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Production of Cold Active Lipase from Bacillus sp.

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Abstract
A cold active lipase producing *Bacillus* sp. strains were isolated from sewage of oil. *Bacillus* sp. strain SY-7 was determined as the best lipase producing isolate. The highest enzyme production was found at 20°C and pH 8.0 on tributyrin media. Analyses of molecular mass of the partial purified lipase was carried out by SDS-PAGE which revealed a single band as 110.5 kDa. The enzyme activity and stability were determined by spectrophotometric and titrimetric methods. The enzyme was active between pH 4.0-10.0 and 5-50°C and showed optimal activity and stability at pH 8.0 and 20°C. In the presence of $BaCl_2$ (4mM), KCl (4mM), $AgNO_3$ (4mM), CuSO4 (4mM), $MgCl_2$ (4mM), $CaCl_2$ (4mM), $ZnCl_2$ (4mM) and NaCl (4mM), the enzyme exhibited the following activities 105%, 100%, 100%, 89%, 105%, 95%, 100% and 100%, respectively. In the presence of Tween-20(5%), Tween-80(5%), detergent-1(5%) and detergent-6(5%) the enzyme saved its original activity. SDS(5%), detergent-2(5%) and detergent-7(5%) increased the activity 10%, 5% and 5%, respectively. Detergent-3(5%), detergent-4(5%) and detergent-5(5%) reduced its activity respectively, 14%, 14% and 52%. According to these results, SY-7 lipase shows alkaline, psychrotrophilic, cold active and stable, chelator and detergent resistant properties. Owing to these properties, this lipase can be useful in detergent industry.

Keywords: Lipase, psychrophilic, alkaline, detergent resistant, Bacillus sp. SY-7

INTRODUCTION

Lipases constitute a group of enzymes defined as carboxylestreases that catalyze the both hydrolysis and synthesis of long chain acylglycerols at the lipid-water interface [1,2,3]. They belong to the structural superfamily of α/β-hydrolases [2,4,5]. Lipases have been found in many species of animals, plants and microorganisms [6]. However microbial lipases (from bacteria, fungi, yeast and actinomycetes) have more attention because of their characteristics such as action under mild conditions, stability in organic solvent and temperature, high substrate specifity and regio- and enantioselectivity [7,8]. Therefore industries have interest in exploring new lipases with unique characteristics and high activities that are derived from microorganisms. Especially cold active lipases are effective for consideration of product stability and energy savings, rendering applications such as detergent formulations, fine chemistry catalysis, specifically food processing [9,10,11].

This study determines isolation of *Bacillus sp*. strain SY-7, characterization and optimization of SY-7 lipase and properties of the enzyme activity in industrial area.

METHODS

Microorganisms and culture conditions

Bacillus sp strain SY-7 was isolated from sewage of olive oil. After isolation of the strain, it was identified by morphological and biochemical tests [12]. *Bacillus* sp strain SY-7 was screened for lipase production on %1 tributyrin agar plates at different temperatures (0-50°C) and pH values (6.0-9.0). Lipase production was determined by development of clear zone around the colonies.

Lipase Production and Partial Purification

Bacillus sp strain SY-7 was inoculated in olive oil media and allowed to grow at 20°C for 72 h with shaking at 150 rpm. The sample was harvested by centrifugation at 10000 rpm for 15 min at 4°C and the supernatant was concentrated by ethanol (1:1) at -20°C for 12 h for partial purification. After alcohol precipitation, obtained precipitat was collected by centrifugation at 10000 rpm for 15 min at 4°C and pellet was dissolved in 50mM Tris-HCl buffer (pH 7.0) [13, 14].

Enzyme assay

Lipase activity was measured by the spectrophotometric method using p-NPP (p-nitrophenyl palmitat) as a substrate. Lipase activity was assayed by measuring the absorbance of liberated p-NPP at 405 nm. One unit of activity was defined as the amount of enzyme needed to release 1 µmol of nitrophenol per min [14,15]. Lipase activity was also assayed by titrating free fatty acids liberated from olive oil. One unit of lipase activity was defined as the activity required to release 1 µmol of fatty acids per min under the above conditions [16,17,18,19,20].

