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Isolation of Phototrophic Bacteria from Water and Soil Samples

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KEYWORDS

phototrophic bacteria, Estuary, bacteriochlorophylls

ABSTRACT:

The main aim of this study was to isolate and identify phototrophic bacteria from water and soil sources. The water samples were collected from the river of Godavari at Rajahmundry near Mangalavaripeta and Estuarian water from Antharvedi, a place where the Godavari River merges with the Bay of Bengal. The Soil samples were collected from Rice fields near Kurmurthy and the selected samples were enriched in Biebl and pfenning media for the isolation of phototrophic bacteria. About twenty-one strains of bacteria were obtained in anaerobic cultures. The pure isolates were tentatively identified by studying the optimum pH, shape of the bacterial cell, colour of the cell suspensions, biomass, pigmentation characters like predominant bacteriochlorophylls and carotenoids. Tentative identification of these strains helps in further screening for desired phototrophic organisms for various applications.

1. Introduction:

Phototrophic bacteria are a bacterial group capable of performing photosynthesis by using sunlight. Unlike plants and algae, which use chlorophyll for photosynthesis, phototrophic bacteria utilize various pigments, including bacteriochlorophyll, to capture light energy and convert it into chemical energy. These bacteria play a vital role in various ecosystems, and their unique ability to convert sunlight into energy contributes to the cycling of nutrients and the overall balance of ecosystems.

Phototrophic bacteria are found in aquatic environments like freshwater lakes, ponds and terrestrial environments with suitable conditions. Some species flourish in extreme environments, such as hot springs and hypersaline environments. Phototrophic bacteria can be either oxygenic or anoxygenic. They are differentiated based on the type of chlorophyll and carotenoid pigments present, photosynthetic electron donor used as and the composition of their photosynthetic machinery.

The phototrophic bacteria are divided into two major groups, Anoxygenic phototrophic bacteria and oxygenic phototrophic bacteria. Anoxygenic photosynthetic bacteria have been divided into three groups based on pigmentation. They are purple bacteria, green bacteria and

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heliobacteria. There are two varieties of purple bacteria. One variety uses sulfur instead of oxygen to carry out the process of photosynthesis, and oxygen can actually inhibit their growth. These bacteria live in aquatic environments with oxygen deficiencies. The other variety of purple bacteria resides in oxygenated environments and requires oxygen for photosynthesis. Like purple bacteria, some green bacteria conduct photosynthesis by using sulfur.

Purple non-sulphur bacteria (PNSB) are phototrophic microorganisms, which increasingly gain attention in plant production due to their ability to produce and accumulate high-value compounds that are beneficial for plant growth. Remarkable features of PNSB include the accumulation of polyphosphate, the production of pigments and vitamins and the production of plant growth-promoting substances (PGPSs).

Anoxygenic phototrophic purple bacteria are the major group of phototrophic bacteria which convert light energy into chemical energy by anoxygenic photosynthesis with the help of Phototrophic pigments. These pigments appear in various colours from brown, red, pink, beige, purple. Phototrophic pigments (bacteriochlorophyll a or b and carotenoids) are situated in the cytoplasmic membrane and internal membrane systems (vesicles, lamellae or membrane stacks) (Imhoff, 2006).

2. Materials and Methods:

1. Place of work

The bacterial culture work studies were carried out in Microbial physiology laboratory of Department of Botany, Osmania University. Water samples were collected from Godavari River and Antarvedi and Soil samples from rice fields of Kurmurthy, for present studies.

2. **Water Sample collection**: For the current study, Water samples were collected from Godavari River at Rajahmundry and Antarvedi Estuary (Godavari and Bay of Bengal Sangam) at Sakhinetipalli. Parameters such as pH and temperature of the site were recorded immediately.

2.1. Geograhical location of Water sample collection:

1. Godavari River at Rajahmundry, Mangalavaripeta, Andhra Pradesh (17.0072096,81.7665708).
2. Antarvedi Estuary (Godavari and Bay of Bengal Sangam) at Sakhinetipalli, East Godavari (16.3157565,81.7207469).

3. **Soil Sample collection:**

Soil samples were also collected from rice fields of Chinna Chinthakunta, Kurmurthy, Mahbubnagar District, Telangana. All the samples were collected in Zip-lock covers and brought to the laboratory to study the physicochemical parameters of the samples. Geographical locations of the sampling regions are given below.

