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Research article

Characterization of lipase produced by *Aneurinibacillus aneurinilyticus* strain LP-II isolated from soil of oil mill

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Abstract

Biotechnological important lipase producing bacterium strain LP-II was isolated from local oil mill soil at Gangakhed (India). Lipase is an important industrially employed enzyme and used in trans-esterification of algae oil to biodiesel. LP-II is Gram positive log rods have showed growth at 40°C and pH 6.7. LP-II has utilized dextrose and fructose as carbon source and produced acid in the medium. LP-II lipase produced lipases 1.5 U/mL at 40°C and 8.0 PH after 48 hours of incubation at 150 rpm. In addition, LP-II have showed positive result for gelatinase, caseinase oxidase and catalase tests at its optimum temperature and pH. Strain LP-II was identified as *Aneurinibacillus aneurinilyticus* using morphological, and biochemical methods. Identification made in former step was confirmed by 16SrRNAgene sequencing method as *Aneurinibacillus aneurinilyticus* strain LP-II (Accession no.: MF696161). LP-II lipases have an ability to work at moderate temperature and slightly high pH. Thus, lipase produced by LP-II strain may find application in various industrial processes and household chemicals.

Introduction

Lipases (triacylglycerol acyl-hydrolases, EC3.1.1.3) are the hydrolysing enzyme that catalyses hydrolysis and synthesis of ester [1]. Lipases have applications in industries such as in dairy for hydrolysis of milk and fats, in detergent industry as an additive in washing powder and textile industries to increase fabric absorbency [2-5]. Additionally, Lipases are uses in transesterification reaction [6, 7], in pulp and paper industry [8], in the synthesis of biodiesel [9]. Commercially significant microbial lipases are produced by varieties of organisms [10-12] include bacteria, fungi, plants and animals. However, microbial lipases are more interesting because their high yield using cost effective raw materials [13-15]. In the present study lipase producing bacterium was isolated from oil mill soil sample by algae oil enrichment technique and lipase production was estimated using qualitative and quantitative methods.

Experimental

Materials and methods

All laboratory aids, chemicals, media and solvent required for the experiments were purchased from S. D. Fine Chemicals Ltd and HiMedia Pvt. Ltd. (India).

Sample collection and enrichment of media

Soil sample was collected from oil mill at Gagakhed (MS), India using random composite sampling method [16, 17]. Soil was cleaned in laboratory. One gram of soil was measured and added to 250 mL Erlenmeyer flask containing 50 mL basal medium (ingredients/L: peptone 1.5 g, yeast extracts 0.5 g, algae oil 1 mL (v/v), pH 7.0. The medium was incubated at 30°C on cooling shaking incubator (HiMedia Pvt. Ltd.) at 150 rpm for 72hours [18].

Isolation and screening of lipase producing microorganism

Enriched medium was used to isolate liapse producing microorganism. Spread plate method was used to isolate lipase producer. 100 µL enrichment broth culture was spread tributyrin agar plates containing 1% tributyrin. Inoculated plates were incubated at variable temperature from 30 to 60°C for 24 to 48hours. Colonies showing zone of clearance was isolated on same agar medium and obtained as pure culture for further experiments. Lipase producers showing highest zone of clearance were chosen for further work [19, 20].

Identification of lipase producing bacteria

Isolated lipase producing bacterium was identified using morphological and biochemical methods. 16S rRNA gene sequencing methods was used for confirmation of identification made in former steps. Gram nature was determined. Isolated species were observed for utilization

of carbon source (glucose, fructose, mannitol, xylose & mannose and their ability to produce lipase, oxidase, catalyse, urease, gelatinise, starch, nitrate reduction, IMViC [21, 22].

Lipase activity

Lipolytic activity was estimated by a spectrophotometric assay using pNPP (para nitro phenyl palmitate) as a substrate. 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 in a water bath for 30 min. After 30 min. 0.25 mL of 0.1M Na_2CO_3 was added to terminate the reaction. The reaction mixture was centrifuged at 10000 rpm for 10 min. 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 [23-25].

Properties of Lipases

The optimum temperature for lipase was evaluated by using lipase activity with 2, 4- dinitrophenolpalmitate at temperature ranged from 30-60°C at pH 7. The effect of pH (6-10) on enzyme activity was analysed by the spectroscopic assay using pNPP as substrate. pH was optimized at optimum temperature recorded in former step. Optimum enzyme activity was measured under standardized enzyme test conditions [26, 27].

Results and Discussion

Screening and isolation of lipase producing microorganisms

Lipase producing bacterium was isolated form soil collected at oil mill in Gangakhed (India). Isolated species were obtained as pure culture and stored at low temperature (4°C). Isolated species were observed for production of lipase enzyme. Bacterial species LP-II showed maximum production of lipase amongst isolated species. Therefore, it was selected for further experiment.

