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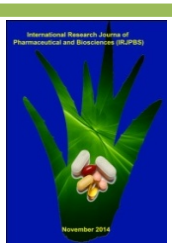

Cultivation of purple phototrophic bacteria using agricultural waste media

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Abstract

Purple phototrophic bacteria are group of heterotrophic bacteria that grow anaerobically using sunlight as energy source and organic compounds as the sources of carbon. These bacteria are not easily cultivated in the laboratory but play significant roles in environment. These are finding biotechnological applications like biopesticides, bioplastics and hydrogen production. The phototrophic pigment i.e. rhodopsin derived from these bacteria is used in many computer parts because of its properties to sense light. The synthetic media allows sufficient growth of these bacteria but for economical productions these bacteria must be grown in natural media. The purpose of present study is to grow these bacteria in agricultural waste media derived from crops. For this study cotton seed cake, soybean waste and molasses have been undertaken. Those agricultural waste media have been quantified. The habitat chosen for the study was Wadali Lake, Amravati. Sufficient growth was observed in cotton seed cake media followed by soybean waste media and lesser growth was observed in media containing molasses. The results showed that the agricultural waste media would be economical grow purple phototrophic bacteria. The bacterium characterized during the study was *Rhodospirillum* spp. Finally, phototrophic pigment rhodopsin was extracted from *Rhodospirillum* spp.

Keywords: Purple bacteria, agriculture waste media, rhodopsin, *Rhodospirillum* spp.

INTRODUCTION

Purple phototrophic bacteria (PPB) or purple non-sulphur phototrophic bacteria (PNSP) are the group of bacteria that grow under anaerobic condition where sufficient amount of light can reach such as beneath the surface of water or at the bottom of the lake. PPB possesses insoluble carotenoid pigments that absorb the light energy. The purple bacteria are recently

finding lot of application both biotechnological and environmental. The photobacterial pigment *i.e*. bacteriorhodopsin has lots of application in computer fields as well. This pigment is being extracted and characterized from a range of photoheterotrophic bacteria [1].

The phototrophic bacterium *Rhodopseudomonas palustris* was cultivated in sago starch processing waste water [2]. The sago effluent contains bark and pith residues. This waste is sometimes used as dietary fibre for animal food or it is burned. This waste was photometabolized by *Rhodopseudomonas palustris* which is a rich source of proteins. These bacteria also produce bioplastics [3,4].

Azad *et al.*, (2001) studied growth and production of biomass of *Rhodovulvum sulphidophilum* in sardine processing waste water. The processed fish water is rich in organic nutrients due to blood, tissue and fish extracts. Such organic waste is used as substrate for the production of phototrophic bacterial biomass [5]. This ultimately leads to control of pollution as well. The species cultivated was *Rhodovulum sulphidophilum* that is rich in protein, lipids, vitamins, minerals, carotenoids and other co-factors. The same species has been used as fish food supplement also [6]. Feeding trials for fishes were carried using photobacteria and algae. When *Rhodovulvum* sp were mixed with algae it was observed that the growth and survival of fin fish larvae were improved than using only algae.

As like fish waste water, swine waste water too supports the growth of purple bacteria. The wastewater was found to be rich in acetate and propionate as the major carbon nutrients. The purple bacterial genera like *Rhodobacter* and *Rhodopseudomonas* were found to be predominant genera. The probable presence of those bacteria was based on the pink colored microbial mats [7]. The biomass obtained in Pfennig's synthetic medium for purple phototrophic bacteria and the medium composed of poultry slaughter house waste were compared. It was found that the biomass obtained from slaughter house waste was relatively higher than synthetic medium [8]. Biomass is also produced from freshwater phototrophic bacteria [9, 10, 11].

Various methods are currently been devised to treat domestic waste water using phototrophic bacteria [12]. *Rhodobacter sphaeroides* was cultivated on palm oil mill effluent. Maximum bacterial biomass of 6.5 g/L dry weight and 72% reduction of COD were achieved after 96-h culture with 20% (v/v) inoculum level [13]. A photo-bioreactor was designed to treat food processing waste. The BOD and COD removal efficiencies were in the range of 51% and 58% respectively. The photo-bacterial biomass yield was 0.6 g dried solid/g BOD with crude protein content of 0.41 g/g dried solid. *Rhodopseudomonas palustris* was the bacterium characterized during the study [14]. The medicinal plant Stevia is widely used for extraction of sweetener. The residues generated during the extraction have been used to grow purple bacteria. The species grown was *Rhodopseudomonas palustris.* These phototrophic bacteria also produce hydrogen which is clean source of

energy [15]. Those photosytnhetic bacteria were isolated from oil contaminated soil of Nacharam, Hyderabad, India. A consortium was prepared for this purpose. Nunkaew *et al*., (2012) introduced rice straw broth as a medium to isolate purple nonsulfur bacteria. Because of anaerobic conditions and sufficient water in the paddy fields; such habitat supports growth photosynthetic bacteria. Rice straw is quite rich in organic substrates like cellulose (32-37%), hemicellulose (29-37%) and lignin (5-15%) [16]. Wide range of phototrophic bacteria are found potent for hydrogen production [17,18,19,20,21].

