

PRODUCTION POTENTIAL OF LOCALLY ISOLATED STRAIN OF *FUSARIUM SOLANI* (MBL 24) FOR EXTRACELLULAR LIPASES

TEHREEMA IFTIKHAR¹, MUBASHIR NIAZ^{1*}, MUHAMMAD ANJUM ZIA²,
MANEELA SADAF¹ AND RUKHSANA JABEEN³

¹Laboratory of Mycology & Biotechnology, Department of Botany, Govt. College University, Faisalabad, Pakistan

²Laboratory of Enzymology, Department of Chemistry & Biochemistry, University of Agriculture, Faisalabad

³Department of Plant Sciences SBKW University, Quetta, Pakistan

Abstract

The present study is focused to exploit the indigenous strain of *Fusarium solani* (MBL-24) for the extracellular lipases production through solid state fermentation technique. In order to exploit the locally isolated fungal strain for the industrial usage, various parameters tested was incubation temperature, screening of substrates, incubation period, size of inoculum, volume of moistening agent, additional oils, additional carbon and nitrogen sources. Wheat bran was surprisingly found to be an ideal source for the growth of the organism and subsequently for enhanced enzyme production as compared to the other substrates. Out of various oils tested brassica oil at 1% was found to be an ideal additional source for the maximum activity (3.78 ± 0.04^a U/mL) of lipases. It was found that 2 mL of inoculum supported highest lipase enzyme (4.32 ± 0.05^a U/mL) production. Maximum activity (5.36 ± 0.10^a U/mL) was obtained when fungus was incubated for two days (48h) and at 1:3 substrate to diluent ratio *i.e.*, (7.0 ± 0.03^a U/mL). As an additional supplement 1% CaCO₃ was proved to be best for maximum extracellular lipases production. Inorganic nitrogen source seems to be better supplement as compared to organic nitrogen sources. Sodium nitrate supported maximum lipase production (5.17 ± 0.07^a U/mL). It is apparent from the optimized conditions that the strain has a good potential for the production of extracellular lipases and can be exploited for several industries keeping in view the optimized temperature and incubation period.

Introduction

Lipases are ubiquitously present in nature and found everywhere on earth. Lipases act upon the ester bonds and industrial lipases have the characteristic feature of hydrolyzing the fats and oils into free fatty acids and glycerol on the oil-water interface (Gilbert, 1993). Lipases are not reported to be involved in any anabolic reaction *In vivo*, therefore lipases can be used at industrial scale as a catalyst (Lacointe *et al.*, 1996; Rajeswari *et al.*, 2011). The lipases which are currently utilized in industries are microbial in origin which occurs widely in nature (Gitlesen *et al.*, 1997; Mark *et al.*, 2001; Hsu *et al.*, 2002; Asad *et al.*, 2011). Thirty four fungal strains were reported to be isolated from oil mill waste by serial dilution technique which proved that ambient temperature supports wide range of fungal strains (Gopinath *et al.*, 2005). Fungal lipases are reported to be produced by solid substrate and submerged fermentation techniques (Khan *et al.*, 2008). It is concluded by the previous workers that solid state fermentation always supports greater enzyme production and utilizing the cheaper substrate (Shukla *et al.*, 2011). Solid state fermentation also takes the edge as it exploits the cheaper agro industrial by products (Divakar *et al.*, 2006). *Fusarium* sp., is also reported to be a pathogen on corn crop (Ahmad *et al.*, 2006). The lipases of microbial origin find extensive applications in pharmaceutical, food, chemical, cosmetic, leather industries and for the purpose of waste water treatment (Gulati *et al.*, 2005; Gunstone, 1999). Fungi being ubiquitous in nature flourish in a long range of environmental extremes and is highly successful due to its versatility (Gopinath *et al.*, 2005). 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 *et al.*, 2008). *Aspergillus niger* is among the well known lipase

producer, mainly used in the industry (Pokorny *et al.*, 1994; Undurraga *et al.*, 2001). There is a shifting trend of industrial set up in Pakistan and it is likely that building hi-tech industry will be the major focus of Pakistani entrepreneur in coming days. There is currently interest to permit its production locally. The need of the time is to explore the local environmental conditions for the development of indigenous cultures. The present study is aimed to optimize the cultural conditions for the biosynthesis of extracellular lipases by a locally isolated strain of *Fusarium solanii* (MBL-24) in order to exploit it for industrial usage.

