Occurrence and Extra cellular Enzymatic Activity Profiles Of Bacterial Strains Isolated From Hot springs Of Western Coastal Districts of Maharashtra, India

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Abstract

Extracellular enzymatic activity profiles of thermophilic bacterial strains isolated from hotsprings of Namboli area of the Western coastal district of Maharashtra have been studied. Bacterial strains of spring water and sediments were enriched in various nutrient mediums and later isolated on the same with gelarite as the solidifying agent. About 98% of the isolates tested showed extracellular enzymatic activity. Out of all the isolates screened, 8 isolates exhibited protease, amylase and cellulase activities respectively.

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.

MATERIALS AND METHODS

The samples were collected from the hotspring of Namboli and Dhapoli area of western coastal districts in (Mumbai and Ratnagiri, recording the temperature of 60°C and 71°C respectively) of Maharashtra, India. Altogether 2 water and 2 sediment samples were collected from each of the hot springs. 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 hot springs aseptically and transported to the laboratory in sterile conditions and processed later on. Weighted sediments samples as well as the water samples were subjected to various enrichment Medias, viz; HS Media, Yeast Soluble Starch broth media, M9 Media, Nutrient Media, Potato Dextrose Media, Thermus Media, Marine Salt Media etc, and later isolated on the same using gelarite as a solubalizing agent. Serially diluted samples were inoculated by spread plate method. The plates were incubated at 60± 2°C to isolate only the thermophilic strains. All the plates were incubated up to 48 hrs.

RESULTS

The plates were assessed for bacterial colonies after 48hrs of incubation. The bacterial colonies appeared on the agar plates were selected based on the morphological characteristics. After isolation the pure cultures of the isolates were maintained on the respective agar slants for further investigation. Altogether 20 strains were screened quantitatively 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 positive cellulase activity is shown as presence of yellow hallo against red background. The strains were screened for proteolytic activity on milk agar plates. Positive protease activity was detected by the presence of clear zone ([8]).The strains were further assayed for their qualitative enzyme activity.

Temperature and pH of the water and sediments were assessed at the time of collection. Temperature and pH of the water were 65°C and 6.1 while for the sediments 65°C and 6.0 respectively. The physiochemical analysis of the water and sediment samples helped in designing the enrichment and isolation Medias (Table-1).

Figure 1

Table 1: Enzyme activity of thermophiles from hot springs

Sr.No	Test Conducted	Result
1	pH	6.05
2	Specific Conductivity (ms/cm)	2.44
3	Chloride as Cl (mg/L)	679
4	Total Alkalinity as HCO_3 (mg/L)	29
5	Sulphate as SO_4 (mg/L)	135
6	Borate as B (mg/L)	0.084
7	Fluoride as F (mg/L)	3
8	Total hardness as $CaCo_3$ (mg/L)	350
9	Calcium as Ca (mg/L)	144
10	Magnesium as Mg (mg/L)	Nil
11	Sodium as Na (mg/L)	270
12	Pottasium as K (mg/L)	8
13	Total dissolved solids (mg/L)	1528
14	Manganese as Mn (mg/L)	Nil
15	Silica as Sio_2 (mg/L)	32
16	Iron as Fe (mg/L)	Nil

Out of the 20 strains screened 18 showed positive activity for all the enzymes tested. A maximum number of 8 isolates showed heavy activity for amylase, cellulose as well as protease activity as represented in (Table-2).

Figure 2

Isolates	Zone of digestion of substrate (mm)			
	Lipase	Amylase	Cellulase	Protease
NB3	0	12.0	5.0	0.9
NB2	0	6.1	2.0	0.7
NBG1	0	11.2	8.6	0.6
GBn	0.1	12.0	2.2	0.2
GBu	0	10.6	3.6	0.8
Tact	0	3.6	6.3	13.0
T1	3.0	4.2	3.8	1.1
GS	0	11.0	7.1	1.3
S	0	1.3	1.6	0

DISCUSSION

The Physiochemical studies infers that Chloride, Sulphate, Sodium and total dissolved solids were found to be in higher concentration as compared to the chemical properties reported by the Geothermal Energy resources of India. The enrichment as well as the growth Medias were designed in accordance to the results obtained. Our study clearly revealed the potential of bacterial strains and their ability of extracellular enzyme production. Almost 98% of the strains have shown extracellular enzymatic activity for all the enzyme screened. 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 Tact and T1 and GS have shown maximum proteolytic activity. Maximum amylase activity has been shown by the isolates NB3 and Gbn, others showed a substantial degree of activity. The amylases from thermophiles are gaining interest in various industrial and biotechnological applications ([15], [16]). The percentage of organisms having cellulytic activity is less in comparison to other enzymes there are only few reports from bacterial strains. But the Thermophilic cultures isolated by us showed diverse activity, isolates NBG1, Tact and GS have shown maximum cellulytic activity ([14]). The cultures dint proved to be Lipase producers, only T1 showed some Lipolytic activity.

CONCLUSION

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.

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