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Research Article

BIOCHEMICAL CHARACTERIZATION OF AN EXTRACELLULAR LIPASE FROM NEW STRAIN OF *RHIZOPUS SP* ISOLATED FROM OIL CONTAMINATED SOIL

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ABSTRACT: In the present study the optimum pH, optimum temperature, pH stability, thermal stability and metal ion stability was determined for the isolated lipase from the new strain of *Rhizopus sp* (TP.St02). It showed optimum pH of 6.0 at 30ºC.The lipase was quite stable at pH 5.5 and 40°C. The enzyme activity was greatly enhanced in the presence of 2mM and 5mM concentrations of Mn^{2+}ions, whereas Hg^{2+} severely inhibited enzyme activity.
Keywords: Temperature, stability, lipase, activity.

INTRODUCTION

Lipases are now attracting an vast attention because of their biotechnological prospective [1,2]. This interest arises from the capacity of these enzymes to catalyse synthetic reactions in non-aqueous media [3,4]. With the aim of improving activity, storage and operational stability, especially in low-water media, their immobilisation on various organic and inorganic supports has been studied broadly [5,6]. The flexibility of lipases leads to multiple industrial applications in foods, flavours, pharma, cosmetics and environmental protection, etc. [7,8]. They have been employed for direct esterification and transesterification reactions in organic media to produce esters having possible applications in fine chemicals, pharma and agrochemicals industries. Moreover, several works reported the ability of lipases to catalyse the synthesis of short chain fatty acids and alcohols used as additives for a variety of perfumes and flavours [9,10], biosurfactants [11,12] and biofuels [13,14]. This work reports on the biochemical characterization of isolated lipase secreted by new strain of *Rhizopus* sp (TP.St02).

MATERIALS AND METHODS

Isolation of fungi from soil and Culture conditions

Soil samples were collected from oil mills near Medbowli, Hyderabad. One gram of soil was collected using a sterile spatula in a sterile Petri plate. The soil was brought to the laboratory and processed immediately. The fungal organisms present in the soil were isolated using soil dilution method [15] and the cultures were maintained on Potato Dextrose Agar Media and Basal media. 1 mL of spore suspension, from a culture in PDA medium after 72 hours incubation, was inoculated into 500 mL Erlenmeyer flasks containing 20 g of wheat bran and water (60:40 w/w). These flasks were incubated at 30ºC for 96 hours and the lipase produced was extracted with distilled water, followed by filtration.

Lipase assay

Lipase activity was assayed quantitatively using 4-nitrophenyl palmitate as the substrate according to the method described by [16]. One enzyme unit was defined as the amount of enzyme that liberated 1 µmol of 4-nitrophenol per minute under the assay conditions.

Biochemical Characterization of lipases

Optimum temperatures for lipases were determined by [16] with little modifications using the at the following assay temperatures (ºC): 20, 30, 35, 40, 45, 50, 55, 60, 70 and 80. Initially, reactions were performed in reaction mixtures containing phosphate buffer 50 mM, pH 7.0.

Once the optimum temperature was known, the same process was repeated at the optimum pH found after the optimum pH assays. Optimum pH for lipases were determined using the previously described replacing the phosphate buffer (50 mM final concentration; pH 7.0) used in the standard reaction mixture by the following buffers (at a final concentration of 50 mM, and prepared as described by [17]: citrate buffer (pH 3.0–4.0), succinate-NaOH buffer (pH 4.0–6.0), phosphate buffer (pH 6.0–7.5), Tris-HCl buffer (pH 7.5–9.0) and glycine-NaOH buffer (pH 9.0–12.0). The assay temperature used was the optimum temperature for the enzyme. Optimum pH assays were repeated when the optimum temperature of the enzyme at the optimum pH obtained changed. For thermostability assays, enzyme solutions prepared in 50 mM phosphate buffer (pH 7.0) were incubated for 3 h at the following assay temperatures (°C): 20-60ºC. Samples were withdrawn at 5-15 min intervals and were assayed at the optimum temperature and pH of the enzyme by spectrophotometer. For pH stability assays, enzyme solutions were prepared in the corresponding buffers (at 50 mM): citrate buffer (pH 3.0−4.0), succinate-NaOH buffer (pH 4.0–6.0), phosphate buffer (pH 6.0–7.5), Tris-HCl buffer (pH 7.5–9.0) and glycine-NaOH buffer (pH 9.0– 12.0), and were incubated for 1-24 h at room temperature. Enzyme solutions were then assayed at the optimum temperature and pH of the enzyme by spectrophotometer. To determine the effect of metal ions on the lipase enzyme, to 0.1ml of the enzyme 2ml of potassium phosphate buffer (pH 7) were added. To this 2mM and 5mM of different metal ions were added. These preparations were taken in a series of test tubes and incubated at 30 ^{0}C for 10min.