Materials and Methods

Microorganism: The fungal culture under study was obtained from Laboratory of Mycology and Biotechnology, Department of Botany, Govt. College University, Faisalabad. The isolated fungal cultures were maintained on 4% potato dextrose agar (PDA) slants.

Substrates used: Different agricultural by-products used in the present study such as brassica meal, poultry meal, almond meal, soybean meal, wheat bran, and banola meal were obtained from the local market.

Fermentation technique: Production of fungal lipases was studied through solid state fermentation as reported by Korn & Fujio (1997). After fermentation the enzyme was extracted by the addition of 100mL phosphate buffer of pH 7. The filtrate was subjected to titrimetric analysis of extracellular lipases by the modified method of Kempka *et al.*, (2008).

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

*Corresponding author: +92419201488, mniaz_prof@yahoo.com

Results and Discussion

Optimization of cultural conditions increases the production potential of the subject organism many folds as compared to the un-optimized conditions. Oils play a vital role to enhance extracellular lipases production. Different oils like coconut oil, sunflower oil, olive oil and brassica oil were used to observe the lipase activity at 28°C (Fig. 1). Lipase activity ranges from 1.44 ± 0.06^d to 3.78 ± 0.04^a U/mL. Maximum activity was obtained when brassica oil was added as an additional supplement (1%) while organism exhibited minimum activity (1.44 ± 0.06^d U/mL) when coconut oil was used as an additive. Addition of oils to growth medium resulted in enhanced lipase production as reported by (Sekhon *et al.* 2006; Shukla *et al.*, 2011). Pogaku *et al.*, (2010) also used different oils to enhance lipase production. Therefore, brassica oil was optimized for further studies.

Inoculum size plays pivotal role with regard to the enzyme production (Fig. 2). Inoculum was varied from 1 mL to 5 mL to achieve maximum extracellular lipases activity. Extracellular lipases activity ranges from 1.36 ± 0.05^e U/mL to 4.32 ± 0.05^a U/mL. Maximum lipases activity (4.32 ± 0.05^a U/mL) was obtained when 2 mL of inoculum was added to 10 g of basal substrate as supporting medium for fungus and minimum activity was noted when inoculum size was 5 mL. Specific activity of enzyme was also highest (33.2 U/mg) when 2 mL inoculum was used. Lower or higher inoculum size did not support higher activity. With the increase in mycelial mass, enzyme production declined due to increase in cell mass formation that caused the exhaustion of nutrients from the fermentation mash. The findings of the D'Annibale *et al.*, (2006) are inline with the present results. Iftikhar *et al.*, (2008) observed maximum lipase production at 1 mL inoculum size which is in contrary to the present findings. Therefore, 2 mL inoculum was optimized for further studies.

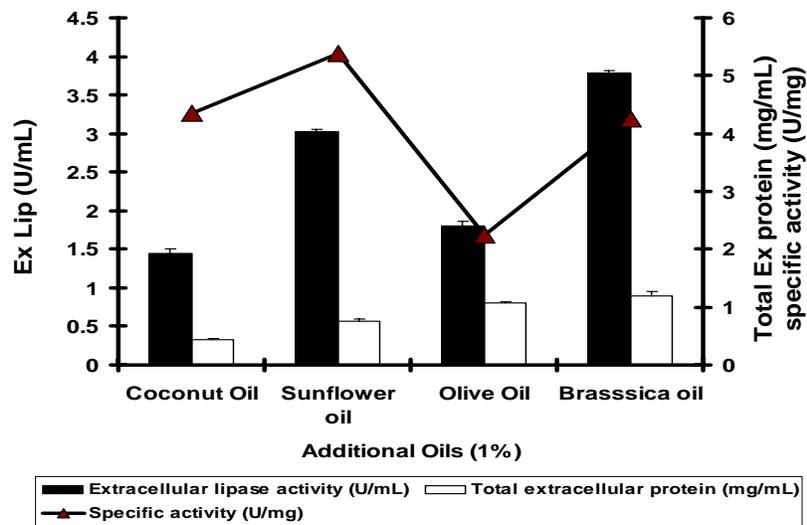


Fig. 1. Effect of various oils on the production of extracellular lipases by *Fusarium solani* (MBL 24) through solid substrate fermentation.

