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Isolation and identification of Novel Lipase-producing Bacterium *Aneurinibacillus aneurinilyticus* LP-II

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ABSTRACT

The aim of this research work was to isolate novel strain of lipase producer from local oil mill soil at Gangakhed. The lipase can be catalysing the transesterification of algae oil in to biodiesel. A sample of soil was collected from oil mill and enrichment the soil sample with basal lipase production media containing 1% Algae oil as a carbon source. Lipase producing microbes were screened on tributyrin agar plates, the activity of crude enzyme were determined. The isolate produced lipase and showed maximum activity 1.5 U/mL at 40°C and at 7.0 PH, after 48 hours of incubation period at 150 rpm. Isolate identified by morphological, biochemical and 16s r-RNA sequencing gene. The 16s r-RNA sequencing revealed it is a new strain of *Aneurinibacillus aneurinilyticus* LP-II.

Keywords: Lipase, isolation, novel strain, 16sRNA, algae oil

Lipases (triacylglycerol acylhydrolases; EC3.1.1.3) are the hydrolysing enzyme that catalyse hydrolysis and synthesis of ester (Haikuan Wang, haojiong Zhong, 2012). Lipases comprises the major group of biocatalyst and possess many industrial applications, specifically in dairy industry for hydrolysis of milk and fats, in detergent industry as a additive in washing powder, textile industries uses lipase to increase fabric absorbency (Sirisha, E.; Rajaseka, N. *et al.*, 2010). Recently lipases are uses in transesterification reaction (Meintanis, C.; Chalkou, K.I. 2006). They are also used in pulp and paper industry (Salameh, M. and Wiegel, J. *et al.*, 2007), in the synthesis of biodiesel (Bhavani, M. and Chowary, G.V. *et al.*, 2012). Lipases occur widely in the environment but only microbial lipases are commercially significant (Senthikumar, R.; Selva Kumar, G. *et al.*, 2008). Lipase are produced by microorganism (bacteria, fungi) plants and animals, however microbial lipases are more interesting because they can be produced in high yield in lower cost and shorter time (Nawani, N.; Khurana, J.; Kaur, J. (2006)). Several bacillus species are the main source of lipase. Lipase are generally produced by microbes when lipids, glycerol, bile salt etc. are supplied in growth medium as chief carbon source. (Vieille, C. and Zeikus, G.J. *et al.*, 2001). The present study was aimed with the objective to isolate lipase producing bacterial strain from oil mill soil sample by algae oil enrichment technique and lipase production was optimized with the culture condition of production medium.

MATERIALS AND METHOD

Enrichment of lipase producing microorganisms

One gram of soil sample were collected from oil mill at Gagakhed India, As described by Haikuan *et al.* 2012,

and was added in to 250 ml Erlenmeyer flask containing 50 ml basal medium for lipase production containing (gL^{-1}) peptone 1.5gm, Yeast extracts 0.5gm, algae oil 1ml, PH 7.0. the media was incubated at 30°C on rotary shaker at 150 rpm for 72 hours. (Tembhukar, V.R. 2012)

Isolation and screening of lipase producing microorganism

Loop full cultures of grown microorganism in the enrichment culture were spotted on tributyrin agar plates containing 1% tributyrin and was incubated at varies temperature from 30 to 60°C for 24 to 48 hours. Then, in order to select the best lipase producer strain the highest zone of clearance producing bacterial colony were isolated for lipase producing bacteria (Sennthikumar, R. *et al.,* 2008)

Identification of lipase producing bacteria

The strain possessing the highest lipase were identified both morphological, biochemical characters and 16sRNA gene sequencing, morphological, biochemical and enzymatic activity (lipases, nitrate reduction, oxidase, catalyse, urease, gelatinise, starch) and a set of six sugar test was used namely D fructose, D glucose, D manitol, Xylulose, Mannose. Morphological studies of best lipase producing were under microscope by Gram staining and the IMViC (Subhas Verma *et al.,* 2014, Nashima, K. 2012).

Lipase activity

Lipolytic activity was determined by a spectrophotometric assay using pNPP (Para nitro phenyl palmitate) as a substrate. (This can be carried as suggested by Mobarak, E. *et al.,* 2011) The reaction mixture consisted of 0.1 ml enzyme extract, 0.8 ml of 0.05 M Tris buffer (pH 7) and 0.1 ml of 0.01M of p-NPP dissolved in isopropanol. The reaction mixture was kept at 40°C for 30 min in a water bath and 0.25ml of 0.1M Na_2CO_3 was added to stop the reaction. The reaction mixture was centrifuged at 10000 rpm for 10 min and the Optical density (O.D.) was determined at 410 nm. One unit of lipase activity was defined as the amount of enzyme which liberated 1 µmol of p-nitro phenol per min from p-nitro phenyl palmitate.

