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Invitro Optimization of Media Parameters for Lipase Enzyme Activity of Cladosporium SPS Isolated from Oil mill Effluent

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Abstract

Lipases (triacylglycerolacyl hydrolases, EC3.1.1.3) are class of enzymes which catalyze the hydrolysis of long-chain triglycerides. The ability of lipases to perform biotransformation has made them increasingly popular in the food, detergent, chemical, and pharmaceutical industries. In the present study lipase producing Cladosporium was isolated from oil effluent and the effect of different physical and chemical parameters on lipase production and activity was evaluated. Enzyme kinetics of lipase was determined at varying pH (4-10) and temperature (20-50 °C). Also it was observed in response to addition of different metal ions, carbon source and nitrogen source. Maximum Lipase activity was observed between pH 6-8 with peak activity (7.85 Units/ml) at pH 6. During Temperature optimization maximum activity (8.71 Units/ml) was observed and at 30° C. Lipase activity was highest (8.12 Units/ml) and (7.75 Units/ml) with addition of 3% carbon source or 2% inorganic nitrogen source respectively. Among the ions KCL showed highest lipase activity (6.88 Units/ml). Therefore, from the results it can be concluded that the reported organism in this study can be exploited further for lipase production and then commercialization.

Keywords: Cladosporium, Lipase, Optimization, Enzyme activity.

1. Introduction

Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) catalyze the hydrolysis of triglycerides to glycerol and free fatty acids as well as catalyze the hydrolysis and transesterification of other esters [1]. Environmental pollution due to oil effluents is one of the major problems and there is need for detoxification and destruction of these contaminants effluents. Different Lipase producing microorganisms are used for the oil effluent remediation process to detoxify and degrade the oil effluents [2]. The Biotransformation ability of lipases to perform very specific chemical transformation has made them established in the food, detergent, chemical, and pharmaceutical industries [3, 4 and 5]. Many microorganisms such as bacteria, yeast and fungi are known to produce lipases and have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil [6]. The industrial demand for lipolytic enzymes continues to stimulate the search for new enzyme source and many microorganisms that are the potential candidates for lipases are yet to be identified and characterized.

Lipases producing fungi found in several habitats, including soils contaminated with oils, wastes of vegetable oils, dairy product industries, seeds, and deteriorated food [7, 8]. Fungal lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity [9, 10]. The lipases production is influenced by nutritional and physicochemical factors such as pH, incubation time, temperature, carbon and nitrogen sources [11, 12 and 13]. Hence, optimization of the components of the fermentation medium and growth conditions is essential for enzyme production and activity. Also, the growth organisms under optimal nutrient and physicochemical conditions for the optimum lipase productions need to be standardized for each specific organism. In this study, an attempt is made to investigate the production of lipase by *Cladosporium sp.* isolated from groundnut and palm oil mill effluent under different growth conditions, including carbon and nitrogen sources, metal ions, pH and temperature.

2. Materials and Methods

2.1 Isolation of Lipolytic organisms

Different effluent sample were collected from groundnut and palm oil mill. The effluent was serially diluted (up to 10^{-7}) and 1.0ml were spread on sterile portato dextrose agar and incubated for 5 days at 28^{o}C. The resulting colonies were individually inoculated on to sterile tributyrin agar media containing 10gms/l Tributyrin, 10gms/l Tryptone, 5gms/l NaCl, 5gms/l yeast extract and 17gms/l agar. The formation of clear zone around the colony indicated lipolytic microbes. The colonies showing maximum zone of hydrolysis was selected as the test organism. Pure culture of these organisms was obtained by repeated streaking and maintained on Potato Dextrose agar with 1.0% olive oil. The organism was identified by morphological characteristics.

2.2 Lipase Enzyme production and Assay

The Lipase production medium contained Olive oil 5%, peptone 5gm/l, yeast extract 5gm/l, glucose 5gm/l, NaCl 3gm/l and MgSO47H2O 0.5gm/l. The lipolytic organism was inoculated in media and incubated at 28^0C for 7 days. The crude enzyme was obtained by centrifugation of production media at 10,000 rpm, 4ºC for 10 min. Lipase activity was determined by spectrophoto-metric method using p-NPP (ρ-nitrophenyl palmitate) at pH 8.0. The coefficient of extinction of p-nitrophenol (p-NPP), 1.5 x 104 L/mol/cm, was determined by measuring absorbance at 410 nm after incubation for 15 min with the enzyme. One unit was defined as the amount of enzyme liberating 1µmol of ρ-nitrophenol per min at 37ºC.

