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Isolation and Characterization of Photosynthetic Bacteria from Municipal Waste

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A B S T R A C T

One of the photosynthetic bacterial isolate L6 grown at 10 °C confirmed as *Rhodoferax* sp. and six isolates showed growth at 30 °C were isolated from municipal wastes in Kashmir and Ladakh valleys. The cells were Gram-negative, pigmented, non-spore-forming, mostly oxidase positive except K2 isolate and nitrate reduction negative except G17 isolate. All isolates showed growth in aerobic-light conditions except K2 and anaerobic-light except L6. All isolates utilized acetate, malate and succinate as carbon source while as benzoate and mannitol were not used except G17. However mannitol was used as carbon source by L6 also. Isolate K2 had shown well defined growth in sulphide medium and was regarded as sulphur bacterium while as isolates G17, L6, L27, K5, S5 and S7 were unable to utilize sulphide and thiosulphate as electron donor and considered as non sulphur bacteria. The morpho-biochemical test further confirmed K2 isolate as *Chlorobium* sp., S5 and L27 as *Rhodospirillum* sp., S7 as *Rhodobacter* sp., G17 as *Rhodopseudomonas* sp., K5 as *Rhodomicrobium* sp. Isolates G17, L6, L27, S5 and S7 were observed the most efficient on the basis of versatility in carbon utilization character. Isolate L6 was regarded as the psychrophillic/psychrotolerant photosynthetic purple non sulphur bacterium.

Introduction

In Jammu & Kashmir, only 1% of 1374 MT of municipal solid waste generated per day gets processed (Ministry of Housing and Urban Affairs, GOI, 2018). Waste generation and its control have taken an important role in our environment. With the doubling of population and changing lifestyle pattern of the inhabitants the quantity of municipal waste generated is increasing in an alarming rate. Municipal solid waste is made up of different organic and inorganic fractions like food, vegetables, paper, wood, plastics, glass, metal and other inert material (Mor, 2006). The waste sites harbour many types of microorganisms beneficial as well as deleterious. Photosynthetic bacteria are

ubiquitous in nature. They are present in aquatic environments and form water blooms. The presence of purple non sulphur bacteria is particularly dependent upon the degree to which water is polluted by organic matter. Their growth contributes to the purification of the heavily polluted water exposed to sunlight e.g, in sewage lagoons (Holm and Vennes, 1970). The photosynthetic bacteria are used in the main purification stage of organic waste water treatment (Kobayashi, 1975). Approaches to understand the types of photosynthetic bacteria growing in the municipal waste, the samples were collected from various sites of Kashmir and Ladakh valleys. The aim of the study was the isolation and characterization of photosynthetic bacteria from these waste samples based on the morphological and biochemical characteristics.

Materials and Methods

Four survey area viz. Gulmarg, Srinagar, Leh and Kargil were selected and from each area two sites were selected. Four dumpy samples of biodegradable wastes which had access to sunlight containing a substantial pigmented growth (soil mixed with waste and water) were collected from four different locations of each site and then composited into one sample per site. So, eight composite samples were collected in sterile zip-lock plastic bags maintaining aseptic conditions from eight waste rich sites, marked according to their source and site and stored at 4 °C. Purposive Method of Sampling was adopted during the collection of samples and the location wise details are furnished in the Table 1.

The dumpy waste samples collected from sites were grown anaerobically under light source of 3000 to 5000 lux in the sterile bottles containing Pfennig's medium (Pfennig Norbert, 1967) for enrichment of purple non-sulfur photosynthetic bacterial colonies till they form "bloom". Once the enrichment had achieved turbidity with the consequent bloom, it was streaked onto plates which were then incubated anaerobically near the light source at 10°C. The initial pH of the medium was adjusted to pH 7.0 using 5M NaOH which was raised to 9.0 on subsequent streaking methods. The isolates were purified by repeated streaking on plates and the pure cultures were kept on agar slants at 4 °C for further use. Also, the growth of the photosynthetic bacteria in the samples was enhanced by incubation under anaerobic conditions and 800 Lux ('anaerobic-light') at room temperature (30 °C) for 4 days. One gram of the incubated sample was diluted in 100 ml of distilled water and plated on Pringsheim's media run in the triplicates and incubated for a week. The clear colonies formed were enriched in G5 medium (Kohlmiler and Gest, 1951). A loopful of pinkish culture broth was streaked onto G5 agar without vitamins. After 7 days' incubation, each colony was picked up and streaked onto new G5 agar plates. This procedure was repeated (on sulfide medium also) until pure cultures were obtained. Sodium succinate or malate was used as an organic carbon source for isolation of purple non- sulphur bacteria because these are not generally used by other bacteria under photoheterotrophic growth conditions with no hydrogen sulfide.

