Extracellular Enzymatic Activity of Bacterial Strains Isolated from a Local Hotspring Tarabalo, Nayagarh District, Orissa, India

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Citation

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Abstract

Studies on the 49 extracellular enzyme producing thermophilic bacteria isolated from a sulphur hotspring Tarabalo, Orissa, India were carried out. Sediment and water sample of the hotspring were assessed on Nutrient agar and MacConkey agar medium for bacterial strains. Out of 49 isolates, 77.75%, 97.95%, 87.75%, 83.67%, 87.71% of the strains showed amylase, protease, caseinase, lipase, cellulase activities respectively by plate assay method at 54˚C.

INTRODUCTION

Thermophilic microorganisms have gained a great deal of attention in the last two decades (Becker et al. 1997, Beg et al. 2000, Lee et al. 1999, Sonnleitner and Fiechter 1983). Enzymes from these microorganisms are of special interest since they are not usually denatured by high temperature, even active at elevated temperature (Adams and Kelly 1998, Fitter and Heberle 2000, Ladenstein and Antranikian 1998, Touzel et al. 2000, Zeikus et al. 1998) and these are also more resistant to chemical reagents and extreme pH values in comparison to their mesophilic homologues (Hough and Denson 1999, Schiraldi and Rosa 2002, Pentazaki et al. 2000). Their thermostability is associated with performing enzymatic reaction at high temperature allows higher substrate concentrations, lower viscosity, fewer risk of contamination and often higher reaction rates (Seatovic et al. 2004). The new potential of using microorganisms as biotechnological sources of industrially relevant enzymes have stimulated a renewed interest in the exploration of extracellular enzymatic activity (EEA) in Thermostable Bacteria (Buzini and Martini 2002).

The aim of this study was to asses extracellular enzymatic activities of thermophilic bacteria in Tarabalo a local sulphur Hotspring of Orissa, India.

MATERIALS AND METHODS

ISOLATION OF BACTERIA

The samples were collected from three sites of the thermal spring of Tarabalo area of Nayagarh district of Orissa, India. It is the second largest hotspring in India, as per the report of Tourism Development Corporation, India. Tarabalo is still a virgin hotspring in regard to microbial wealth, exploration. Temperature and pH of the sediment and water sample were measured in the field after collection. Soil and water samples were collected from three different sources in Tarabalo Hotspring aseptically and transported in to the laboratory in sterile polythenebags (Sediment samples) and autoclaved glass vials (Water samples) respectively. One gram of the sediment sample was suspended in 5 ml of sterile distilled water that served as stock sample, which was streaked on Nutrient agar (NA) and MacConkey Agar (MA) plates. Where as one loop of water sample was directly streaked on the above said plates. (Media were prepared by addition of NB and MB+1.5%Agar+50% distilled water+50% hotspring water for NA and MA respectively). The plates were packed in polythenebags to avoid drying and were incubated in 54C. After 24-48 hours of incubation, the bacterial colonies were selected based on their morphological characteristics and picked up for pure culture on modified NA, medium. Isolated colonies were preserved on NA slants at room temperature for future use. Further, sediment and water samples were serially diluted (10 fold dilution) and subjected for total plate count through spread plate and pour plate method for enumeration of bacteria on NA plates followings the method of Rath and Subramanyam (1998).

SCREENING PROCEDURES

All the 49 isolates were assessed for their extracellular enzymatic activity. The strains were screened on solid agar media at two different temperatures 38C and 54C in triplicates.

Freshly grown cultures of test bacteria were spot inoculated on starch agar plates by the help of a sterile loop. Amylase activity was observed by incubating the plates and exposing to iodine vapours (Rath and Subramanyam 2000). Protease activity of the isolates was studied by growing isolates on skimmed milk agar plates (Rath 1996). Similarly caseinase activity was also studied by observing the zone of clearance around the colonies where the isolates were incubated at 54C, after spot inoculation on casein (1%) nutrient agar plates. Lipase activity of the isolates was reported by inoculating the isolates into tributryn agar plates, following the method of (Rath 1999). Cellulase activity of the isolates was studied on carboxy methyl cellulose (CMC1%) agar plates following the method of (Rath and Subramanyam 1997)

