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Research Article

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Extraction of PHB and Bacteriorhodopsin from Anoxygenic Phoheterotrophic Bacteria Isolated from Wadali Lake

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ABSTRACT

Decrease in seasonal rainfall is threat to aquatic life including bacteria. Mesophilic aerobic bacteria in aquatic bodies are easy to cultivate in laboratory than anaerobic bacteria. Cultivation of anaerobic bacteria requiring light demands careful protocols and appropriate nutrients. Present work deals with this unique group referred to as anoxygenic photoheterotrophic bacteria from Wadali Lake, Amravati, Maharashtra, India. These bacteria were cultivated on mineral salt succinate agar in anaerobic gaspac jar exposed to light of 2400 lux for 8-10 days. Of the twenty isolates extracted from these lakes ten bacteria were further fully characterized based on their abilities to produce polyhydroxy butyrate (PHB). PHB was extracted from these bacteria and was detected spectrophotometrically. The efficient PHB producing genera belongs to Rhodobacter, Rhodopseudomonas and Rhodospirillum. This work was further extended to isolation and characterization of rhodopsin which is a characteristic pigment of anoxygenic phototrophic bacteria. This pigment has wide range of application most significant being artificial retina preparation. After extraction of this photosynthetic pigment, comparative studies were carried where this pigment was found in higher quantities in Rhodopseudomonas culture broth which suggest the suitability of this culture for rhodopsin extraction. Though rhodopsin is found in wide range of fungi, animals and bacteria but latter group is preferable with regards to ease in isolation of the pigment. Regarding PHB production, the highest levels of PHB were found in Rhodobacter species. The production of both PHB and rhodopsin from photoheterotrophic bacteria are economical processes as the energy source of these bacteria is light.

Keywords: *Anoxygenic; PHB; photoheterotrophs; production; rhodopsin.*

INTRODUCTION

Phototrophic bacteria were studied for the first time by Ehrenberg way back in 1835[1]. Major observations to purple non-sulphur bacteria (PNSP) were contributed by Winogradsky (1988) through studies on purple and lilac spots formed by photosynthetic bacteria[2]. The names designated by Winogradsky are still in Bergey's Manual of Systematic Bacteriology. In seventh edition of Bergey's Manual of Systematic Bacteriology the bacteria capable of photosynthesis were combined into one suborder, *Rhodobacteriace,* and divided into three families i.e. *Thiorhodaceae, Athiorhodaceae* and *Chlorobacteriaceae*. The name green sulfur bacterium was coined by G.A. Nadson in 1906. These were isolated from Lake Saki and were named as *Chlorobium limicola*[3]. Year later Molish assigned these pigmented sulfur and nonsulfur bacteria to the order *Rhodobacteria*[4]. Their role in environmental, industrial and medicinal fields is proven to be significant because of versatility of shifting from phototrophic to organotrophic. From India, many novel species have been introduced by C.V. Ramana and Ch. Sasikala, few of them includes *Rhodobacter vinaykumarii, Rhodobacter aestuarii* and *Rhodobacter changlensis*[5,6,7]. It is mentioned by Gorlenko with regards to phototrophic bacteria "a new reservoir (habitat) means a new microorganism" as there is a continuous introduction of new species in this group particularly from lakes[1]. These phototrophs are of immense field of interest which is mentioned in upcoming para.

Environmentally these phototrophs are quite significant. The phototrophic iron oxidizing bacteria are considered one of the primitive organisms on earth as there was abundance of iron and light at the beginning of life. Removal of iron can be done using these bacteria[8]. Photoheterotrophs plays a major role in deodorization by consuming odorous volatile organic compounds[9]. Similar studies were carried by Myung et al using *Rhodopseupdomonas palustris*[10]. Oil waste can also be degraded using these bacteria. Species of *Rhodobacter* were found to degrade cooking oil into various fatty acids such as propionic and acetic acids[11]. Similarly phenol wastewater is also detoxified by photosynthetic bacteria[12]. The COD and BOD of latex rubber sheet water were reduced to 90% using *Rhodopseudomonas blastica*[13]. Reduction of selenite was also studied by Kessi. Selenite is a product of coal mine which can cause various health hazards. *Rhodospirillum rubrum* and *Rhodobacter capsulatus* are found to reduce selenite from the environment[14]. Photo-bioreactor system was adopted for treatment of pharmaceutical waste water by purple non-sulfur bacteria[15].

