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SHORT COMMUNICATION

Crystallization of *Aspergillus japonicus* lipase by hanging drop method

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Abstract

Lipase purified from *Aspergillus japonicus* isolated from *Ropalidia marginata* paper nest was attempted for crystallization by hanging drop method. 2-methyl- 2, 4-pentanediol (MPD) was used as the precipitating agent and single crystals of *A. japonicus* lipase were obtained by using protein concentrated to 10 mg/mL and a reservoir solution containing 10-20% MPD buffered with 150 mM cacodylate buffer (pH, 6.8). Small crystals of 0.05 x 0.05 x 0.03 mm dimension resulted at the end of a week.

Keywords: Lipase, *Aspergillus japonicus,* hanging drop method, precipitating agent, cacodylate buffer.

Introduction

Lipases (Triacylglycerol acyl hydrolase E.C.3.1.1.3) are involved in the breakdown and mobilization of lipids within the individual organisms as also their migration from one organism to another (Beisson, 2000; Muralidhar *et al*., 2001). Microorganisms are known to produce emulsifying agents or biosurfactants to help solubilize lipids (VanDyke, 1991). Thousands of enzymes with different substrate specificities are known, although only a few have been isolated in pure crystal form, and virtually little is known about their structure and function. Microbial lipases have assumed a great deal of importance as industrial enzymes in view of their potential for use in various biotechnological processes. Fungi are important enzyme producers since they produce enzymes extracellularly (Jaeger and Eggert, 2002).

Lipases are being exploited owing to their low cost of extraction, thermal and pH stability, substrate specificity, and activity in organic solvents. Lipases are the most widely used enzymes in organic syntheses and more than 20% biotransformations are performed with lipases (Gitlesen *et al*., 1997). In view of the variety in applications, there has been a renewed interest in the development of sources of lipases. Numerous species of bacteria, yeasts and moulds produce lipases with different enzymological properties and specificities but moulds are known to be more potent lipase producers (Choo *et al.,* 1998). These microorganisms produce lipases both by solid substrate and submerged fermentations (He *et al.,* 2004). Because of huge variation in applications, the availability of lipases with specific characteristics has still been a limiting factor. Thus, to search for new lipases with different characteristics and to improve their production continue to be new goals for researchers. Most commercially viable lipases are synthesized by fungi and yeasts.

Commercial lipolytic enzymes are produced from *Rhizopus delemar* (Espinosa *et al.,* 1990; Cruz *et al.,* 1993; Shimada *et al.,* 1996); *Humicola lanuginosa* (Morinaga *et al.,* 1986; Ivanova *et al.,* 2002), *Penicillium chrysogenum* (Ferrer *et al.,* 2000), *Fusarium heterosporum* (Nagao *et al.,* 1998), *Rhizopus chinensis* (Nakashima *et al.,* 1988) and *Candida rugosa* (Valero *et al.,* 1988; Obradors, 1993). A number of extracellular fungal lipases have been purified and the physico-chemical properties ascertained. Many lipases have been extensively purified and characterized in terms of their activity and stability profiles relative to pH, temperature, and effects of metal ions and chelating agents. In many cases, lipases have been purified to homogeneity and crystallized. Against these backdrops, in this little piece of investigation, lipase purified from *Aspergillus japonicus* isolated from *Ropalidia marginata* paper nest was attempted for crystallization by hanging drop method.

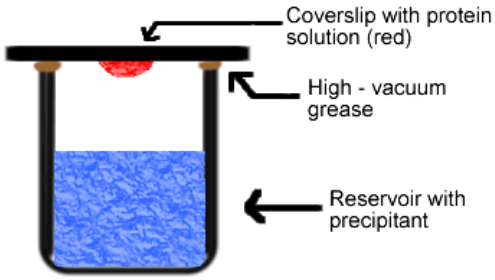
Materials and methods

Purified Aspergillus japonicus lipase: Lipase from *Aspergillus japonicus* isolated from *Ropalidia marginata* paper nest was purified to homogeneity by Q-sepharose and Sephadex G-100 chromatography (Jayaprakash and Ebenezer, 2012).

Crystallization of A. japonicus lipase by hanging drop method: Among the crystallization micro method (McPherson, 1982; 1990; Carter, 1990), hanging drop technique uses diffusion to bring about equilibrium and requires only small amount of protein. In this method a drop of 3-5 µL of protein solution of purified *A. japonicus* lipase was mixed with the same volume of precipitant solution suspended on a siliconized microscope glass cover slip. The cover slip placed upside down over a small well containing 0.5 to 1.0 mL of precipitating solution.