RESULTS

Water and sediments showed a chaterstics hydrogen sulphide odour. During sample collection the temperature and pH of water and sediments were measured, to be 56.6 C, 8.5 and 56.1C, 8.45 respectively. Bacteria were more in sediment in comparison to the hotspring water, (Table-1). Forty-nine bacteria were selected based on morphological features and growth on NA and MA plates named HCTB-1 through HCTB-49. All 49 isolates were screened for different extracellular enzymatic activities. The strains were assessed for enzymatic activity in terms of zone sizes and substrate digestion at the temperature mentioned below Table-2 & Fig.1.

Figure 1

Table-1: Total Plate Count (TPC) of bacteria

Samples	No of sample	Bacteria CFU/gm or ml of sample on NA plate	
		Spread plate	Pour plate
Sediment1	1	8.5×10^6	7.1×10^4
Water1	1	5.5×10^4	4.3×10^3
Water2	1	5.1×10^4	4.8×10^3

Figure 2

Table-2: Enzyme activity of thermophiles

Isolates	Zone of digestion of the substrate (mm)									
	Amylase		Protease		Casinase		Lipase		Cellulase	
	38°C	54°C	38°C	54°C	38°C	54°C	38°C	54°C	38°C	54°C
HCTB1	-	-	12.0±2.2	28.0± 1.8	7.0±0.8	21.0±2.3	8.0±0.0	12.0±1.1	6.0±0.0	7.0±0.0
HCTB2	-	-	-	-	6.0±1.0	13.0±1.0	12.0±0.6	16.0±1.5	4.0±1.0	8.0±1.1
HCTB3	-	-	10.0±1.8	21.0±2.1	-	-	-	-	3.0±1.2	8.0±2.5
HCTB4	-	-	14.0±1.2	18.0±0.8	13.0±1.1	20.0±2.3	-	-	5.0±0.8	10.0±0.0
HCTB5	10.0±0.0	15.0±2.5	11.0±0.5	26.0±1.0	7.0±1.4	13.0±2.1	9.0±0.5	18.0±1.0	3.0±0.4	6.0±1.3
HCTB6	8.0±0.8	14.0±3.0	12.0±0.8	28.0±1.9	5.0±1.3	11.0±1.3	6.0±0.0	16.0±0.7	3.0±1.0	6.0±1.4
HCTB7	-	-	15.0±1.0	26.0±2.6	1.0±0.0	14.0±1.8	5.0±0.0	13.0±0.5	3.0±1.1	5.0±0.8
HCTB8	6.6±0.6	13.5±2.5	11.0±0.0	19.0±1.2	11.0±0.6	18.0±1.3	8.0±0.6	15.0±1.9	4.0±1.8	13.0±1.0