Properties of Lipases

The optimum temperature for lipase was evaluated by using lipase activity with 2-4 dinitophenol palmitate (Sd Fine-Chem. Ltd,) at various temperature from 30-60°C under variable PH 6- 10. Optimum enzyme activity was measured under standard enzyme test condition. As per the methodology described by (Heshman *et al.,* 2005) the optimal PH and temperature of the lipase was studied and determined in buffer solution by incubating lipase substrate. The effect of pH on enzyme activity was analysed by the spectroscopic assay using pNPP as substrate.

RESULTS AND DISCUSSION

Isolation and screening of lipase producing micrograms

Based on morphological and biochemical properties (Table 1) the isolate was identified as *Aneurini bacillus* sp. To conform the test result for isolate, 16sRNA gene was sequenced and analysis clearly indicated that the strain LP-II was a member of genus *Aneurini bacillus* and showed maximum (99.45%) similarity with 16s RNA sequence ATCC12856(T). This sequence data has been deposited in GeneBank database and given the accession number MF696161.

Qualitative screening for lipase

Qualitative assay of lipase enzyme was reported by Bhavani *et al.* (2012) and Prasana *et al.* (2013) their study showed that novel isolate produced maximum zone of clearance. In our study the novel algae oil hydrolysing lipase showed 30mm zone (Fig. 1).

Characterization of Lipases

(I) Effect of temperature on lipase activity

In this investigation crude lipase assay was done, the lipase activity at 40°C showed the maximum enzyme activity of 1.823U/ML. At lower temperatures and (30°) the enzyme activity is lower 0.892U/ML, and at higher temperature activity of enzyme was decreased to 1.810 (at 50°C) and at 60°C the activity remarkably decreases to 0.302U/ML, this is because of

Fig. 1: Lipase production by isolate LP-II on tributyrin agar

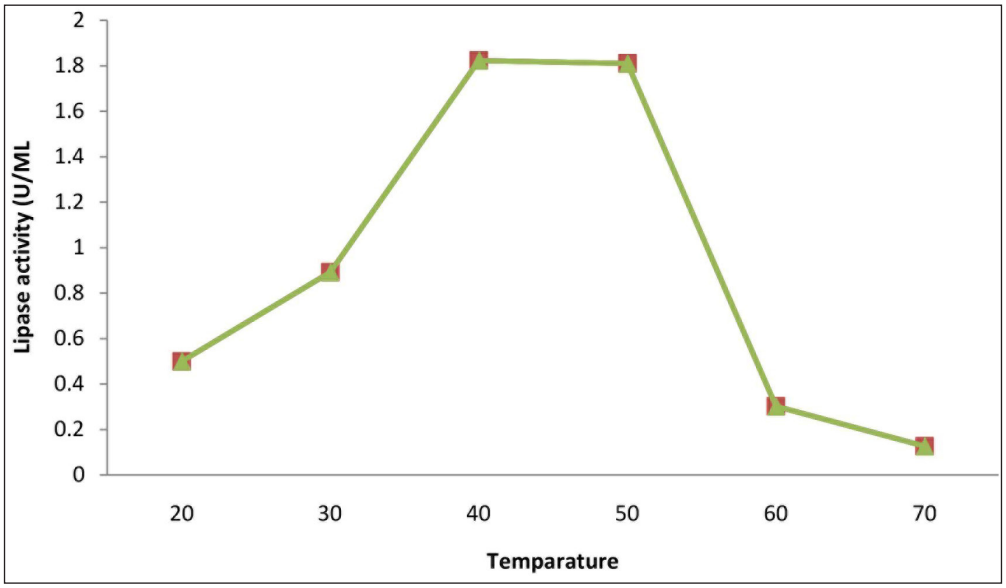

Fig. 2: Effect of temperature on Lipolytic Activity

denaturation of enzymes molecule (Fig. 2). It has been reported that most of the lipase produced by bacillus and pseudomonas have optimum temperature 37°C to 45°C. (Jaeger, K.E. and Reetz. 1998, Narshima, E.K. *et al.,* 2012) has been reported that mutant bacillus species showed 1.358 U/ml (Nasima, K. 2012). Lipolytic activity of *Aneurini bacillus danicu* NBRC 102444**,** *Aneurini bacillus thermoaerophilus* DSM 10154T (AB112726) was detected as (0.512U/mL). Panagiota M. Stathopoulou 2013), Hesham. M. Saeed *et al.* (2005), Borker P.S. and Khobragade C.N. (2009) also showed the effect of PH and temperature on the lipase activity.

