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# Isolation of Anoxygenic Phototrophic Bacteria from Soil and Water Samples

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***Abstract---*** *An oxygenic phototrophic bacterium is the gram-negative bacteria that can use light as an energy source and they are anaerobic as they do not evolve oxygen during photosynthesis. Aerobic an oxygenic phototrophic bacterium is another group of this bacteria which are obligate aerobes that capture energy that light by an oxygenic photosynthesis. Bacterial isolation was done from the soil and water sample collected, followed by the serial dilution and pure cultures were obtained from the cultures grown. Bacteria isolated were purple sulphur bacteria (Chromatium, Ectothiorodospira, Thioapsa), purple non sulphur Bacteria, Green sulphur Bcteria (Chlorobium, Petodictyon, Green Non Sulpher bacteria (Chlorofixus). Were tend to be obtain from the soil and water samples further identification of the bacteria and utilization of Bacteria should be classified in the further studies.*

***Keywords---*** *Bacterial Isolation, Soil and Water Samples, Phototrophic Bacteria.*

## I. INTRODUCTION

Phototrophic Bacteria are encountered in almost every day of water as well as in the soil. The existence of phototrophic bacteria uncovered by Christian Gottfried Ehrenberg. The develops of purple sulfer bacteria depend, as a rule. On the present of light, $H_2$s and other organic substances. Photo trophic bacteria are an oxygenic confined to oxygen free. Aquatic habitats receiving sufficient solar radiation to support photosynthesis. The environment such as ponds, polluted ponds, the environment hypolimion of lakes, have the nutrients like $H_2S$, $H_2$& simple organic compounds. Specially required by anoxy genicphototrophs, thereby making the photo trophic bacteria predominant. The phototrophic bacteria are ubiquitous in nature. Their autotrophic made of nutrition is now exploited to isolate strains with efficient carbon sequestration capacities and applied in fields of biotechnology. Phototroophic bacteria consist of four phenotypically and phylogenetically distinguished groups with ability to perform anoxygenic photosynthesis (Johannes F Imhoff et.al.,)

These are

Chlorobiales (Green Sulsphur bacteria)

Heliobacteriaceae

Chloroflexales (green filamentous bacteria)

Purple bacteria (α, β and ϒ of Proteobacteria)

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Separaiton of these groups is based on 16SrDNA

Sequences, phenotypic, physiological characteristics and on chemical composition of cell constituents.

***Classification***

Table 1: Classification of Bacteria

| | *Organism* | *Family* | *Charecterists* |
|---|---|---|---|
| Purpule Sulphur | Chromatium | Chromatiaceae | Photolithotrophs |
| | Ectothiorodospira | | |
| | Thioapsa | | |
| Purpule Non-Sulphur Bacteria | Organism | Rhodospirillaceae | Photoorganotrophs |
| Green Sulfur Bacteria | Chlorobium, Petodictyon | Chlorobiceae | Photolithotrophs |
| Green non-sulfur Bacteria | Chlorofixus | Chlorofixeacae | Photolithotrophs |

Aim of the current work is too isolate Phototrophic Bacteria (both Sulfur and Non-sulfur bacteria) from the soil samples of various agricultural fields.

## II. MATERIALS AND METHODS

***Collection of Samples***

The both samples soil and water were collected Agriculture fields in Nalgonda and Godavari water. The water sample was collected in a 250 ml reagent bottle from the fresh waters of Godavari. The samples were brought to the laboratory of Plant and microbial physiology lab, Department of Botany, University College of Science, Osmania University, Hyderabad-500007 for isolation of PNSB.

***PSB (Photosynthetic Bacteria) media preparation Material***

Egg, monosodium glutamate (tasting powder), Chinese salt (sea salt).

**Procedure:** One egg is taken in a beaker, then one spoon of monosodium glutamate is added to it and then one spoon of sea salt is added. These are mixed and thoroughly. Then half glass of distilled water is added and mixed thoroughly, now it is stored in a bottle. 10 ml of the water sample is taken in a clean bottle of 500 ml and is filled with distilled water. Then take 1 gram of the soil sample into a separate bottle of 500 ml and fill it with distilled water.

One spoon of the egg solution is taken into each bottle, closes the caps of the bottles and is covered with parafilm. Now the bottles are kept in the culture room for the growth of bacteria. The temperature should be maintained 28°C.The bottles are shaken daily and the changes are observed.