The isolates were identified according to the procedures described by Watanabe *et al.* (1981). The stock cultures of the isolates were stored at 4°C overlying with sterile liquid paraffin under anaerobic light condition for 2-3 months. The isolated photosynthetic bacteria were characterized on the basis of colony morphology, colour, growth characteristics, Gram staining, spore forming ability, mobility, catalase, oxidase, urease, nitrate reduction and carbohydrate utilization tests (Masanobu, 2003). The compositions of

selective media used for the isolation of photosynthetic bacteria are given in Table 2.

Results and Discussion

Six isolates of non-sulphur and an isolate of sulphur photosynthetic bacteria were purified, characterized and stored for further use. Isolate K2 had shown well defined growth in sulphide medium and was regarded as sulphur bacterium while as isolates G17, L6, L27, K5, S5 and S7 were unable to utilize sulphide and thiosulphate as electron donor and considered as non sulphur bacteria.

The morpho-biochemical test further confirmed K2 isolate as *Chlorobium* sp., S5 and L27 as *Rhodospirillum* sp., S7 as *Rhodobacter* sp., G17 as *Rhodopseudomonas* sp., K5 as *Rhodomicrobium* sp. and L6 has shown growth at 10 ^{o}C confirmed as *Rhodoferax* sp. Isolates G17, L6, L27, S5 and S7 were observed the most efficient on the basis of versatility in carbon utilization character. Isolate L6 was regarded as the psychrophillic/psychrotolerant photosynthetic purple non sulphur bacterium. The results are presented in Table 3 and 4.

Table.1 Location of samples collected from Kashmir and Ladakh for analysis

S.No.	Survey area	Site	Sample Site Coordinates
1.	Gulmarg	Near Hotel Hill Top	34º 2′ 46.57″N 74º23′ 15.82″E
2.		Gulmarg Meadow	34º 4′ 9.08″N 74º22′ 24.93″E
3.	Srinagar	Dargah Hazratbal	34º 7′ 38.16″N 74º50′ 18.06″E
4.		Achan Landfill Area	34º 9′ 18.41″N 74º49′ 0.20″E
5.	Leh	HMAARI	33º 58′ 46.13″N 77º41′ 56.03″E
6.		Leh Golf Course	34º 8′ 23.26″N 77º34′ 50.05″E
7.	Kargil	KVK	34º 32′ 17.13″N 76º09′ 02.38″E
8.		New Sabzi Mandi	34º 33′ 37.03″N 76º7′ 33.03″E
	Total Composite Sample = 08		

Table.2 Composition of media used for isolation of Photosynthetic bacteria

S.No.	Media used	Composition/ litre of Distilled H_2O
1.	Pringsheim's Medium	K NO_3 = 0.2 g; Mg SO_4. $7H_2O$ = 0.01 g; $(NH_4)_2$ HPO_4 = 0.02g; $CaCl_2.6H_2O$ = 0.005 g; $FeCl_3$ = 0.0005 g.
2.	Pfennig's medium	KH_2PO_4 = 0.33 g; $MgSO_4.7H_2O$ = 0.33g; NaCl = 0.33g; NH_4Cl = 0.5g; $CaCl_2.2H_2O$ = 0.05 g; Sodium succinate = 1g; Yeast extract = 0.02 g. pH 6.8-7.2 After autoclaving, sterile solutions of Trace salts solution = 1.0 ml and 0.02% $FeSO_4.7H_2O$ solution = 0.5 ml were added. Trace Salt Solution ($ZnSO_4.7H_2O$ = 10 mg; $MnCl_2.4H_2O$=3mg; H_3BO_3 = 30 mg; $CoCl_2.6H_2O$ = 20 mg; $CuCl_2.2H_2O$ = 1mg; $NiCl_2.6H_2O$ = 2mg; Na_2MoO_4 = 3mg; Distilled H_2O = 1000 ml. pH= 3-4). For solid medium preparation Sodium succinate = 1g; Yeast extract = 1g and Agar = 15g were additionally added to the medium.
3.	G5 medium	Peptone = 5g; Yeast extract = 5g; L- glutamic acid = 4g; Malic acid = 3.5 g; KH_2PO_4 = 0.12 g; K_2HPO_4 = 0.18g; Agar = 15 g. Initial pH = 7.0 by using 5M NaOH
4.	Sulfide medium	Na_2S = 0.1g; Na_2HCO_3 = 0.02g; $(NH_4)_2$ SO_4 = 0.132g; Basal medium (Nicotinic acid = 1.0 mg; *p*-aminobanzoic acid = 1.0 mg; thiamine = 1.0 mg; biotin = 0.001 mg) Distilled H_2O = 100 ml. pH= 6.8