(II) Effect of PH on lipase activity

The effect of PH on enzyme activity was determined covering the range (6 -10), this study was conducted

Table 1: Morphological and Biochemical characterisation of LP-II isolate

Test	Result
Gram stinging	Gram positive
Shape	Long rod
Temperature for growth	20-40 (40 Maximum)
PH	6.5-7.0
Nitrate reduction	Positive
Lipase	Positive
Oxidase	Positive
Catalase	Positive
Caseinases	Negative
Gelatinise	Positive
Sugar fermentations	Negative
D-glucose	Negative
D fructose	Positive
Dextrose	Negative
Indol	Positive
Methyl red	Negative
Citrate	Positive

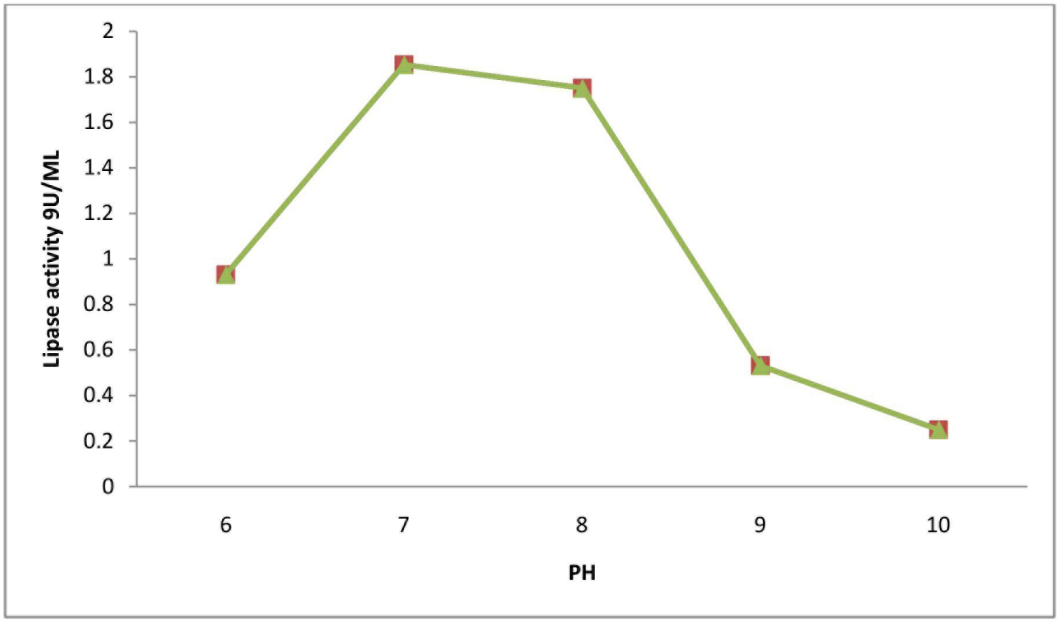

Fig. 3: Effect of PH on Lipolytic Activity

between the range from PH 6 to 10. Enzyme showed maximum activity 0.932U/ML at PH 7.0, while enzyme activity decreased when PH rinsing from 7-10 (Fig. 3). The effect of PH on *Bacillus* sp. FH5h *bacillus* was reported by Nawani, N.; Khurana, J.; Kaur, J. (2006) they showed that the maximum lipolytic activity gained at ph 8-9 and affected by PH 10.), Maytham Ayuob *et al.* (2016) reported the effect of PH on lipase activity, our study also recorded the similar finding.

CONCLUSION

Soil is a culture of a huge and diverse kinds of microorganisms, enrichment techniques is one of most promising techniques to isolate the desirable microbes. In our study we successively isolated *Aneurini bacillus aneurinilyticus* LP-II bacterial culture that has potential to produce lipase enzyme which selectively hydrolyse algal lipids at 40°C and stable at PH 7. This is best enzyme to be used in the transesterification reaction, which mostly carried above 40°C. The lipase of this novel species can be stable during transesterification reaction.

COMPETING INTEREST

Authors have declared that no competing interest exists.

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AUTHORS' CONTRIBUTION

This work was carried out by both the authors and involves in protocol, analysis, and the proof read the manuscript.

CONFLICT OF INTEREST

No conflict of interest

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