A slow but significant contribution of Purple non-sulphur bacteria (PNSP) cannot be denied in agriculture fields. Aminolevulinic acid (ALA) is produced by PNSP which is emerging as selective biodegradable herbicide and insecticide. ALA is produced extracellularly making it an economical and more productive process[16]. Medicinally also ALA has been studied for anti-tumor action which caused necrosis of the tumours. First time it was reported by Grant *et al*,[17]. An unnoticed role of phototrophic bacteria to fix the nitrogen is a process found in many habitats including paddy fields[18]. One more economical application of these phototrophic bacteria is their use as food supplement for fishes[19]. *Rhodopseudomonas palustris* was particularly found high in protein content and is suggested as source of food for fishes[20].

The industrial application of PNSP is in production of polyhydroxy butyrate (PHB). Large numbers of samples from tannery effluent were screened for purple non-sulphur bacteria in Warangal district belonging to South India. Various bacteria were isolated of which *Rhodopseudomonas palustris* was found efficient for PHB production[21]. Properties of these PNSP bio-plastic can be regulated by varying the composition of the copolymer[22].

Another important compound produced by PNSP is bacteriorhodopsin which is light driven proton pump that harness energy from light to create a proton gradient across the bacterial membrane to drive energetic processes. These were discovered by Stoeckenius *et al*, in 1979 from *Halobacterium salinarium*[23]. The biological applications of rhodopsin include artificial retina preparation, photo detectors for defense purposes; current and most important application is generation of electricity[24]. It is emerging as one of the important compounds in computer fields also viz. light modulators, optical memories, colour photochromic sensors as well as papers and ink[26,27,28].

In this study we have characterized photoheterotrophic bacteria from a pond situated in Amravati namely Wadali Lake. Further, PHB and bacteriorhodopsin pigment from these bacteria were extracted and comparative analysis carried to suggest efficient bacteria.

MATERIALS AND METHODS

Enrichment and isolation of PNSP

Samples from Wadali Lake were grown on mineral salt succinate agar. Plates were incubated for 6-8 days anaerobically in a gas pack jar under light. The pH was kept neutral and temperature ranged from 30 to 38^0 C. Black colored and red colored colonies appeared on the media of which former belonged to anaerobic sulfur bacteria which were eliminated. After tedious screening; typical red colored colonies on mineral salt succinate agar were undertaken for morphological and biochemical characterization.

Extraction of PHB

Broth cultures were grown anaerobically under light for 8-10 days. After sufficient turbidity cell pellet was suspended in sodium hypochlorite solution. Granules were centrifuged at 10,000 rpm for 20 min which were washed with water, acetone and alcohol. Finally polymer was dissolved by extraction with boiling chloroform followed by filtration and filtrate was used for assay of PHB. This was heated with sulphuric acid for 10 min, at 100^0C in water bath. Solution was cooled and absorbance measured at 235 nm.

Extraction of Rhodopsin:

Phototropic bacteria were grown in nutrient solution anaerobically and broth was centrifuged at 8000 rpm for 15 minutes. Cell pellet was separated & ethanol was added followed by centrifugation at 10,000 rpm for 10 minutes. Cell debris separated and ethanol was again added and centrifuged at 10,000 rpm for 10 minutes. Absorbance was read at 500-600 nm the range at which rhodopsin absorbs maximally.

RESULTS AND DISCUSSION

Enrichment and isolation of PNSP

Fig.1 and Fig.2 Few of anoxygenic phototrophic bacteria showing characteristic red colored colonies on mineral salt succinate agar