Care should be taken such that the chamber is sealed properly by applying grease to the circumference of the well before the cover slip is placed. The hanging drop setup is shown in Fig. 1. The initial precipitant concentration in the droplet is less than that in the reservoir. Over the time the reservoir pulls water from the droplet in a vapor phase until the surface tension of drop and reservoir are equilibrated. During this equilibration process the sample is also concentrated, increasing the relative super-saturation of the sample in the drop. Equilibration is reached when the reagent in the drop is approximately the same as in the reservoir (Rhodes, 1993; McRee, 1993).

Fig. 1. Hanging drop vapour diffusion.



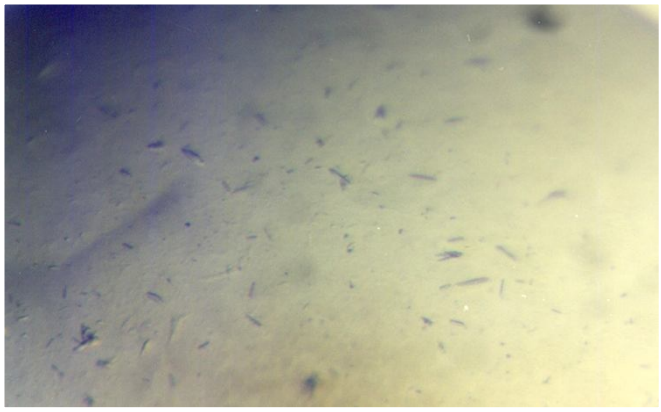
Results and discussion

The purified lipase from *A. japonicus* was crystallized by hanging drop vapour diffusion method. 2-methyl- 2, 4-pentanediol (MPD) was used as the precipitating agent. Single crystals of *A. japonicus* lipase were obtained by the hanging drop method using protein concentrated to 10 mg/mL and a reservoir solution containing 10-20% MPD buffered with 150 mM cacodylate buffer (pH = 6.8). Two µL of the protein was mixed with 2 µL of reservoir solution. Small crystals of 0.05 x 0.05 x 0.03 mm dimension resulted at the end of a week (Fig. 2). The dimensions of crystals for certain fungi are given as- *Geotrichum candidum;* 0.6 × 0.6 × 0.3 mm (Schrag and Cygler 1993), *Humicola lanuginose;* 0.4 × 0.4 × 0.2 mm (Brzozowski *et al*., 1991), *Rhizopus niveus;* 1.2 × 0.3 × 0.3 mm (Kohno *et al*., 1994), and *R. delemer;* 0.4 × 0.4 × 0.2 mm (Derewenda *et al*., 1992). The crystal size for *A. japonicus* is also characteristic to this fungus.

Conclusion

Lipase purified from *Aspergillus japonicus* isolated from *Ropalidia marginata* paper nest was attempted for crystallization by hanging drop method. 2-methyl- 2, 4-pentanediol (MPD) was used as the precipitating agent and single crystals of *A. japonicus* lipase were obtained by using protein concentrated to 10 mg/mL and a reservoir solution containing 10-20% MPD buffered with 150 mM cacodylate buffer (pH, 6.8). The crystals obtained were of 0.05 x 0.05 x 0.03 mm dimension.

Fig. 2. Crystals of *Aspergillus japonicus* lipase (x 6).