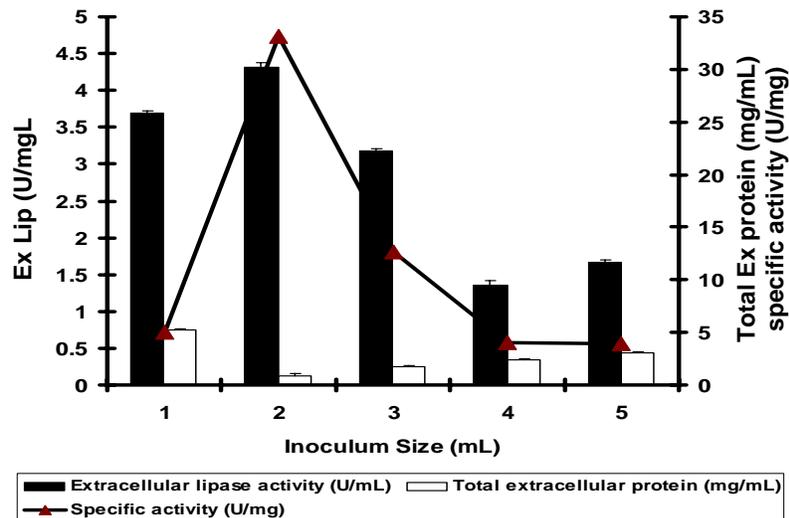


Fig. 2. Effect of size of inoculum on the production of extracellular lipase by *Fusarium solani* (MBL 24) through solid substrate fermentation.

Nitrogen sources play an important role to enhance the extracellular lipase activity. In the present study different organic and inorganic nitrogen sources were tested with regard to their effect on the product production. The supplementary nutrients used were yeast extract, peptone, urea, NH_4Cl and NaNO_3 . As Lipase activities ranges from 2.20 ± 0.04^e U/mL to 5.17 ± 0.07^a U/mL (Fig. 3). Maximum lipase activity (5.17 ± 0.07^a U/mL) was obtained when NaNO_3 was used and minimum lipase activity was

observed when peptone was used as an additional nitrogen source. Maximum extracellular protein was also noted by using Sodium nitrate (0.28 ± 0.02^a mg/mL). Rifaat *et al.*, (2010) reported that peptone, malt extract and casein were optimal sources for maximum lipase activity by *Fusarium oxysporum* which are not completely in accordance with the present findings. Therefore, NaNO_3 @ 1% was optimized for further studies.

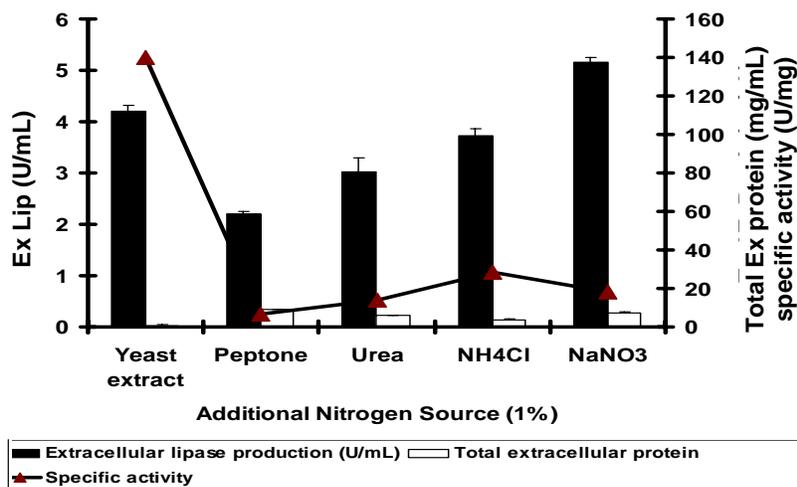


Fig. 3. Effect of nitrogen sources on lipase production by *Fusarium solani* (MBL 24) through solid substrate fermentation.