Agricultural waste media offers economical cultivation of photobacteria. Cotton, soybean and sugarcane are the major crops in Maharashtra, India. The waste products of these crops i.e. molasses, cotton seed cake and soybean waste have been undertaken in the study and the efficiency of those media is reported along with extraction of phototrophic pigment.

MATERIAL AND METHODS

Enrichment and isolation of Anoxygenic phototropic bacteria

The sediment samples were collected from Wadali Lake, Amravati. For maintaining anaerobic conditions and light the transparent anaerobic jar was used. After 4-7 days the growth was observed in the form of reddish bloom. This growth was then subcultured on different type of waste media like cotton seed cake, soybean and molasses with varying concentration of media.

Identification of bacteria

Identification of Isolates was based on Morphological, cultural and biochemical characteristics. Morphological characteristics**:** The morphological Characters were examined under microscope by performing gram staining & motility. Biochemical Characteristics**:** The Biochemical characters were examined by conducting the following test.

Production and extraction of rhodopsin

The Production of rhodopsin was carried out by inoculation of red colonies into the crude media containing cotton seed cake, soybean and molasses. The broth was centrifuged at 8000 rpm for 15 min and the cell pellet was separated. Sodium lauryl sulphate was added to the cell pellet which disrupts the cell membrane. This preparation was centrifuged at 8000 rpm for 10 min. The supernatant was discarded and disrupted cell pellet containing rhodopsin was dissolved in ethanol followed by centrifugation at 8000 rpm for10 min. The absorption maxima were noted.

RESULT AND DISCUSSION

Cultivation of anoxygenic phototrophic bacteria

Enrichment of phototropic bacteria was done by inoculation of serially diluted sediment sample into the succinate broth medium at 30^0c for 4-7 days in anaerobic jar. Characteristic

reddish growth was observed in broth which was obtained in pure form on succinate agar plate (Fig 1).

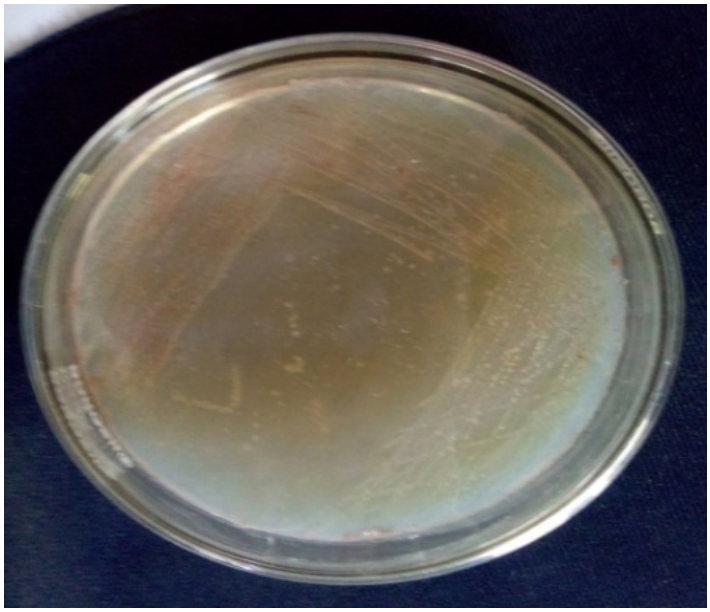


Fig1. Red coloured colonies are indicative of phototrophic bacteria

Fig 2. Culture broth from left to right a) Growth on cotton seed cake media b) Growth on soybean media c) Growth on molasses media

Subculturing was done in different waste media like cotton seed cake, soybean and molasses and observed for maximum growth (Fig 2). Then maximum growth was observed in cotton seed cake, moderate growth was observed in soybean and lowest growth was observed in molasses containing media.