RESULT AND DISCUSSION

In the present investication to determine the optimum temperature the isolated lipase enzyme was subjected to different temperatures in the range of 20-80ºC. The maximum lipolytic activity from TP.St-02 was shown at 30ºC (Fig 1).

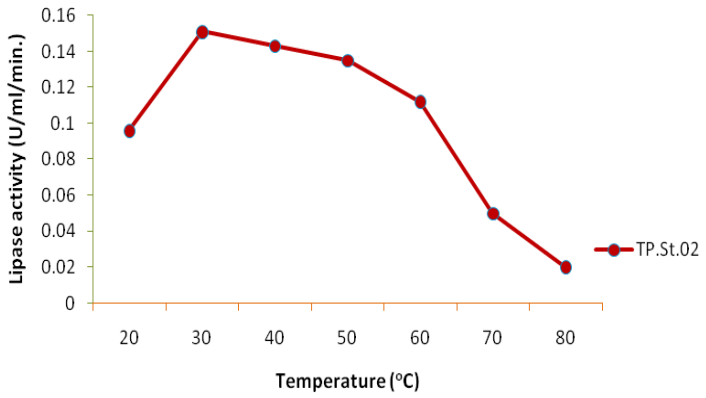

Fig.No.1 Optimum temperature for Lipase activity

Aspergillus carneus lipase showed optimum activity at 37ºC [18] and according to [19] very few fungal lipases exhibit temperature optima above 40ºC, thus the extra cellular lipase from *Rhizopus* sp. may have potential in high temperature reactions. According to [20], the interest in running bioprocesses at high temperatures lies in the favorable changes in most physical properties of fats in elevated temperatures and in the stability of thermostable lipases in organic solvents. The enzyme was stable at temperatures below 50ºC keeping at least 40% of its activity after 1h treatment. Further increase in the incubation temperature, the activity of the enzyme was greatly inhibited. It might be due to the denaturation of the enzyme at high temperature. The extra cellular lipolytic activity from the isolates TP.St-02 was active at pH 6.0 (Fig 2).

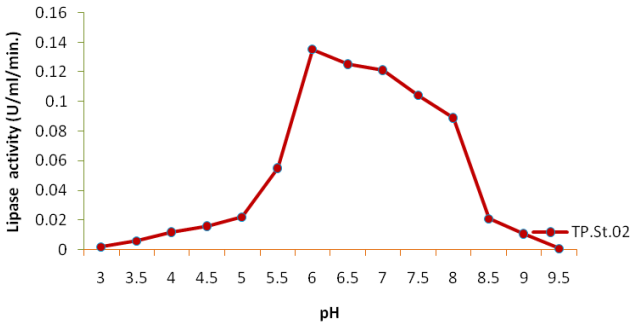

Fig.No.2. Optimum pH for Lipase produced

The pH stability of the isolated lipase enzyme was determined by incubating the enzyme in buffers of different pH ranging from 2–10. The lipase enzyme isolated from this *Rhizopus sp* (TP.St02) was quite stable up to pH 5.5 and retained 90% of its activity (Fig 3). Decline in activity was observed with increase in pH and at pH 7.5 the enzyme retained nearly 20% of its original activity.

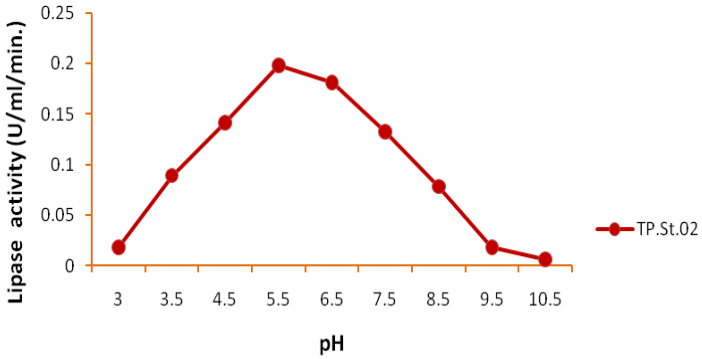

Fig.No. 3 pH stability of the Lipase produced

In order to determine the thermal stability of the enzyme, the isolated lipase enzyme from fungal species was incubated at different temperatures ranging from 20°C to 60°C. The lipase enzyme isolated from the *Rhizopus sp* (TP.St02) was fully stable and showed 90% activity at 40°C. Thereafter, the activity declined and was 50% and 20% of the original activity at 45^{0}C and 50^{0}C. This may be due to denaturation of the enzyme. The enzyme was completely inactivated above 60^{0}C (Fig 4).