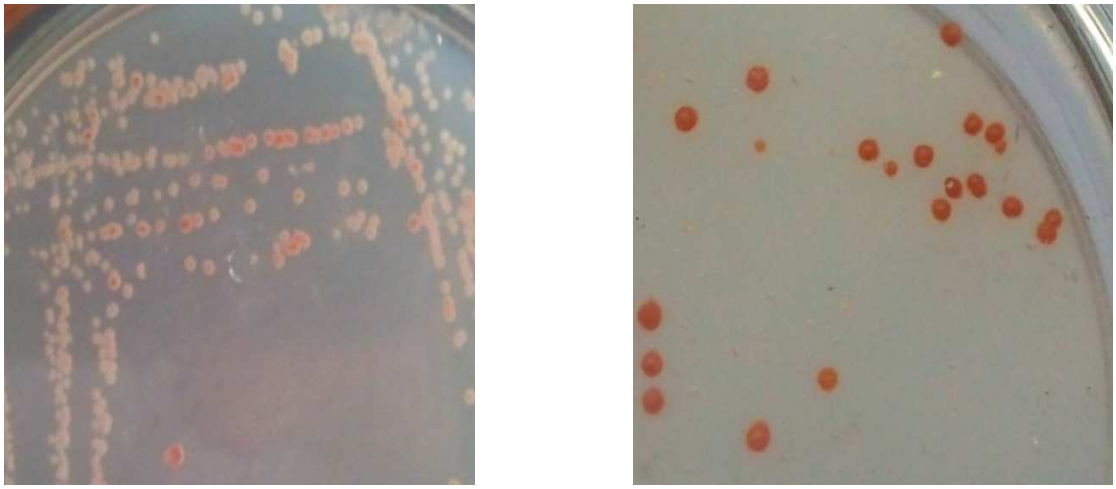
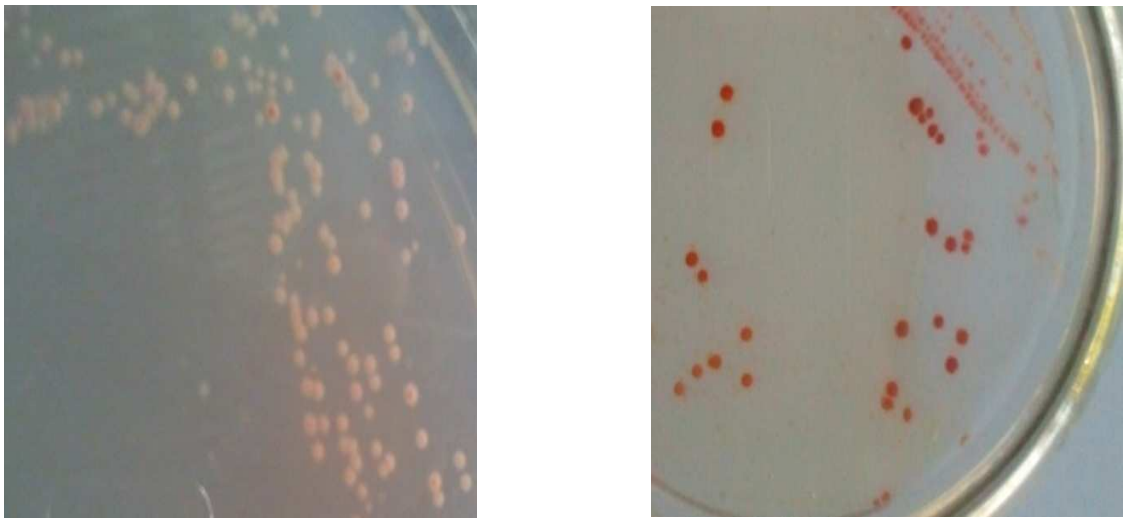


Fig.3 and Fig.4 Red colored colonies of anoxygenic phototrophic bacteria on mineral salt succinate agar



Both sulfur and purple bacteria appeared on mineral salt succinate agar. The black colored colonies belonged to anaerobic sulfur bacteria which were eliminated. After tedious screening; typical red colored colonies on mineral salt succinate agar were undertaken for morphological and biochemical characterization (Fig1 to Fig4). Color to these bacterial colonies is because of the red pigment rhodopsin which is responsible for photosynthesis. Morphological and biochemical characteristics of those cultures are shown in table1.

Table1:Characterization of phototrophic bacteria isolated from lake

Culture designation	Cultural characteristics							Morphological properties				Biochemical characteristics														Identified Bacterial species
	Color	Size	Shape	Margin	Elevation	Opacity	Consistency	Gram stain	Shape	Endospore	Motility	Catalase	Indol	MR	VP	Citrate	Sucrose	Arabinose	Dextrose	Fructose	Lactose	Mannitol	Rhamnose	Salicin	Xylulose	Cellobiolose	
M1	Red	1 mm	Circular	Regular	Convex	Opaque	Moist	-	Short rod	NE	M	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	*Rhodospirillum sp.*
M2	Pink	0.5 mm	Circular	Regular	Convex	Opaque	Moist	-	Long rod	NE	M	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	*Rhodopsecudomonas sp.*
M3	Red	0.5 mm	Circular	Irregular	Convex	Opaque	Moist	-	Short rod	NE	M	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	*Rhodobactor sp.*
M4	Red	1 mm	Circular	Regular	Convex	Opaque	Moist	-	Short rod	NE	M	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	*Rhodobactor sp.*
M5	Pink	1 mm	Circular	Regular	Convex	Opaque	Moist	-	Long rod	NE	M	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	*Rhodopsecudomoas sp.*
M6	Red	1 mm	Circular	Regular	Convex	Opaque	Moist	-	Short rod	NE	M	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	*Rhodobactor sp.*
M7	Red	0.1 mm	Circular	Regular	Convex	Opaque	Moist	-	Short rod	NE	M	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	*Rhodobactor sp.*
M8	Pink	0.1 mm	Circular	Regular	Convex	Opaque	Moist	-	Long rod	NE	M	-	-	-	-	+	+	-	-	+	-	+	+	-	-	-	*Rhodopsecudomoas sp.*
M9	Red	0.5 mm	Circular	Irregular	Convex	Opaque	Moist	-	Short rod	NE	M	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	*Rhodospirillum sp.*
M10	Red	0.7 mm	Circular	Regular	Convex	Opaque	Moist	-	Short rod	NE	M	-	-	-	-	+	+	-	+	-	-	+	+	-	-	-	*Rhodobactor sp.*