2.3 Optimization for Lipase Production

Different concentrations (0.5%, 1%, 1.5%, 2%, 2.5% and 3%) of Honge (Karanja) oil were added to production media as a sole carbon source and lipase activity was determined. Different concentrations (1%, 2%, 3%, 4% and 5%) of inorganic nitrogen (Ammonium tartarate) were added to production media and lipase activity was determined. The production medium was adjusted to different pH (4, 5, 6, 7, 8, 9, and 10) and lipase activity was determined. The production media was incubated at various temperatures (20, 25, 30, 35, 40, 45 and 50 °C) and lipase activity was determined. Different metal ions ($Cacl_2$, $Mgcl_2$, $Zncl_2$, $Mncl_2$, $Cocl_2$, $Hgcl_2$, $Bacl_2$, KCl, Agcl, $Fecl_2$, $Cucl_2$ and $Nicl_2$) with final concentration of 1 % were added to production media and lipase activity was determined.

3. Results and Discussion

The Lipolytic microorganism was isolated from groundnut and palm oil mill effluent and identified as *Cladosporium* on the basis of morphological characterization (Figure 1). Laboratory scale optimization was carried out for lipase production and activity by isolated *Cladosporium*. Lipase activity was observed after addition of different concentration (0.5-3 %) of Honge (Karanja) oil as sole carbon source (Figure 2). Lipase activity steadily increased with addition of carbon source and maximum lipase activity (8.12 Units/ml) was observed at 3% carbon source. This oil was found to be better stimulant for enhanced lipase activity. In inorganic nitrogen source optimization, maximum lipase activity (7.75 Units/ml) was observed at 2% concentration of Ammonium tartarate (Figure 3). Further addition of nitrogen source negatively affected lipase activity. Lipase activity was estimated at pH (4-10) and maximum lipase activity (7.85 Units/ml) was observed at pH 6 (Figure 4). Neutral pH was better suited for Lipase activity and extreme acidic and alkaline pH negatively affected lipase activity. Among the different temperatures maintained in the present study (20°C to 50°C) maximum lipase activity (8.71 Units/ml) was observed at 30 ^{0}C (Figure 5). Lipase activity was significantly reduced at higher or lower temperature than 30 ^{0}C. The lipase activity in media supplemented with KCL showed highest lipase activity (6.88 Units/ml) during ion optimization (Figure 6).

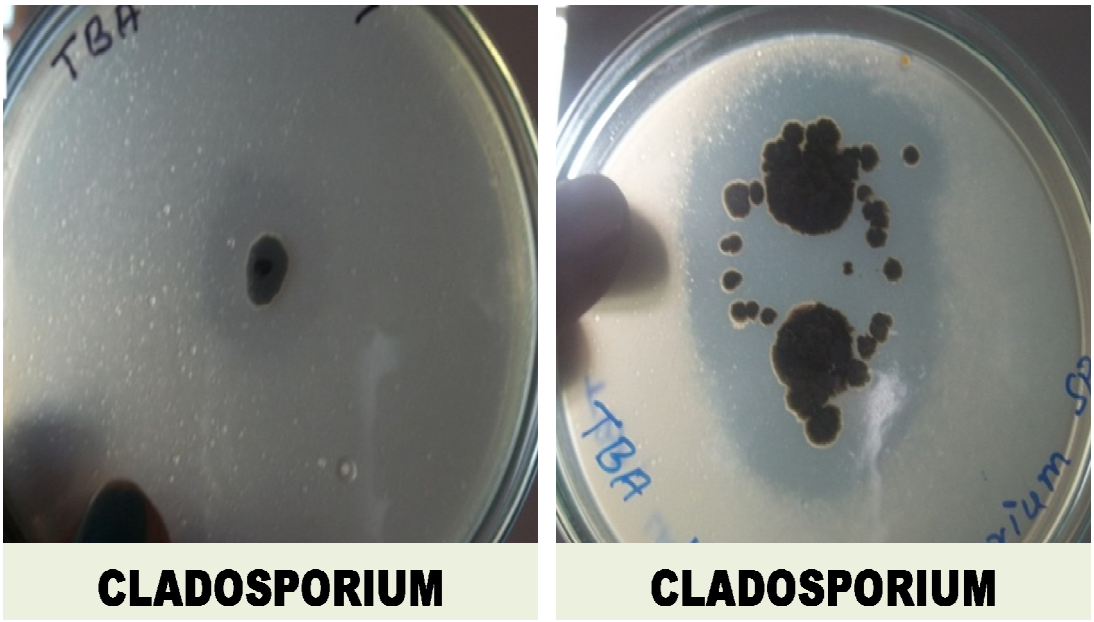

Figure 1: Lipolytic activity of Oil mill effluent sample and isolated Cladosporium.

