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Original Research Article

Inducers for the enhanced production of lipase by *Streptomyces* isolated from mangrove ecosystem

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A B S T R A C T

Among 105 isolates of *Streptomyces* that were screened for lipase production, *S. halstedii* strain ST 70 exhibited higher activity. Different types of Tween and triglycerides (vegetable oils) were used as inducer substrate in the medium as well as substrate to evaluate the activity of lipase enzyme. Factorial experimental design (23) was formulated with 2 incubation period for *Streptomyces* culture, 2 nutrient medium (Starch casein and Lipase medium), 2 incubation period for enzyme activity (1 hr and 4 hr). Total 720 analyses were performed with enzyme samples obtained from different treatments. Overall, 7 days incubation period for growth of *Streptomyces halstedii* strain ST 70 in starch casein medium was required to produce 1.6 IU/ml lipase enzyme active upon Tween 20 in presence of any Tween (20, 40, 60, and 80). This strain could be able to degrade olive oil and sunflower oil too. Lengthy incubation period for enzyme (4 hrs) proved to best for its higher activity.

Keywords

Lipase, *Streptomyces*, Tween, Lipid, Mangrove

Introduction

Lipases are important due to their ability to catalyze with wide spectrum of lipid substrates and stability under varied temperature and pH. They have been used as an additive in food processing, pesticide, drug formulations, etc. (Li *et al.,* 2001; Vallin *et al.,* 2005). Several organisms have been reported as producer of intracellular and extracellular lipase including *Streptomyces* that are well known for the production of biopharmaceuticals and other metabolites (Gupta *et al.,* 2002; Nutan *et al.,* 2002; Kamini *et al.,* 1997; Levadoux *et al.,* 2002). However, they are poorly studied for lipases (Lescic *et al.,* 2001; Abramic *et al.,* 1999). In the present study, *Streptomyces halstedii* strain ST 70 was isolated from mangrove ecosystem. Which is India's second largest mangrove ecosystem and did not explored well as far as the lipase producers are concerned. In the present study, an experiment was planned to evaluate the effect of different substrates useful for enzyme induction and activity under different cultural conditions.

Materials and Methods

Source of organisms: *Streptomyces halstedii* strain ST 70 was isolated from different samples (water, soil, plants) collected from Bhitarkanika mangrove of Orissa.

Identification of strain: slide culture was prepared on starch casein medium and ISP 3 medium incubated at 30 ºC and 37 ºC by using cavity slides. Periodical observations regarding spore morphology were recorded by using Nikon Japan Trinocular Research Microscope Model 50i. Different biochemical tests for carbohydrate utilization, nitrogen utilization, growth in different stress conditions, antibiotic resistance, and amino acid degradation, enzyme activity like, amylase, protease, asparaginase, antifungal activity and phosphate solubilization were analyzed for characterization. Finally data were used for the identification of *Streptomyces* isolate using Probabilistic identification of bacteria (PIBwin). Strains of *Streptomyces* were screened for lipase activity through plate culture assay on lipase test medium. The quantitative analysis of lipase of positive strains was done by titrimetric method (Kamini *et al.*, 1997). In which, 25 ml of olive oil was homogenized with 75 ml of 2% polyvinyl alcohol and used as the substrate. The substrate emulsion (5ml) and 4 ml of 0.1 M sodium phosphate buffer, pH 7.0 were pre incubated at 37º C for 10 min and 1 ml of the enzyme solution was then added and incubated at 37º C for 20 min. The reaction was terminated by the addition of 20 ml of acetone and titrated against 0.01 M sodium hydroxide. The heat-inactivated enzyme was added to the reaction mixture as control. One unit of lipase activity was defined as the amount of enzyme that released free fatty acids in 1 min under standard assay conditions. For further experiment, 9 different types of substrates viz., Tween 20, 40, 60 and 80, mustard oil, sunflower oil, soyabean oil, ghee and olive oil were used as inducers substrate into the medium (1 ml / 25 ml of medium) and substrate for enzyme activity. Two medium i.e. starch casein medium and lipase test medium were taken and cultures were incubated for 7 days and 15 days Culture filtrate of the different media were used as enzyme and assayed in two different incubation time i.e. 1 hr and 4 hr by above procedure.

