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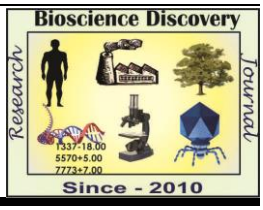

Exploration of Tural hot water spring for thermostable Lipase producer

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Abstract

Two thermophilic bacteria were isolated from Tural hot water spring, located at Sangmeshwar tehsil of Ratnagiri district in Maharashtra, India. Of these, one efficient lipase producing Gram positive rod shaped, motile bacterium was selected and designated as G2. The isolate was identified as *Bacillus species* on the basis of cultural, microscopic and biochemical characters. It showed optimum growth on nutrients agar medium at pH 7 and temperature 60˚C.

INTRODUCTION

Lipases are prevalent enzymes produced by all biological systems, viz. animals, plants and microorganisms. Microorganisms produce wide spectrum of lipases that differ in their enzymatic characteristics such as substrate specificity, pH, temperature activity and stability profile (Anurag Sekhon *et al.*, 2006). Lipases must be reasonably thermostable and active in organic solvents if they are to be used in wide range of synthetic reactions. Thermostability is important for resistance towards the chemical modifications caused by the high temperatures employed in various industrial lipase-catalyzed reactions (Fairolniza Mohd *et al.*, 2011). Lipases constitute the most important group of biocatalysts for the synthesis of biopolymers and biodiesel, synthesis of fine chemicals, the production of enantiopure pharmaceuticals, and are also important in the dairy industry, in detergents and in paper manufacturing. Thermostable lipase producers therefore have their wide application in industrial uses, such as digestive aids, food additives for flavor, reagents for the synthesis of useful compounds and treatment of domestic sewage (Sharma *et al.*, 2009, Pathak *et al.*, 2016). Present investigation is therefore aimed at Exploration of Tural hot water spring for isolation of thermostable lipase producer.

MATERIALS AND METHODS

Sample collection

Water sample was collected by grab sample collection method from Tural hot water spring, located at Sangmeshwar tehsil of Ratnagiri district in Maharashtra. Samples were collected in clean, presterilized plastic bottles without any air bubble and transported in laboratory within 24 hours and maintained in refrigerator at 4^0C. The temperature and pH of the samples were measured while sample collection with standard digital thermometer and pH meter respectively. (Pathak *et al.*, 2016)

Isolation

Water sample was serially diluted and spreaded on nutrient agar plates. The plates were incubated for 24 hours at 60˚C. After incubation morphologically different colonies appeared on the medium were selected for further screening. (Sonalkar *et al.*, 2015, Khairnar *et al.*, 2012)

Screening of isolates for lipolytic activity

Lipase producing organisms were screened by qualitative plate assay method. Isolates were grown on Tributyrin agar plates containing 0.5% (w/v)

peptone, 0.3% (w/v) yeast extract, 1% (v/v) Tributyrin and 2% agar having pH 7.0 and incubated at 60°C for 2 days. After incubation plates were observed for zone of clearance around colonies due to hydrolysis of tributyrin. (Malihe *et al.*, 2012, Pathak *et al.*, 2016)

Table no.1: Morphological Characteristic	
Characters	**Observed Character**
Size	4mm
Shape	Circular
Color	White
Margin	Undulate
Surface	Smooth
Elevation	Semi Raised
Consistency	Sticky
Opacity	Opaque
Grams Nature	+ve, rod
Motility	Motile

Culturing and Characterization of the Isolates

The isolate showing remarkable zone of clearance was selected for further analysis. Morphological and biochemical characteristics of the isolate was studied for the identification of the isolate (Khairnar *et al.*, 2012, Pathak *et al.*, 2016)

RESULTS AND DISCUSSION:

The hot spring water was odorless. The temperature and pH at the time of sample collection was recorded as 65^0C and 7.6 respectively. Incubated plates showed various colonies in the range of 1 to 5 mm large in size. Four morphologically distinct colonies were selected and designated as G1 G2, G3 and G4. Out of these two isolates G1 and G2 showed zone of clearance on Tributyrin agar plates. Further fast growing opaque, white colony of G2 was selected for next processes. Selected isolate showed a remarkable zone of clearance on Tributyrin agar plates and was identified by microscopic feature, cultural characteristics, biochemical tests and sugar profile as shown in table 1 and table 2 respectively. The selected isolate was identified as *Bacillus* species by comparing with Bergey's Manual of systemic bacteriology volume.

Table no.2: Biochemical Characteristics					
Test	Result	Enzyme profile	Result	Sugar profile	Result
Catalase	+	Amylase	+	Glucose	+
Indole production	_	Urease	+	Ribose	+
Methyl red	+	Lipase	+	Maltose	+
VP	_	Cellulase	_	Lactose	+
Citrate utilization	_	Pectinase	_	Sucrose	+
Asculin Hydrolysis	_	Laccase	_	Xylose	_
Nitrate Reduction	_	Oxidase	_	Fructose	_

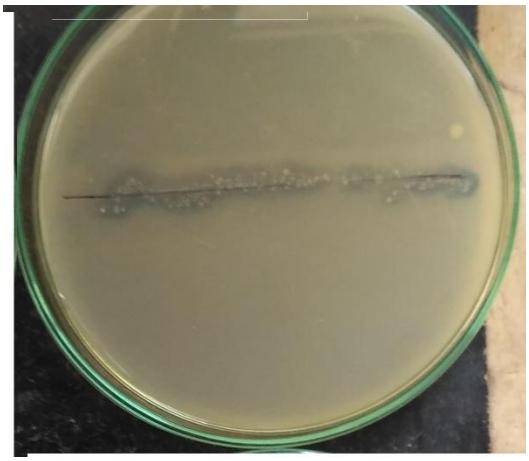

Fig. 1 Tributyrin Hydrolysis test of G2

An efficient lipase producer was isolated from Tural hot water spring and identified as *Bacillus* species. As lipase production by G2 was carried out at high temperature, it can be used in different industries, where lipolytic process is carried out at high temperature.

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