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Effect of Carbon Source and Lipase Inducers on Extracellular Lipase Production by *Bacillus* sp. BK-L40

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The bacteria isolated from soil and screened for lipase by spirit blue agar medium. Among the isolated bacterial strains, one strain (BK-L40) was exhibited higher clear zone (higher lipase activity). Further, the selected strain was identified as *Bacillus* sp. based on the morphological and physiological characteristics and the 16S rRNA sequencing. The highest production of enzyme was obtained in medium supplemented with 1% lactose as carbon source in LB broth on 48 h of incubation. The lipase activity was decreased in all the tested lipase inducers, but the activity was completely nil in the case of Tween 80. This result confirmed that the carbon source is able to stimulate and the lipid source is repressed the synthesis of lipase.

Key words: *Bacillus*, screening, lipase, optimization.

Lipases are triacylglycerol acylhydrolases (EC.3.1.13) that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids (Sharma *et al*., 2001). The microbial lipases have increased a great deal of attention due to their potential uses in industries (Macrae and Hammond, 1983). Particularly, the bacterial lipases have wide applications in various industries such as food, detergent, cosmetic and pharmaceutical (Jaeger *et al*., 1994; Gupta *et al*., 2004). The microbial lipases have gained importance due to their easy cultivation, low cost of extraction, pH and thermal stabilities and substrate specificity compared with plant and animal lipases (Lawson *et al*., 1994).

The production of lipase by microorganisms is generally influenced by media composition, incubation time, pH, temperature, carbon sources and lipase inducers. In biotechnological enzyme production process, even small improvements have been significant for commercial success. The bacterial lipases are induced into the medium which contained proper lipid sources (Sharma *et al*., 2001). However, a few investigators have reported that the lipase activity was repressed when the presence of lipid source into the medium by *Pseudomonas aeruginosa* EF2 and *Acinetobacter calcoaceticus* (Gilbert *et al*., 1991; Mahler *et al*., 2000). The extracellular lipase was previously isolated from different species of *Bacillus* (Ertugrul *et al*., 2007; Takac and Marul, 2008). The present report was carried out by the isolation, screening and identification of a new lipase producing bacteria. Further, the optimization was carried out for the production of higher level of extracellular lipase.

MATERIALS AND METHODS

Isolation and screening of lipase producing microorganisms

The bacteria were isolated from soil contained detergent waste (South Korea) and screened for the production of lipase using spirit blue agar containing lipase reagent (Marshall, 1992). Briefly, the bacteria were streaked onto spirit blue agar (Tryptone, 10 g/l; yeast extract, 5 g/l; agar, 20 g/l; spirit blue, 0.15 g/l; lipid reagents-

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containing a mixture of tributyrin and Tween 80, 30 ml/l; pH, 6.8). The selected strain was maintained on LB agar plates at 4 ºC and stored at -80 ºC in glycerol.

Identification of the selected strain

The bacterial strain BK-L40 was identified based on morphological and biochemical characterization. The identification was further confirmed by the 16S rRNA gene sequencing method. The 16S rRNA gene sequence was then compared with sequences available in the nucleotide database by using the BLAST algorithm at the NCBI (www.ncbi.nlm.nih.gov).

Lipase assay

Lipase activity was assayed quantitatively using 4-nitrophenyl palmitate as the substrate according to the method described by Winkler and Stuckmann (1979). In brief, 10 ml of isopropanol containing 30 mg of 4-nitrophenyl palmitate (pNPP) was mixed with 90 ml of 0.05M phosphate buffer (pH 8.0) containing 207 mg of sodium deoxycholate and 100 mg of gum arabic. A total of 2.4 ml of freshly prepared substrate solution was then pre-warmed at 37 ºC and mixed with 0.1 ml of enzyme solution. After 15 min of incubation at 37 ºC, the absorbance was measured at 410 nm against an enzyme free control. One enzyme unit was defined as 1 µmol of 4-nitrophenol enzymatically released from the substrate (ml/min).

