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Isolation and screening of thermophiles producing Lipase enzyme

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ABSTRACT

Samples were collected from Aravali, Unhale and Unhavare hot water springs and subjected to isolation of thermophiles. The thermophilic isolates obtained were screened out to check their ability to produce Lipase enzyme and named. Only one isolate was found worth studying further on the basis of yield of lipase. The isolate was identified as *Bacillus sonorensis* SGRP 3.

Key words : *Thermophiles, Lipase, Bacteria*

Introduction

Thermophiles are those organisms which grow above 40°C, having optimum growth temperatures between 50 to 55°C. (Gleeson *et al.,* 2013). Hot water spring is one of the special niches which harbours thermoduric and thermophilic organisms (Khaled *et al.,* 2022; Elkazzaz *et al.,* 2020). Recently, thermostable lipases are in great demand due to their ability to function at relatively high temperature, which aids in accelerating reaction rate, substrate solubility and also prevent contamination by mesophiles. Lipases belong to triacyl glycerol ester hydrolase EC3.1.1.3 class. Lipases break down triglycerides into free fatty acids and glycerol by breaking down ester bond. Though lipases are produced by animal and plants, microbial lipases have received attention due to their wide variety of biotechnological applications, diversity of catalytic activities, high yields, ease of genetic manipulation (Hansan *et al*., 2006). Amongst microbes producing lipases, thermophiles are specially important because some fats have melting points higher than room temperature where the thermostable lipases produced by these thermophiles come to rescue (Lee *et al.,* 1999).

Materials and Methods

Collection of samples

Aravali, Unhale (Rajapur), Unhavare hot water spring samples were collected aseptically in presterilized container, brought to laboratory and stored at 4 °C.

Isolation of thermophiles

0.1 mL of hot water spring sample was spread inoculated on Thermus agar (ATCC medium 697) (Sikdar *et al*., 2015; Elkazzaz *et al.,* 2020) and then the plates were incubated at 50 °C for 24-48h. After incubation well isolated representative colonies were purified and preserved for further research work.

Screening of Lipase producing organisms

The suspension of isolate was spot inoculated on Tributyrin agar and then the plates were incubated at 50 °C for 24-48 h. The isolate that showed zone of hydrolysis of fats around the growth was consid-

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ered as positive for Lipase production. Relative enzyme activity (REA) was calculated using formula given below (Suresh *et al.,* 2020)

$$REA = \frac{\text{Diameter of zone of hydrolysis (mm)}}{\text{Diameter of growth (mm)}}$$

The isolate which exhibited maximum REA was selected for further study.

Production of Lipase enzyme

100 mL of Tributyrin broth was inoculated with 1% inoculum of promising isolate selected from screening study. The broth was then incubated for 24-48 h at 50 °C in shaker incubator.

Enzyme assay (Sarkar and Chourasia, 2017) (Thimmaiah, 1999)

After specified period of incubation, the production medium was centrifuged and supernatant was used as crude enzyme sample. Enzymatic assay was carried out by titrimetric method where initial and final titration readings were noted. For final reading separate set was run where to 25ml D/W, 100mg sodium taurocholate and 2 ml of oil mixture, 15 ml enzyme sample was added kept on vortexed for 30 mins after which it was titrated against 0.1N NaOH solution using phenolphthalein indicator. Similar separate set was run for initial titration without addition of enzyme sample. This was done to estimate the amount of free acids present if any in the oil.

Identification of Isolate

The isolate was identified by 16S r RNA gene sequencing. The closest strain of the isolate was determined by BLAST and phylogenetic analysis. Afterwards the gene sequence was deposited in NCBI and accession no was obtained.

Results and Discussion

Isolation of thermophiles

Total 15 thermophilic isolates were obtained from all the hot water samples. Out of these 15 isolates, five isolates each were obtained from Aravali, Unhale and Unhavre each.

