.6×105	2.8×10^5	6.1×10^6
13	1.3×10^4	1.3×10^5	1.7×10^5	1.2×10^6	2.7×10^6	5.1×10^6

CFU= Colony forming units

Enrichment of water and sediment soil samples

Out of (84) water samples kept for enrichment, 71 samples showed positive enrichment. However some of the enrichments of water samples from inlet (8), culture ponds (3) and draining channel (2) were negative and all the soil sediment samples (84) showed positive enrichment, with the appearance of brown / brownish-red to purple colour (Fig.2a-3).

Fig.2. (a) and (b) enrichments of water samples from shrimp ponds

Fig-3Enrichments of soil sediment samples from shrimp ponds

Purification of PNSB

All the (155) total positive enrichments obtained from water and soil sediment samples, were subjected to purification. After repeated streaking on agar slants, using modified Biebl and Pfennig s (1981) medium, 210 pure cultures of purple nonsulfur bacteria were obtained. Based on morphological and cultural characterization, the purified colonies were grouped under 12 PNSB strains (Fig. 4-) with alphanumeric strain code viz. BRP (Brown, Red, Purple) and numbers ranging from 1 to 12 viz. BRP1 to BRP12. The details regarding the number of purified colonies representing different strains of PNSB are given in table 8.

Fig.4. Agar slants containing purified PNSB strains

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