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Lipase production by *Fusarium culmorum* in solid state fermentation

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Abstract: *Fusarium* is a large genus of filamentous fungi which cause some of the most important diseases in agricultural and horticultural crops. This fungus is considered to be a useful producer of enzymes from an industrial point of view. In the present study, lipase production by *Fusarium culmorum* SY6 was investigated under solid-state fermentation (SSF). Among the several agronomic wastes, corn cob hulls and tomato pulp supported the highest yield of lipase (170 and 165 U/g of dry substrate, respectively) after five days of incubation. It was determined that pH 9 and 60°C gave optimum enzyme activity. The *F. culmorum* SY6 strain grown in SSF in a simple medium proved to be a promising microorganism for lipase production.

1. Introduction

Lipase (triacylglycerol acylhidrolases, EC 3.1.1.3) is an extra cellular enzyme which catalyses the hydrolysis of triglycerides to free fatty acids and glycerol (Singh and Mukhopadhyay, 2012). According to its various industrial applications, biotechnological uses for lipases are steadily increasing (Stamatis *et al.,* 1999). Although lipases can be obtained from bacteria and yeasts (Jaeger *et al.,* 2000; Kulkarni *et al.,* 2002), the enzymes from fungi generally meet industrial demand since they are usually excreted extracellularly, facilitating extraction from fermentation media (Hiol *et al.,* 2000; Abbas *et al.,* 2002).

Fusarium is a large genus of filamentous fungi, and most of *Fusarium* species are harmless saprobes and relatively abundant members of the soil microbial community (Summerell *et al.,* 2001). This ecological habitat of the fungus implies that *Fusarium* would be a useful resource of extracellular enzymes. Several different enzymatic activities were investigated in isolates of *Fusarium* species, including lipase (Burkert *et al.,* 2004; Bakri *et al.,* 2013, 2014).

Solid-state fermentation (SSF) technique involves the growth and metabolism of microorganisms on moist solids without any free flowing water. SSF has many advantages over submerged fermentation, including an economical use of space that is required for fermentation, simplification of the fermentation media, superior yields, and no requirement for complex machinery (Pandey, 1994). However, enzyme production is related to the type and concentrations of nutrients and growth conditions (Prazeres *et al.,* 2006).

To reach commercial feasibility, enzyme production must be increased by introducing more potent strains, and by optimising culture conditions (Singh and Mukhopadhyay, 2012). Since the effect of carbon sources on lipase production by the fungus *F. culmorum* has not been investigated so far, a study toward this aim was conducted on the new *F. culmorum* strain SY6 cultured under solid state fermentation (SSF).

2. Materials and Methods

Microorganism

The organism used was *F. culmorum* SY6, isolated in our laboratory and having the ability to produce lipase enzyme (Bakri *et al*., 2014). The strain was grown on Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI. USA) with 13 mg/l kanamycin sulphate added after autoclaving and incubated at 23°C for 10 days in the dark to allow mycelial growth and sporulation. The cultures were maintained on silica gel at 4°C until needed.

Cultural conditions

The strain *F. culmorum* SY6 was grown in 250-ml Erlenmeyer flasks containing (g/l): $Na_2HPO_4 \cdot 2H_2O$ 10; KCl 0.5; $MgSO_4 \cdot 7H_2O$ 0.15 and yeast extract 5, as nitrogen source. The mineral salt was added in such a way that the final substrate-to-moisture ratio was 1:5. The pH was adjusted to 6.5 before sterilization. The influences of different carbon sources (wheat bran, corn cobs hulles, beet pulp, tomato pulp, soya cake, cotton seed cake and wheat

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straw) on lipase production were tested (Fig. 1). The contents were sterilized by autoclaving at 121°C for 15 min. After cooling, the sterilized medium was inoculated with spores (10^6/ml) from a seven-day-old culture. The flasks were incubated at 30°C for five days.

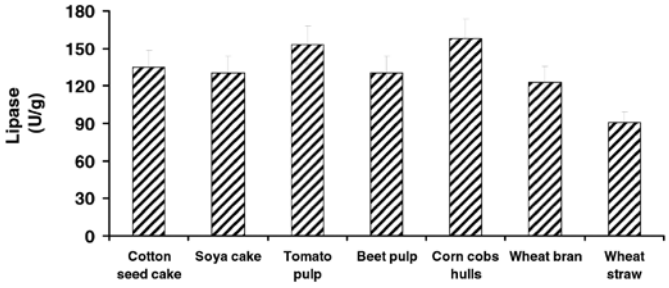

Fig. 1 - Effect of some agro-industrial wastes as carbon source on lipase production by *F. culmorum* SY6.

Enzyme assay

Liplolytic activity was determined titirmetrically on the basis of olive oil hydrolysis (Macedo *et al*., 1997) with some modifications. Olive oil emulsion substrate was prepared by mixing 10 mL olive oil and 90 ml from 5% Arabic gum solution. The reaction mixture contained 5 mL olive oil emulsion substrate and 1 mL of crude enzyme. The enzyme substrate mixture was incubated on an orbital shaker with a shaking speed of 150 rpm at 50°C for 20 min. To stop the reaction, 10 ml ethanol acetone mixture (1:1) was added to the reaction mixture. Liberated fatty acids were titrated with 0.05 mol/L NaOH. One lipase unit (U) was defined as the amount of the enzyme that released one µmol fatty acid per min.

Effect of pH

The effect of pH on lipase activity was measured at different pH values ranging from 4 to 10. The pH of the reaction mixture was varied using various buffers (citrate buffer, phosphate buffer, and borate buffer).

Effect of temperature

Temperature effect on lipase activity was determined at different temperatures in the range 40-80°C. Crude enzyme and substrate were tested by pre-incubating at various reaction temperatures to determine the optimal incubation temperature.