Incubation period is also an important factor in extracellular lipase production. Rate of incubation varied from 24h to 96h at an interval of 24 h (Fig. 4). It is evident from the results that lipase showed maximum activity (5.36 ± 0.10 U/mL) after 48h by using *Fusarium solani* under solid substrate fermentation conditions. Minimum activity (1.5 ± 0.04 U/mL) was observed at 96h of incubation. Any

change in the incubation period from 48h, lowers the enzyme activity. It might be due to the exhaustion of nutrients. This finding is in accordance with Korn & Fujio, 1997. Iftikhar *et al.*, (2008) also observed maximum lipase production when fungus was incubated for 48 h. Similar results were also observed by Pogaku *et al.*, (2010). Therefore, 48h was optimized for further studies.

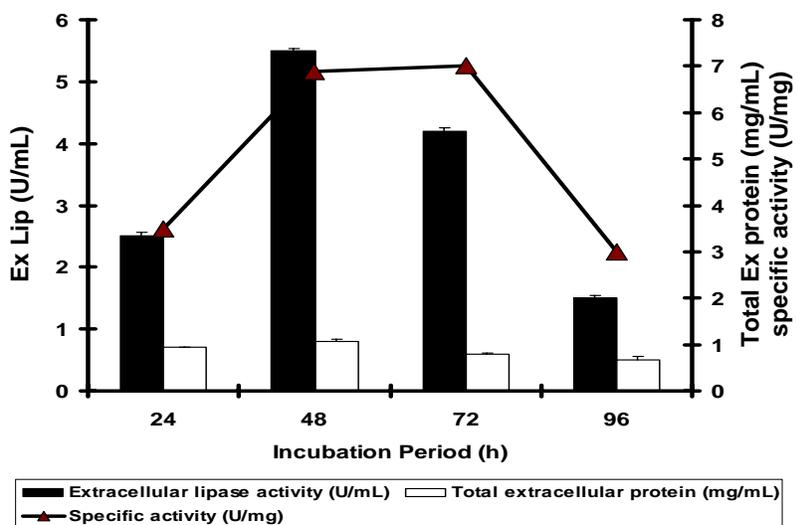


Fig. 4. Effect of incubation period on the production of extracellular lipase by *Fusarium solani* (MBL 24) through solid substrate fermentation.

Incubation temperature is an important environmental factor that greatly effects the lipase production. A range of incubation temperatures was applied with regard to their effect on extracellular lipases production by *Fusarium solani* through solid substrate fermentation (Fig. 5). Incubation temperatures ranged from 20°C to 40°C at an interval of 5°C. Maximum lipase production (6.23 ± 0.10^a U/mL) was noted at 30°C while minimum extracellular lipase production (4.11 ± 0.11^c U/mL) was observed at 35°C and maximum specific activity (124.62) was also observed at 30°C. Activity was decreased above and below this temperature. Balaji & Ebenezer (2008) revealed that optimum temperature for lipase production by *Colletotrichum gloeosporioides* was 25°C and high temperature may possibly lead to denaturation of enzyme. Therefore, 30°C was optimized for further studies.

Screening of substrates plays an important role under solid state fermentation conditions. Different agricultural

byproducts such as brassica meal, poultry meal, almond meal, wheat bran soybean meal and banola meal were used as solid substrates to support the growth of *Fusarium solani* for the production of industrially valuable lipases. Fig. 6 reveals that lipases production ranges from 2.37 ± 0.05^e U/mL to 6.74 ± 0.16^a U/mL. Maximum lipase production (6.74 ± 0.16^a U/mL) was observed when wheat bran was used as a basal substrate while minimum lipase production (2.37 ± 0.05^e U/mL) was observed with almond meal. That seems surprising, it might be due to high starch contents present in the wheat bran which fulfills the nutritional needs of the organism in much better way as compared to the others. Divakar *et al.*, (2006) also used different agro-industrial materials for microbial growth and product formation while wheat bran proved superior to other substrates. Therefore, wheat bran was selected for further studies.

