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STUDIES ON CHLOROPHYLL SPECTRUM AND ANTIBIOGRAM PROTEIN PATTERN OF PHOTOSYNTHETIC BACTERIA

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Abstract
The isolation of photosynthetic bacteria from Luangwa river sediments, the classical methods were adopted for the isolation green sulphur bacteria and purple sulphur bacteria. It took around 6-8 weeks in Winogradsky Column method and 15 days for the isolation using enrichment medium. It is observed that at low watts exposure the appearance of Green sulphur bacteria and at high light intensity the appearance of red colour colonies. Then spectral analysis was done for the presence of chlorophyll and carotenoid pigments. The possibility of antimicrobial activity was checked and it proved positive only against Klebsiella spp and Bacillus spp. As far as the Thin Layer Chromatography was concerned all strains showed R_f values, which ranged in maximum activity.

Keywords*: Green sulphur bacteria, Purple sulphur bacteria, Winogradsky column, chlorophyll and carotenoid pigments.*

Introduction

Photosynthetic bacteria is a group of independent, self-supporting microbes which synthesize useful substances from secretion of roots, organic matter and harmful gases (e.g. Hydrogen sulphide) by consuming light from the sun and the soil heat sources of energy. Plant growth and development will promote the substances developed by these microbes include amino acids, nucleic acids, bioactive substances and sugars. The metabolites developed by these microorganisms are absorbed directly into plants and act as substrates for increasing beneficial population. The light-dependent production of hydrogen by photosynthetic purple bacteria was first observed with cultures of R. rubrum[8]. Purple non-sulphur bacteria, on the other hand, rarely appear in visible concentrations; their presence in nature, therefore, can only be evaluated from results obtained by enrichment techniques[1] or by membrane filtration[2, 15].

Chemical analysis has shown that in addition to proteins, the pigments (bacterio chlorophylls, carotenoids), and the isoprenoid quinines[7], chlorosomes also contain lipids that are thought to form a surface monolayer known as the envelope. In addition to the major light-harvesting bacterio chlorophylls, which can be either Bacterial Chl c, d or e depending on the species, isolated chlorosomes also contain small amounts of Bacterial Chl a. However, the major part of the cellular Bacterial Chl a which is found either in the Fenna ± Matthews ± Olson a protein (FMO-protein) that is located between the chlorosome and the cytoplasm membrane, or else is associated with the reaction center polypeptides in membrane[9].

Materials and Mathods

Antibacterial Activity of Photosynthetic Bacteria

Isolation of photosynthetic bacteria from sediments of Luangwa river using classical Winogradsky column and sub culturing using different enrichment media. The photosynthetic bacteria were identified by using gram's staining method. About 5 ml of sample was taken. It was then centrifuged and residue was collected. Then it was then mixed with methanol. Mixed residues was impregnated with filter paper disk (5mm diameter) and dried. Furthermore placed in pathogen-cultivated plates (Klebsiella spp, Bacillus subtilis, and Vibrio spp) and kept in 37°C for 48 hours. Inhibition zone was noted and measured.

TLC Analysis of Photosynthetic Bacteria

Qualitative analysis of antibacterial components of photosynthetic bacteria in the experimental sample was carried out by using Thin Layer Chromatography (TLC). Then, applying slurry made by silica gel G for TLC grade and applied over the glass slides, TLC slides were made. This was dried at 60°C for an hour of period. The dried slides were pre-activation base was drawn on the TLC slides 1cm away from the base line on the portion of the TLC slides.

After the samples were spotted on the baseline of the TLC slides at 1cm, allowed to dry at room temperature. Sample applied in TLC slides were placed in pre-saturated TLC chamber contains mobile phase of two combination solvents like Acetone: Methanol (1:9), Acetone: Petroleum ether (1:9), Acetone: Benzene (1:9) and the chromatogram was developed up to a mark. Chromatogram marked slides were taken out dried for few minutes. Using iodine vapour the slides were stained and spots were marked. The distances travelled by each spot in base line and relative R_f values were calculated.

$$R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

Spectral Analysis
The samples were centrifuged and then the residue was collected. It was then mixed with acetone then taken for spectral analysis at 200-800nm using UNICAM UV–Visible spectrophotometer.