Effect of pH and temperature on lipase activity and stability

The optimal pH of the lipase was determined by measuring lipase activity at 20°C at different pH levels (pH 4.0-10.0): sodium asetat (pH 4.0-5.0), phosphate (pH 6.0-7.0), Tris HCl (pH 8.0-9.0) and glycine (pH 9.0-10.0). The pH stability was measured by incubating the partial purified enzyme in different pH between 4.0-10.0 for 1 h at 20°C. The residual activity was assayed under standart conditions. The optimum temperature of SY-7 lipase was determined by measuring the enzyme activity at various temperatures (5-50°C) in Tris-HCl buffer (pH 8.0.) Thermal stability was determined by incubating the enzyme in Tris-HCl buffer (pH 8.0) for 24 h at different temperatures (5-50°C) followed by

measuring residual activity. The activity of untreated enzyme was considered as a control (100%).

Effect of metal ions, chelating agents, inhibitors and detergents on lipase activity

The effect of various chemicals on lipase activity was assayed by pre-incubating the enzyme at 20°C for 60 min and the residual activity was measured under standart method conditions. The activity of untreated enzyme was taken as 100%.

Determination of molecular mass

SDS-PAGE was performed to determine molecular weight and homogenity of the lipase by using 10% resolving and 5% stacking gel. The electrophoresis was performed with 25 mA. The molecular weight of SY-7 lipase was estimated using a standart molecular weight marker (bovine serum albümine, 66 kDa).

RESULTS

Strain SY-7 was gram positive, rod shaped, spor forming and an aerobic bacterium. According to the results of various morphological and biochemical characteristics, it was identified as genus *Bacillus*. *Bacillus* sp strain SY-7 grew and produced lipase enyzme at pH 6.0-9.0 with an optimum pH of 8.0, and at 5-50°C with an optimal temperature of 20°C on tributyrin agar plates (Fig. 1). Determination of molecular mass of the partially purified enzyme was carried out by SDS-PAGE. Result of this application, the enzyme revealed one band as 110.5 kDa (Fig. 2). The purified lipase was active in the range of pH 4.0-10.0 and in the temperature range of 5-50°C with maximal activity at pH 8.0 (Fig. 3) and 20°C (Fig. 4). The activity of lipase significantly decreased when the pH was increased to 10.0. The enzyme showed stability over a pH range 7.0-9.0 (Fig. 5). When the temperature was raised to 30°C, the activity of lipase sharply decreased. The enzyme was highly stable at 10-20°C with a residual activity greater than 90% of its initial activity (Fig. 6). The enzyme was incubated with chemicals and the remaining activity was measured at 20°C. In the presence of $BaCl_2$ (4mM), KCl (4mM), $AgNO_3$ (4mM), $CuSO_4$ (4mM), $MgCl_2$ (4mM), $CaCl_2$ (4mM), $ZnCl_2$ (4mM) and NaCl (4mM), the enzyme exhibited the following activities; 105%, 100%, 100%, 89%, 105%, 95%, 100% and 100%, respectively (Fig. 7). Besides, SDS(5%), detergent-2(5%) and detergent-7(5%) increased the activity 10%, 5% and 5%, respectively. However, detergent-3(5%), detergent-4(5%) and detergent-5(5%) reduced its activity respectively, 14%, 14% and 52% (Fig. 8).

DISCUSSION

In this study, we showed that properties of lipase from *Bacillus* sp. SY-7. Temperature and pH that the best grown of *Bacillus* sp. strain SY-7 which isolated from sewage oil and the most produced of lipase was 20°C and pH 8.0 respectively. Ertuğrul et al. [21] isolated *Bacillus* sp. from sewage water of olive oil plant and its optimum lipase production was at 30°C and pH 6.0. Kiran et al. [22] produced lipase from *Pseudomonas* sp. (MSI057) that optimum activity has at 30°C and pH 9.0.

Since highest activity of this enzyme was pH 8.0, it was identified as an alkaline enzyme. Besides the lipase exhibited highest activity at 20°C. So, we concluded that it is a *psychropilic enzyme*. Chen et al. [23] showed that the optimum activity of lipase from *Psychrobacter* sp. C18 was pH 8.0 and at 30°C. Gökbulut and Arslanoğlu [3] revealed that lipase from *Pseudomonas fluroescens* was active at a temperature range of 15-65°C and it exhibited maximum acitivity pH 8.0 and at 45°C.

