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EXTRACTION, PURIFICATION AND CHARACTERIZATION OF THERMOSTABLE LIPASE FROM *THERMUS SPP.*: AN INDUSTRIAL EFFLUENT ISOLATE

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ABSTRACT
Two thermophilic organisms were isolated from soil samples which were collected from vicinity of Local oil extraction industry, Dairy industry, Pulp paper industry, MIDC, Nanded, Maharashtra, India by composite sample collection method. Of these, one efficient lipase producing Gram negative rod shaped, non motile bacteria was selected and designated as TS 6. The isolate was identified as *Thermus 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. Production and partial purification of lipase was carried out and it was recorded as 63.7 U/ml.

Keywords: Thermophiles, soil sample, Lipase.

INTRODUCTION

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are widely used as biocatalyst due to their ability to catalyse the hydrolysis of triacylglycerides in aqueous solutions and also synthetic reactions in organic media. 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-catalysed reactions.(Mahile et.al 2013) Thermostable lipases from thermophiles have received attention due to their potential application in enzymatic processing of lipids, because some lipids are solid at room temperature and their processing in liquid form is possible after melting. This process requires thermostable lipase which is stable at high temperature (Pathak and Rathod 2016, Shirisha et.al 2010). 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 2015'b'). Present investigation is therefore aimed at Exploration of industrial area for isolation of thermostable lipase producer.

MATERIALS AND METHODS

A) Sample collection

Soil samples were collected by composite sample collection method from vicinity of Local oil extraction industry, Dairy industry, Pulp paper industry, MIDC, Nanded, Maharashtra, India. The

samples were collected in sterilized polythene bags and transported to the laboratory. (Pathak et.al 2014, Hingole & Pathak2013)

B) Isolation

Soil samples were 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, Rathod and Pathak 2014)

C) Screening of isolates for lipolytic activity

Lipolytic organisms were screened by qualitative plate assay method. Isolates were grown on Tributyrin agar base plates (0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (v/v) Tributyrin and 2% agar, pH 7.0) and incubated at 60°C for 2 days. Incubated plates were observed for zone of clearance around colonies due to hydrolysis of tributyrin.(Imamura S. and Kitauras 2000, Aires-Barros M.R., 1994)

D) Culturing and Characterization of the Isolates

The isolate showing maximum zone of clearance hereby referred as TS6 was selected for further analysis. Morphological and biochemical characteristics of the isolate were studied for the identification of the isolate (Khairnar et.al 2012, Pathak et.al 2015'a').

E) Production and partial purification of lipase

For production of lipase, selected culture was grown in medium containing yeast extract (10g/l), NaCl (5g/l), peptone (30g/l) and 1% (w/v) olive oil at 60^{o} C for 48 hrs. The culture broth was harvested after incubation by centrifugation at 12,000 rpm for 30min at 4°C. The collected supernatant was used as crude enzyme solution and assayed for lipolytic activity. (Pathak and Rathod 2016, Polkade et.al 2015)

F) Lipase assay

Lipase activity was measured by titrimetric method using olive oil as a substrate. Olive oil (10% v/v) was emulsified with gum Arabic (5% w/v) in 100mM potassium phosphate buffer pH 7.0. 100 µl of enzyme was added to the emulsion and incubated for 15 minutes. The reaction was stopped and fatty acids were extracted by addition of 1.0ml of acetone: ethanol solution (1:1). The amounts of fatty acids liberated were estimated by titrating with 0.05M NaOH using a phenolphthalein indicator.

One unit of enzyme is defined as the amount of enzyme required to hydrolyze µmol of fatty acids from triglycerides. (Lee et.al 1999, Pathak et.al 2015'b')

RESULTS AND DISCUSSION

Soil samples were black in color and showed neutral pH. Incubated plates showed various colonies in the range of 1 to 3 mm large in size. Six morphologically distinct colonies were selected and designated as TS 1 –TS 6. Out of these Two isolates TS 2 AND TS 6 showed zone of clearance on Tributyrin agar plates Further fast growing translucent, white colony of TS 6 was selected for further processes and designated as TS 6. 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 *Thermus species* by comparing with Bergey's Manual of systemic bacteriology volume I. Further production and partial purification of lipase by selected isolate was carried out and it produced 63.7 U/ml of lipase after 48h of incubation at 60^{o}C.

Table no.1: Morphological Characteristic

Characters	Observed Character
Size	1.5mm
Shape	Circular
Color	White
Margin	Entire
Surface	Smooth
Elevation	Raised
Consistency	Sticky
Opacity	Translucent
Grams Nature	-ve, rod
Motility	Non motile

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**	+

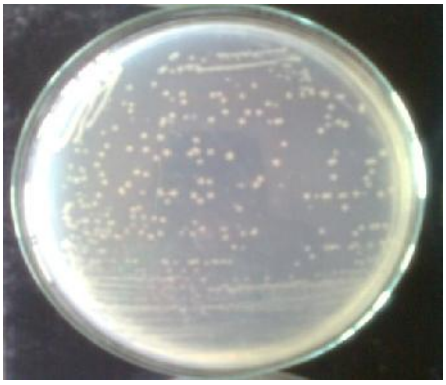

Fig. 1 Isolated colonies

Fig. 2 Tributyrin Hydrolysis test of TS 6

CONCLUSION

An efficient lipase producer was isolated from vicinity of Industrial Effluent and identified as *Thermus species.* As production of lipase by TS6 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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