African Journal of Biotechnology Vol. 8 (1), pp. 073-076, 5 January, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

A comparative study of the lipase yield by solid state and submerged fermentations using fungal species from biopharmaceutical oil waste

V. Mohanasrinivasan[1]*, P. Dhrisya[1], K. P. Dipinsha[1], C. Manu Unnithan[1], K. Megha Viswanath[1] and C. Subathra Devi[2]

[1]Department of Biotechnology, MET'S School of Engineering, Mala, Thrissur-680735, India.
[2]School of Biotechnology, Chemical & Biomedical Engineering, VIT University, Vellore, Tamilnadu, India

Accepted 3 November, 2008

A comparative study of lipase enzyme yields by solid state fermentation (SSF) and submerged fermentation (SmF) was performed here. Three fungal colonies were isolated from biopharmaceutical oil waste collected from "Oushadhi" (The Pharmaceutical Corporation (IM) Kerala Ltd). The pure colonies were then used as inoculums for solid state and submerged fermentation of lipase. Strains were identified as *Aspergillus* sp., *Trichoderma* sp. and *Penicillium* sp. The oil waste itself was used as a substrate for SSF and it was supplemented with ($(NH_4)_2SO_4$ 5.0 g/l; Na_2HPO_4 6.0 g/l; KH_2PO_4 2.0 g/l; $MgSO_4.7H_2O$ 3.0 g/l and $CaCl_2$ 3.0 g/l) at pH 6. The composition of production media (pH 6) used for SmF was glucose-10, peptone-20, NaCl-5 and yeast extract-5 (g/l).The comparison of enzyme yields showed that the yield in SSF was higher than that of SmF in most cases. Out of the three fungal strains, lipase produced by *Aspergillus* sp. showed the highest enzyme activity on assay by olive oil substrate emulsion method.

Key words: Lipase, SSF, SmF, *Aspergillus* sp, *Trichoderma* sp and *Penicillium* sp.

INTRODUCTION

Lipases (triacylglycerol acylhydrolase; E.C.3.1.1.3) are ubiquitous enzymes produced by most biological systems including animals, plants and microorganisms. In eukaryotes, lipases are involved in various stages of lipid metabolism and are found in energy reservoir tissues. Lipases have become one of the prominent industrial enzymes they act over a wide range of pH and temperature, possess high specificity, do not require co-factors, and can catalyze a wide range of reactions.

The lipases produced by microbes such as bacteria, fungi and yeast are most suitable for industrial applications because of their ease of production, relatively inexpensive fermentation techniques, wide variety and stability in organic solvents, which enables their use in organic synthesis. They hydrolyze triglycerides to diglycerides, monoglycerides, glycerol and fatty acids. In addition, they also serve as biocatalysts for alcoholysis, acidolysis, esterification and ammonolysis. The common mode of action of lipases is as shown:

Fats or Oils + Water $\rightarrow$ Fatty acids + Glycerol

With the rapid development of enzyme technology, many new potential biotechnological applications for lipases have been identified in the areas of detergent industry, food industry, paper manufacturing industry, biosurfactant synthesis, organic synthesis of cosmetics and pharmaceuticals. They have also proved effective and promise a vital role in separating enantiomers, possessing high regio and stereo selectivity (Razors and Voss, 2001).

Each industrial application requires lipase with properties with respect to specificity, stability, temperature, pH dependence and ability to catalyze synthetic ester reactions in organic solvents. The hunt for such enzymes goes on. Solid state fermentation processes are of special economic interest for countries with an abundance of biomass and agro-industrial residues. SSF is gaining more and more attention in recent years due to the possibility of using cheap agro-industrial waste as substrate.

*Corresponding author. E- mail: mona_mic78@yahoo.co.in.

Here a comparative study of lipase enzyme yields by SSF and SmF was performed.

METHODOLOGY

Sample collection and site description

The materials utilized as substrate for solid state fermentation was procured from 'Oushadhi', The Pharmaceutical Corporation (IM) Kerala Ltd. Oushadhi is a fully Government owned enterprise which produces ayurvedic medicines, food supplements, cosmetics etc. Oushadhi has a state-of-the-art manufacturing facility at Kuttenellur, 8 km East of Thrissur. While following traditional ayurvedic medicine manufacturing systems, this factory combines the elements of modern technology by using advanced machinery. With more than 450 popular products, Oushadhi meets the entire requirements of Government hospitals and dispensaries in Kerala and other states. The products include Asavas, Arishttas, Dhravams, Choornams, Khashayas, Thailas (medicated oil), and Chyavanaprasams. The process of production produces a vast amount of waste materials-both oily and non-oily. Here we have taken oil-containing waste as substrate for lipase production.

Isolation of fungal strains from oil waste

The fungal strains were isolated from the substrate (oil waste). After incubating the substrate in the open air environment for a week, the substrate containing plate was noticed for the growth of fungal strains. Pure cultures of fungal strains were obtained by sub culturing onto the Potato Dextrose Agar (PDA) medium.