Fig. 1: PSB (Photosynthetic Bacteria) Media Prepared and Incubated

Fig. 2 : Colour Change was Observed Every Day

***Observations***

The samples were taken in separate bottles and the photosynthetic bacterial media was prepared and the samples were kept at 28°C to isolate the bacteria in the culture room of Plant and microbial physiology laboratory, Botany department, University College of Science, Osmania University, Hyderbad-500007.The samples were kept for 17 days, during these days there was changes in the color of the culture media due to the growth of bacteria, as the bacteria were growing and they were producing more pigments and the color was changing. On the second day there no color was seen in the color of the culture media but on the third day, first the turbidity was seen in the water sample but no change in the color of the soil sample. On the fifth day the color of water sample was changed to slight pink but no change was seen in the soil sample; but on the eighth day the color of the soil sample also was changed to pink and the bacteria was growing very fast. As the bacteria were growing, the color of the culture media day by day was changing to purple due to the more production of pigments. After seven ten days, the media was prepared for the streaking and purifying of the bacteria and the streaking was done with the soil sample.

**Streaking:** Streaking was done on the bacteria specific media**.**

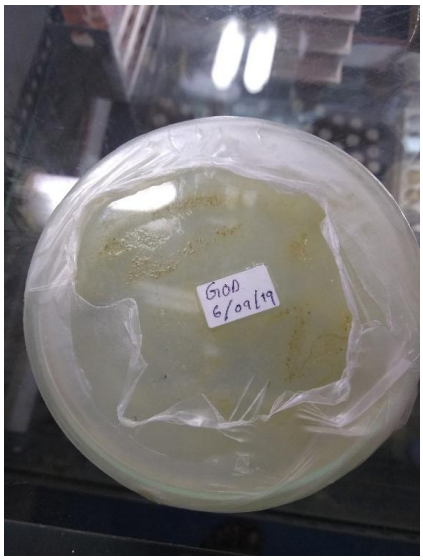
**Composition of modified Btebl and Pfennig's (1981) medium used for the growth of purple non-sulfur bacteria:**

Table 2: Composition per 1000.0ml

| *Ingredients* | *g/liter or ml/ liter* |
|---|---|
| $KH_2PO_4$ | 0.50mg |
| $MgSO7H_2O$ | 0.2mg |
| NaCl | 0.4mg |
| $NH_4C1$ | 0.6mg |
| $CaCl_2.2H_2O$ | 0.05mg |
| Organic compound (succinate) | 3g |
| Yeast extract | 0.3g |
| Ferric citrate solution (0.1%) | 5ml |
| Micronutrient solution (SL7) | 1ml |
| $NaHCO_3$ (10.0%) | 10ml |
| $Na_2S.9H_2O$ (24.7%) | 2ml |
| Vitamin $Bi_2$ (2mg/ 100ml) | 1ml |

Table 3: Composition of SL7

| *Ingredient* | *(mgL-$^{1}$)* |
|---|---|
| Distilled water | 1000.0ml |
| HC1 (25%) | 7.7ml |
| FeSO7$H_2O$ | l-5g |
| ZnCb | 70mg |
| MnCh.4$H_2O$ | lOOmg |
| $H_3BO_3$ | 300mg |
| CoC12-6H2O | 190mg |
| NJC12-6H2O | 24mg |
| NaaMoO 2$H_2O$ | 36mg |
| CuCl2-2$H_2O$ | 2,0mg |



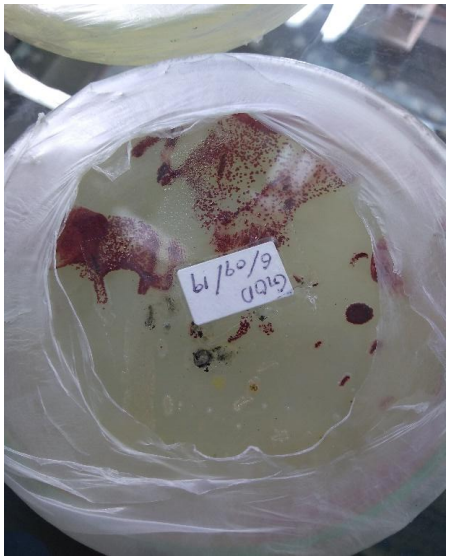


Fig. 3: Streakin of Bacteria on the Media Fig. 4: Growth of Bacteria

Fig. 5: Bacteria Colonies are Growing Well

***Purification***

***Serial Dilution***

For serial dilution 500 ml of the culture media is prepared.