Screening of isolates

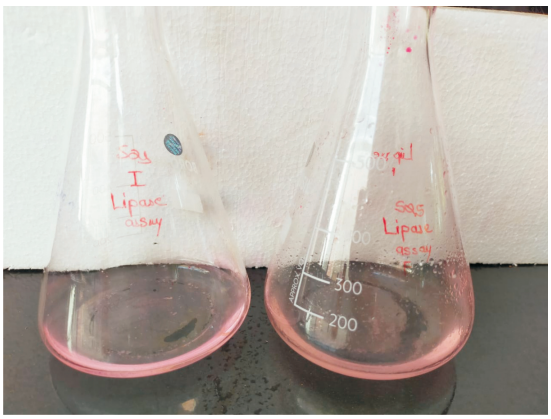
When the isolates were subjected to screening test, eleven isolates were found to produce lipase enzyme. When the Relative lipase activity was determined, one isolate was found to be promising. This isolate was then used for production of enzyme.

Table 1. Screening of Isolate for Lipase production

Sr.No	Isolate	Collectionsite	Lipase activity	REA
1	IS1	Aravali	+	1.28
2	IS2	Aravali	+	1.28
3	IS3	Aravali	+	1.25
4	IS4	Aravali	+	1.25
5	IS5	Aravali	-	-
6	IS6	Unhale	-	-
7	IS7	Unhale	-	-
8	IS8	Unhale	+	1.28
9	IS9	Unhale	+	1.25
10	IS10	Unhale	+	1.83
11	IS11	Unhavare	+	1.28
12	IS12	Unhavare	-	-
13	IS13	Unhavare	+	1.33
14	IS14	Unhavare	+	1.33
15	IS15	Unhavare	+	1.25

Enzyme assay

The selectedpomising isolate was found to produce 0.57 U/ mL/min of lipase enzyme. (Photoplate 1)



Photoplate 1: Lipase assay-Titration Initial and Final

Identification of isolate

Isolate was found to have maximum similarity with *Bacillus sonorensis* in phylogenetic analysis of the 16Sr-RNA gene sequence and named as *Bacillus sonorensis* SGRP 3. (Fig. 1) After submitting 16Sr RNA gene sequence to the NCBI genebank, the Accession no: OQ121796 was obtained. Nerurkar *et al.* have reported production of lipase enzyme by mesophilic *Bacillus sonorensis* strain isolated from marine clams from Kalbhadevi estuary, India. In another study, *Bacillus sonorensis* 4R isolated from Thar desert was found to produce hyperthermostable alkaline lipase. Prior to this *Bacillus sonorensis* was re-

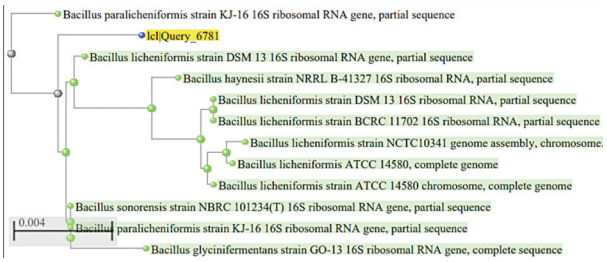


Fig. 1. Phylogenetic tree

ported to be isolated from Sonoran dessert and Kalbhadevi estuary (Bhosale *et al.*, 2016). Analysis of above reports makes it clear that different strains of *Bacillus sonorensis* can produce lipase at different temperatures ranging from Mesophilic to hyperthermophilic limits.

Conclusion

Five thermophilic isolates were obtained from each of samples collected from each of three hot water spring viz Aravali, Unhale and Unhavare making the sum total of fifteen isolates. Out of these fifteen isolates eleven were found to Lipase producers. Amongst them only one isolate (Isolate 10) was worth studying further on the basis of quantum of hydrolysis strain and named as *Bacillus sonorensis* SGRP 3. The isolate was identified as *Bacillus sonorensis* SGRP 3 on the basis of 16S rRNA gene sequencing and phylogenetic analysis. This isolate was found to produce 0.57U/mL/min of lipase enzyme. Hence, it can be concluded that hot water sample can serve as potential source of thermophilic isolates producing lipase enzyme. Such sites should be explored more and more to search out biotechnologically potential variety of isolates. The isolate *Bacillus sonorensis* SGRP 3 obtained should be subjected to optimization study so as to obtain higher yield. The thermophilic lipase possesses industrial applications.

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Conflict of interest

The authors declare that they have no conflict of interest.

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