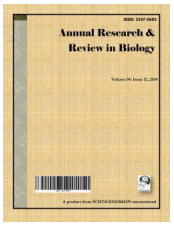

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Process Optimization for Lipolytic Bacterial Fermentation: Value Addition to the By-products of Oil Seeds

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Authors' contributions

This work was carried out in collaboration between both authors. Author AS designed the study, performed the statistical analysis and decided the protocol. Author SS managed the analysis, literature searches of the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Lipase enzyme has wide application in industries, particularly food and detergent, but high production cost has always limited its use. Extensive studies are underway on production of high quality and low cost lipase enzyme in large amounts, for which microbial sources have been found to be the best.
Aim: To estimate the potential of oil cakes for bacterial lipase production.
Methodology: By-products of different oil seeds viz. neem, sesame, flax, mustard, coconut, castor, and groundnut were used for the preparation of fermentation media to culture lipolytic *Pseudomonas aeruginosa*. Optimization of growth condition was done with respect to different parameters such as fermentation time, nitrogen supplements, carbon additives, and lipid sources.
Results: A good lipolytic *P. aeruginosa* JCM5962 (T) strain was isolated from soil of sugarcane field. Results of the study showed that coconut, sesame, neem, flax and mustard oilcakes induced

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good lipolytic activity from bacteria. Negligible lipase activity was obtained when organism was cultured in castor and groundnut oilcake medium. 1% ammonium nitrate as an additional nitrogen supplement was found to be ideal parameter for improved production.
Conclusion: According to present work, lipases could be economically produced by *P. aeruginosa* using low cost oil cakes as potent substrate for fermentation medium.

Keywords: Lipase enzyme; oil cakes; P. aeruginosa; semi-solid state fermentation.

1. INTRODUCTION

Crop residue as fruit seeds, bran, bagasse and husk are utilized as potential raw materials in bioprocesses. They provide an excellent substrate for the growth of organism supplying the essential nutrients to them [1-3]. Oil cakes are by-products of oil seeds, obtained after oil extraction from it. Oil cakes composition depends on their extraction methods, variety, and growing condition. They are economically cheap, stable and dependable sources available in large quantities throughout year [4]. In semi solid state fermentation (SmSSF), the nature of the solid support material is an important parameter that influences the product yield and consequently, there is a continuous search for newer and better substrates [5]. India is the world's leading oilseeds producing country. Oilcakes have high nutritional value, especially high protein content. A very large scale lipase production has been observed in many studies with oil cake extract [6]. Moreover, the blending of oil seed by-products shows better performance, by providing more suitable environment for microbial growth. SSF has attracted increasing attention for the production of antibiotics, enzymes, bio molecules, metabolites, etc., due to several biotechnological advantages such as higher end-product concentration, higher fermentation productivity, lower catabolic repression, and higher product stability.

Lipase enzymes act in aqueous-organic interfaces, producing glycerol, free fatty acids and catalyzing the cleavage of ester bonds in triglycerides. Apart from animal sources, lipases are also reported in various microorganisms and plants. Over the last decades interest in these enzymes has increased markedly, in view of their diverse applications in medicines, food additives, clinical reagents and for synthesis of biodiesel and biopolymers [7]. Microbial lipases have special industrial attention because of their selectivity, stability and broad substrate specificity [8,9]. Microbial enzymes are highly stable in comparison of plant and animal enzymes and their production is also more safe and convenient [10]. Therefore it is important to increase the production by optimizing culture conditions and at the same time reduce the production cost. A mostly employed strategy for this is the submerged fermentation and semi solid state fermentation for microbial growth. However SmSSF is the most appropriate process due to its various benefits and bioconversion parameters [11,12]. Oilcakes have shown great benefit in such fermentation processes as support and nutrient source for production of enzymes, vitamins, antioxidants, antibiotics etc. [13].

Previous studies done in our laboratory have identified a novel high lipolytic strain of *P. aeruginosa* JCM5962(T) [14], isolated from soil of sugarcane field. Present work was undertaken to study the potential of different oilcakes as semi solid state fermentation medium for improved production of extracellular lipase from *P. aeruginosa* JCM5962(T).

2. MATERIALS AND METHODS

2.1 Lipolytic Microbial Strain

P. aeruginosa JCM5962(T) isolated from sugarcane soil was used throughout the study. It produced an extracellular lipase of 31kDa. The enzyme was characterized to be stable at 50ºC and at pH 8.0. Working culture was prepared on nutrient broth by incubating it for 24 h at 37°C. The appropriate dilution of these cultures was used as inoculum.