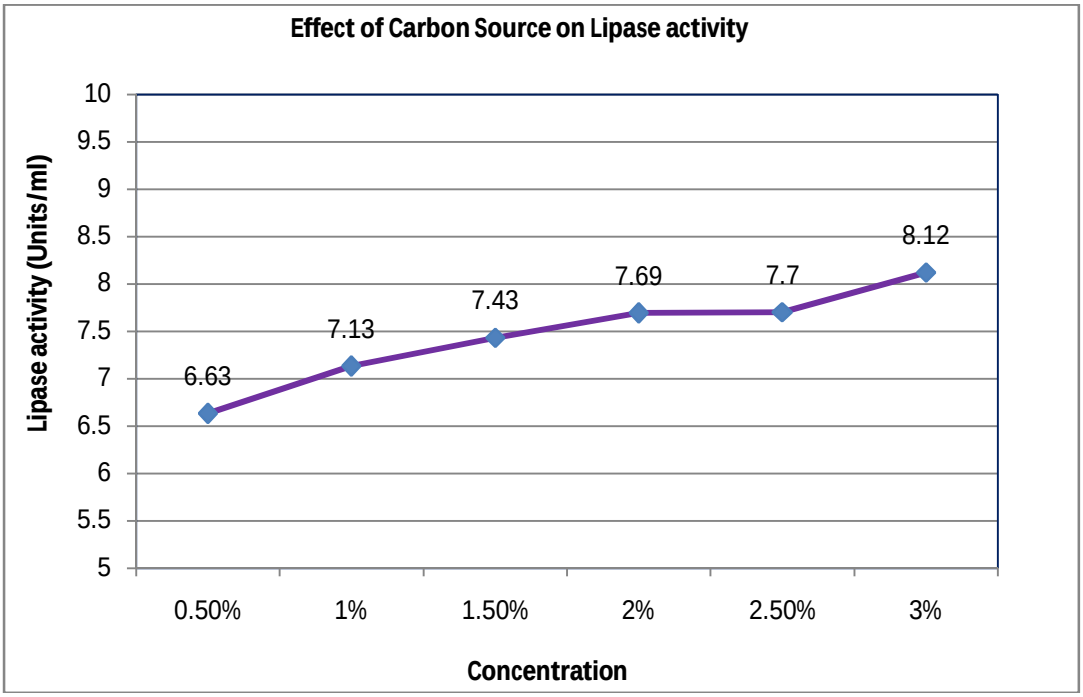

Figure 2: Effect of Different Concentrations of Carbon Source on Lipase Activity.