Abreviations: M- Motile, NE- Non-endospore, +- positive,- - negative

Extraction of PHB

Fig5: Culture flask before incubation (left) and after incubation (right)

Fig6:Characteristic red colored growth of PNSP on succinate broth

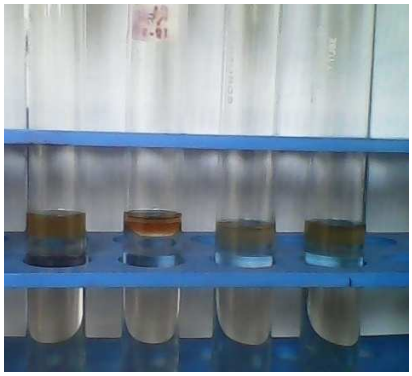


Fig. 7 and 8 Extraction of PHB

Table2: Spectrophotometric detection of PHB at 235 nm

Organism	O. D.
Rhodospirillum sp.(M1)	0.858
Rhodopseudomonas sp.(M2)	0.870
Rhodobacter sp.(M3)	0.972
Rhodobacter sp.(M4)	0.968
Rhodopseudomonas sp.(M5)	0.892
Rhodobacter sp.(M6)	0.968
Rhodobacter sp.(M7)	0.960
Rhodopseudomonas sp.(M8)	0.917
Rhodospirillum sp.(M9)	0.867
***Rhodobacter sp.*(M10)**	**0.974**

Extraction of Rhodopsin:

Table3. Spectrophotometric detection of rhodopsin pigment at 530 nm

Identified bacteria	Absorbance at530 nm
Rhodospirillum sp.(M1)	0.100
Rhodopseudomonas sp.(M2)	0.164
Rhodobacter sp.(M3)	0.200
***Rhodobacter sp.*(M4)**	**0.214**
Rhodopseudomonas sp.(M5)	0.189
Rhodobacter sp.(M6)	0.184
Rhodobacter sp.(M7)	0.142
Rhodopseudomonas sp.(M8)	0.206
Rhodospirillum sp.(M9)	0.122
Rhodobacter sp.(M10)	0.140

Of the ten isolates five *Rhodobacter* spp, three *Rhodopseudomonas* spp and two *Rhodospirillum* spp were obtained. Cultures were grown in broth for bulk production of PHB and rhodopsin along with a control kept in dark. *Rhodobacter* spp M10 showed maximum rhodopsin production (O.D.-0.974) than *Rhodobacter* spp (M3) (0.972). *Rhodobacter* spp M4 and *Rhodobacter* spp M6 (0.968) displayed more rhodopsin production than *Rhodopseudomonas* spp (M8) (0.917) and less than *Rhodobacter* spp (M3). *Rhodobacter* spp (M7) (0.960) showed more rhodopsin production than *Rhodopseudomonas* spp (M5) (0.892) and less than *Rhodopseudomonas* spp (M8).Whereas *Rhodopseudomonas* spp (M5) displayed more rhodopsin production than *Rhodopseudomonas* spp (M8) (0.870). Whereas *Rhodospirillum* spp (M9) (0.867) showed more rhodopsin production than *Rhodospirillum* spp M1 (0.858) and lesser than *Rhodopseudomonas* spp (M2). With regards to PHB production it was confirmed that *Rhodobacter* spp (M10) produced highest amount of PHB than other species of *Rhodobacter*, *Rhodopseudomonas* and *Rhodospirillum* spp. Thus, highest rhodopsin production was observed in *Rhodobacter* spp (M4), *Rhodopseudomonas* spp and lowest in *Rhodospirillum* spp.

CONCLUSION

In this study presence of anoxygenic photoheterotrophic bacteria is revealed in Wadali Lake, Amravati which has potential for rhodopsin and PHB production. Among isolates characterized three genera of anoxygenic photoheterotrophic bacteria were predominant i.e. *Rhodopseudomonas* spp, *Rhodobacter* spp and *Rhodospirillum* spp as studied by morphological and biochemical properties. Further, their PHB producing abilities were studied spectrophotometrically which showed all isolates were able to produce PHB efficiently. *Rhodobacter* spp (M10) was the most efficient genera for the production of PHB followed by *Rhodopseudomonas* spp and *Rhodospirillum* spp. Rhodopsin a characteristic pigment of these bacteria was also isolated in higher amount with ease from *Rhodobacter* spp (M4).

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