Results and Discussion

During screening of 105 strains of *Streptomyces*, 18 strains were positive for lipase production in extracellular condition. Among them, *S. halstedii* ST 70 was found to be good producer of lipase in liquid culture i.e. 1.6 IU/ml^{-1}. In present study synthetic and natural oils were used as inducer substrate and enzyme substrate. The lipase produced by 7 day old culture prepared in starch casein medium containing Tween and soybean oil separately showed higher activity at one hour duration for Tween 60. However, natural oil worked well as an inducer to produce enzyme that was active for olive oil only (Fig. 1A and B). The highest enzyme activity i.e. 1.6 IU/ml at 4 hr incubation with Tween 20 was recorded in the growth medium aided with Tween 80 as inducers followed by Tween 60 (1.575 IU/ml^{-1}) and Tween 40 (1.425 IU/ml^{-1}). Natural oil i.e. mustard oil, sunflower oil, soybean oil, ghee and olive oil induced the enzyme that was active maximally for sunflower and olive oil (Fig. 1B). The lengthy incubation period i.e. 15 days for growth of organism in starch casein medium neither show much difference in substrate specificity nor effect of inducers. Again, the lipase produced in presence of synthetic inducer substrates preferred synthetic substrates for their better activity than natural oils (Fig. 1C). Four hours of incubation period of this enzyme did not show much effect as compared to 1 hr incubation period when natural oils were used as inducer or substrates (Fig. 1D). Among two nutrient medium used in this study starch casein medium produced more

active enzyme as compared to lipase test medium. The enzyme produced from 7 days old culture in lipase test medium containing synthetic substrates as inducers did not exhibit any enhancement in enzyme activity on synthetic substrates where as natural oil, as inducers, were more active on synthetic substrates (Fig. 2A). Exceptionally, lipase produced in medium induced by Tween 80 showed higher activity with all Tween substrate used for enzyme activity. The lipase produced in lipase test medium was more active in 4 hr. incubation period as compared to 1 hr. of incubation (Fig. 2B). At the same time, lengthy incubation period of organism produced negative effect on enzyme activity than 7 days of incubation of organisms in lipase test medium (Fig. 2C and D). Natural oils induced lipase production that was more active on sythetic substrates rather non synthetic substrates where as synthetic substrates induced lipase found to be active for both type of synthetic and non synthetic substrates. Over all, starch casein medium was found superior than lipase test medium. The 7 days culture of *S. helstedii* showed good activity as compared to longer incubation period. The enzyme produced in presence of inducer substrate exhibited good activity in four hour incubation at 37 ºC. However, one-hour incubation of enzyme with different substrate has shown comparatively higher activity than non induced enzyme.

Fig.1A Effect of substrate on lipase activity in starch casein medium (7 days growth, 1 hr enzyme incubation)

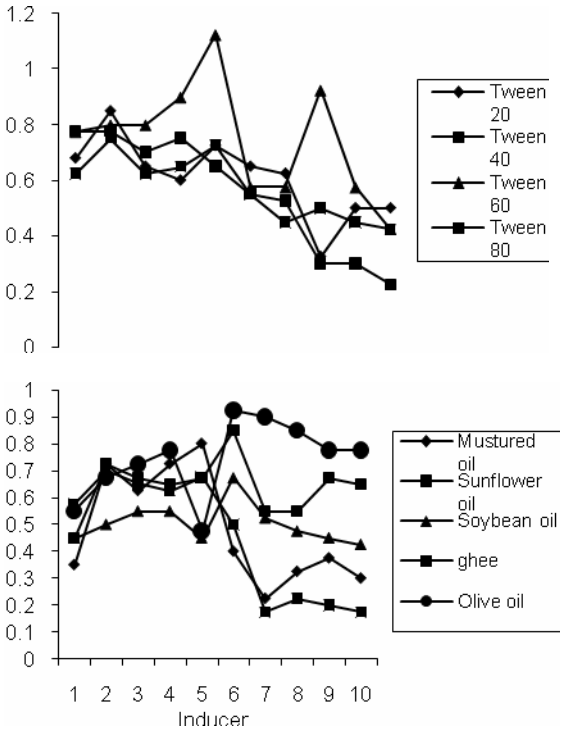


Fig.1B Effect of substrate on lipase activity in Starch casein medium (7 days growth, 4 hr. enzyme incubation)