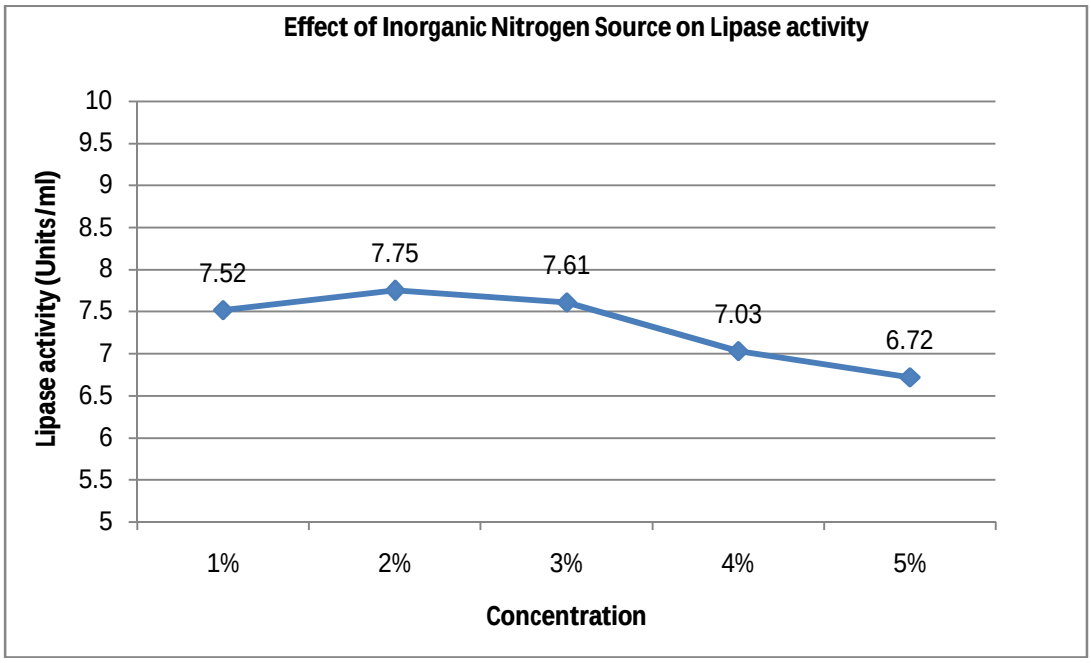

Figure 3: Effect of Different Concentrations of Inorganic Nitrogen on Lipase Activity.

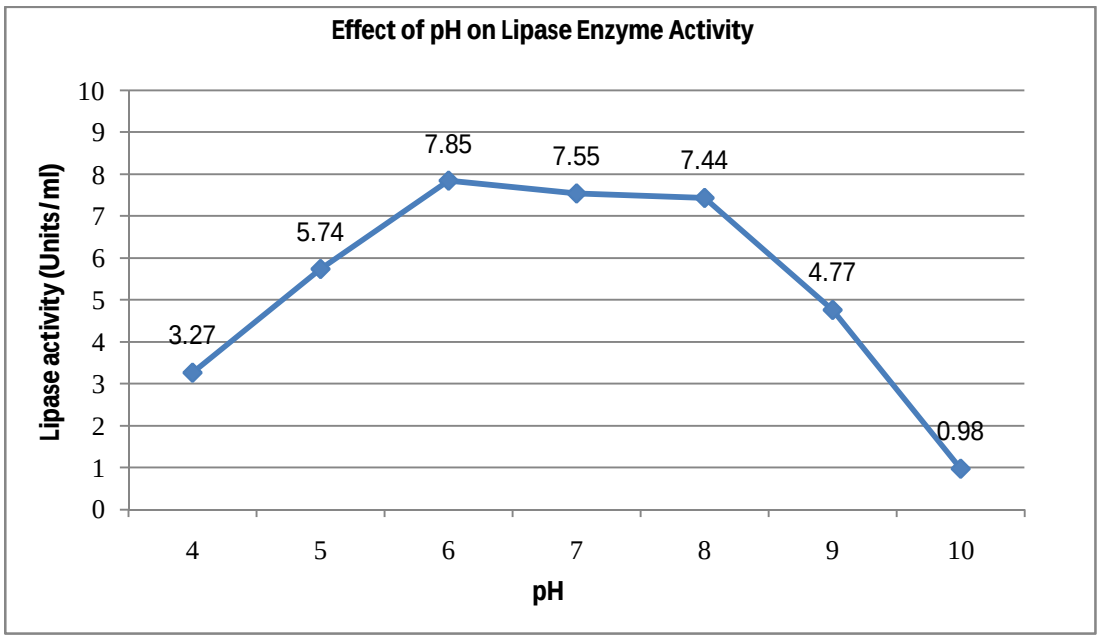

Figure 4: Effect of pH on Lipase Enzyme Activity.

