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Occurrence and Extracellular Enzymatic Activity Profiles of Bacterial Strains Isolated from Hotspring of West Kameng District of Arunachal Pradesh, India

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Abstract: Extracellular enzymatic activity profiles of thermophilic bacterial strains isolated from hotspring of dirang area of west kameng district of arunachal Pradesh have been studied. Bacterial strains of spring water and sediments were assessed on nutrient agar medium. About 92% of the isolates tested showed extracellular enzymatic activity. Out of the 24 isolates screened 21, 19, 15 and 9 isolates exhibited protease, lipase, amylase and cellulase activities respectively.

Key words: Hotsprings · Thermophiles and Extracellular enzymes

INTRODUCTION

The potential of microorganisms as biotechnological sources of industrially relevant enzymes has stimulated a renewed interest in the exploration of microorganisms for extracellular enzymatic activity. The enzymes both active and stable at high temperature are of great technological potential [1]. The extreme environments as source of isolation and selection of useful microorganisms have been highlighted [2] and the progress in this area have been possible with the isolation of large number of thermophilic microorganisms from different exotic ecological ecological zones of the earth and subsequent extraction of useful enzymes from them [3]. The advances in genetics and microbial physiology have a strong impact enzyme production; screening programmes for the selection of microorganisms able to produce bioactive molecules continue to be an important aspect of biotechnology.

The samples were collected from the hotspring of dirang area of west kameng district of arunachal Pradesh, India. The hotspring is located on the bank of river Dirang-chu at an height of 1495m asl. Altogether 2 water and 2 sediment samples were collected from the hotspring. Water and sediments showed characteristic hydrogen sulfide odour. Temperature and pH of the water and sediments were assessed during sample collection [4]. Water and sediments were collected from the springs asceptically and transported to the laboratory in sterile conditions and processed later on. Weighted sediments samples were shaken and allowed to settle for 5 minutes. Serially diluted samples were centrifuged at 5000 rpm for 15 minutes as pretreatment to make the extraneous matter to settle. Nutrient agar medium (HIMEDIA) was used to isolate the bacterial strains (the medium was sterilized by autoclaving at 15lbs pressure, 121°C for 15 minutes). Serially diluted samples were inoculated by spread plate method. The plates were incubated at 47± 2°C to isolate the thermophilic strains. All the plates were incubated up to 48 hrs.

The plates were assessed for bacterial colonies after 48 hrs of incubation. The bacterial colonies appeared in the agar plates were selected based on the morphological characteristics. After isolation the pure cultures of the isolates were maintained on nutrient agar slants for further investigation. Altogether 24 strains were screened for extracellular enzymatic activity. The strains were screened for the enzymatic activity at the same temperature mentioned above. Cultures were screened for their ability to hydrolyze starch on nutrient agar medium (Peptone 5; Yeast extract 1.5; beef extract 1.5; NaCl 5; agar 16; distilled water 1000 ml; pH 7.2) with 1% soluble starch as substrate. Later the plates were flooded with 1% iodine in 2% KI. The clear zones around the colony indicated the amylase activity [5]. The strains were screened for lipase activity on Tributyrate agar medium (Nutrient agar with 1% Glycerol tributyrate as substrate. A clear zone developed around the colony indicated lipase activity [6]. The strains were screened for cellulose activity in agar medium with 1% CMC (Carboxy methyl cellulose) as substrate. The plates were incubated and stained with Congo red dye and destained [7]. The

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positive cellulase activity is shown as presence of yellow hallo against red background. The strains were screened for proteolytic activity soft agar overlay containing non fat milk powder as substrate. Positive protease activity was detected by the presence of clear zone [8].

The numbers of hotsprings occurrence are large but the knowledge on hotspring microflora is scanty [9]. Screening of microbial strains from the northeastern region of India deserves special attention to explore the vast potentialities of endemic and diverse microflora of this region for tier commercial usage as north east India has been identified as the indoburma mega biodiversity hotspot by conservation international [10]

Temperature and pH of the water and sediments were assessed at the time of collection. Temperature and pH of the water were 42°C and 8.1 while for the sediments 41°C and 7.9°C, respectively. Thermophiles are more in sediments samples plated in nutrient agar medium than water samples. Out of the 24 strains screened 22 showed positive activity for anyone of the enzyme tested. A maximum number 19 isolates showed lipase activity followed by 21 showing protease activity, 15 have shown amylase activity and 9 have shown cellulase aqctivity represented in Table 1.

Our study clearly revealed the potential of bacterial strains and their ability of extracellular enzyme production. Almost 92% of the strains have shown extracellular enzymatic activity for anyone of the enzyme screened. The highest lipolytic activity was shown by the thermophile by DH4 based on the zone of clearance. The lipases from thermophilic bacteria are gaining interest with application in detergent and diary industries [11-12]. In the recent years there is an increasing interest in proteases from thermophilic bacteria due to its inherent ability in various industrial and biotechnological applications [13-14], the isolates DH3 and DH10 have shown maximum proteolytic activity. Maximum anylase activity has been shown by the isolate DH7. The amylases from thermphiles are gaining interest in various industrial and biotechnological applications [15-16]. The percentage of organisms having cellulytic activity are less in comparison to other enzymes there are only few reports from bacterial strains. DH8 has shown maximum cellulytic activity [14].

The above study clearly revealed new and interesting perspectives showing that bacterial strains isolated from hotsprings, represents a source of several enzymes that can be exploited potentially for biotechnological purpose.

Table 1: Enzyme activity of thermophiles from hot springs

	Zone of Digestion of the substrate (mm)			
Isolates	Lipase	Amylase	Protease	Cellulase
DH1	0.0	0.0	12.4	7.4
DH2	12.0	0.0	10.2	0.0
DH3	0.0	6.2	18.2	0.0
DH4	12.0	8.2	14.0	0.0
DH5	16.8	8.0	10.4	0.0
DH6	0.0	12.4	0.0	8.0
DH7	12.0	16.2	18.4	0.0
DH8	15.0	8.0	10.4	8.4
DH9	8.0	8.2	6.0	4.0
DH10	14.0	6.0	18.2	0.0
DH11	12.0	0.0	10.4	0.0
DH12	14.0	0.0	6.0	10.0
DH13	10.0	12.2	16.0	8.0
DH14	14.2	0.0	12.2	2.8
DH15	0.0	0.0	0.0	0.0
DH16	8.0	6.2	10.0	0.0
DH17	14.0	8.0	6.4	0.0
DH18	9.2	5.2	7.0	0.0
DH19	8.0	6.0	8.4	0.0
DH20	0.0	0.0	0.0	0.0
DH21	6.0	0.0	4.0	3.0
DH22	11.2	14.0	8.4	10.4
DH23	11.2	10.4	9.6	0.0
DH24	10.0	0.0	7.4	0.0

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