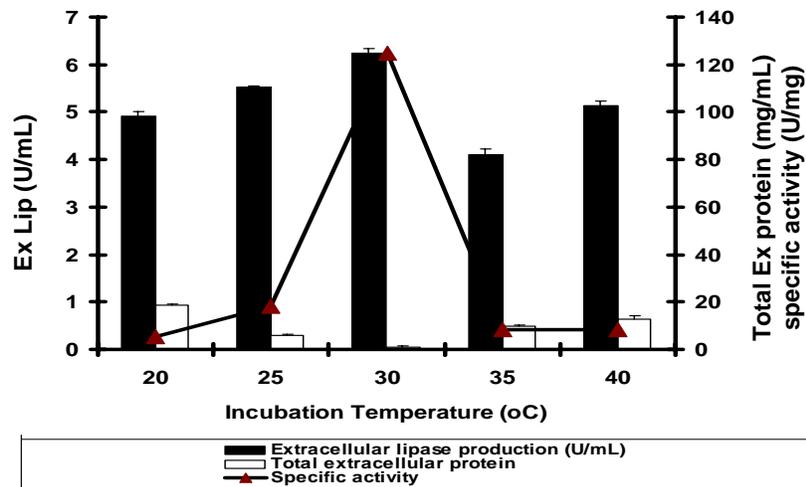


Fig. 5. Effect of temperature on the production of extracellular lipase by *Fusarium solani* (MBL 24) through solid substrate fermentation.

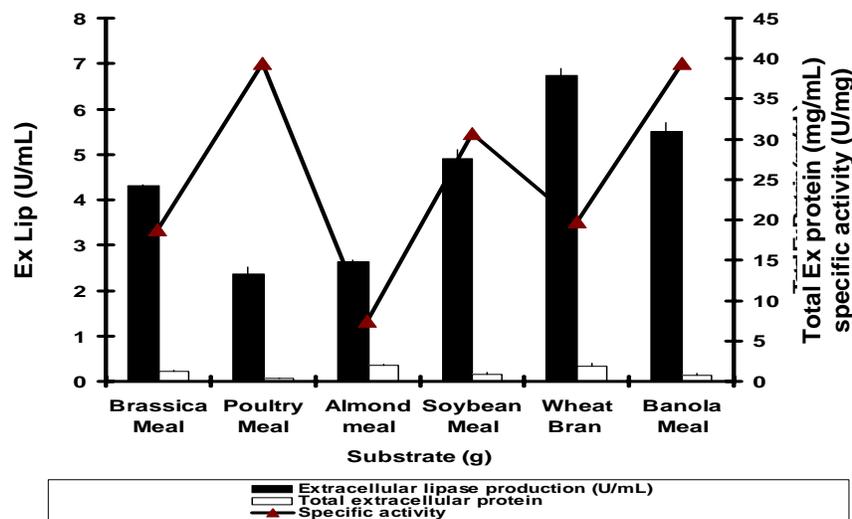


Fig. 6. Effect of different substrates on the production of extracellular lipase by *Fusarium solani* (MBL 24) through solid substrate fermentation.

Substrate to diluents ratio play important role towards enhanced enzyme production. For this purpose 1:1, 1:2, 1:3, 1:4 and 1:5 substrate to diluents ratio was employed (Fig. 7). The results showed that lipase activities range from 4.46 ± 0.05^e U/mL to 7.0 ± 0.03^a U/mL. Maximum extracellular lipase activity was obtained when (1:3) 30mL volume of moistening agent was applied and minimum activity was observed when (1:5) 50mL volume was used. Suitable moisture contents are necessary for proper microbial growth. Amount of water is very limited in solid state fermentation while optimum amount of water is necessary as it has a valuable effect on the productivity of SSF processes (Imandi *et al.*, 2010). Babu & Rao (2007) reported maximum lipase activity at 80% moisture contents by using *Yarrowia lipolytica* as lipase source. In solid state fermentation, high moisture contents cause decrease porosity and change the wheat bran particle structure. On the other hand low moisture contents cause restriction to the solubility of nutrients present in wheat bran (Babu & Styanarayana, 1996). Therefore, 30mL of moistening agent was optimized for further studies.