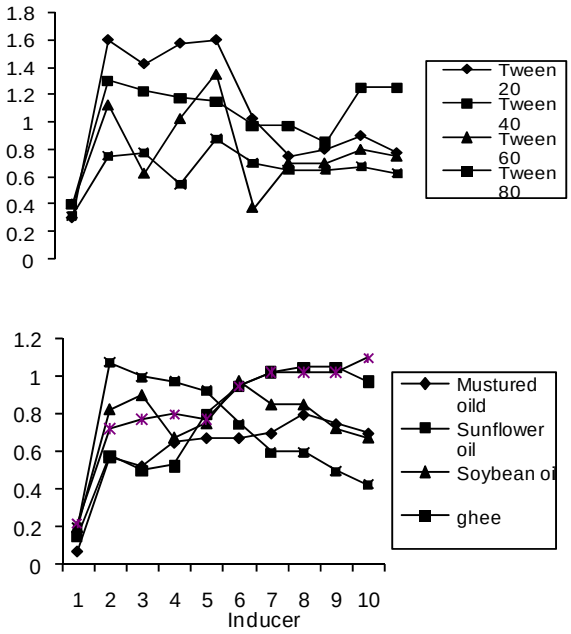


Fig.1C Effect of substrate on lipase activity in starch casein medium (15 days growth, 1 hr enzyme incubation)

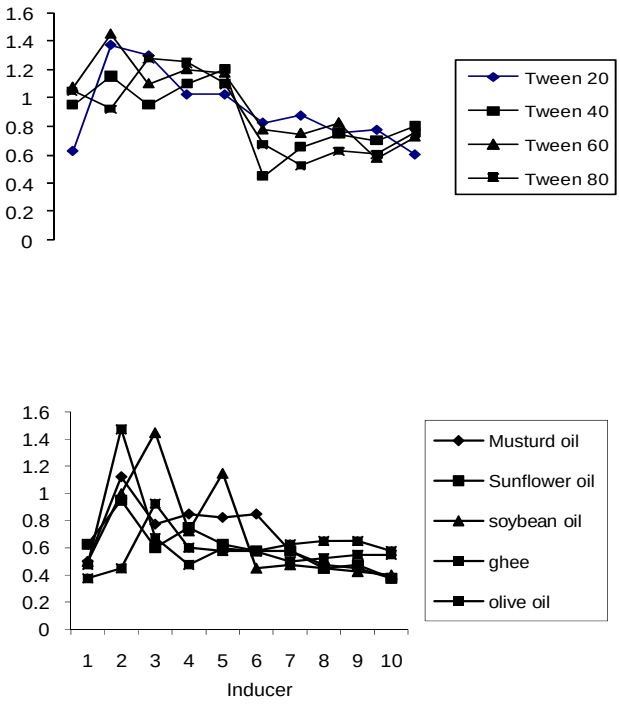


Fig.1D Effect of substrate on lipase activity in starch casein medium (15 days growth, 4 hr enzyme incubation)

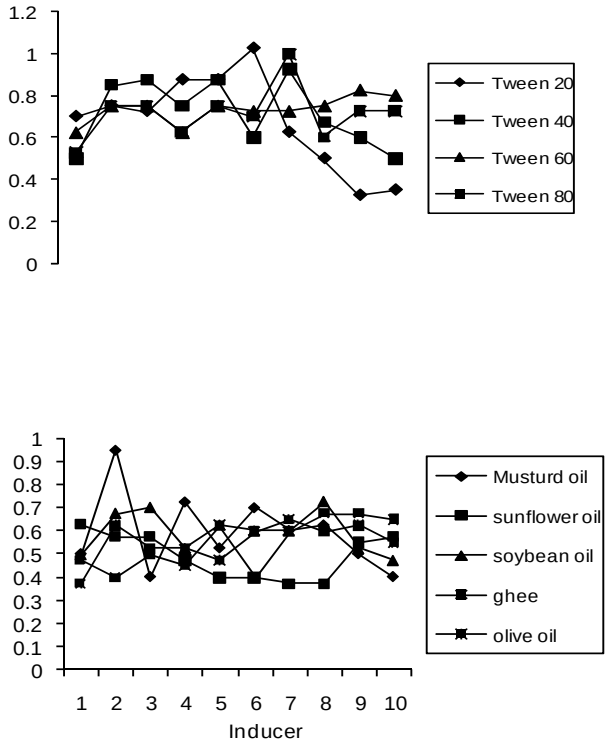


Fig.2A Effect of substrate on lipase activity in lipase test medium (7 days growth, 1 hr enzyme incubation)