3.1. Geograhical location of Soil sample collection

1. Rice fields of Chinna Chinthakunta, Kurmurthy, Mahbubnagar District, Telangana (16.456781, 77.823497).

4. Isolation and Enrichment

4.1. Experimental organisms: Purple nonsulphur bacterial strains isolated from Water samples collected from the Godavari River at Rajahmundry, Antarvedi at East

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Godavari district and the soil samples collected from the Rice fields of Chinna Chinthakunta, Kurmurthy are the experimental organisms. **4.2. Culture media**: Modified Biebl and Pfennig media (1981) was used for the isolation and enrichment of Anoxygenic Phototrophic bacteria.

Table 1: Composition of modified Biebl and Pfennig's (1981) medium used for the growth of Anoxygenic Phototrophic bacteria

Ingredients	**mg. L^{-1} or ml. L^{-1}**
KH_2PO_4	500
$MgSO_4.7H_2O$	200
NaCl	400
NH_4Cl	600
$CaCl_2.2H_20$	50
Organic compound	3.0g
Yeast Extract	300
Ferric citrate solution (0.1%, w/v)	5ml
*Micronutrient solution (SL7)	1ml
Vitamin B12 (2mg/100ml)	#1 ml
SL 7	*1ml

*SL7: (mg L^{-1}): HCl (25%, v/v) – 1 ml; $ZnCl_2$ (70); $MnCl_2.4H_2O$ (100); H_3BO_3 (60); $CoCl_2.6H_2O$ (200); $NiCl_2.6H_2O$ (20); $Na_2MoO_4.2H_2O$ (40); $CuCl_2.2H_2O$ (20). # Vitamin B_{12} (2 mg) was dissolved in deionised water (100 ml) and filter sterilized by using 0.22 µm pore-sized Millipore cellulose acetate membrane filter into a sterile screw cap tube; Final pH of the medium was adjusted to 7.0 to 7.5 with sterile HCl (1 N)/ NaOH (1 N).

4.3. Enrichment and purification of PNSB:

4.1.1. Purple non-Sulfur Bacteria (PNSB): Modified Biebl and pfennig's media was used for the enrichment and isolation of purple non-sulfur bacteria. For the enrichment of these bacteria cultures must be incubated under the illumination of about 3200±100 lux and a temperature of 30±2^{0}C along with anaerobic conditions has to be maintained. In the current studies, ultra pure argon gas has been used for maintaining the anaerobic condition. Purging the head space of the test tube with argon ensures anaerobic conditions. Modified Biebl and Pfennig media(1981) described for the selective enrichment of phototrophic sulphur bacteria was used.

4.1.2. Purification: Purification of enriched purple non-sulfur bacteria was carried out using the routine serial dilutions and streaking plate technique on agar slants. Enriched cultures were serially diluted using the modified Biebl and Pfennig media. Serial dilutions were continued until visually uniform coloured homogenous suspension was obtained. Using 10^{-6}, 10^{-7}, 10^{-8} dilutions, streak plating was done on agar slants. Biebl and Pfennig media along with 2% agar were used to prepare agar slants. Agar slants were

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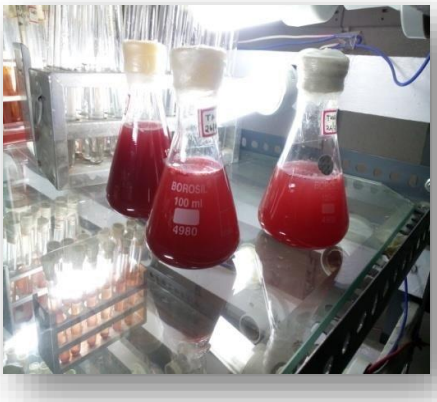
preferred over plates as PNSB has to be maintained in anaerobic condition. Anaerobic conditions were maintained using argon gas in 15 x 150 mm test tubes. Streaking was continued until colonies appeared on two successive slants were identical.

30±2oC temperature. Anaerobic conditions were maintained by flushing ultrapure argon or nitrogen gas into the tubes. Isolated strains of Photosynthetic sulfur bacteria were enriched and purified in the same media by serial dilutions and repeated plating of the cultures in the agar medium.