HCTB9	11.0±1.9	16.5±1.5	12.0±1.3	25.0±2.6	6.0±0.5	10.0±1.9	-	-	5.0±2.1	6.0±0.0
HCTB10	-	-	11.0±1.8	29.0±2.8	5.0±0.4	8.0±0.6	5.0±0.0	4.0±0.0	4.0±2.0	5.0±0.0
HCTB11	-	-	10.0±2.0	21.0±2.0	9.0±0.8	22.0±2.4	11.0±1.0	25.0±1.5	8.0±2.3	14.0±2.8
HCTB12	10.0±2.8	16.5±2.6	20.0±2.5	22.0±1.6	5.0±0.5	16.0±1.3	9.0±0.6	16.0±1.1	5.0±1.6	6.0±1.7
HCTB13	-	5.0±2.1	15.0±1.6	36.0±1.3	4.0±0.6	12.0±1.2	3.0±0.0	12.0±0.6	5.0±1.4	5.0±2.1
HCTB14	8.3±2.3	11.5±2	9.0±0.8	19.0±1.0	7.0±0.6	16.0±0.6	4.0±0.5	10.0±1.7	4.0±0.4	6.0±2.4
HCTB15	9.3±1.3	13.0±0.0	9.0±0.6	19.0±0.0	9.0±0.4	18.0±1.7	3.0±0.6	10.0±0.7	3.0±0.5	7.0±1.7
HCTB16	16.0±1.7	9.5±1.5	17.0±1.8	29.0±2.3	3.0±0.0	5.0±0.0	4.0±1.1	7.0±0.0	4.0±0.8	5.0±2.2
HCTB17	12.3±3.1	20.5±3.5	13.0±1.1	31.0±3.6	6.0±0.0	16.0±1.6	12.0±0.7	26.0±0.8	9.0±1.3	13.0±3.6
HCTB18	12.6±2.2	17.5±3.2	15.0±1.4	26.0±3.1	12.0±1.0	21.0±1.7	8.0±0.6	17.0±1.5	11.0±1.6	15.0±3.1
HCTB19	13.0±0.0	21.5±4.1	11.0±2.1	23.0±1.1	5.0±0.8	10.0±1.1	7.0±0.8	7.0±1.5	7.0±1.0	8.0±1.3
HCTB20	7.3±0.8	13.3±2.2	10.0±0.6	21.0±2.2	7.0±0.7	12.0±1.8	4.0±0.0	8.0±1.9	3.0±0.0	6.0±1.8
HCTB21	-	-	12.0±1.3	25.0±2.4	-	-	5.0±0.0	6.0±0.0	4.0±0.0	5.0±2.3
HCTB22	14.0±0.0	16.0±2.3	11.0±1.2	21.0±1.9	6.0±1.0	11.0±1.0	8.0±0.8	21.0±2.6	2.0±0.6	6.0±1.0
HCTB23	14.6±2.2	13.3±1.6	18.0±2.8	39.0±1.0	5.0±1.1	9.0±1.1	7.0±0.0	5.0±0.6	-	-
HCTB24	10.0±1.2	16.0±2.0	15.0±0.8	27.0±1.7	7.0±1.5	15.0±1.7	-	17.0±0.8	3.0±0.8	3.0±0.0
HCTB25	5.3±0.0	9.0±0.0	12.0±0.0	22.0±2.2	5.0±0.7	6.0±0.0	8.0±2.1	16.0±1.0	3.0±0.9	3.0±1.1
HCTB26	7.0±1.0	13.0±1.4	11.0±0.4	21.0±2.6	11.0±0.0	14.0±2.3	11.0±1.5	14.0±1.2	2.0±0.0	3.0±1.0
HCTB27	7.6±1.4	13.0±1.2	8.0±0.0	17.0±2.0	8.0±0.6	8.0±0.6	9.0±1.7	14.0±2.0	3.0±0.5	4.0±1.4
HCTB28	4.6±1.1	10.0±1.0	15.0±0.0	36.0±0.0	11.0±1.1	13.0±0.9	10.0±1.8	7.0±0.6	-	-