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References

1. Beisson, F., Arondel, V. and Verger, R. 2000. Assaying *Arabidopsis* lipase activity. *Biochem. Soc. Trans.* 28: 773–775.
2. Brzozowski, A.M., Derewenda, U., Derewenda, Z.S., Dodson, G.G., Lawson, D.M., Turkenburg, J.P., Bjorkling, F., Huge-Jensen, B., Patkar, S.A. and Thim, L. 1991. A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex. *Nature*. 351: 491–494.
3. Carter, W. 1990. Protein and nucleic acid crystallization, Academic Press, New York.
4. Choo, D.W., Kurihara, T., Suzuki, T., Soda, K. and Esaki, N.A. 1998. Cold adapted lipase of an Alaskan psychrotroph, *Pseudomonas* sp., Strain B 11-1: Gene cloning and enzyme purification and characterization. *Appl. Environ. Microbiol*. 64(2): 486-491.
5. Cruz, P.M., Christen, P. and Farres, A. 1993. Medium optimization by fractional factorial design for lipase production by *Rhizipus delemar*. *J. Ferm. Bioengg*. 76: 94–97.
6. Derewenda, U., Brzozowski, A.M., Lawson, D.M., Derewenda, Z.S., 1992. Catalysis at the interface: The anatomy of a conformational change in a triglyceride lipase. *Biochem.* 31: 1532–1541.
7. Espinosa, E., Sanchez, S. and Farres, A. 1990. Nutritional factors affecting lipase production by *Rhizopus delemar* CDBB H313. *Biotechnol. Lett*. 12: 209–214.
8. Ferrer, M., Plou, F.J., Nuero, O.M., Reyes, F. and Ballesteros A. 2000. Purification and properties of a lipase from *Penicillium chrysogenum* isolated from industrial wastes. *J. Chem. Technol. Biotechnol*. 75: 569–576.
9. Gitlesen, T., Bauer, M. and Adlercreutz, P. 1997. Adsorption of lipase on polypropylene powder. *Biochemica. et. Biophysica. Acta.* 1345: 188-196.
10. He, Y.Q., Wang, B.W. and Tan, T.W. 2004. Fermentation production technology of lipase with *Candida* sp. *Chinese J. Biotechnol*. 20(6): 921-926.

11. Ivanova, M., Svendsen, A., Verger, R. and Panaiotov, I. 2002. Action of *Humicola lanuginosa* lipase on long-chain lipid substrates: 1. Hydrolysis of monoolein monolayers. *Colloids Surfaces B. Biointerfac*. 26: 301–314.
12. Jaeger, K.E. and Eggert, T. 2002. Lipases for biotechnology. *Curr. Opin. Biotechnol.* 13: 390-397.
13. Jayaprakash, A. and Ebenezer, P. 2012. Purification and characterization of *Aspergillus japonicus* lipase from a pig fat production medium. *J. Acad. Indus. Res*. 1(1): 1-7.
14. Kohno, M., Kugimiya, W., Hashimoto, Y. and Morita, Y. 1994. Purification, characterization and crystallization of two types of lipases from *Rhizpous niveus*. *Biosci. Biotechnol. Biochem.* 58: 1007–1012.
15. McPherson, A. 1982. Preparation and analysis of protein crystals, Wiley, New York.
16. McPherson, A. 1990. A current approach to macromolecular crystallization. *Euro. J. Biochem*. 189: 1-23.
17. McRee, D.E. 1993. Practical protein crystallography. San Diego: Academic Press, pp. 1-23.
18. Morinaga, T., Kanda, S. and Nomi, R. 1986. Lipase production of a new thermophilic fungus, *Humicola lanuginosa var. catenulata. J. Ferm. Technol*. 64: 451–453.
19. Muralidhar, R.V., Chirumamila, R.R., Marchant, R. and Nigam, P. 2001. A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochem. Engg. J.* 9: 17-23.
20. Nagao, T., Shimada, A., Sugihara, A. and Tominaga, Y. 1998. C-Terminal peptide of *Fusarium heterosporum* lipase is necessary for its increasing thermostability. *J. Biochem*. 124: 1124–1129.
21. Nakashima, T., Fukuda, H., Kyotani, S. and Morikawa, H. 1988. Culture conditions for intracellular lipase production by *Rhizopus chinensis* and its immobilization within biomass support particles. *J. Ferm. Technol*. 66: 441–448.
22. Obradors, N. and Montesinos, J.L. 1993. Effects of different fatty acids in lipase production by *Candida rugosa. Biotechnol. Lett*. 15(4): 357-360.
23. Rhodes, G. 1993. Crystallography made crystal clear. San Diego: Academic Press, pp. 8-10, 29-38.
24. Schrag, J. and Cygler, M. 1993. A refined structure of the lipase from *Geotrichum candidum*. *J. Mol. Biol*. 230: 575-591.
25. Shimada, Y., Sugihara, A., Maruyama, K., Nagao, T., Nakayama, S., Nakano, H. and Tominaga, Y. 1996. Production of structured lipid containing docosahexaenoic and caprylic acids using immobilized *Rhizopus delemar* lipase. *J. Ferm. Bioengg.* 81: 299–303.
26. Valero, F., Ayats, F., Lopez-Santin, J. and Poch, M. 1988. Lipase production by *Candida rugosa*: Fermentation behaviour. *Biotechnol. Lett*. 10: 741–744.
27. VanDyke, M.I., Lee, H. and Trevors, J.T. 1991. Applications of microbial surfactants. *Biotechnol. Adv*. 9: 241–252.

