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SHORT COMMUNICATION

Psychrotrophic Hydrolytic Bacteria from Antarctica & Other Low Temperature Habitats

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ABSTRACT

Samples of water, soil, lake sediments and blue-green algal mats from Antarctica were processed for enumeration, isolation and screening of psychrotrophic hydrolytic bacteria. Amylolytic bacteria were preponderant (75 per cent) in the blue-green algal mat samples. Protease, lipase, amylase and urease producing bacteria were also isolated from the samples. Biochemical characteristics indicated that the isolates mainly comprised *Pseudomonas* and *Bacillus* species. Proteases and lipases of antarctic bacterial strains preferably hydrolysed denatured protein substrate and water soluble monomeric synthetic lipid substrates, respectively.

1. INTRODUCTION

Biodegradation offers a rapid, effective and convenient means of organic waste disposal. The rate of biodegradation gets reduced drastically at extreme low temperatures due to decreased rate of growth of microorganisms[1]. Cold-active bacteria (psychrophilic/psychrotrophic) have been isolated and investigated less often[2]. Bacteria that grow at low temperatures can be made use of for enhancing biodegradation in extreme cold habitats. Psychrophiles were defined as bacteria which have an optimum growth temperature of 15 °C or lower with an upper growth temperature around 20 °C. Psychrotrophs are able to multiply in the temperature range of 0° to 15 °C and grow optimally at around 25 °C. Cold-active bacteria and their enzymes have assumed considerable importance due to their biotechnological applications[3]. Various forms of bacteria have been isolated from the extreme low temperature habitats of Antarctica and other snowbound habitats[4]. In this regard, selection of samples, techniques for isolation of bacterial strains and screening for enzymes constitutes an important aspect of studies on cold-active bacteria. The present communication reports the isolation of psychrotrophic bacteria from samples of soil, water and decaying organic matter collected from Antarctica and some snowbound regions at high altitudes (Leh and Siachen). The isolated bacterial strains were

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screened for their capability to produce hydrolytic enzymes.

2. MATERIALS & METHODS

All microbiological media and media components used were procured from HiMedia Co., Bombay. All the chemicals used in media preparation were of the highest purity available commercially. Azocasein and Hammerstan casein were obtained from Sigma Chemical Co., USA and Sisco Research Laboratories, India, respectively. API biochemical gallery strips were purchased from La Balme Les Groltes, France. Nutrient agar, gelatin agar, skimmed milk agar, Hammerstan casein agar, tributyrin agar and starch agar were prepared as per standard procedures[5].

2.1 Sample Collection

The samples were collected around *Maitri* station in Antarctica. These included five soil, seven mud, six lichen and six blue-green algal mat samples taken from lakes and water bodies. Collections were done during summer season in 1994 (14th Antarctica Expedition). Care was taken to see that the sampling sites were 1-2 km away from the *Maitri* station, where there was no human activity. These samples were collected in 4-5 outings.

High altitude samples were obtained from the Siachen glacier region at an altitude of 4000 m during December 1997. The site from where samples were collected was about 1 km away from human activity. Three leaf litter, two mud and four soil samples were collected, each in 50 g lots. All samples were packed into sterile screw cap vials and brought to the laboratory in a refrigerated container.

2.2 Isolation of Bacteria

One gram quantity of each sample was suspended in 5 ml nutrient broth. Ten-fold dilutions were prepared again in nutrient broth. Samples of 0.1 volume were spread over nutrient agar in duplicate. These plates were incubated up to 10th day at 10 °C and 20 °C to obtain bacterial growth. However, bacterial counts were taken after 4-5 days of incubation. The resulting colonies were further sub-cultured on standard nutrient agar media.

2.3 Maintenance of Cultures

Each isolated culture was sub-cultured on nutrient agar slants in five replicates and stored at 5 °C in a refrigerator.