HCTB29	7.6±2.2	14.0±2.0	11.0±2.1	29.0±3.5	6.0±0.4	12.0±0.7	7.0±2.1	13.0±0.0	4.0±1.2	6.0±1.8
HCTB30	9.0±3.1	12.6±1.8	20.0±2.2	22.0±0.5	11.0±0.8	18.0±1.6	6.0±1.6	8.0±0.7	3.0±1.0	5.0±1.6
HCTB31	7.6±0.0	9.5±3.5	26.0±1.6	27.0±1.7	7.0±0.0	6.0±0.0	-	-	-	-
HCTB32	9.3±2.0	15.5±3.1	12.0±0.6	22.0±0.8	10.0±1.1	11.0±1.0	8.0±1.4	14.0±0.9	-	-
HCTB33	8.0±2.4	13.3±2.4	22.0±1.9	31.0±1.0	7.0±1.2	8.0±0.4	-	-	-	-
HCTB34	9.6±2.1	17.0±2.1	8.0±0.0	18.0±3.1	9.0±1.4	13.0±0.6	11.0±0.7	23.0±1.7	4.0±1.0	13.0±2.9
HCTB35	7.6±1.8	15.5±2.0	9.0±0.4	12.0±2.4	-	-	3.0±0.0	16.0±1.4	-	-
HCTB36	-	-	13.0±1.2	25.0±1.2	8.0±0.5	17.0±0.8	6.0±0.5	6.0±0.8	2.0±0.0	3.0±1.0
HCTB37	-	-	12.0±1.6	26.0±1.8	7.0±0.3	13.0±1.3	4.0±0.0	5.0±0.3	5.0±2.2	2.0±0.0
HCTB38	7.6±0.9	14.0±2.3	9.0±1.6	13.0±2.3	5.0±0.6	16.0±1.9	6.0±0.9	10.0±2.1	4.0±1.7	2.0±1.1
HCTB39	7.7±0.7	14.0±1.2	15.0±1.9	30.0±1.4	-	-	10.0±0.4	16.0±3.4	9.0±2.7	16.0±3.4
HCTB40	-	10.0±1.0	12.0±2.2	13.0±0.7	7.0±0.0	8.0±1.0	-	-	8.0±2.2	2.0±1.0
HCTB41	-	-	15.0±2.7	33.0±1.4	-	-	5.0±0.0	4.0±0.0	3.0±1.1	3.0±1.3
HCTB42	10.0±1.4	14.0±0.0	16.0±2.9	24.0±1.6	11.0±0.8	16.0±1.1	-	-	2.0±0.0	3.0±0.0
HCTB43	6.0±0.6	13.0±0.0	9.0±1.1	16.0±2.1	5.0±0.0	11.0±1.0	12.0±1.0	16.0±0.5	3.0±0.5	4.0±1.6
HCTB44	8.0±0.8	12.0±3.8	12.0±2.4	26.0±1.9	6.0±0.6	12.0±2.1	6.0±1.2	12.0±1.0	4.0±0.8	4.0±2.1
HCTB45	10.0±1.2	16.0±2.7	14.0±0.8	29.0±1.3	8.0±0.8	10.0±2.3	11.0±1.8	-	3.0±0.4	3.0±1.5
HCTB46	11.0±1.8	13.0±2.1	13.0±1.2	24.0±2.1	-	-	12.0±1.1	6.0±0.0	5.0±1.8	4.0±2.0
HCTB47	7.0±0.6	11.0±1.2	12.0±3.1	20.0±1.2	11.0±1.0	16.0±0.6	8.0±1.7	18.0±0.4	5.0±1.4	9.0±3.0
HCTB48	7.5±0.8	15.0±2.4	10.0±2.6	18.0±1.7	8.0±1.0	12.0±0.7	7.0±0.8	13.0±1.8	-	-
HCTB49	9.0±0.0	14.0±2.1	12.0±1.7	22.0±1.2	9.0±1.5	13.0±0.8	6.0±0.6	12.0±2.1	3.0±0.0	4.0±1.2