2.2 Preparation of Fermentation Media

The oilcake substrates used for the production of lipases were procured directly from the oil spillers. Dry oil cakes were added with 100 ml of distilled water. The medium were mixed and autoclaved for 15 min at 121°C.

2.3 Enzyme Extraction and Assay

Phosphate buffer of pH 8 was added to the medium in 1: 1 ratio after fermentation and

enzyme was extracted by centrifugation at 4°C at 10,000 rpm for 10 min. The clear supernatant obtained was used as crude enzyme.

Lipase activity was done using *p*-nitrophenylpalmitate as a substrate [15]. Substrate solution containing phosphate buffer (pH 8.0) with gum Arabic (Hi Media) and sodium deoxycholate (Sigma Aldrich, USA) along with *p*-nitrophenylpalmitate (Sigma Aldrich, USA) in isopropanol was pre-incubated with crude enzyme at 37°C. The Release of *p*-nitrophenol was spectrophotometrically measured at 405 nm. One unit of lipase activity was defined as the amount of enzyme releasing 1 µmol *p*NP under standard assay conditions.

2.4 Optimization of Production Parameters for Semi Solid State Fermentation

To obtain maximum lipase production to suit industrial processes, optimization of parameters of medium was done. Various parameters were optimized for maximal lipase yield with incubation time (24, 48, 72 and 96 h). The effect of carbon sources (1% w/v of glucose, fructose, sucrose, lactose and maltose) in growth medium was determined. In another set of experiments, additional nitrogen sources (1% w/v of ammonium nitrate, potassium nitrate, peptone, beef extract, yeast extract) were added in the media and lipase activity was measured. Similarly, the effect of lipid supplementation by mixing 0.5% v/v of flax, mustard, sesame, soybean, and olive oils in all SmSSF was also studied.

3. RESULTS AND DISCUSSION

Selection of a suitable substrate for the production of enzyme is a primary-key factor and an extremely significant step. The present study deals with the production of lipase from *P. aeruginosa* JCM5962(T) using different oilcakes under semi solid state fermentation.

3.1 Optimization of Incubation Time

To determine incubation time for maximum lipase production, cultures were kept at 37ºC for 24, 48, 72 and 96 hours. As demonstrated in Fig. 1, in earlier stages of incubation a low level of lipase activity was obtained, which gradually increased with the passage of time.

Lipolytic activity was found to be maximum in coconut, sesame and flax oil cakes fermentation media after 72 hr incubation and in mustard oilcake after 48 hr of incubation whereas groundnut oilcakes did not induce significant activity. Therefore in further experiments groundnut oilcakes was not used. Several reports showed the effect of incubation time on lipase production. According to one study, *Micrococcus roseus* gave an yield of lipase (1.66 U/gds) in SmSSF using groundnut oilcake [15]. *Penicillium fellutanum* [16] showed a good lipase activity after 24 hr of incubation, whereas *Serratia marcescens* showed a good lipase activity after 6 days of incubation [17].

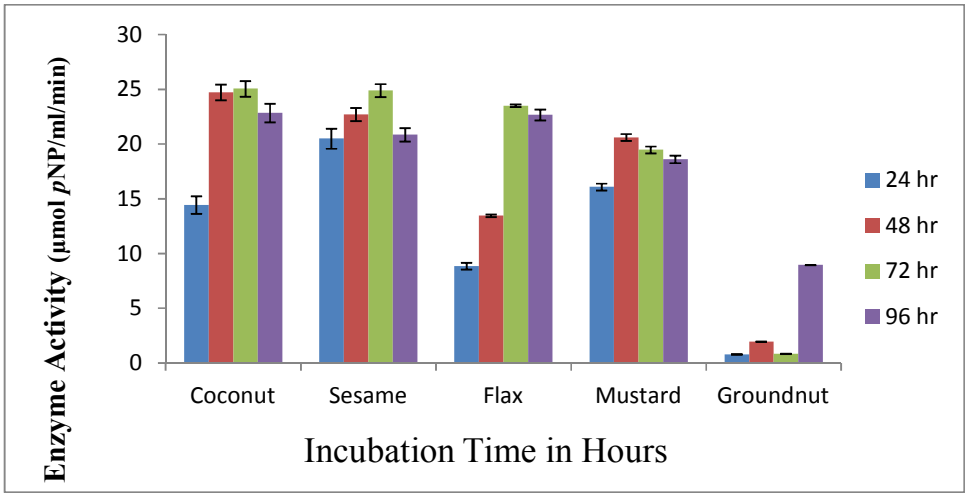

Fig. 1. Effect of different incubation time for different oil cake medium (24 – 96 hr) on lipase activity by *P. aeruginosa* JCM5962(T)