Table 4: Composition per 500 ml of the Culture Media

| *Ingredients* | *Gram or ml* |
|---|---|
| $KH_2PO_4$ | 0.25mg |
| $MgSO_4.7H_2O$ | 0.1 mg |
| NaCl | 0.2mg |
| NH4C1 | 0.3mg |
| $CaCk.2H_2O$ | 0.025mg |
| Organic compound (succinate) | 1.5g |
| Yeast extract | 0-15g |
| Ferric citrate solution (0.1%) | 2.5ml |
| Micronutrient solution (SL7) | 0.5ml |
| $NaHCO_3$ (10.0%) | 5ml |
| $Na_2S.9H_2O$ (24.7%) | 1ml |
| Vitamin $B_{12}$ (2mg/ 100ml) | 0.5ml |

In one of the test tubes 5 ml of the culture media is taken and a single colony of the bacteria is taken from the petri plate with the help of a loop and is inoculated into the test tube. 10 other test tubes are taken and are labeled from 1 to 10, in each test tube 9 ml of the culture media is added. 1 ml of the inoculated culture media is taken with the help of a micropipette and serial dilution is done.

Pure culture was grown in the media prepared, after growth of the bacteria in the culture media, 1000 ml of the culture media contains bacteria is divided into two. They are taken into centrifuge tubes and centrifuged for 15-20 min at 6000 rpm to get the pellets.

## III. RESULTS AND DISCUSSION

Isolation of the Photosynthetic Bacteria involves selection of the sites with elevated atmospheric $CO_2$ i.e. open top chambers erected at CRIDAl, Hyderabad for carbon sequestration and climate change studies'; Inoculations of the cultures are done in a laminar air flow with burner. Enrichment medium was filled completely into the screw cap test tubes and a small amount of the inoculum was introduced into each test tube. Elevated atmospheric $CO_2$ stimulates the photosynthesis of plants (Ainsworth, 2008). This in turn results in more carbon input into soil by stimulated root exudates and the decomposition of increased plant biomass (Rogers *et al*., 1994; Daepp *et al*., 2000; Jastrow *et al*., 2000). The studies reveal that elevated atmospheric $CO_2$ leads to the increases in SOC by 14% (Feng *et al*., 2009) and dissolved organic C (DOC) by 42% (Cheng, L.,) in soil. These screw cap test tubes were kept under the illumination of 2500 lux at room temperature around 30°C. The growth of the different strains of Photo synthetic Bacteria isolated from the soil samples, was evitable by change of color of the medium in the test tubes to either pink or purple. Analysis of the soil samples has been carried out for physico-chemical parameters from which photosynthetic bacteria are isolated. (Table-5). Plating & Streaking methods were followed (on the agar medium in petriplates) to separate different colonies of photosynthetic bacteria. Serial dilution method was followed for further purification of the various strains (10 dilutions). Identification and characterizations of these

photosynthetic bacterial strains were done to some extent (up to family level). Studies on morphological characters, *in vivo* absorption spectral characters, color of the cell suspension, predominant bacteriochlorophyll, type of carotenoid gives an idea about the family to which the particular bacteria belong (Table-6).

## IV. CONCLUSION

Bacterial isolation was done from the soil and water sample collected, followed by the serial dilution and pure cultures were obtained from the cultures grown. Bacteria isolated were purple sulphur bacteria (Chromatium, Ectothiorodospira, Thioapsa), purple non sulphur Bacteria, Green sulphur Bcteria (Chlorobium, Petodictyon, Green Non Sulpher bacteria (Chlorofixus). Were tend to be obtain from the soil and water samples further identification of the bacteria and utilization of Bacteria should be classified in the further studies.

Table 5

| *S. No* | *Treatment t* | *PH* | *Avg temp* | *Relative Humidit* | *Carbonate sppm* | *Sulfate sppm* | *Nitrate sppm* | *Chloride sppm* | *Org. matter* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Ambient control | 6.9 | 28°C | 50 | 101 | 195 | 5 | 287 | 0.13 |
| 2 | 550ppm | 6.8 | 28.5°C | 42 | 126 | 203 | 6.5 | 275 | 0.18 |
| 3 | 700ppm | 6. 8 | 29.1°C | 38 | 140 | 228 | 8 | 320 | 0.22 |

Table 6

| *S. No.* | *Motility +/-* | *Cell size in urn* | | *Color of cell suspension* | *Predominant* | *Predominant* | *Family, to which the PSB belong* |
|---|---|---|---|---|---|---|---|
| | | *Width* | *Length* | | | | |
| 1 | + | 1.0 | 2.4 | Orange | Bchl-a | Lycopene & Rhodopene | Chromatiaceae |
| 2 | + | 1.6 | 2.3 | Dark Pink | Bchl-a | Okenone | Chromatiaceae |
| 3 | + | 1.0 | 1.8 | Pink | Bchl-a | Rhodopene | Chromatiaceae |
| 4 | + | 1.2 | 2.6 | Purple Violet | Bchl-a | Rhodopinal series | Chromatiaceae |
| 5 | + | 1.8 | 2.5 | Orange brown | Bchl-a & c | Normal sprilloxant hin | Chromatiaceae |

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