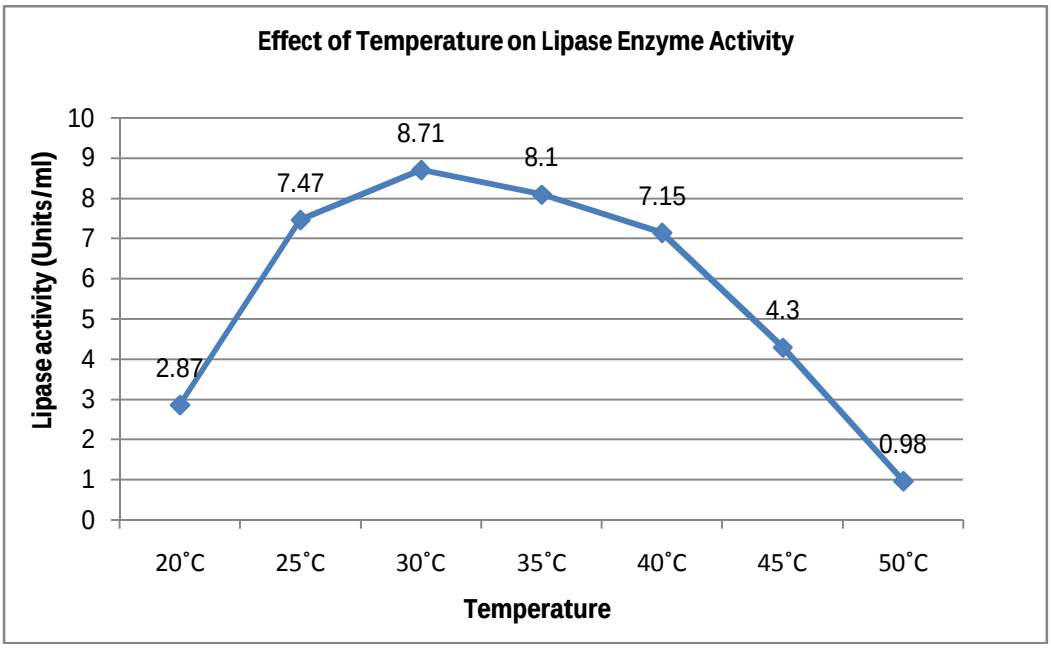

Figure 5: Effect of Temperature on Lipase Enzyme Activity.

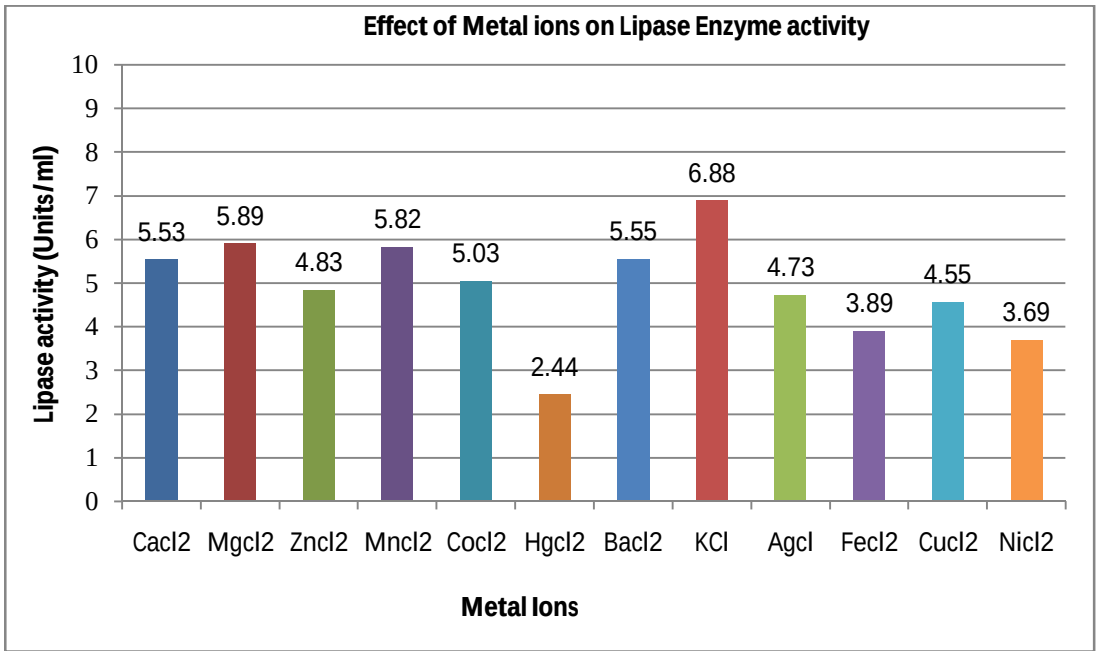

Figure 6: Effect of Metal Ions on Lipase Enzyme Activity.

Conclusion

The present study was focused on novel lipase-producing microorganisms and optimization of the medium composition for enhanced lipase activity. Higher Lipase activity was detected with 3% carbon source, 2% nitrogen source and KCL ion addition. Also, average pH for maximum lipase activity was observed in the range of pH 6-8 and at temp 30 ^{0}C. The current findings suggest that isolated *Cladosporium* from oil effluent can be exploited for further study and industrial commercialization.

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