Thirty eight, forty eight, forty three, forty one and forty two isolates out of 49 isolates screened, showed positive activity for amylase, protease, caseinase, lipase and cellulase respectively, though enzymatic activities were observed at 38C, an increased enzymatic activity in terms of zone sizes was reported at higher temperature (54C), indicating the thermophilic nature of the isolates as well as the thermostability of the enzymes. Surprisingly HCTB-13 and HCTB-40 did not show amylase activity at low temperature (38C), but the activity was reported at higher temperature (54C) with a zone diameter of 5mm and 10mm respectively (table-2). Similarly HCTB-24 represented lipase activity at 54C with a zone diameter of 17mm, where as no activity was reported at lower temperature (38C). No such distinctions were reported while studying the protease and cellulase activities of the isolates during the investigation.

But the isolate HCTB-45, which showed lipase activity at 38C, did not show activity at 54C. A maximum of 48 isolates showed protease activity on skimmed milk agar plates. The % enzymatic activities of the isolates are presented in Table-3.

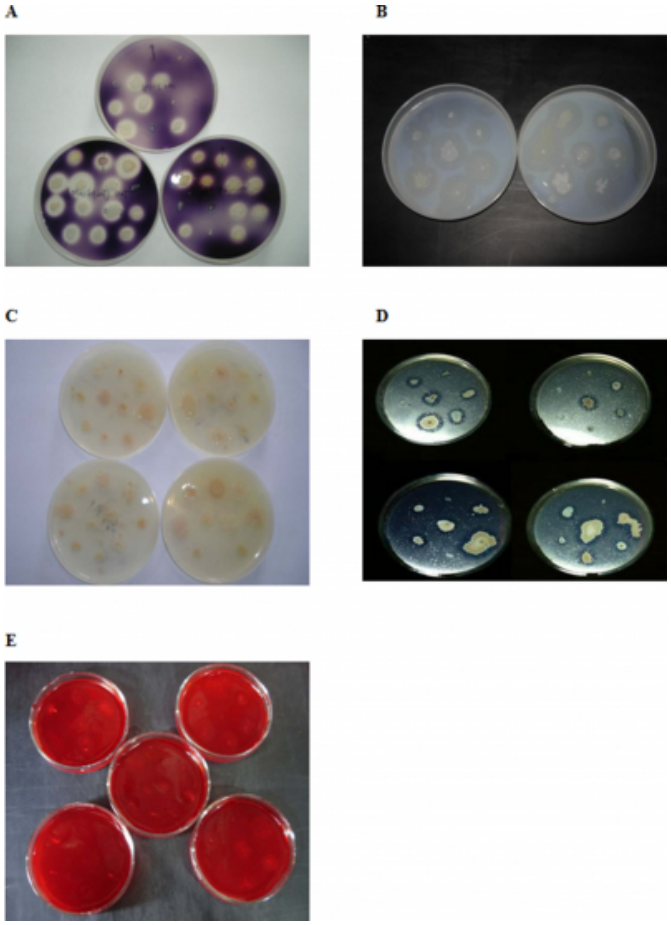
Figure 3

Table-3: Percentage of isolates showing different enzymatic activities by plate assay method.

Enzymes	% of isolates showing activity	
	38^0C	54^0C
Amylase	73.46	77.55
Protease	97.95	97.95
Caseinase	87.75	87.75
Lipase	83.67	83.67
Cellulase	87.71	87.71

Figure 4

Fig.-1. Extracellular enzymatic activity of the isolates at 54C. A. Amylase activity B. Protease activity C. Caseinase activity D. Lipase activity E. Cellulase activity.



DISCUSSION

In this investigation we clearly recorded the occurrence of bacterial strains, in the hotspring Tarabalo, in the distinct of Nayagarh in state of Orissa, with industrially important extracellular enzymatic activities. It is worth to mention that most of the amylolytic enzymes produced by various microbial strains are not able to function efficiently under the condition prevailing in industrial process. Hence there is a constant search for better performers (Kelly and Fogarty 1983). In this regarded we recorded amylase activity of 38 bacterial isolates at 54C could be attractive candidates for commercial amylase. The smaller zone sizes on solid agar plates are presumably because of low amounts of lipase molecules released by the colonies (Kouker and Jaeger 1987). It is well established that the logarithim of the lipase activity is linearly related to the zone diameter there by fulfilling a requirement of a valid agar assay (Lawrence et al. 1967). Lipases from thermophilic microorganisms are gaining interest with application in detergent and diary industries (Rath 1999). Because of their inherent ability in different industrial and biotechnological application protease from thermophilic bacteria are gaining interests in recent years (Coolbear et al. 1991&1992, Rath and Subramanyam 1996). The percentages of organisms having cellulolytic activity are less in comparison to other enzymes studied from bacteria (Rath and Subramanyam 1997, Bora and Kalita 2007). In this investigation we reported 87.71% of the isolates showing extracellular cellulolytic activity at 54C, could be novel sources for different industrial processes using cellulases as a catalyst.

CONCLUSION

The above investigation clearly revealed thermostable extracellular enzymatic activity of bacterial strains isolated from a local Hotspring. To the best of our knowledge, perhaps this study is first of its kind to explore the bacterial wealth of Tarabalo Hotspring in the state, Orissa, India. Studies such as this is a prerequisite for tapping the biotechnological potential of the microbes from this unique ecosystem.

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