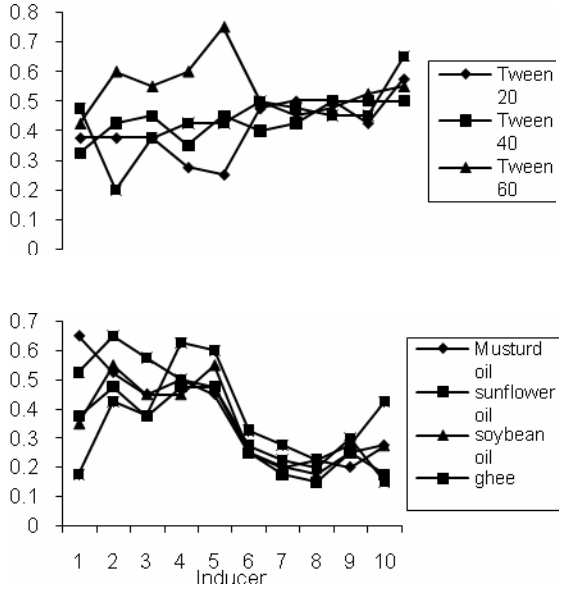
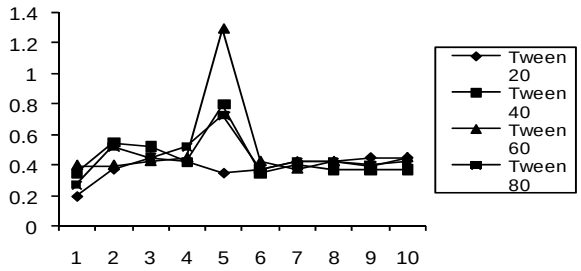


Fig.2B Effect of substrate on lipase activity in lipase test medium (7 days growth, 4 hr. enzyme incubation)



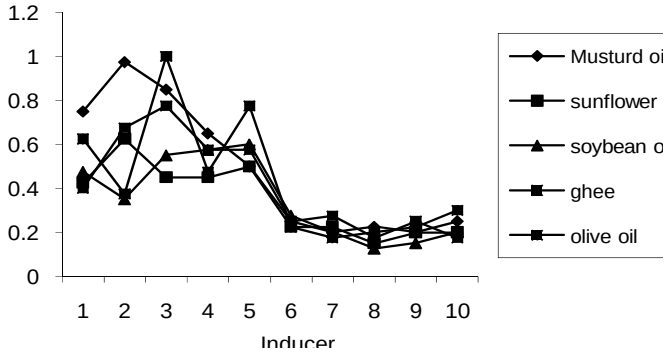
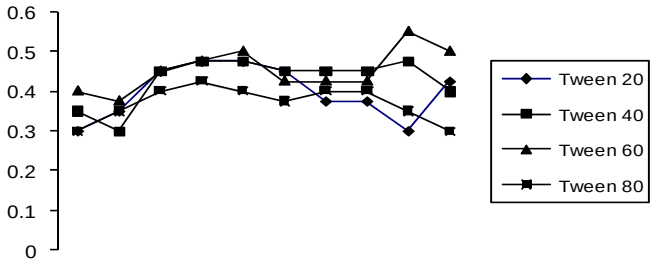


Fig.2C Effect of substrate on lipase activity in lipase test medium (15 days growth, 1 hr enzyme incubation)



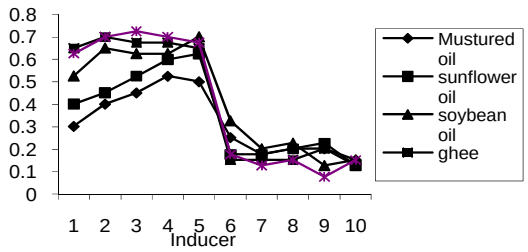
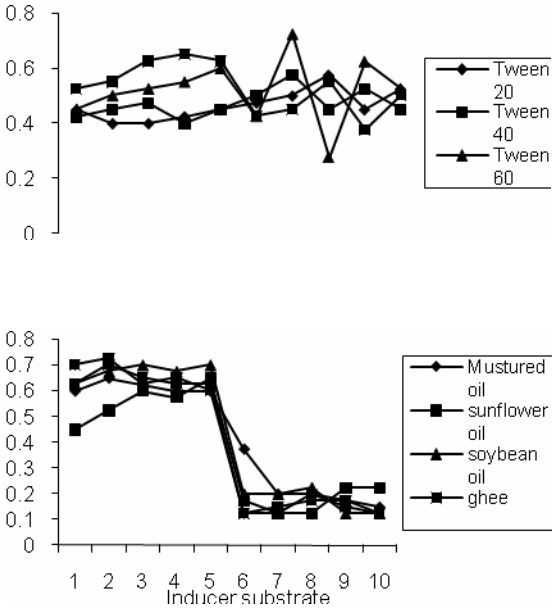


Fig.2D Effect of substrate on lipase activity in lipase test medium (15 days growth, 4 hr enzyme incubation)



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