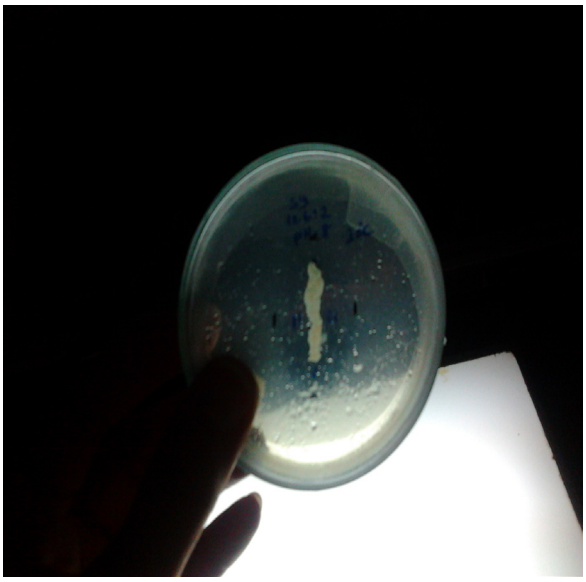

Fig. 1. Lipase synthesis of *Bacillus* sp. SY-7

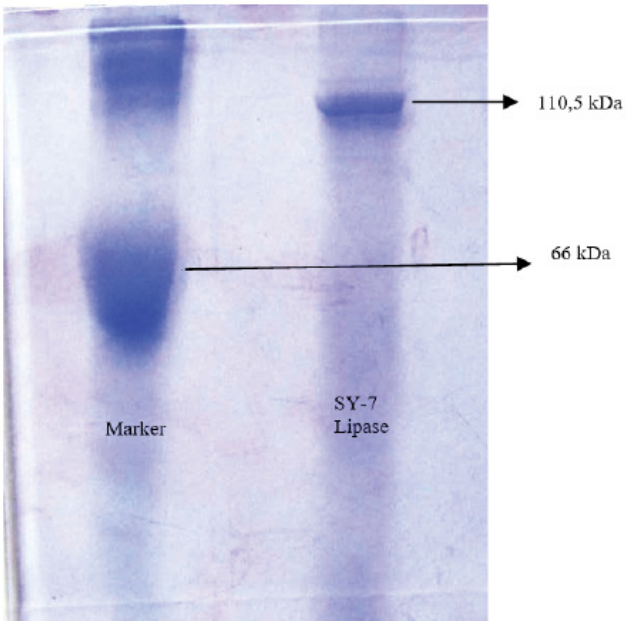

Fig. 2. SDS-PAGE analysis of SY-7 lipase

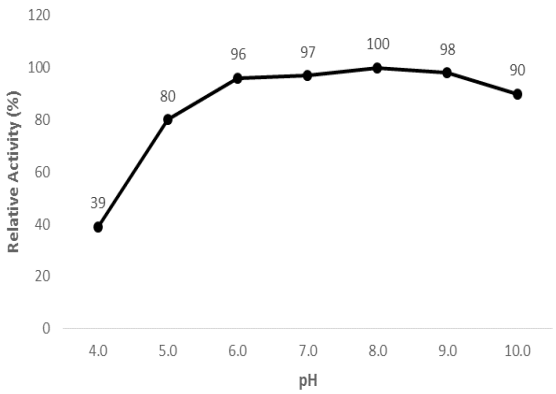

Fig. 3. Effect of pH on the activity of *Bacillus* sp. SY-7 lipase

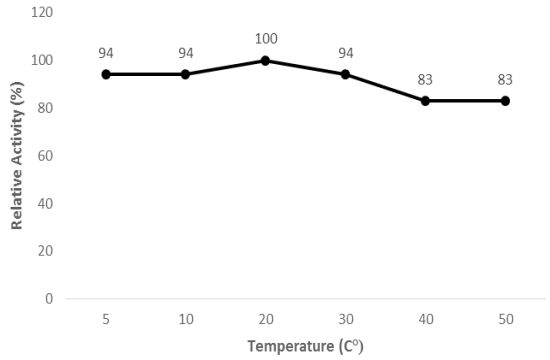

Fig. 4. Effect of temprature on the activity of *Bacillus* sp. SY-7 lipase

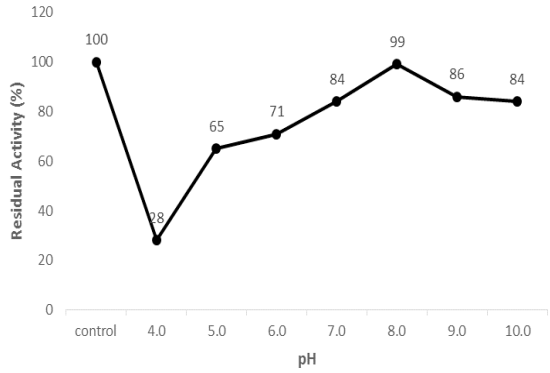

Fig. 5. Effect of pH on the stability of *Bacillus* sp. SY-7 lipase

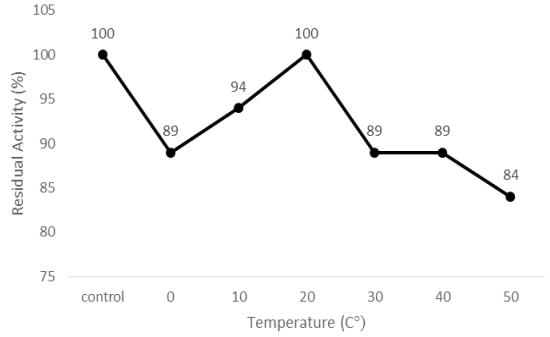

Fig. 6. Effect of temprature on the stability of *Bacillus* sp. SY-7 lipase

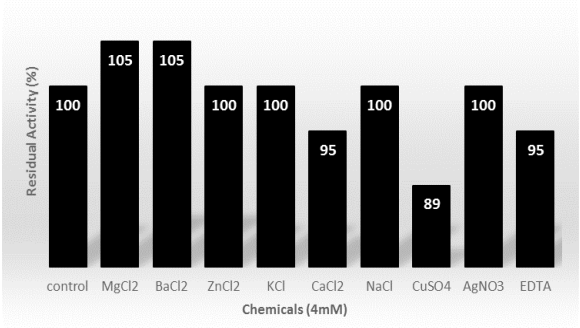

Fig. 7. Effect of metal ions, chelating agents, inhibitors on the activity of *Bacillus* sp. SY-7 lipase

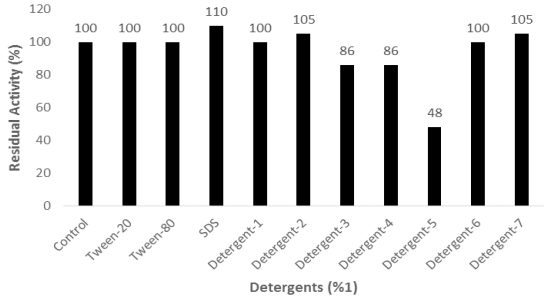

Fig. 8. Effect of various detergents on the activity of *Bacillus* sp. SY-7 lipase

When we analysed the stability of lipase, firstly, SY-7 lipase was active in a broad range between pH 4.0 and 10.0, with an optimum pH 8.0. Whereas its activity was saved with higher pH values (9.0-10.0), it significantly decreased with lower pH values (4.0-5.0). Therefore, this enzyme determined as an alkaline tolerant enzyme. Secondly, lipase from *Bacillus* sp. SY-7 was active between 5-50°C with an optimum 20°C. As a result of temperature assays, this enzyme was defined as cold active lipase. Gökbulut and Arslanoğlu [3] revealed that the lipase enzyme secreted by *Pseudomonas fluorescens* KE38 exhibited high stability retaining 100% and 70% of its activity after incubation at 45 °C and pH 8.0, respectively.