Effect of different culture media on lipase production

The following culture media were used for optimization studies with the compositions : (1) Nutrient broth: NaCl, 5 g/l; peptone, 5 g/l; beef extract, 3 g/l; (2) LB broth: tryptone, 10 g/l; yeast extract, 5 g/l; NaCl, 10 g/l. For the production of lipase, an Erlenmeyer flask (250 ml) containing 50 ml of medium was inoculated with fresh culture. The inoculated flasks were then incubated at room temperature with constant shaking at 180 rpm. The cell-free supernatant was recovered by centrifugation (10,000 rpm, 10 min at 4 ºC) and the clear supernatant was used to determine the lipase activity.

Effect of different carbon sources and lipase inducers on lipase production

The production of lipase was evaluated in different carbon sources (1%) such as glucose, sucrose, maltose, lactose, and corn starch. The carbon sources were sterilized separately and then aseptically added to the autoclaved medium. The following substances were used as lipase inducers (1%): Olive oil, soybean oil, tributyrin and Tween 80. Medium that contained without lipase inducer was used as a control.

RESULTS AND DISCUSSION

Isolation and identification of lipase producing bacteria

The bacteria isolated from soil and were screened for extracellular lipase by spirit blue agar medium. Among the different bacterial strains, one strain (BK-L40) was exhibited higher clear zone, its indicating the higher lipase activity. Further, the selected strain was identified based on morphological, physiological and biochemical characterizations. The results showed that the BK-L40 is gram positive, aerobic and spherical shape. The preliminary identified bacterial strain was further confirmed by 16S rRNA sequencing. Approximately, 1379 bp sequences was obtained from BK-L40 and aligned with other 16S rRNA sequences available in GenBank database. The phylogenetic tree was constructed using the neighbor-joining method only with the culturable *Bacillus* species (Fig 1) and the phylogenetic analysis indicated that the strain was showed close sequence identity (99%) to *Bacillus* sp. PCWCSI7 (NCBI accession No. GQ284382). The data of the 16S rRNA sequence is available in GenBank under the accession number FJ999624.

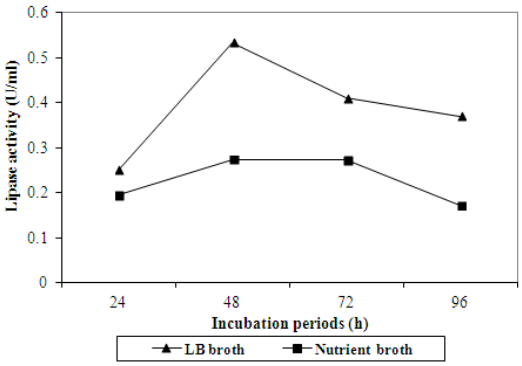

Fig. 1. Effect of different culture media on lipase production

Production of extracellular lipase

Effect of different culture media on lipase production

All of the microorganisms have own nutritional and physico-chemical requirements for their growth and enzyme production. Of the two different media tested, the highest lipase was obtained in LB broth on 48 h of incubation (Fig. 2). The enzyme activity was declined after 48 h due to the nutrient depletion or feed back inhibition. The optimum incubation period was maintained throughout the optimization studies. This incubation time was similar to other study (Kiran *et al*., 2008).

Effect of different carbon sources on lipase production

Among the different carbon sources tested, the highest lipase activity was detected in