A loopful pure culture of fungi was suspended into few drops of lactophenol-cotton-blue solution on a microscope slide and covered with a cover slip. The preparation was examined under a microscope and the observations were recorded in order to identify the fungi.

preparation of the inoculum for lipase production

A spore suspension was prepared by adding 5 ml sterile distilled water to 72 h culture ($1x10^8$ spores per ml), and added to 45 ml inoculum media that consist of (g/l) glucose 10, peptone 20, NaCl 5 and yeast extract 5, pH 6 in 250 ml Erlenmeyer flasks, incubated on rotary shaker at 30°C for 48 h.

Production of lipase by solid state fermentation

The production was carried out in 1000 ml Erlenmeyer flasks. 40 g of the solid substrate and 4 ml of salt solution (pH 6.0) with the composition (g/l) $(NH_4)_2SO_4$ 5.0; Na_2HPO_4, 6.0; KH_2PO_4 2.0, $MgSO_4.7H_2O$ 3.0 and $CaCl_2$ 3.0 was taken in 3 flasks. The moisture content was maintained at 70% by addition of distilled water. The flasks were autoclaved and cooled. 10% inoculum was added to each of the flasks and incubated at 30°C for 96 h.

Production of lipase by submerged fermentation

The process was carried out in 1000 ml Erlenmeyer flasks. A 10%level of inoculum was added to 500 mL production medium and incubated at 30°C for 96 h. The samples were withdrawn after 96 h and assayed for their lipolytic activity.

Lipase extraction from solid state fermentation

After the completion of 96 h of fermentation the enzyme was extracted and the activity determined as follows: The biomass with substrate was agitated with 50 ml of distilled water on a magnetic stirrer for 30 min. The contents were filtered through muslin cloth. Residue was again treated with 50 ml of distilled water and extracted. The filtrates were pooled together and centrifuged at 10,000 rpm for 15 min. The supernatant obtained after centrifugation served as the enzyme source. The total protein concentration was estimated by Lowry et al. (1993) method.

Lipase extraction from submerged fermentation

After the completion of 96 h of fermentation, 50 ml of biomass with broth was agitated on a magnetic stirrer for 30 min. The contents were filtered through muslin cloth. Residue was treated with 50 ml of distilled water and extracted. The filtrates were pooled together and centrifuged at 10,000 rpm for 15 min. The supernatant obtained after centrifugation served as the enzyme source. The total protein concentration was estimated by Lowry et al. (1993) method.

Lipase assay (Mustranta et al. 1992)

The activity of lipase enzyme was determined using olive oil substrate emulsion method. The assay mixture consisted of 1.0 ml of the substrate emulsion (70.0 ml emulsifying reagent with 30.0 ml olive oil homogenized for 5 min; the emulsification reagent comprising NaCl 17.9 g, KH_2PO_4 0.14 g, glycerol 540.0 ml, gum Arabic 10.0 g and distilled water to a total volume of 1.0 L.), 0.8 mL of 0.2 M potassium phosphate buffer (pH 7.0) and 0.2 ml of this enzyme was incubated at 37°C for 15 min. The reaction was terminated by adding 2.0 ml of acetone – ethanol mixture (1:1, v/v). The amount of fatty acid liberated was determined by titration with 0.01 N NaOH. One unit (U) of lipase activity is defined as the amount of enzyme required to liberate one micromole equivalent fatty acid/minute/ml under the above assay conditions.

RESULTS AND DISCUSSION

Growth of three fungal colonies was observed. Strain 1 showed the characteristic brown colored colonies with black colored spores; strain 2 showed bluish-green colored colonies; and strain 3 showed yellowish green colored colonies given in Figure 1 The pure cultures (Figure 2) of fungal strains were stained using lacto phenol cotton blue stain. According to the microscopic observation and known data described by Cappuccino and Sherman (2004), the fungal strains were identified as strain 1 *Aspergillus* sp., Strain 2 *Penicillum* sp. and strain 3 *Trichoderma* sp.

In the present study we had compared the lipase yield by SSF and SmF using the three fungal species given in Figures 3 and 4. For different fungal species the results are shown in Tables 1, 2 and 3. As shown in Table 1, total enzyme activity for SSF and SmF was 213.165 U and 119.628 U, respectively. From this it was evident that the lipase yield is higher in case of SSF when compared to SmF using *Aspergillus* sp. Table 3 shows the comparison of enzyme yield by SSF and SmF using *Penicillium* sp.; it is clear that lipase yield is higher in case of SSF. On the contrary Table 2 shows that the enzyme yield from SmF had higher yield when compared to SSF, using *Trichoderma* sp. Vazquez et al. (1993) compared SmF systems and SSF systems for lipase

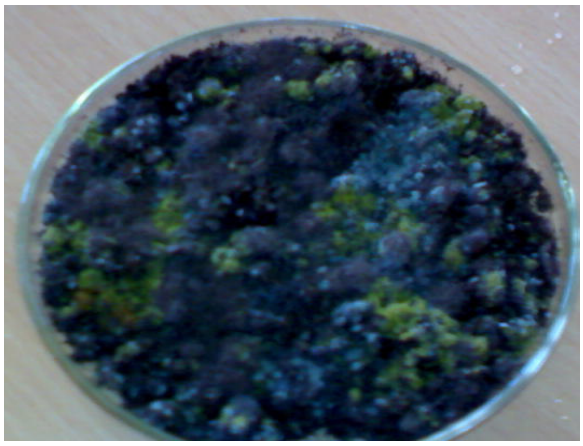

Figure 1. The three different fungal colonies growing on the oil waste.