3.2 Effect of Carbon Sources

The impact of additional carbon sources (1% w/v) was studied (Fig. 2). In this study, the enhancing effect of carbon sources was found to be limited. Among the tested carbon sources, it was found that, sucrose and lactose sugars increased lipase production in sesame oilcake only reaching 12.38% and 25.32% more than the control respectively. In all other test system, no increase in enzyme production was observed. Only lactose was found to be lipase inducing carbon source with oilcakes for *P. aeruginosa* to some extent. So in the study enhancing effect in lipase production by supplying different carbon sources was limited. Many studies also showed that the glucose-containing compounds have a negative influence on lipase production [18]. Whereas de Azeredo et al. [19] stated that these carbon sources could influence the lipase activity in *Penicillium restrictum* using solid state fermentation. Another study had reported maximum lipase production by *P. aeruginosa PseA* in sucrose supplied medium [20].

3.3 Effect of Nitrogen Sources

Nitrogen source is another important factor in any fermentation process for successful production of bio molecules. The effect of nitrogen sources in SmSSF is shown in Fig. 3.

Ammonium nitrate, potassium nitrate, peptone, beef extract and yeast extract (1% w/v) when used as additional sources, increased the production of lipase from *P. aeruginosa* upto 193.24%. Ammonium nitrate was found to be the best additional nitrogen source as it increased 193.24% in coconut oilcake, 81.13% in flax oilcake and 81.3% lipase activity in sesame oilcake in comparison to control. However, supplementation of peptone was found to have no significant role in increasing the production in all tested oilcake medium. According to Alkan et al. [21] ammonium nitrate was the best nitrogen source for lipase production by *Bacillus coagulans*. An increase in lipase production when ammonium nitrate was added as inorganic nitrogen source to the *Geotrichum candidum* has been shown [22]. Lipase production enhanced in case of *P. citrinum* with supplementation of vegetable oil waste with ammonium chloride and ammonium sulphate [23].

3.4 Effect of Lipid Sources

The supplementation of different triglycerides, i.e. flax, sesame, mustard, olive, and soybean oil at the concentration of 0.5% were added to the oil cake SmSSF medium and lipase activity was detected. The results in the present study revealed that only sesame (45.72%) and olive oil (18.24%) increased lipase activity marginally in coconut oilcake when compared with control, whereas rest of the oil supplements suppressed the lipase production (Fig. 4). The reason for this may be the presence of required lipids in oil cake itself. According to Falony et al. [24] the production of lipase was more significant in culture medium added with lipids as the carbon source than in the culture medium without lipids. Extracellular lipase production by different microorganisms on lipids has been extensively reported [25].

Previous studies also demonstrated standardisation of non-edible oil cake of *A. indica* as SmSSF for high lipase production and maximum yield for this was obtained after 96 hour incubation in four percent neem oil cake medium [26].

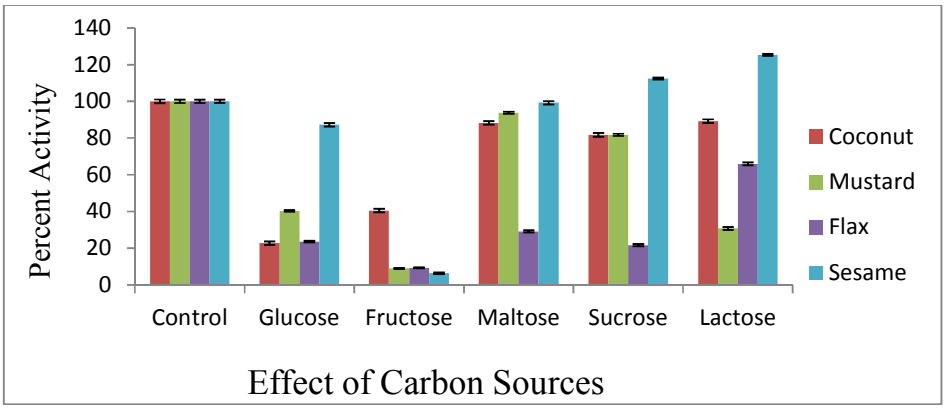

Fig. 2. Effect of different carbon sources on lipase activity by *P. aeruginosa* JCM5962(T)

