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Research Article

Isolation and biochemical Characterization of potential thermostable Lipase producer from industrial effluent of oil, dairy and paper industry

Anupama Prabhakarrao Pathak*, Gautam T Kamble, Swati R Jadhav and Mayuri S Sarsar.

School of Life Sciences (DST- FIST, UGC-SAP Sponsored)

Swami Ramanand Teerth Marathwada University, Dnyanteerth, Vishnupuri,

Nanded, Maharashtra, India - 431606.

Abstract

Three thermophilic organisms were isolated from soil sample 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 Cocci, oval shaped, motile bacteria was selected and designated as TS1. The isolate was identified on the basis of morphological and biochemical characters as *Nisseria ovis*. It showed optimum growth on nutrients agar medium at pH 7 and temperature 50˚C. TS1 have produced 66U/ml thermostable lipase and it was determined by titrimetric assay method. TS1 have produced 66U/ml thermostable lipase and it was determined by titrimetric assay method.

Keywords: Thermophiles, soil sample, Lipase.

INTRODUCTION

Lipases are glycerol ester hydrolases that act on acylglycerols to liberate fatty acids and glycerol. Lipases can hydrolyze long chain water-insoluble triglycerides into diglycerides, monoglycerides, glycerol and fatty acids[1]. The lipases are widely used in the pharmaceutical, food, detergent, leather and cosmetic industry due to because of their unique characters of stereospecificity, substrate specificity, regioselectivity and ability to catalyze a heterogeneous reaction at the interface of water insoluble and water soluble systems[2,3]. Out of the commercially available lipases, most of them are microbial lipases which are extracellular in nature. The lipase producing micro organisms have been found in diverse habitat such as hot springs, compost heaps, decaying food, vegetable oil processing factories, dairies, soil contaminated with oil and oil seeds[4-5]. In this paper an attempt has been made to study the isolation and biochemical Characterization of potential thermostable Lipase producer from industrial effluent of oil, dairy and paper industry with reference to previous reports.[6,16].

MATERIALS AND METHODOLOGY

A) Sample collection

Soil samples were collected from vicinity of Local oil extraction industry, Dairy industry, Pulp paper industry, MIDC, Nanded, Maharashtra, India by composite sample collection method. The samples were collected in sterilized polythene bags and transported to the laboratory.[7-9]

B) Isolation

1 gm of soil was transferred to a 250 ml flask containing 100 ml sterile distilled water and kept in shaking incubator at 120 rpm for 20 min. Serial dilution were made and 0. 1 ml of dilution a were spread on plates containing Nutrients Agar medium. The plates were incubated for 24 hours at 50˚C and

morphologically different colonies appearing on the medium were isolated.[7,8]

C) Biochemical tests

For the identification of isolate the biochemical tests were performed such as Catalase and oxidase activity, indole production, citrate utilization, lipase and H_2S production, hydrolysis of gelatin, casein, starch, cellulose and urea were observed, MRVP test and carbohydrate fermentation tests were also performed using standard procedures.[17]

D) Screening of isolates for lipolytic activity:

The predominant bacteria in the nutrient agar plate were isolated and screened for lipolytic activity on agar containing Tributyrin (1% w/v). Incubated plates were observed for zone of clearance around colonies. [10]

E) Culturing and Characterization of the Isolates

The isolate showing maximum zone of clearance hereby referred as TS1 was selected for further analysis. Morphological and biochemical characteristics of the isolate were studied for the identification of the isolate.[10-12]

F) Lipase enzyme production:

Isolate TS 1 was inoculated in production medium containing (%w/v) peptone 0.2; $NH_4H_2PO_4$ 0.1; NaCl 0.25; $MgSO_4 \cdot 7H_2O$ 0.04; $CaCl_2.2H_2O$ 0.04; olive oil 2.0 (v/v); pH 7.0; 1-2 drops Tween 80 as emulsifier. Erlenmeyer flasks containing 100 ml of liquid medium were incubated at 50°C in shaking incubator (150 rpm). After 24 hours of incubation, the culture was centrifuged at 10,000 rpm for 20 min at 4ºC and supernatant was used as the sources of extracellular enzyme. The lipase activity in the supernatant was determined by the titrimetric assay method.[11-14]

G) 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 at room temperature. The reaction was stopped and fatty acids were extracted by adding 1.0 ml of acetone: ethanol solution. The amount of fatty acids liberated were estimated by titrating with 0.05M NaOH till attainment of pH 10.5 using phenophathelin indicator. One unit of enzyme was defined as the amount of enzyme required to hydrolyse µmol of fatty acids from triglycerides.[11,12]

Table 1
Morphological Characters

Characters	Observed Character
Size	2.5mm
Shape	Circular
Color	White
Margin	Entire
Surface	Smooth
Elevation	Raised
Consistency	Sticky
Opacity	Transparent
Grams Nature	-ve, Cocci
Motility	Motile

