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Mineral Constituents of Culture Medium for Lipase Production by *Rhizopus oligosporous* Fermentation

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Abstract: Solid-state fermentation for lipase production was carried by *Rhizopus oligosporous* ISU^{UV}-16. Almond meal was used as basal substrate with distilled water as moistening agent at substrate to diluent ratio of 1:0.7. Fermentation was carried out at 30°C and culture was incubated for 48 h. Maximum lipase activity under solid-state condition was obtained when Tween 80 at the level of 0.5 % was used as additional carbon source. Best organic nitrogen sources for optimal lipolytic activity was ammonium sulfate (4 %). This study would improve the further optimization steps on the bioprocess development track.

Key words: Solid-state fermentation, lipase, *Rhizopus oligosporous*, mineral constituents, carbon source, nitrogen source

Introduction

Lipases constitute the most important group of biocatalysts for biotechnological applications. Novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals, and flavor compounds (Jaeger and Eggert, 2002). The advantages of the enzymatic hydrolysis over a chemical process are less energy requirements and higher quality of the obtained product. Solid-state fermentation is a conventional and an efficient method for lipase biosynthesis. The solid-state fermentation holds tremendous potential for the production of enzyme as it fulfills the demand of industrial environment and food biotechnology as compared to stirred fermentation (Pandey *et al.*, 1999). Different kinds of concentration of carbon and nitrogen sources have a significant effect on lipase yield. Tween 80 was used as a principal carbon source for the maximum production of lipase and found to be among the highest positive significant variables affecting lipase activity (Abdel-Fattah *et al.*, 2002). Polypropylene powders as the adsorbent for organic solution containing n-hexadecane and olive oil were employed as the carbon source for producing an alkaline lipase from *Acinetobacter radioresistens* (Liu and Tsai, 2003). Benjamin and Pandey (1997) reported ammonium sulfate as best nitrogen source for lipase productivity. The highest enzyme productivity was obtained in a medium with olive oil as carbon source and a combination of ammonium sulfate and peptone as nitrogen source (Gao and Breuil, 1995; Zhou *et al.*, 2000).

Materials and Methods

Organism and culture maintenance: *Rhizopus oligosporous* ISU^{UV}-16, a UV treated mutant was used in present study. The culture was maintained on agar malt medium.

Fermentation technique: Production of fungal lipase was studied by solid-state fermentation technique (Korn and Fujio, 1997). Ten grams of almond meal with 7 ml of diluent was added in 250 ml cotton wool plugged conical flask. The flasks were autoclaved at 15-lb/inch² pressure (121°C) for 15 min. and cooled at room temperature. One ml of the spore suspension (2.7×10^6 spores ml⁻¹) was aseptically transferred to each flask and flasks were then placed in an incubator at 30±2°C for 48 h. The flasks were run parallel in triplicates.

Assay protocol

Enzyme extraction: After 48 h 100 ml of phosphate buffer (pH 7.0) was added to each flask. The flasks were rotated at the rotary shaker at 200 rpm for one hour at 30°C. After one hour the ingredients of the flask were filtered and filtrate was used for estimation of lipase activity.

Lipase activity: A unit lipase is defined as “the amount of enzyme, which release one micromole fatty acid per minute under specified assay conditions”. Lipase activity (U/g) in the fermented meal was determined titrimetrically on the basis of olive oil hydrolysis (Kundu and Pal, 1970). One ml of culture supernatant was added to the reaction mixture containing 10% homogenized olive oil in 10% gum acacia,

0.6% CaCl_2 solution and phosphate buffer (pH 7.0). Liberated fatty acid was titrated against 0.1N NaOH using phenolphthalein as indicator. The end point was pink color.

Results

Effect of additional carbon sources: Different carbon sources were used as additional carbon sources for the enhancement of lipase activity by strain of *Rhizopus oligosporus* ISU^{UV}-16 (Fig. 1). Additional carbon source at the level of 1% was added to the almond meal substrate. Glucose, sucrose, starch, olive oil, mustard oil and Tween 80 gave lipase activity 32.56, 36.84, 27.54, 46.05, 31.09 and 62.84 Ug^{-1} , respectively. Hence maximum production of lipase was observed when Tween 80 was used as carbon source. Tween 80 was optimized as the best carbon source for lipase activity.

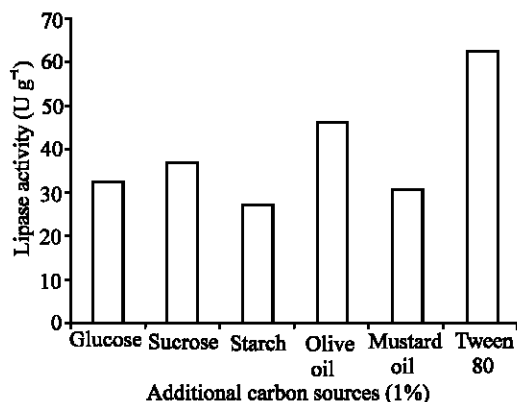


Fig. 1: Influence of different additional carbon sources on lipase production by *Rhizopus oligosporus* ISU^{UV}-16

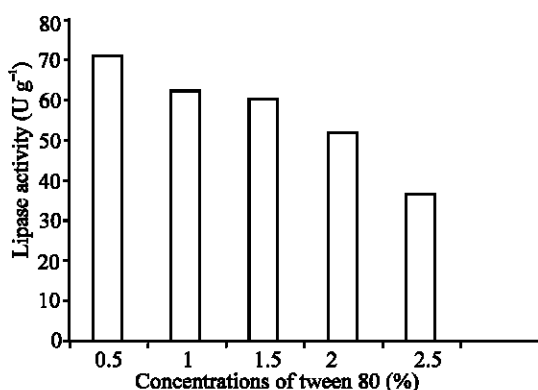


Fig. 2: Optimal concentration of Tween 80 for lipase production by *Rhizopus oligosporus* ISU^{UV}-16.