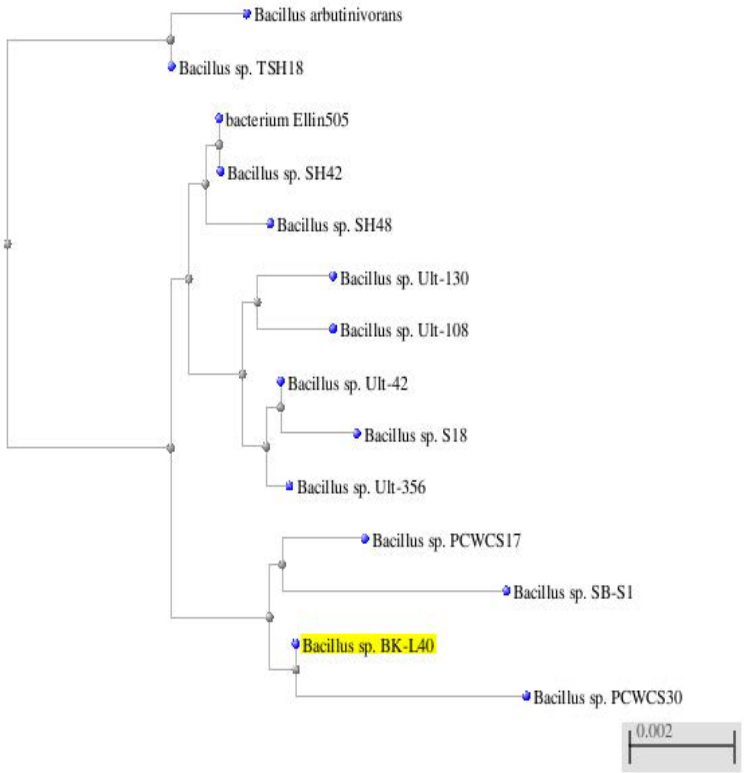

Fig. 2. Phylogenetic tree based on the 16S rRNA gene sequence generated using the neighbor-joining method showing the relationships between the strain BK-L40 and other related *Bacillus* species

the case of lactose (Fig. 3). The other carbon sources were produced lipase in descending order: sucrose > maltose > corn starch > glucose. Similarly, Joseph *et al*. (2006) have reported that the lipase production was improved after addition of lactose into the medium by *Staphylococcus epidermis*. The utilization of the particular carbon source for microbial growth and enzyme synthesis were varied from microorganism to microorganism.

Effect of different lipase inducers on lipase production

Of the different lipase inducers, the enzyme activity was decreased in all the tested lipase inducers compared than control (lactose). However, the enzyme activity was completely nil in the case of Tween 80 (Fig. 4). Joseph *et al*. (2006) have reported that Tween 80 was poor induction for lipase production in *Staphylococcus epidermis*. In contrast, Tween 80 was reported to be the best inducer for synthesis of lipase by *Acinetobacter* sp. (Anbu *et al*., 2011). Many researchers have reported that the lipase inducers were increased the enzyme activity (Gao *et al*., 2004; Joseph *et al*., 2006; Anbu *et al*., 2011). This result confirmed that

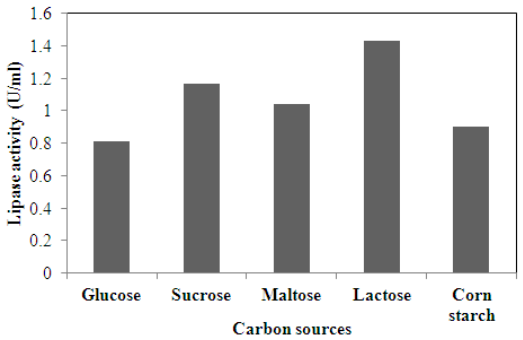

Fig. 3. Effect of various carbon sources on lipase production

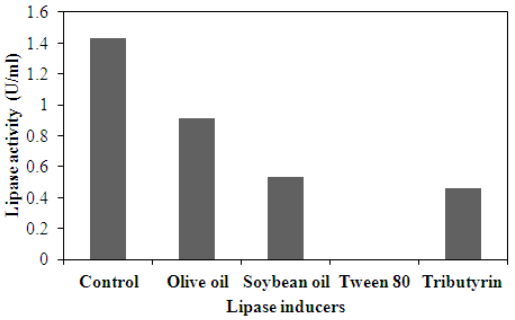

Fig. 4. Effect of various lipase inducers on lipase production

the lactose act as a carbon source as well as inducer for lipase production.

