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PRODUCTION PROCESS OF EXTRACELLULAR LIPASES BY *FUSARIUM* SP. USING AGRICULTURAL BYPRODUCTS

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Abstract

The present study piece of study is designed to optimize the environmental conditions for the production of extracellular lipases by *Fusarium* sp. The fungal culture was checked in order to test its potential for lipases biosynthesis. Almond meal was optimized to be the ideal basal support for the enzyme production using solid state fermentation technique. Ten grams of almond meal supported maximum extracellular lipases (3.48±0.01[a] U/mL) production . Other cultural conditions like level of inoculum, quantity of moistening agent, rate of fermentation, fermentation temperature, nitrogen supplements, carbon additives, addition of various oils were also optimized. One millilitre inoculum, 10 mL of moistening agent, 30°C incubation temperature, 1% peptone as additional organic nitrogen supplement, 1% Glucose and 1% olive oil was optimized as the ideal parameters for the maximum production of the extracellular lipases. Maximum units of the extracellular lipases (56.7±0.01[a] U/mL) was obtained when incubation period was optimized at 72h.

Introduction

Lipases are special enzymes which hydrolyze glycerides to free fatty acids and glycerol (Gilbert, 1993). Lipases catalyses ester synthesis in liquid media (Lacointe *et al*., 1996). Microbial lipases are significantly important due to its application in various industries (Mark *et al*., 2001; Hsu *et al*., 2002; Ramini *et al.,* 2010). Lipases is most frequently used enzyme in biotransformations (Gitlesen *et al*., 1997). Chemical, pharmaceutical, cosmetic, food & leather industries involved the use of this enzyme (Gulati *et al*., 2005; Gunstone, 1999). Fungi being the most versatile tool, frequently used for various enzymatic products (Gutarra *et al.,* 2007). The ability of fungi to produce extracelllar enzyme is of great survival value and make it a potent source among the other microbial flora (Gopinath *et al*., 2005). Fats and oils are recognized as essential nutrients in both human and animal diet. Beneficial fatty acids are made available to the organisms after the action of lipases therefore, it serves as a vehicle for fat soluble vitamins and important components of cell membrane. Among all the microorganisms, fungi especially *Rhizopus* sp., *Mucor* sp., *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp., are preferable lipase sources. (Gracheva *et al*., 1980; Iftikhar, 2009). *Aspergillus niger* is among the well known lipase producer, mainly used in the industry (Pokorny *et al*., 1994; Undurraga *et al*., 2001). The present study is aimed to optimize the cultural conditions for the production of extracellular lipases by a locally isolated strain of *Fusarium* sp..

Materials and Methods

Microorganism: The fungal culture under study was obtained from Mycology and Biotechnology Research Laboratory, GCU Faisalabad. Microscope (MEIJI Model: ML2100) was calibrated and various measurements were taken for the identification after morphological examination of strains after Kirk (2009). Identifications were then confirmed by Prof. Dr. Syed Qaiser Abbas (Ahmad, 1956; Ahmad, 1960 & Ahmad, 1962). The isolated fungal cultures were maintained on 4% potato dextrose agar (PDA) slants.

Substrates used: Different agricultural byproducts used in the present study such as almond meal, brassica meal, rice meal, and poultry meal were obtained from local market.

Fermentation technique: The fermentation technique used in the present study was solid state fermentation (Korn & Fujio, 1997). Preliminarily, 7 mL of diluent (distilled water) was used to moisten the 10 g of basal substrate. The flasks were autoclaved at 15-lb/inch² pressure (121°C) for 15 minutes and cooled at room temperature. One mL of the spore suspension prepared in monoxal O.T (Di-octylester of sulfosuccinic acid) in order to break the clumps and was aseptically transferred to each conical flask and flasks were then placed in an incubator at 30 ± 2°C for 48 hours (Iftikhar *et al.,* 2009). The experiment was run parallel in triplicate.

Buffer preparation: Buffers of various pH were prepared in the required composition, after consulting the web site (http://delloyd.50megs.com/moreinfo/buffers2.html#acetate).