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

Identification lipase producing bacterium

Morphological and biochemical methods (Table 1) were used to identify bacterium LP-II. As per the Baergy's Manual of Systematic Bacteriology isolated bacterium LP-II was identified as *Aneurinibacillus aneurinilyticus*. 16S rRNA gene sequencing methods was used to confirm identification of bacteria made in former steps. The identified strain LP-II showed 99.45% similarity type strain *Aneurinibacillus aneurinilyticus* ATCC12856(T).

Accession number of *Aneurinibacillus aneurinilyticus* strain LP-II

16S rRNA gene sequence was deposited in NCBI Gene Bank database under the accession number MF696161.

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

Parameter	Result
Gram nature	Gram +ve
Shape	Long rod
Optimum Temp.	40°C
PH	7.0
Glucose	-
Fructose	+
Dextrose	-
Caseinase	-
Catalase	+
Gelatinise	+
Lipase	+
Oxidase	+
Nitrate reduction	+

Qualitative screening for production of lipase

Qualitative plate test showed that algae oil hydrolysing lipase producing strain LP-II showed zone of clearance 30 mm (Figure 1). Similar types of results were reported by many people's [9, 18].

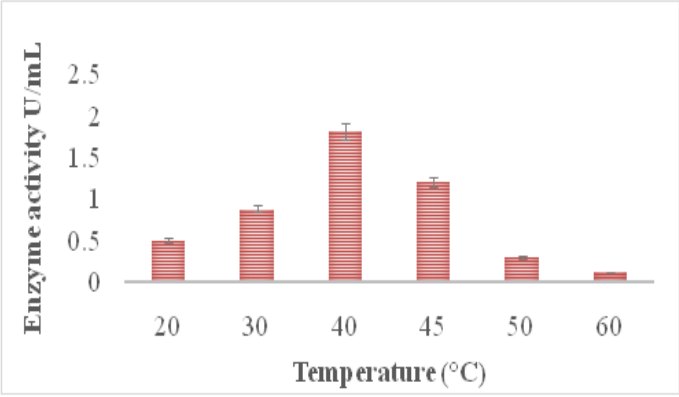

Figure 2. Effect of temperature on activity of lipase.

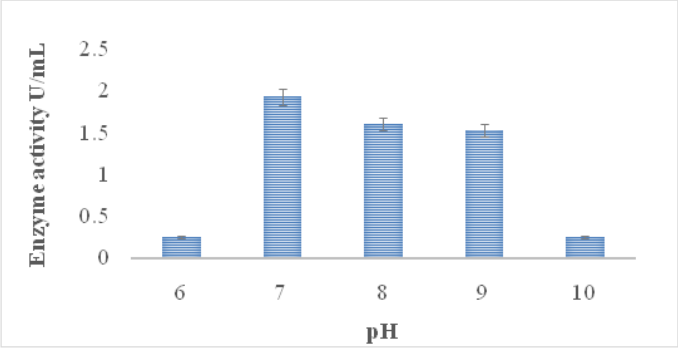

Figure 3. Effect of pH on activity of lipase.

Characterization of Lipases (Quantitative test)

Effect of temperature on lipase activity was carried out at temperatures 30 to 60°C. Crude lipase showed maximum activity at 40°C (1.82 U/mL). LP-II lipase showed remarkable activity at 60°C (Figure 2). Many research groups worldwide have reported that lipase produced by *Bacillus* and *Pseudomonas* have optimum temperature 37°C to 45°C. Mutants of *Bacillus* species showed 1.358 U/mL lipase production [7, 14].

The effect of pH (6-10) on lipase was determined. It was recorded that lipase produced by LP-II showed maximum activity 1.93U/mL at pH 7.0. LP-II lipase showed considerable activity at pH 9 and pH 10 (Figure 3). Similar types of results were reported by in the case *Bacillus* sp. FH5h [14, 28]. It was recorded that *Bacillus* sp. FH5h showed activity up to pH 8-9 but there was significant drop in activity at pH 10 [29]. Therefore, LP-II lipase produced by *Aneurinibacillus aneurinilyticus* strain LP-II have advantages over the previously reported strains.

Lipase produced by LP-II showed considerable and maximum lipolytic activity than earlier reported strains such as *Aneurinibacillus danicu* NBRC 102444**,** *Aneurinibacillus thermoaerophilus* DSM 10154T (AB112726) produced lipase about 0.512U/mL. These reports confirms that *Aneurinibacillus aneurinilyticus* strain LP-II isolated by us have considerable industrial potential than the previously reported lipase producing bacteria.

Conclusion

Aneurinibacillus aneurinilyticus strain LP-II is capable of producing industrially significant lipase enzyme. LP-II lipase can selectively hydrolyse algal lipids at 40°C and more stable at pH 8. This is prerequisite for the best enzyme to be used in the transesterification reaction, which mostly carried above 40°C. LP-II produced by *Aneurinibacillus aneurinilyticus* strain LP-II may find applications many industries.

Conflicts of interests

Authors have declared that there is no any conflicts of interest exist.

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Author (s) contribution statement

This work was carried out by DC as a part of his Ph.D. thesis. CK and DC both the authors are involves in designing protocol, laboratory analysis and writing the manuscript. DC and CK have equally contributed in writing and proof reading of MS in this final form.

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