Culture of PNSB

Serial dilution

Fig.1.Anaerobically growing isolates in screwcap and subasealed test tubes

Fig.2. Cultures grown on slants for purification

4.1.3. ISOLATION AND ENRICHMENT OF SAMPLES:

Immediately after the collection, the water samples were transferred into the test tubes containing modified Biebl and Pfennig media (1981) for selective isolation of Photosynthetic sulphur bacteria and incubated at 3200lux of light intensity and

5. Screening of Purple Non-Sulfur Bacteria for Growth and Biomass.

PNSB forms homogenous suspension in liquid medium. Once the cultures are inoculated, their increasing optical density (OD) values measured at 660nm can be a reliable source to calculate the generation

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time of bacteria. Bacteria inoculated into modified Biebl and Pfennig media and OD values were monitored for every one hour which was plotted on to graph to obtain the generation time of each strain. The biomass yield was estimated in terms of the dry weight of the cells (Arunasri, 2004). The observation of bacteria under scanning electron microscope was done at National Institute of Nutrition, Hyderabad**.** For the extraction of genomic DNA CTAB method proposed by Murray and Thompson, in 1980 was used. PNSB strains were harvested by centrifugation (10000rpm) for 15mins.

6. Results:

About twenty-one strains of bacteria were obtained in anaerobic cultures. The pure isolates were tentatively identified by studying the optimum pH, shape of the bacterial cell, colour of the cell suspensions, pigmentation characters like predominant bacteriochlorophylls and carotenoids. These strains were tentatively identified using Bergey's Manual of Determinative Bacteriology (1994). The results are summarized in table 1.

Table 1: Tentative identification of the Anoxygenic Phototrophic bacteria strains

S. No	Strain No	Source	Cell shape	Colour of the suspension	Bacterial chlorophyll	Carotenoids	Tentative genus	Biomass g/l
1	SR101	Godavari Water Sample Rajahmundry	Slightly ovoid	Pink to Rose red	a or b	Spirilloxanthin series	*Rhodobacter sp.*	1.5
2	SR102		Rods	Purple red	a	Okenone series	*Chromatium sps*	1.8
3	SR103		Rods	Red	a or b	Spirlloxanthin series	*Ectothiorhodospira sps.*	1
4	SR104		Rods	Brown red	a or b	Normal Spirilloxanthin series	*Chromatium sp.*	1.3
5	SR105		Rods	Reddish Brown	a	Normal Spirlloxanthin series	*Rhodospeudomonas Sp.*	3.6
6	SR106		Rods	Red	a or b	Spirlloxanthin series	*Rhodospeudomonas sp.*	1.5

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7	SR107		Spherical	Pink to Rose red	a or b	Spirilloxanthin series	*Rhodospeudomonas bacter sp.*	1.4
8	SR108		Rods	Brown red	a or b	Normal Spirilloxanthin series	*Rhodospeudomonas sp.*	2.3
9	SR109		Rods	Purple red	a	Spirilloxanthin series	*Rhodospeudomonas sp*	2.9
10	SR110	Godavari water sample Antharvedi	Spherical	Pink to Rose red	a or b	Spirilloxanthin series	*Rhodospeudomonas sp.*	1.5
11	SR111		Rods	Red	a or b	Spirilloxanthin series	*Rhodospeudomonas sp.*	0.9
12	SR112		Rods	Red	a or b	Spirilloxanthin series	*Rhodobacter sp.*	3.5
13	SR113		Spherical or slightly ovoid	Purple red	a	Okenone series	*Thiocystis sp.*	1.9
14	SR114		Spherical or slightly ovoid	Pink to Rose red	a or b	Spirilloxanthin series	*Rhodobacter sp.*	1.1
15	SR115		Spherical or slightly ovoid	Pink to Rose red	a or b	Spirilloxanthin series	*Rhodospirillum sp.*	2.2
16	SR116	Rice fields of Kurmurthy	Spherical or slightly ovoid	Purple red	a	Okenone series	*Thiocystis sp.*	1

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17	SR117	Spherical or slightly ovoid	Pink to Rose red	a or b	Spirilloxanthin series	*Rhodospeudomonas sp*	2.6
18	SR118	Spherical or slightly ovoid	Pink to Rose red	a or b	Spirlloxanthin series	*Amoebobacter sp*	1.6
19	SR119	Rods	Brown red	a or b	Normal Spirilloxanthin series	*Chromatium sp*	1.8
20	SR120	Rods	Red	a or b	Okenone series	*Thiocystis sp.*	2
21	SR121	Rods	Red	a	Spirilloxanthin series	*Rhodospeudomonas sp.*	3.9

The characteristic colour of cell suspension of isolates varied from red to brown red and pink to reddish pink. According to colour of cell suspension dominant carotenoid pigments can be known. For maximum strains, spirilloxanthin series was found to be the dominant pigment. The shape of the isolates varied from spherical to rods. Tentative identification of these strains helps in further screening for desired organisms. All the cultures were purified by repeated serial dilution and by streaking on agar slants until pure cultures were obtained and the bacterial strains showing higher carotenoid content and biomass can be selected and utilized for various applications of phototrophic bacteria.

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