Extraction of enzyme: After 48 hours 100 mL of phosphate buffer (pH 7.0) was added to each flask. The flasks were rotated on the rotary shaker at 150 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 in the fermented meal was determined titrimetrically as reported by Iftikhar *et al.,* (2009). Experiments were performed in triplicate and average values were taken for data analyses.

Statistical Analysis: All the experimental data were analysed by co-stat software.

Results and Discussion

In the present study four different agricultural by products such as brassica meal, rice bran, poultry feed and almond meal @ 1% were studied for their effect on the production of extracellular lipase by *Fusarium* sp. (Fig. 1). It was found that production of extracellular lipase ranged from 1.25 ± 0.01[d] U/mL to 3.48 ± 0.01[a] U/mL. Almond meal gave maximum enzyme activity (3.48 ± 0.01[a] U/mL) and was found to be the most suitable one among all the substrates. Although specific activity was

higher in rice bran but overall protein production was very low. Thus, almond meal was selected as the optimized source of carbon and nitrogen. This data was found not in conformity with findings of Rao *et al.*, (1993) and Kamini *et al.,* (1998) who worked with other substrates and obtained higher titers of lipase.

The effect of different incubation temperature was studied on production of extracellular lipases by *Fusarium* sp. and results are shown in Fig. 2. A range of different temperatures i.e., from 20 to 60°C with 10 ^{o}C difference of each temperature applicatione was applied to evaluate temperature optima for the production of extracellular lipases. It was found that production of extracellular lipase ranged from 2.4±0.1^e U/ mL to 8.16±0.01^a U/ mL. The highest lipase yield (8.16±0.01^a U/ mL) was achieved at 30°C and the production dropped on both sides of this temperature application Higher temperatures appreciably proved to be deleterious for enzyme production. this decrease in the lipase activity might be due to the fact that the enzyme denatured at higher temperatures. The optimum growth temperature for lipase production in this study is inline with the findings of Iftikhar *et al*., (2010). Therefore, 30°C was optimized for further studies.

The effect of size of Inoculum was also optimized for the production of extracellular lipase by *Fusarium* sp. and the data is presented as Fig. 3. Different dilution of spores as described earlier,i.e.,., 1, 2, 3, 4 and 5 mL were added to each fermentation flask. The results show that production of extracellular lipase ranged from 4.12±0.01^c U/ mL to 9.47 ±0.04^a U/ mL. The highest units of extracellular (9.47±0.01^a U/ mL) were obtained with 1 mL of inoculum size. While minimum unit of extracellular lipase (4.12±0.01^c U/ mL) was obtained at 5 mL of inoculum size. There are differential report on this aspects optimization studies in literature by different researchers working with other fungi. Ushio *et al*., (1996) also optimized 1.0ml of inoculum for maximum lipase production. Imandi *et al*., (2010) reported a 2mL of inoculum. Therefore, 1 mL inoculum was selected for further studies.

The effect of volume of diluent on the production of extracellular lipase was studied by *Fuasrium* sp. (Fig. 4). For this purpose, different amount of diluent e.g., 10, 15, 20, 25, and 30 mL were added in each fermentation flask. The results shows that production of lipase enzyme ranged from 9.35±0.01^e U/ mL to 17.27±0.01^a U/ mL. Maximum lipase production (17.27±0.01^a U/ mL) was recorded at 10 mL of diluent volume and minimum production of lipase (9.35±0.01^e U/g) was recorded at 30 mL. which could be due the resason that lipase production decreased at very higher moisture content which may be ascribed to the decrease in porosity and hence interfered with gaseous exchange leading to suboptimal growth and less enzyme production as indicated by Silman *et al*., (1979). Less lipase activity was observed at lower moisture content due to the reduction in the solubility of nutrients of the substrate that lowered the degree of swelling, and created higher water tension as suggested by Guerra *et al*., (2003). The present results are in accordance with the results of Mahanta *et al.*, 2008; Mateus *et al*., 2009. Therefore, 10mL volume of diluent was optimized for further studies.