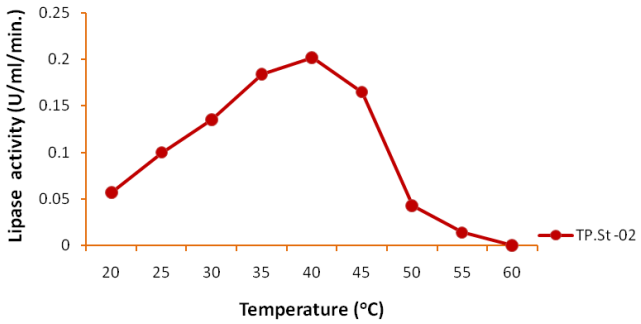

Fig.No. 4 Thermal stability of the Lipase produced

[21] also optimized 40 ° C temperatures for the optimum activity of lipases. Our findings are in agreement with the work of [22]. The enzyme activity was greatly enhanced in the presence of 2mM and 5mM concentrations of Mn^{2+} ions, whereas Hg^{2+} severely inhibited enzyme activity (Fig 5).

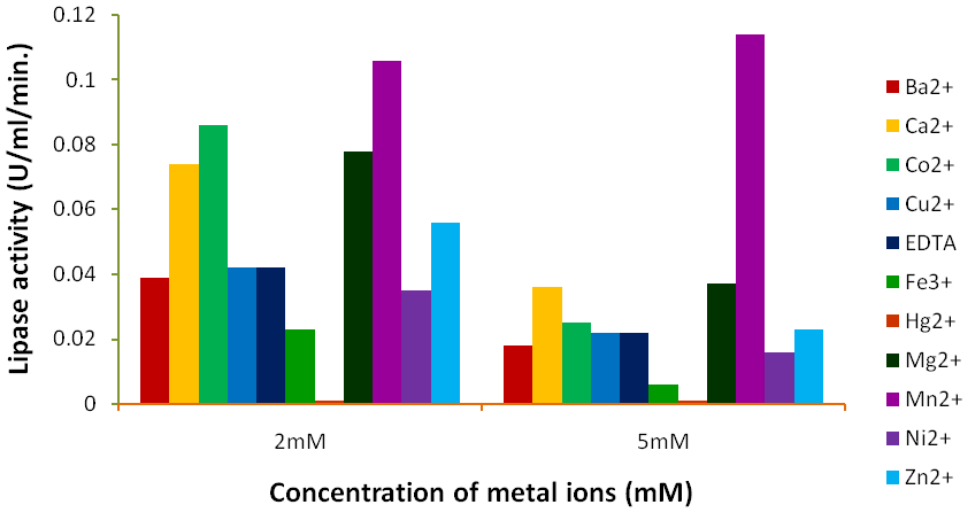

Fig.No.5 Metal ion stability of the Lipase produced

Hg^{2+} strongly inhibited the lipase activity and Mn^{2+} enhanced it. This behavior was also observed for the *Aspergillus carneus* lipase [18]. EDTA inhibited about 60% of the enzyme activity, which indicates the partial requirement for metal ions. The result of the present study confirms the above findings as the lipase activity was enhanced in the presence of Mn^{2+}. Like Co^{2+}, Cu^{2}+, Mg^{2+} and Ca^{2+} and stimulated the activity of lipases by both wild and mutant strains as previously reported by [23]. Both at 2mM and 5mM concentrations the TP.St02 showed the same response of inhibition of enzyme activity in the presence of Hg^{2+}. Similar kind of work has also been reported by [24, 25].

CONCLUSION

Fungi constitute naturally immobilized lipases which have high catalytic power and stability. Out of 7 fungal strains isolated from the oil contaminated soi1 1 strain exhibited maximum lipase activity. *Rhizopus sp* proved to be the best lipase producer. Various physicochemical parameters were studied to determine the optimum conditions for its lipase production. The obtained results showed that the TP.St02 strain which is closely related to *Rhizopus sp* has optimum pH and Optimum temperature of 6.0 and 30ºC respectively. The pH stability, temperature and metal ion stability of the lipase was found to be5.5 ,40ºC and 2mM and 5mM concentration of Mn^{2+}enhanced the lipase activity. The Hg^{2+} strongly inhibited the enzyme activity.

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