Table 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	-	**Amylase**	+	**Glucose**	-

RESULT AND DISCUSSION

Total three morphologically distinct bacterial colonies were appeared on nutrient agar plates at 50˚C. These colonies were designated as TS1, TS2 and TS3. Isolate TS1 showed luxuriant growth beside largest zone of clearance due to hydrolysis of tributyrin and selected for further study. Morphologically TS1 was motile, Gram negative Coccus that developed white circular transparent colony with raised elevation and sticky consistency. When biochemically characterized TS1 strain was positive for catalase, while Indole, Methyl Red, citrate utilization and nitrate reduction were negative. It showed positive result for amylase production. TS1 have utilized Maltose as a carbon source however Glucose, Sucrose, Lactose, Xylose, Ribose were not unitized. On the basis of biochemical characterization the isolate TS1 was identified as a *Nisseria ovis* by comparing its morphological and biochemical characteristic with Bergeys Manual of systemic bacteriology. The production and purification of lipase by this isolate TS 1 was carried out. TS1 have produced 66U/ml thermostable lipase and it was determined by titrimetric assay method.

CONCLUSION

Lipase producing bacteria was isolated and identified as *Nisseria ovis* from vicinity of Industrial Effluent. Partially purified 66U/ml lipase was obtained after production. The organism shows the optimum growth at 50˚C. The enzyme produced can stable at high temperature and can be used for the various industrial applications as the thermostable lipases have great importance.

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REFERENCES

1. Joseph BP, Ramteke PW and ThomasG, Cold active microbial lipases: Some hot issues and recent developments. Biotech Advances, 2008, 26: 457-470.
2. BrockmanH, BorgstormB, Lipases. Elsevier, 1984. Amsterdam.
3. JaegerK, Reetz T, Microbial lipases from versatile tools for biotechnology. TIBTECH, 1998,16, 396–403.
4. SughiharaA, SenooT, EnokiA, ShimadaY, NagaoT andTominagaY, Purification and charactrization of a lipase from Pichiaburtonii, Appl.Microbiol Biotechnol, 1995, 43, 277-281.
5. JaegerKE, RansacS, DijkstraBW, HenrelCC and MissetO , Bacterial lipases. FEMS Microbiol, 1994, Rev. 15, 29-63.
6. SugiharaA, UeshimaM., ShimadaY, TsunasawaS. and TominagaY, Puri¢cation and characterization of a novel thermostable lipase from Pseudomonas cepacia. J. Biochem, 1992, 112, 598-603.
7. Reeve JN, Thermophiles in New Zealand. ASM News, 1994, 60, 541-545.

8. PathakAP, RathodMG, Exploration of Unkeshwar hot springs in Maharashtra for thermostable amylase producer**.** Res. Rev. Biosci 2014, 8 (7) 269–276.
9. PathakAP, RathodMG and RampurkarVV, An eco-friendly approach for thermostable amylase production using *Bacillus firmus* APP6: a hot spring isolate. Asiatic J. Biotech. Res. 2014, 4 (4): 101-105. ISBN 0976-4992. Pacific publishers.
10. PathakAP and DeshmukhKB, "Alkaline protease production extraction and characterization from alkaliphilic *Bacillus licheniformis* KBDL4: A Lonar Soda Lake Isolate" Indian J. Expt. Biol., 2012, (50) 569-576 (CSIR-NISCAIR) publication (IF 1.29).
11. AiresBarrosMR, TaipaMA and CabralJMS, Isolation and purification of lipases.In: Wooley P, Petersen SB (eds) Lipases-their structure, biochemistry and application. Cambridge University Press, Cambridge, 1994**,** 243-270
12. SirishaE, RajasekarN and LakshmiNarasuM, Isolation and Optimization of Lipase Producing Bacteria from Oil Contaminated Soils**,** Advances in Biological Research,2010, 4 (5): 249-252, ISSN 1992-0067
13. ImamuraS and KitauraS, Purification and characterization of a monoglyceryl lipase from the moderately thermophilic Bacillus sp. H-257, J. Biochem., 2000, 127, 419-425.
14. LinkoYY, LamsaM, WuEX, UosukainenJS and LinkoP, Biodegradable products by lipase biocatalysis. J. Biotechnol, 1998,66(1): 41-50
15. FarihaHasan, ShahAamerAli and HameedAbdul, Industrial applications of microbial lipases. Enzyme and Microbial Tech, 2006, 39(2): 235-251.
16. GuptaMN and Roy, Enzymes in organic media: Forms, functions and applications,Eur. ,J. Biochem, 2004, 271,2575-2583.
17. AnejaKR, experiments in microbiology plant pathology and biotechnology, forrh edition, new age publication, 2004, 245-275.