The effect of additional nitrogen sources was investigated for the production of extracellular lipase by *Fusarium* sp. (Fig. 5). For this purpose 1% of different nitrogen sources, *i.e.,* peptone, yeast, NH_4NO_3, $NaNO_3$ and Urea were added to the fermentation flasks. The result showed that peptone @1% supports maximum extracellular lipase production (27.32±0.01^a U/mL). Our work is not in accordance with D'Annibale *et al*., (2006) whereas Edwinoliver *et al*., (2010) found that additives had no effect on lipases' production. The results are also in line with the findings of Ramani *et al*., (2010) who found that urea has inhibitory effect on lipase production whereas Imandi *et al.,* 2010 reported that urea showed higher lipolytic activity. Therefore, peptone @ 1% was optimized for further studies.

The effect of additional carbon sources was tested for the production of extracellular lipase by *Fusarium* sp. (Fig. 6). For this study 1% of different carbon sources such as glucose, sucrose, $CaCO_3$, KHCO3 and starch were added to the fermentation substrate. It was found that production of extracellular lipase ranged from 15.66±0.01^e U/ mL to 28.32±0.01^a U/ mL. The maximum production was achieved when glucose @ 1% was added. While other carbon sources didn't show an increase in lipases activity, that could possibly be due to the contamination problems and also increase the cost of production as reported by Imandi *et al*., (2010). The present work supported by the findings of Iftikhar *et al.,* (2010). Therefore, glucose was optimized as the additional carbon source for further studies.

The effect of different oils was investigated for the production of extracellular lipase by *Fusarium* sp. (Fig. 7). For this purpose 1% of different oils such as olive oil, vegetable oil, coconut oil and brassica oils were added to the fermentation medium. The result shows that extracellular lipase production ranged from 21.12±0.01^c U/mL to 28.28±0.01^a U/mL. Maximum lipase activity (28.28±0.01^a U/mL) was obtained when olive oil was added to the fermentation flask. The present results are not in accordance with D'Annibale *et al*., (2006) as in his work every oil has an inducing effect (5-7-fold increase) whereas in the present work *Fusarium* sp. did not exhibited remarkable increase in extracellular lipase production. Although in the presence of olive oil there is no inhibitory effect on enzyme production but no remarkable increase, therefore, it is suggested that oils do not have great influence on enzyme production in the present study.

The effect of rate of fermentation was studied on the production of extracellular lipase by *Fusarium* sp. (Fig. 8). For this purpose, fermentation flasks were place in incubator for different time duration, i.e., 24, 48, 72, 96 and 120 hours. It was found that production of extracellular lipase ranged from 25.67±0.001^e U/ mL to 56.7±0.01^a U/ mL. Maximum lipase production was obtained after 72 h of incubation. It might be due to the exhaustion of nutrients in substrate, which resulted in the inactivation of enzyme. Decline in exponential curve might be due to the exhaustion of nutrients or loss of moisture. The results are in line with the findings of (Edwinoliver *et al.,* 2010 and Kamini *et al*., 1998). A decline in the biosynthesis of enzyme showed after 48h of cultivation as reported by Haq *et al*., (2001). Therefore, 72 h was optimized for further studies.

From the present studies it can be concluded that the local fungal species of *Fusarium* sp. can be a good candidate on account of its local availability. However, more comprehensive studies like probable pathological aspects of the fungus, enzyme thermodynamic and kinetic studies,etc. may be done before its recommendation as an alternative biotic source for lipase production.

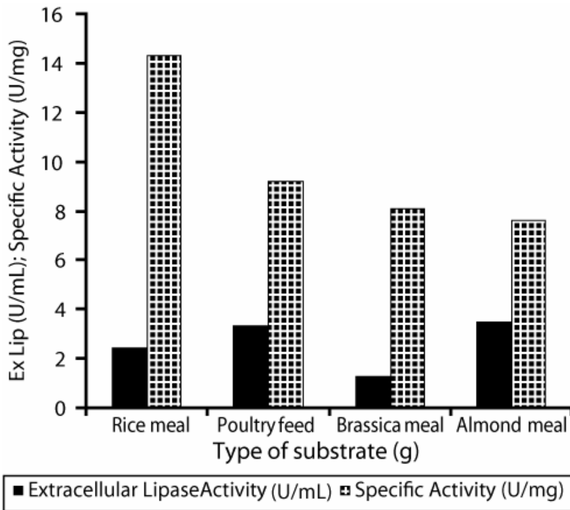

Fig. 1. Selection of substrate for the production of extracellular lipase by *Fusarium* sp. through solid substrate fermentation technique.