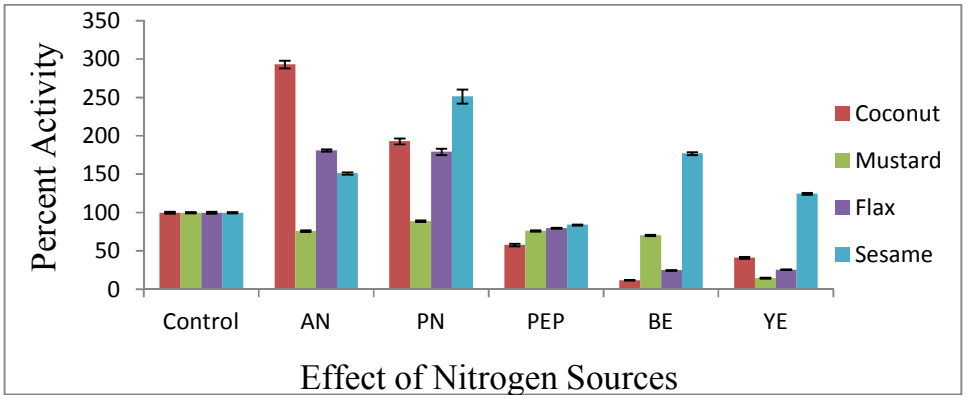

Fig. 3. Effect of different Nitrogen sources on lipase activity by *P. aeruginosa* JCM5962(T)
(AN - Ammonium nitrate, PN - potassium nitrate, PEP - peptone, BE - beef extract, YE - yeast extract)

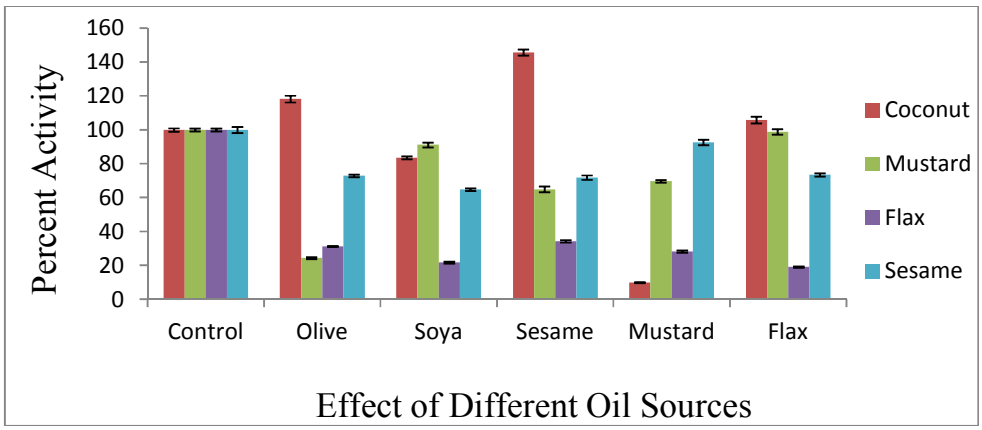

Fig. 4. Effect of different edible oils on lipase activity by *P. aeruginosa* JCM5962(T): Olive oil, soybean oil, sesame oil, mustard oil and flax oil (.5%) were used as additional triglycerides sources for all the medium

4. CONCLUSION

The potential of lipases in food and other industries shows the need to develop novel cost-effective technologies for increased production, scaling up and purification of this versatile enzyme. Agricultural waste utilization for industrial processes is one of the developing areas in modern industrial biotechnology and oilcakes offer potential benefits when used as substrates in developing bioprocesses for the production of organic chemicals and enzymes. According to present work, the extracellular lipases could be economically produced by a novel high lipolytic *P. aeruginosa* JCM5962(T) by semi-solid state fermentation using easily available, edible or non edible and low cost oil cakes as potent substrate. The results of this study demonstrated the addition of nutrient supplements were not much required rather than 1% ammonium nitrate to oil cake produces significantly high lipase from *Pseudomonas aeruginosa*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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