Characterization of phototrophic bacteria

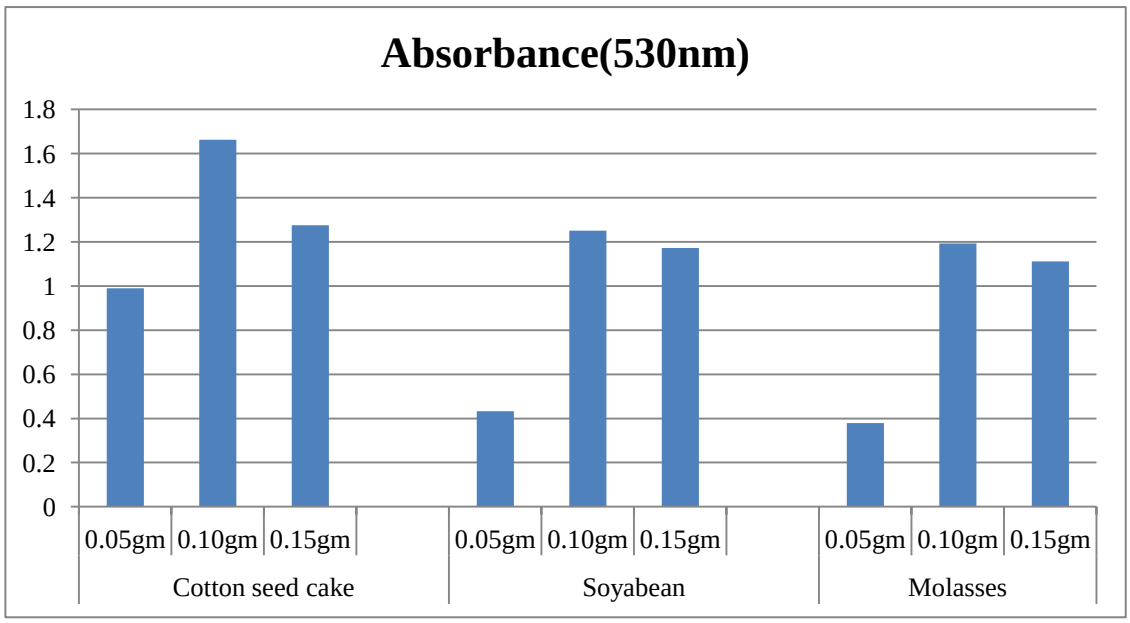
Table1. Biochemical properties of phototrophic bacteria:

Sugar	Acid
Dextrose	Positive
Fructose	Positive
Sucrose	Positive
Cellobiose	positive
Lactose	Negative
Mannitol	Positive
Sucrose	Negative
Rhamnose	Negative
TSI	positive
Salicin	Positive
Arabinose	Negative

In present study morphological, cultural and biochemical characteristics one of the prominent bacterium was carried out on the basis of strong reddish bloom in succinate broth. On the basis of these characteristics the anoxygenic, phototrophic bacterium was identified as *Rhodospirillum spp.* This isolate was subjected to the production of rhodopsin (Table1).

Production and extraction of rhodopsin from different waste media

Graph 1: UV-Visible absorption spectra of rhodopsin at different concentration of media



Rhodopsin production was low at initial days and high at seventh day. This rhodopsin production was observed by taking absorbance at 530nm on spectrophotometer. The absorbance was directly based on the rhodopsin production. Highest rhodopsin production was observed in cotton seed cake, moderate in soybean while lowest in molasses. Thus, cotton seed cake was good rhodopsin producers (Graph1).

Three concentrations of these media were used i.e. 0.05, 0.1 and 0.15 of which 0.10 g/100ml shows maximum rhodopsin levels. In cotton seed cake (C1) the absorbance was observed at 0.9898 in 0.05g of cotton seed cake in 100ml of broth while in soybean (S1) shows absorbance at 0.433 and molasses (M1) at 0.3784 in same amount of medium that is at 0.05. In 0.1gm/100ml cotton seed cake (C2) shows absorbance at 1.6624, soybean (S2) 1.25 and molasses (M3) at 1.1928. In third set of inoculation in which waste medium was taken 0.15gm/100ml cotton seed cake (C3) shows absorbance at 1.275, soybean (S3) at 1.172 and molasses (M3) at 1.1109. From the above observation in all waste medium and different amount of media cotton seed cake was observed to be optimized media for the extraction of rhodopsin. The entire media shows highest growth at amount 0.10g/100ml but maximum in cotton seed cake media. Hence cotton seed cake is best medium for extraction of rhodopsin than any other.

CONCLUSION

In present study presence of phototrophic bacteria is revealed in Wadali Lake, Amravati. One of the prominent bacterium was characterized as *Rhodospirillum* species. Economical media composed of soybean waste, cotton seed cake and molasses were designed. Maximum growth was observed in cotton seed cake media, moderate growth in soybean and lowest growth was observed in molasses. Further rhodopsin was extracted from broth and inoculated on different amount of media that is 0.05g, 0.10g and 0.15g/100ml and rhodopsin was efficiently extracted from medium containing 0.10g/ n100ml of cotton seed cake. As rhodopsin has wide applications this medium would be helpful in obtaining economical production of rhodopsin.

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