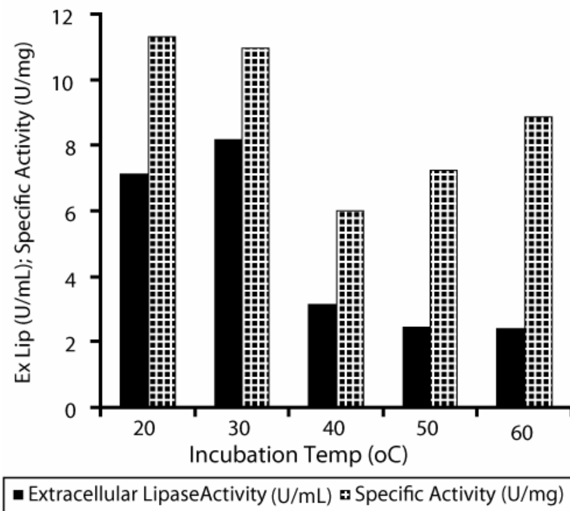

Fig. 2. Effect of incubation temperature on the production of lipase by *Fusarium* sp. through solid substrate fermentation technique.

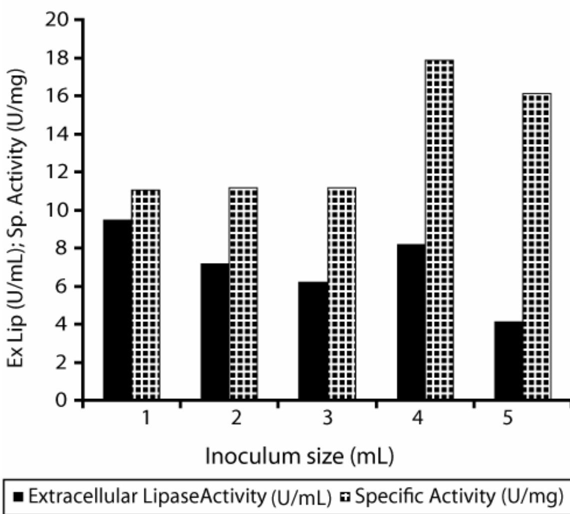

Fig. 3. Effect of size of inoculum on the production of lipase by *Fusarium* sp. through solid substrate fermentation technique.

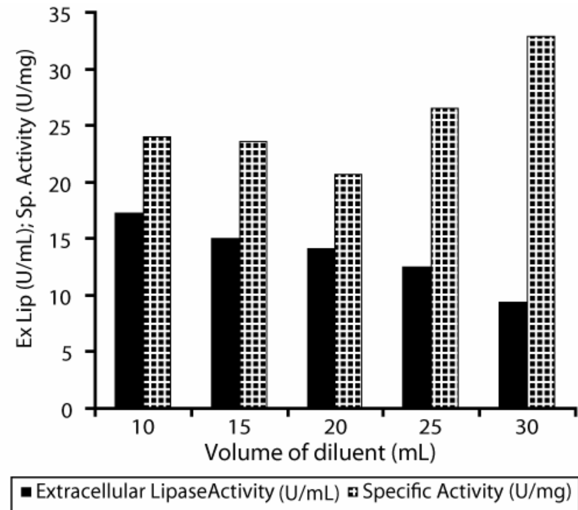

Fig. 4. Effect of volume of diluent on the production of lipase by *Fusarium* sp. through solid substrate fermentation technique.