2.4 Detection of Enzymes

Pure cultures were streaked on starch agar/ azocasein/skimmed milk agar/gelatin/tributyrin agar. Plates were incubated at 10 °C for 10 days. The zones of hydrolysis were detected by flooding the plates with (a) iodine for amylase and (b) $HgCl_2 + HCl$ for protease. Lipolytic zones were clearly visible. Cultures were also grown in liquid media, and their cell-free broths were obtained by centrifugation at 10,000 rpm for 10 min. Enzyme activity in the broths was studied by agar well diffusion method[6]. In the case of synthetic lipase substrates, α-naphthyl acetate, hydrolysis was noticed by flooding the plates with Fast blue BB salt prepared according to the method of Lambrechts and Galzy[7].

2.5 Identification of Hydrolytic Isolates

From each group, a bacterial strain was selected for studying the biochemical characteristics (Table 1). The gram-negative, rod-shaped bacteria were identified using API 20E strips. A majority of the isolates could be typed to the genus level using this system. Tests available on the API 20E strips were also useful for identifying gram-positive bacteria. The isolates were identified using the results by reference to Bergey's Manual of Determinative Bacteriology[8]. Depending on the colony, cell morphology and biochemical properties, the gram-negative, rod-shaped motile bacteria with ability to grow aerobically and possessing the property to produce acids from glucose, mannose, galactose and fructose were identified as strains that belong to the genus *Pseudomonas*. The gram-positive, rod-shaped bacteria producing spores and hydrolysing gelatin, lipid and starch were assigned to the genus *Bacillus*. The gram-negative, motile, rod-shaped and citrate utilising isolate from the glacier region, fermenting

Table 1. Morphological and biochemical properties of isolates showing their ability to produce hydrolytic enzymes

	Pseudomonas sp.	*Bacillus* sp.	*Serratia* sp.	*Arthrobacter* sp.
Gram staining		+		
Cell morphology	Rod	Rod	Rod	Rod
Dimensions(μ)	0.5–1.0	0.6–1.0	0.5–0.8	0.6–1.0
Mobility				
Aerobic				
Spore former				
Oxidase	+			
Catalase	+			
β-galactosidase				+
Urease				
Indole production				
H_2S from cysteine				
Fermentation				
Glucose				
Mannose				
Galactose				
Fructose				
Mannitol				
Sucrose				
Gelatin hydrolysis	+			
Starch				
Tributyrin				
Tween 80				

glucose, sucrose and mannitol with β-galactosidase was identified as *Serratia* sp. A gram-positive isolate showing both coccus and rod-shaped cell morphology at different stages during the life cycle, with urease and gelatinase activities was assigned to *Arthrobacter* sp. The isolate produced cell-associated protease and urease. Therefore, only the whole culture broths produced hydrolytic zones at 5–30 °C.

3. RESULTS

Antarctic samples contained bacterial counts in the range of 10^3–10^4/g of sample (Table 2). The counts of amylolytic, proteolytic and lipolytic bacteria in each sample was enumerated. Bacterial growth on agar medium was noticed at 10 °C and 20 °C within 4-5 days.

3.1 Screening of Isolates for Enzymes

A total number of isolates obtained from the samples was 152. Of these, 41 isolates were proteolytic, 24 isolates were lipolytic and 8 isolates were ureolytic in nature. However, 22 isolates (Table 3) revealed the presence of both lipase and

Table 2. Enumeration of bacterial numbers from decaying blue-green algal mat samples

Sample No.	Total count	Bacterial count/g sample		
		Amylolytic	Proteolytic	Lipolytic
BGA S1	21×10^2	25×10^0	16×10^1	40×10^1
BGA S2	23×10^2	17×10^2	36×10^1	11×10^1
BGA S3	96×10^3	30×10^3	51×10^0	35×10^0
BGA S4	30×10^3	39×10^1	80×10^0	91×10^0
BGA S5	16×10^4	44×10^1	24×10^1	20×10^1
BGA S6	15×10^4	28×10^1	25×10^1	24×10^1