Effect of different amount of Tween 80: The data of Figure 2 shows the effect of different amount of Tween 80 on the

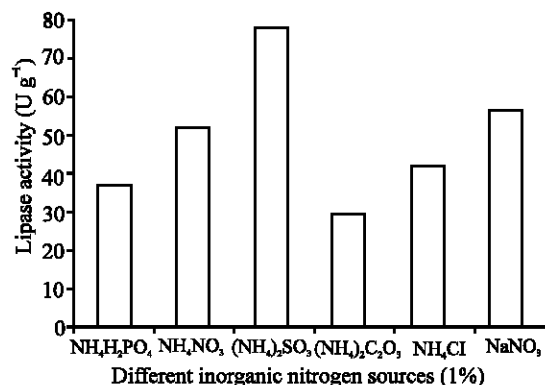


Fig. 3: Influence of different inorganic nitrogen sources on lipase production by *Rhizopus oligosporus* ISU^{UV}-16

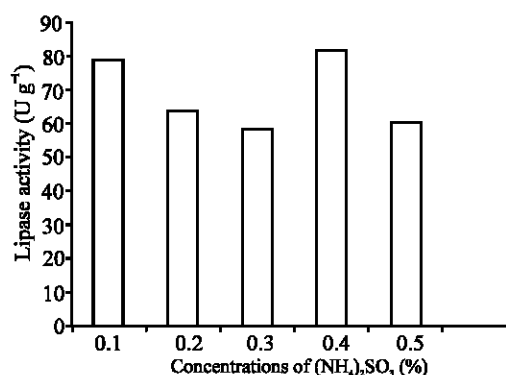


Fig. 4: Optimal concentration of ammonium sulfate for lipase production by *Rhizopus oligosporus* ISU^{UV}-16

production of lipase by strain of *Rhizopus oligosporus* ISU^{UV}-16. Tween 80 at a level of 0.5, 1.0, 1.5, 2 and 2.5% gave lipase activity 71.65, 62.87, 60.61, 51.65% and 36.69 Ug^{-1} , respectively. Maximum production of lipase (71.65 Ug^{-1}) was observed when 0.5% was added in the substrate as an additional carbon source.

Effect of different inorganic nitrogen sources: Different amounts of nitrogen sources (inorganic) on the production of lipase by *Rhizopus oligosporus* ISU^{UV}-16 were also studied (Figure 3). $\text{NH}_4\text{H}_2\text{PO}_4$, NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{C}_2\text{O}_4$, NH_4Cl and NaNO_3 gave lipase activity 37.31 Ug^{-1} , 52.45 Ug^{-1} , 79.10 Ug^{-1} , 30.07 Ug^{-1} , 43.24 and 57.72 Ug^{-1} , respectively. Hence, $(\text{NH}_4)_2\text{SO}_4$ was optimized as the best inorganic nitrogen source for lipase activity.

Effect of different amounts of ammonium sulfate: Ammonium sulfate at a level of 0.1%, 0.2%, 0.3%, 0.4% and 0.5% was used for lipase activity (Fig. 4). Maximum

production of lipase (81.22 Ug^{-1}) was observed when 0.4% $(\text{NH}_4)_2\text{SO}_4$ was added in the culture medium as an additional inorganic nitrogen source.

Discussion

Different carbon sources were used for extracellular lipase production by strain of *Rhizopus oligosporus* ISU^{uv}-16. Tween 80 was found to be the best carbon source, as compared to others. Since it was miscible with water and did not generally inhibit fungal growth (Martinez *et al.*, 1993; Abdel-Fattah *et al.*, 2002). Handelsman and Shoham (1994) also reported the production of extracellular lipase by addition of Tween 80 as best carbon source. Variation in the concentration of Tween 80 was also effective for enhanced lipase production. Maximum lipase activity (71.65 Ug^{-1}) was obtained at 0.5% concentration of Tween 80 as it provide optimum amount of carbon. Enzyme level however decreased with further increase in Tween 80 concentration. It might be due to the increase in fatty acid accumulation through hydrolysis of substrate, suppressing lipase synthesis (Sidhu *et al.*, 1998). Different inorganic nitrogen sources were used for extracellular lipase production by strain ISU^{uv}-16. $(\text{NH}_4)_2\text{SO}_4$ gave maximum production as compared to other inorganic nitrogen sources. It might be due to the optimum growth of mycelium when $(\text{NH}_4)_2\text{SO}_4$ was used as nitrogen source. According to Gao and Breuil (1995), ammonium sulfate gave the best lipase production. 0.4% concentration of $(\text{NH}_4)_2\text{SO}_4$ was found to be the best for lipase production by ISU^{uv}-16.

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