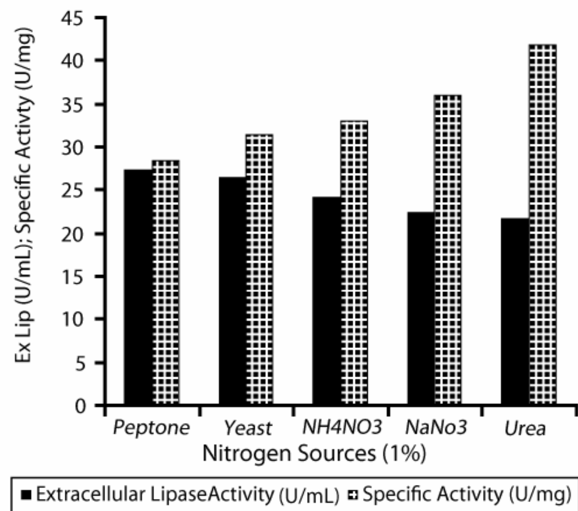

Fig. 5. Effect of additional nitrogen sources on the production of lipase by *Fusarium* sp., through solid substrate fermentation technique.

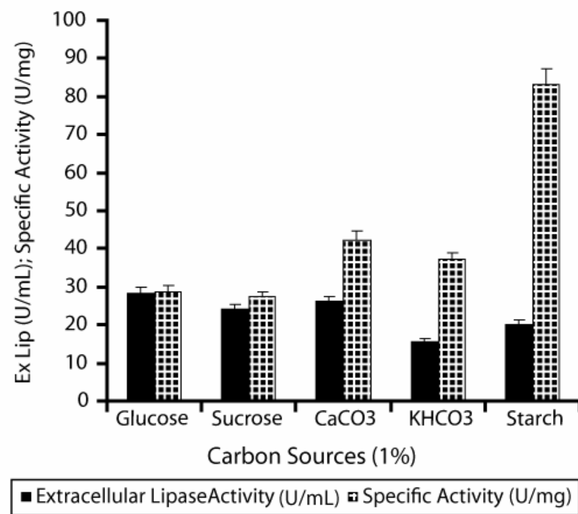

Fig. 6. Effect of additional carbon sources on the production of lipase by *Fusarium* sp. through solid substrate fermentation.

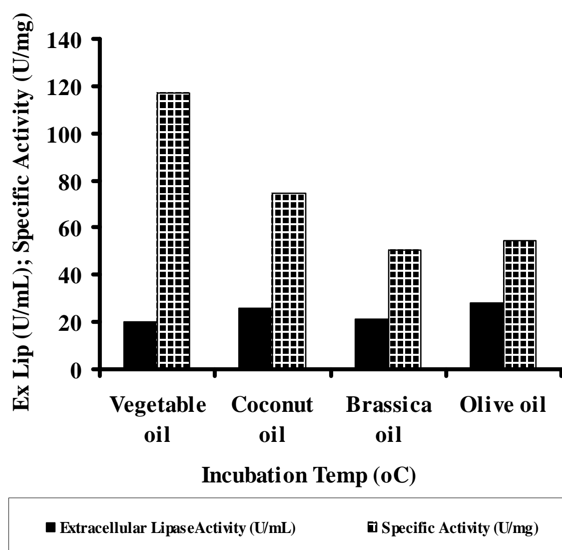

Fig. 7. Effect of different additional oils on the production of lipase by *Fusarium* sp. through solid substrate fermentation.

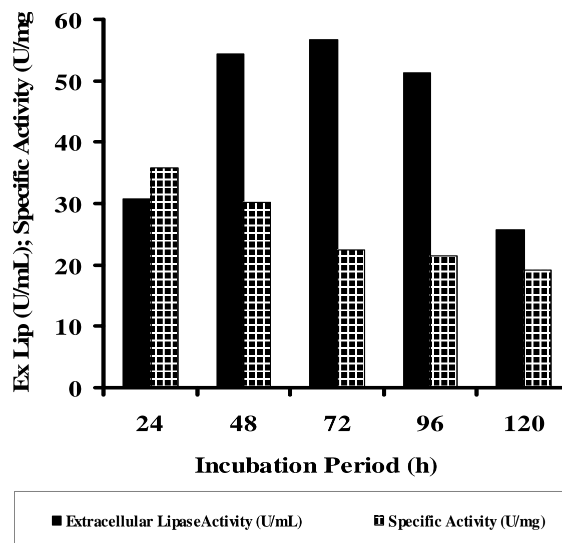

Fig. 8. Effect of rate of fermentation on the production of lipase by *Fusarium* sp., through solid substrate fermentation technique.

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