Table.3 Differentiating characteristics of photosynthetic bacterial isolates

Characteristics	G17	L6	L27	K2	K5	S5	S7
Growth Temp. (°C)	30	10	30	30	30	30	30
Aerobic-light	+	+	+	-	+	+	+
Aerobic-dark	+	+	+	-	-	+	+
Anaerobic-light	+	-	+	+	+	+	+
Anaerobic-dark	-	+	+	-	-	+	+
Growth at Ph	7.2	6.5	7.2	6.0	6.8	7.2	7.2
Colony Morphology	Orange, large, mucoid	Brown, Smooth, circular & convex	Purple,	Green,	Red, tiny, rough, hard	Purple,	Red, small
Cell shape	curved, knobby rods	Curved rods	Curved rods	Long chains of cocci	oval cells connected by filaments	Curved rods	Spherical
Mobility	Motile	Motile	motile	Motile	Non motile	Motile	Motile
Gram staining	-	-	-	-	-	-	-
Spore forming ability	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Positive/Utilization: (+) ; Negative/Non Utilization : (-)

Table.4 Differentiating characteristics of photosynthetic bacterial isolates

Characteristics/(Carbohydrate fermentation)	G17	L6	L27	K2	K5	S5	S7
Catalase test	+	-	+	-	+	+	+
Oxidase test	+	+	+	-	+	+	+
Urease test	+	-	+	-	+	+	+
Motility test	+	+	+	+	-	+	+
Nitrate Reduction test	+	-	-	-	-	-	-
Acetate	+	+	+	+	+	+	+
Benzoate	+	-	-	-	-	-	-
Citrate	+	-	+	-	-	+	+
Ethanol	+	+	+	-	+	-	-.
D-fructose	+	+	+	+	-	+	-
D-Glucose	+	+	+	-	-	+	+
Glutamate	+	-	+	-	v	+	+
Glycerol	+	+	+	-	+	-	-
Malate	+	+	+	+	+	+	+
Maltose	+	-	+	-	-	+	+
Mannitol	+	+	-	-	-	-	-
Succinate	+	+	+	+	+	+	+
Sulphide	-	-	-	+	-	-	-
Thiosulphate	-	-	-	+	-	-	-
Probable genus	*Rhodopseudomonas* sp.	*Rhodoferax fermentans*	*Rhodospirillum* sp.	*Chlorobium* sp.	*Rhodomicrobium* sp.	*Rhodospirillum* sp.	*Rhodobacter* sp.

Positive/Utilization: (+) ; Negative/Non Utilization : (-); Variable : (v)

In looking for the purple non-sulfur bacteria, sodium succinate or malate was used as an organic carbon source because these are not generally used by other bacteria under

photoheterotrophic growth conditions with no hydrogen sulfide.

In conclusion, seven different photosynthetic bacteria were isolated from the eight composite samples of municipal wastes collected from Ladakh and Kashmir divisions of Himalaya range. Out of them one isolate L6 was psychrophilic/ psychrotolerant photosynthetic purple non sulphur bacterium. The colonies of L6 were brown, smooth, circular and convex; Gram-negative, curved rods, motile, non-spore-forming cells; catalase, urease and nitrate reduction negative. All isolates showed growth in aerobic-light conditions except K2 and anaerobic-light except L6. All isolates utilized acetate, malate and succinate as carbon source. Isolate K2 had shown well defined growth in sulphide and thiosulphate media and was regarded as sulphur bacterium while as isolates G17, L6, L27, K5, S5 and S7 were unable to utilize sulphide and thiosulphate as electron donor and considered as non sulphur bacteria. The isolate L6 was confirmed as *Rhodoferax* sp., K2 isolate as *Chlorobium* sp., S5 and L27 as *Rhodospirillum* sp., S7 as *Rhodobacter* sp., G17 as *Rhodopseudomonas* sp., K5 as *Rhodomicrobium* sp. on the basis of varied morpho-biochemical characters. Isolates G17, L6, L27, S5 and S7 were observed the most efficient on the basis of versatility in carbon utilization character.

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