SDS – PAGE
SDS-PAGE is done by using a gel apparatus. This apparatus consist of notched and rectangular glass plates, 1mm thick spacers, gel casting stand and Teflon comb and power pack. In order to proceed the electrophoresis, the separating gel mix (10%), stacking gel mix (5%), sample treatment buffer, tank buffer, coomassie brilliant blue stain, destain I and destain II should be prepared.

Procedure
The glass plates were cleaned thoroughly and assembled using spacers. The slides were then sealed using 2% agar solution. The assembled glass sandwich was checked for any leakage before casting the gel. The separating gel mixture was prepared and gently mixed. The mixer was then poured to the glass sandwich assembly to a level of about 3cm from the notch, separating the gel overlay solution was added over it and allowed to polymerize.

The stacking gel mixture was similarly prepared and dehydrated, after which APS and TEMED were added and gently mixed separating gel overlay solution was poured off and the glass sandwich was filled with the stacking gel mixture. Slowly the comb was inserted without any formation of air bubbles. The mixture was then allowed to polymerize.

Preparation of sample for SDS-PAGE
The cultures exposed to the light were centrifuged separately and their residues were collected. To the residues 1ml of 10% Trichloroacetic acid (TCA) was added and centrifuged. The supernatant was discarded and to the residue, 0.5 ml of sample buffer was added. From this 20µl of each sample was taken and loaded into the gel. Along with this a standard marker was also loaded.

The apparatus was switched on, after the initial pre-run at 50v; the voltage was increased to 100v. The run was carried out till the marker reaches the bottom of the gel. The glass sandwich was then dismantled and the gel was removed which was then placed in staining container overnight. The gel was then destined and observed on a white light Tran-illuminator, after which it was dried and documented.

Results and Discussion
The photosynthetic organism was isolated from the samples collected from Luangwa river by using the classical Winogradsky column and enrichment medium. Based on the Gram's staining the organism was identified to be Gram negative rods. The sample collected was subjected to the physiochemical analysis in which the parameters like temperature, salinity, pH and dissolved oxygen was checked. It was observed that the temperature was about 25°C, which is quite normal for the soil sample present in lakes. The salinity was 2 ppt, pH of the soil sample was 7.9 and dissolved oxygen was 3.5 mg/l the results were tabulated. It shows that temperature, salinity, pH, dissolved oxygen was highly suitable for the survival of photosynthetic bacteria (Table 1). However it has shown that generally in pure laboratory culture all of the strains of photosynthetic bacteria demonstrated typical mesophilic properties and grew optimally at 28°C to 30°C[13].

The method adopted for isolation of photosynthetic bacteria using the classical Winogradsky column and sub cultured using enrichment media. It is found that for the isolation by Winogradsky column is made to provide enrichment culture for anaerobic photosynthetic bacteria from a natural assemblage of organisms and substrates (lake sediment). This column is made from the sediment mixed with certain nutrients (cellulose, phosphate, sulfate and carbonate) and placed under certain physiological conditions so that the growth of photosynthetic anaerobes is encouraged. Then it is made sealed with was and exposed to light. Then it was left for several days allowing the aerobic heterotrophy to "Bloom" and utilize up all the available oxygen in the column thereby making it anaerobic.

Each group of microbes will accumulate in particular regions of the column establishing different physiological areas and gradients: that is the column will contain many "Niches". After the first bloom of aerobic heterotrophy making the column anaerobic, then the column is unwrapped. After removal of oxygen, there will be occurrence of a succession of anaerobes. Anaerobic species respires will also grow utilizing sulfate or thiosulfate as their terminal electron acceptor and reducing them.

The growth of the photosynthetic anaerobes (occurred with either purple or green patches) appeared will be examined and cultured further.

The green and red colonies which were later sub-cultured using enrichment media. It may be the Chlorobium spp and Chromatium spp. Still pure culture work has to be carried out. The antimicrobial activity of the photosynthetic bacteria strain isolated from soil samples of Luangwa river was checked. These mixed cultures of photosynthetic bacteria showed positive against Klebsiella spp only and Bacillus spp. They did not show any zone of inhibition against Vibrio spp (Table-2). The inhibition zone proves that these strains showed effective antagonism against pathogens. Further, purification of components can be done and be studied for individual compound's activity against any other pathogens[6].