Statistical analysis

All the experiments were repeated twice, and the means were analyzed statistically with the analysis of variance using the STAT-ITCF program; LSD= least significant differences at $P<0.05$.

3. Results and Discussion

Effect of carbon sources and lipase production

The results showed that significant differences ($P<0.05$) in the mean lipase yield values existed among carbon sources, with values being consistently higher on corn cob hulls and tomato pulp (170 and 165 U/g, respectively) after five days of incubation, whereas, wheat straw exhibited lowest activity. These results might be attributed to the fact that the presence of more available carbon increases both mycelium growth and its activity. Moreover, corn cob granulate shows excellent adsorption characteristics (Damaso *et al.,* 2008). However, findings these agree with those of Abbas *et al*. (2002), but not with those of Burkert *et al*. (2004). In addition, our results are in agreement with those of Gombert *et al.* (1999) and Falcony et *al.* (2006) in SSF, who reported that lipase enzyme produced by fungi, could be enhanced under SSF.

Although quantitative comparison of lipase activities reported in literature is not always possible because no standard enzyme substrate has been adopted yet, the lipase productivity from *F. culmorum* SY6 observed in this work was higher than optimum productivities reported in the literature for some microorganisms grown in SSF (Table 1).

Effect of PH

Generally, enzymes are sensitive to the concentration of hydrogen ions present in the reaction mixture; therefore, pH is considered an important factor that determines the enzyme activity. The pH- relative activity of *F. culmorum* SY6 lipase was determined in the range 4.0-10.0 pH (Fig. 2). The optimum pH was found to be 9. Most microbial lipases have their optimum activity at a pH range of 7.0-9.0 (Zhang *et al*., 2005; Ulker *et al*., 2011). An optimum pH of 7.0 for *Rhizopus oryzae* lipase (Hiol *et al*., 2000), pH of

Table 1 - Optimum lipase activities produced by filamentous fungi grown in SS

Microorganism	Substrate	Lipase (U/g)	Reference
Aspergillus niger J-1	wheat bran	9.14	Falcony *et al.,* 2006
Penicillium restrictum	babassu oil cake	30.3	Gombert *et al.,* 1999
Fusarium culmorum	cobs hulls	170	This work
Rhizopus oligosporous	almond meal	48	Ul-Ha *et al.,* 2002
Rhizomucor pusillus	olive oil cake and sugar cane	79.6	Cordova, 1998

8.5 for *Trichoderma harziamum* (Ulker *et al*., 2011), and pH 9.0 for *Penicillium caseicolum* lipase (Saxena *et al*., 2003) has been reported.

Effect of temperature

Optimization of temperature is vital for enzyme activity. In our work, the activity of F. culmorum SY6 lipase was investigated at different temperatures and the results obtained are shown in figure 3. The data reveal that the optimum temperature for lipase activity was at 60°C, followed by 70 and 80°C However, when the temperature increased or decreased from 60°C, the activity of lipase gradually reduced. Prazeres et al. (2006) and (Ulker et al., 2011) reported that Fusarium oxysporum and Trichoderma harzianum presented lipase with maximum activity at 55 and 40°C respectively.

4. Conclusions

The present study reveals that *F. culmorum* SY6 strain is a potential and promising microorganism as it produced a high level of lipase under solid state fermentation. Adding corn cob hulls and tomato pulp significantly increased the enzyme production (170 and 165 U/g, respectively) after five days of incubation compared to 117 U/g reported by Bakri *et al.* (2014) in which this compound was not used. Moreover, the basic parameters such as pH and temperature were found to exert a marked influence on the activity of lipase.

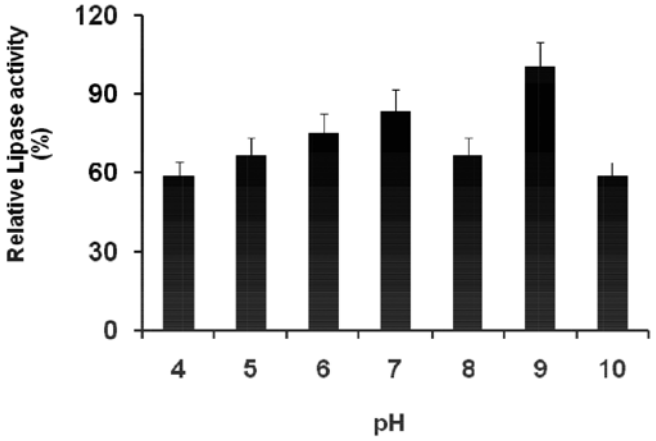

Fig. 2 - Effect of different pH values on the activity of *F. culmorum* SY6 lipase.

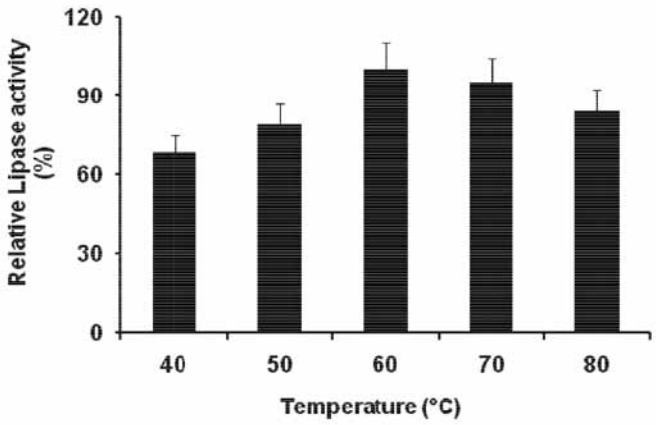

Fig. 3 - Effect of temperature on the activity of *F. culmorum* SY6 lipase.

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