Table 3. Hydrolysis of various substrates by the isolate

	tease	ipase	Urease
(a) Antarctic isolates			
Pseudomonas fluorescens	+++		
Pseudomonas sp.	++++		
Pseudomonas sp. BGA2	++	+++	
Pseudomonas sp. BGA3	+++		
Pseudomonas sp. BGA4	++		
Bacillus sp.	++++	++	+
Bacillus sp. DL1	+		
Bacillus sp. DL3	++++		
Bacillus sp. FL1	++		
Bacillus sp. BT	+++	+	
Bacillus sp. KS	+++		
Bacullus sp. BL2	++		
Soil isolate DRD1	++		
Soil isolate DRD2	++	++	
Soil isolate U1	–		
Soil isolate U2			
Soil isolate U3	++		
Isolate BGA5	+	+	
Isolate BGA6	+		
Clarigester 1	++		
Clarigester 2	++		
(b) Leh, Laddakh isolates			
Serratia sp.	++		
Pseudomonas sp. DS	+		
(c) Siachen glacier isolates			
Serratia liquefaciens	++++	++++	+
Pseudomonas sp.	++	+	

* The markings indicate the diameter of the zone of hydrolysis. +, mm; ++, 8 mm; +++, 8-15 mm and ++++, >15-25 mm.

protease enzymes activities. Three isolates showed the ability to produce all the three enzymes-protease, lipase and urease, simultaneously. Isolates showing about 1 mm hydrolysis zone are shown in Table 3. The maximum activity of the hydrolytic bacteria (zone of hydrolysis) determined by this method was in range 15–25 mm. In contrast, culture supernatant of bacterial strain isolated from glacier regions ity and oliv

4. DISCUSSION

Psychrophilic and psychrotrophic bacteria can be isolated from almost all cold habitats. In the present study, samples consisting of soil, mud, blue-green algal mats and lichen were processed for the isolation of psychrophilic and psychrotrophic bacteria. The bacterial numbers enumerated in six decaying and live blue-green algal mat samples reveal the presence of amylolytic bacteria (74 per cent) followed by proteolytic and lipolytic bacteria. This indicates that the substrates for these

enzymes occur in the biomass of blue-green algae and are utilised by bacteria with the intervention of hydrolytic enzymes. Most of the isolates had shown growth on agar medium at 10–20 °C within 4-5 days. Both the *Pseudomonas* and *Bacillus* species were prevalent organisms in cold habitats of Antarctica which was due to their ability to utilise a wide range of carbon and nitrogen sources. Seven isolates were identified as *Pseudomonas* sp. strains. Gram-positive rod-shaped bacteria were identified as *Bacillus* sp. strains, depending on their biochemical characteristics. Two strains were found to be *Serratia* sp. on the basis of their biochemical properties. Among all the isolates, one *Pseudomonas* sp., one *Bacillus* sp. and one *Serratia* sp. were found to produce maximal levels of protease and lipase. Most of the proteases from these isolates (*Pseudomonas* sp., *Arthrobacter* sp. *and Bacillus* sp.) were unable to hydrolyse hammerstan casein. However, hydrolysis of azocasein and gelatin suggests that they are active against denatured protein substrates. Protease produced by *Serratia* sp. was active against hammerstan casein as well as azocasein and gelatin. Since the rate of hydrolysis of tributyrin and olive oil by the isolates was low, α-naphthyl acetate substrates proved useful in enumerating the isolates into lipolytic bacteria even at sub-ambient temperature. A wide variation was found among the hydrolytic abilities of bacterial isolates from Antarctica and high altitude snowbound areas of the Himalayas in terms of enzymie activity and substrate specificity. Several strains (Table 3) exhibited the ability to produce protease and lipase simultaneously in liquid medium and can be used to produce these enzymes for commercial use. Since *Serratia liquefaciens* and *Arthrobacter* sp. are able to produce β-galactosidase, they provide the source for purification and characterisation of enzymes from cold-active bacteria. Further work on purification and characterisation of these enzymes is in hand to explore the possibility of utilising them in the biodegradation of organic wastes at extreme low temperatures.

5. CONCLUSION

The present study indicates the presence of hydrolytic bacteria in extreme cold habitats of Antarctica and Himalayan regions. These isolates may find use in the biodegradation of organic wastes at low temperatures and for elucidating the cold-active adaptation of these enzymes.

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