The spectrophotometric analysis was done in which the majority of the peaks appeared from 350–665nm. It clearly proved the presence of various chlorophylls like a, b, c, d and e as well as carotenoids (Table-3). As far as the spectrophotometric data is concerned majority of the peaks appeared from 350–665nm. It clearly proved presence of various chlorophyll types as well as carotenoids[4-5].

The unknown protein profile data and the expression of bands clearly indicated the interaction proteins with pigment complexes like chlorophyll and carotenoids. The pigment proteins of photosynthesis are responsible for the absorption of light energy and the primary steps in its conversion to other form of energy. The results are tabulated in Table 4.

According to the protein profile is concerned, the expression of bands clearly indicated the interaction proteins with pigment complexes like chlorophyll and carotenoids. The pigment proteins of photosynthesis are responsible for the absorption of light energy and the primary steps in its conversion to other forms of energy. All of the pigment proteins bind both chlorophylls (Chl) and carotenoids. The Chls do most of the light harvesting whereas carotenoids protect against excess light energy. In some marine organisms, the latter also contribute significantly to light harvesting[10]. Both Chls and carotenoids are essential for correct folding of the proteins that bind them[3].

The main light-harvesting bacterio chlorophyll species occur as large self-assembling rod shaped aggregates dominated by pigment-pigment interactions. Chlorosomes also contain a number of low molecular mass proteins, although these are present in lower amounts, relative to pigments, than is the case with other photosynthetic antenna systems: a protein to pigment ratio of 0.5 (w/w) which has been estimated in chlorosomes isolated from Chlorobium tepidum[12]. Studies involving protease susceptibility and their interaction with the specific antibodies suggest that the major chlorosome proteins are located in the envelope[11-14]. The present study was observing the appearance of number of bands. This clearly indicates that core complex is a mixture of proteins and pigments. A still further study has to be done on the identification of individual colonies and pigment isolation. It all concludes that photosynthetic bacterial isolation is difficult and its usage is highly essential.

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Table 1: Physico Chemical Parameters Of Luangwa River

Sample	Temperature	Salinity (ppt)	pH	Dissolved Oxygen (mg/1)
Soil sample	25°C	2	7.9	3.5

Table 2: Antimicrobial Activity of Photosynthetic Bacteria Strains Isolated Soil Samples of Luangwa River

Strains	Pathogens		
	Klebsiella spp	Bacillus sp	Vibrio spp
AQB – P_1	+(<10mm)	-	-
AQB – P_2	-	0.45	-

Table 3: Spectrum of Photosynthetic Bacteria of Soil Samples Isolated From Luangwa River

Sample	Wavelength (nm)	Absorbance peak value
AQB - P_1	661, 590, 344, 37, 284, 259, 231, 210	0.0326, 0.0629, 0.2249, 2.0068, 0.2782, 2.0439, 0.3191, 1.4181
AQB - P_2	578, 347, 308, 226, 208	0.0413, 0.1105, 3.9999, 0.2268, 1.0304

Table 4: Documentation Results of SDS-PAGE from Soil Samples of Photosynthetic Bacteria Strains Isolated In Luangwa River

MARKER		AQB - P_1		AQB - P_2	
NW (kd)	R_f Values	MW(KD)	R_f Values	MW(KD)	R_f Values
80	0.171	79.869	0.175	78.931	0.201
66	0.381	75.034	0.283	76.916	0.248
56	0.454	71.556	0.329	75.967	0.267
50	0.54	69.426	0.351	69.93	0.346
40	0.651	58.831	0.432	66.313	0.378
29	0.689	55.037	0.463	54.161	0.471
25	0.826	50.142	0.535	50	0.54
20	0.874	46.986	0.612	48.052	0.6
17	0.925	23.798	0.749	37.191	0.661
14	0.942	23.798	0.814	23.798	0.783
		21.28	0.86	19.76	0.878
		16.455	0.929	12.071	0.953