In conclusion, the lipase producing strain (BK40) was isolated from soil and identified as *Bacillus* sp. Further, the culture conditions (media, incubation time, carbon source and lipase inducers) were optimized for production of extracellular lipases. The highest production was observed in LB broth on 48 h of incubation and lactose (1%) as the carbons source. About 1.5 fold of lipase activity was increased in the optimized media compared with non-optimized media. The lipase activity was improved by using carbon source and repressed by lipase inducers.

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REFERENCES

1. Anbu, P., Noh, M.J., Kim, D.H., Seo, J.S., Hur, B.K., Min, K.H. Screening and optimization of extracellular lipases by Acinetobacter species isolated from oil-contaminated soil in South Korea. *Afri. J. Biotechnol.*, 2011; **10**: 4147-4156.
2. Ertugrul, S., Donmez, G., Takac, S. Isolation of lipase producing Bacillus sp. from olive mill wastewater and improving its enzyme activity. *J. Hazard. Mater.*, 2007; **149**: 720-724.
3. Gao, L., Xu, J.H., Li, X.J., Liu, Z.Z. Optimization of Serratia marcescens lipase production for enantioselective hydrolysis of 3-phenylglycidic acid ester. *J. Ind. Microbiol. Biotechnol.*, 2004; **31**: 525-530.
4. Gilbert, E.J., Drozd, J.W., Jones, C.W. Physiological regulation and optimization of lipase activity in Pseudomonas aeruginosa EF2. *J. Gen. Microbiol.*, 1991; **137**: 2215-2221.
5. Gupta, R., Gupta, N., Rathi, P. Bacterial lipases: an overview of production, purification and biochemical properties. *Appl. Microbiol. Biotechnol.* 2004; **64**: 763-781.
6. Jaeger, K.E., Ransac, S., Dijkstra, B.W., Colson, C., Hauvel, M.V., Misset, O. Bacterial lipases. *FEMS Microbiol. Rev.*, 1994; **15**: 29-63.
7. Joseph, B., Ramteke, P.W., Kumar, P.A. Studies on the enhanced production of extracellular lipase by Staphylococcus epidermidis. *J. Gen. Appl. Microbiol.*, 2006; **52**: 315-320.
8. Kiran, G.S., Shanmughapriya, S., Jayalakshmi, J., Selvin, J., Gandhimathi, R., Sivaramakrishnan, S., Arunkumar, M., Thangavelu, T., Natarajaseenivasan, K. Optimization of extracellular psychrophilic alkaline lipase produced by marine *Pseudomonas* sp. (MSI057). *Bioprocess Biosyst. Eng.*, 2008; **31**: 483-492.
9. Lawson, D.M., Brzozowski, A.M., Dodson, G.G., Hubbard, R.E., Huge-Jensen, B., Boel, E., Derewenda, Z.S. Lipases: Their structure, biochemistry and application. In: Woolley, P., Peterson, S.B. (eds), UK: Cambridge University Press, 1994.
10. Macrae, A.R., Hammond, R.C. In: Fogarty W.M (ed): Microbial enzymes and Biotechnology. Applied Science Publishers, 1983.
11. Mahler, G.F., Kok, R.G., Cordenons, A., Hellingwerf, K.J., Nudel, B.C. Effects of carbon sources on extracellular lipase production and lipA transcription in Acinetobacter calcoaceticus. *J. Ind. Microbiol. Biotechnol.*, 2000; **24**: 25-30.

12. Marshall, R.T. Standard methods for the examination of dairy products. 16th ed. Am Publ Health Assoc, Washington, DC, 1992.
13. Sharma, R., Chisti, Y., Banerjee, U.C. Production, purification, characterization and applications of lipases. *Biotechnol. Adv*., 2001; **19**: 627-662.
14. Takac, S., Marul, B. Effects of lipidic carbon sources on the extracelular lipolytic activity of a newly isolated strain of *Bacillus subtilis. J. Ind. Microbiol. Biotechnol.*, 2008; **35**: 1019-1025.
15. Winkler, U.K., Stuckmann, M. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens. J. Bacteriol*., 1979; **138**: 663-670.