Carbon is used as an additional nutrient source for improved enzyme production. For this purpose, different carbon sources such as glucose, sucrose, starch, KH_2CO_3 and CaCO_3 were applied @ 1% to enhance the extracellular lipase activity. Different carbon sources showed different results (Fig. 8). Lipase activity ranges from 3.23 ± 0.10^e U/mL to 7.66 ± 0.29^a U/mL. Glucose presented lowest lipase activity (3.23 ± 0.09^e U/mL) and CaCO_3 showed maximum lipase activity (7.66 ± 0.29^a U/mL) by *Fusarium solani*. It might be due to the reason that calcium ions induces the release of cell bound lipases (Gopinath *et al.*, 2005) Maximum total extracellular proteins (0.64 ± 0.02^a mg/mL) was obtained when sucrose was used as additional carbon source. It may be due to the availability of suitable carbon contents required for maximum lipase production. Babu & Rao (2007) observed that glucose proved to be best carbon source for maximum lipase production which is not supporting the present findings. Therefore, CaCO_3 @1% was optimized as an additional carbon source.

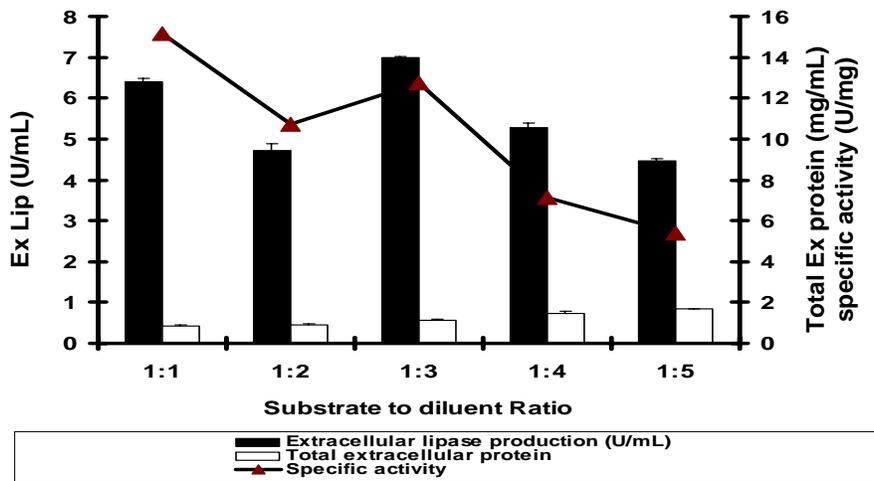


Fig. 7. Effect of moistening agent on the production of Extracellular lipases by *Fusarium solani* (MBL 24) through solid substrate fermentation.

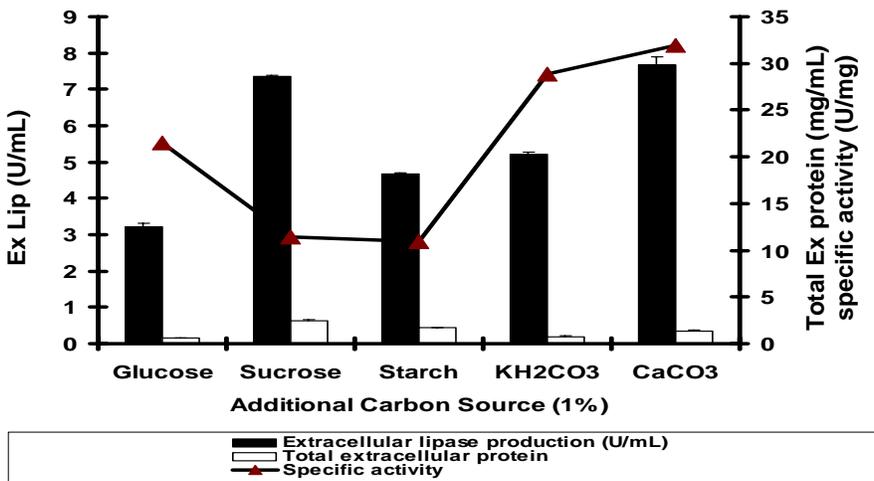


Fig. 8. Effect of carbon sources on the production of extracellular lipase by *Fusarium solani* (MBL 24) through solid substrate fermentation. *Each value is an average of three replicates, error bar denotes standard error among replicate

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