According to the results of various detergents and chemicals treatments, SY-7 lipase is useful enzyme for detergent industry. Chen et al. [23] reported that the activity of lipase from *Psychrobacter* sp C18 which is a psycrothrophic bacteria increased with $CuSO_4$, $FeSO_4$, $CaCl_2$ KCl and $MgSO_4$. When SY-7 lipase was exposed to Tween-20(5%), Tween-80(5%), detergent-1(5%) and detergent-6(5%), the enzyme saved its original activity. Liu et al. [24] showed that the activity of lipase produced from *Fusarium solani* N4-2 was stable in the presence of Tween-80, sodiumcholat, sodium tauracholat. On the other hand, it exhibited more than 75% of its activity with various commercial detergents (OMO, Ariel, Panda, Whitecat, Tide).

SY-7 lipase was detected as 110 kDa by SDS-PAGE. According to some previous studies, molecular weight of lipase is lower than SY-7 lipase. This result may be interest with its partial purification. It is necessary that SY-7 lipase should purify and do zymogram analyze at future studies. Chen et al. [23] determined that the molecular weight of lipase from *Pseudomonas* sp. S5 was measured as 60 kDa by SDS-PAGE.

CONCLUSION

According to these results, SY-7 lipase shows cold active, alkaline, alkali-stable and chelator resistant properties. As it can be understood from these results, SY-7 lipase may find potential applications in detergent formulations.

Acknowledgements

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