A

B

Figure 2. Pure culture of *Aspergillus* sp. (A) and *Trichoderma* sp. (B).

Figure 3. Solid state fermentation flask.

Figure 4. Submerged fermentation flask.

production using several filamentous fungi. Enzyme titers by SSF processes were higher and stable. Among the tested microbial strains, *Petalostemon candidum*, *Petalostemon camembertii*, and *Mucor miehei* proved the best for lipase production. Yet, in another comparative study on lipase production in SmF and SSF systems, Christen et al. (1995) observed a 5-fold increase in lipase productivity in SSF system. In contrast to this, however, Ohnishi et al. (1994) reported less lipase production from *Aspergillus oryzae* using SSF, when compared to SmF where high enzyme yields were obtained.

Raw cake supported the growth and lipase synthesis by the yeast culture. However, supplementation with additional C- and N-sources increased enzyme titers. Bhusan et al. (1994) reported lipase production in SSF system from an alkalophilic yeast strain belonging to

Table 1. Assay of lipase produced by *Aspergillus* sp. using biopharmaceutical oil waste as substrate.

Enzyme sample	Total volume (ml)	Protein concentration (mg/ml)	Total protein (mg)	Specific activity (U/mg of protein)	Total activity (U)
Crude SSF 1	80	12.167	973.36	0.219	213.165
Crude SmF 1	80	9.969	797.52	0.150	119.628

Table 2. Assay of lipase produced by *Trichoderma* sp. using biopharmaceutical oil waste as substrate.

Enzyme sample	Total volume (ml)	Protein concentration (mg/ml)	Total protein(mg)	Specific activity (U/mg of protein)	Total activity (U)
Crude SSF 2	80	11.080	886.40	0.105	93.072
Crude SmF 2	80	11.198	895.84	0.178	159.459

Table 3. Assay of lipase produced by *Penicillium* sp. using biopharmaceutical oil waste as substrate.

Enzyme sample	Total volume (ml)	Protein Concentration (mg/ml)	Total protein (mg)	Specific activity (U/mg of protein)	Total activity (U)
Crude SSF 3	80	7.173	573.84	0.348	199.696
Crude SmF 3	80	6.457	516.56	0.206	106.411

Candida sp. Rice bran and wheat bran, oiled with different concentrations of rice bran oil were used as the substrate. Rice bran supplemented with oil gave higher lipase yields on comparison with wheat bran. In the present study, we had used biopharmaceutical oil waste as substrate for SSF which consisted of plant matter containing cellulose, medicinal oil, etc. It was found that in most cases SSF gave higher enzyme productivity on comparison to SmF. The SSF using *Aspergillus* sp. showed high lipase activity of 213.165 U. *Trichoderma* sp. showed the lipase activity of 93.072 U. *Penicillum* sp. showed lipase activity of 199.696 U. The total protein concentration obtained from SSF was also comparatively high. It was 12.167 mg/ml for *Aspergillus* sp., 11.080 mg/ml for *Trichoderma* sp., and 7.173 mg/ml for *Penicillum* sp.

The utilization of biopharmaceutical oil waste on one hand provides alternative substrate and on the other, helps in solving pollution problems.

REFERENCES

Bhusan B, Dosanjih NS, Kumar K, Hoondal GS (1994). Lipase production from an alkalophilic yeast sp. by solid state fermentation, Biotechnol. Lett, 16: 841-842.

Cappuccino GJ, Sherman N (2004). Microbiology: A Laboratory Manual, pp. 213-225

Christen P, Angeles N, Corzo G, Farres A, Revah S (1995). Microbial lipase production on a polymeric resin. Biotechnol. Tech. 9: 597-603.

Lowry OH, Rosenbrouugh NJ, Farr AL, Randal RJ (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Mustranta A (1992). Use of lipase in the resolution of raceme ibuprofen. Appl. Microbiol. Biotechnol. 38: 61-66.

Ohnishi K, Yoshida Y, Sekiguchi J (1994). Lipase production of Aspergillus oryzae. J. Ferment. Bioeng. 77: 490-495.

Rasors JP, Voss E (2001). Enzyme catalyzed processes in pharmaceutical industry, Appl. Catal. A: Gen. 221: 145-158.

Vazquez OE, Granados-Baeza M, Rivera-Munoz G (1993). Substrates used for solid substrate fermentation. Biotechnol. Adv., 11: 409-416.