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Original Article

OPTIMIZATION OF LIPASE PRODUCTION FROM DIFFERENT AGROINDUSTRIAL WASTES BY MARINE ACTINOMYCETES

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ABSTRACT

Objective: To identify the production of extracellular lipase by solid state fermentation (SSF) using coconut cake and groundnut cake using thermophilic strain of marine actinomycetes.

Methods: From the isolated strains of marine actinomycetes a comparative study has been evaluated on different intends such as pH, temperature, carbon sources, nitrogen sources and additive sources were optimized for maximum yield.

Results: Five different pH was used from 5 to 9 and on the other hand 3 different temperatures were maintained, sucrose, fructose and dextrose were used as carbon sources while urea, peptone and Ammonium nitrate was used as nitrogen sources similarly Tween 20 was used as additive sources. On comparing coconut oil cake and groundnut oil cake for all these different intends, there was maximum extracellular lipase specific activity was obtained in groundnut oil cake.

Keywords Marine Actinomycetes, Lipase, Coconut cake, Groundnut cake.

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis and synthesis of esters formed from glycerol and long chain fatty acids. Lipases are activated only when adsorbed to an oil-water interface [1]. Commercially useful lipases were usually obtained from microorganisms which produce a wide variety of extracellular lipases. Around the world, only about 2% of microorganisms have been tested as enzyme sources. Microbial lipases are produced mostly by submerged culture [2] but solid-state fermentation methods can also be used [3]. In general solid state fermentation is a well adapted and cheaper process than submerged fermentation for the production of a wide spectrum of byproducts (animal feed, enzymes, organic acids, biopulp, aroma compounds, antibiotics, composts, biopesticide, biofertilizers etc). Solid state fermentation is a high recovery method for the production of industrial enzymes [4].

It has been reported that in many bioproductions the amount of products obtained by solid-state fermentation was many fold higher than those obtained in submerged cultivations. In addition, the products obtained have slightly different properties (e. .g., more thermotolerance) while when produced in solid-state fermentation and submerged fermentation. Therefore, solid-state fermentation variables are well controlled and the purity of the product can be defined, this technology may be a more competitive process than is commonly thought. Solid-state fermentation offers many advantages over submerged fermentation for production of the enzyme lipase.

Coconut cake has been used as a potent substrate for production of lipase by marine actinomycetes in solid-state fermentation [5]. Coconut oil cake is obtained as a waste byproduct after oil extraction from dried coconut. Generally coconut oil cake is used as poultry and fish feed since it is rich in soluble sugars, soluble proteins and lipids [6].

MATERIALS AND METHODS

Sample collection and processing

Marine sediment samples were collected at 4-5 feet depth with the help of sterile spatula in a sterile plastic bag from the coastal area of kanyakumari district (8.0780° N, 77.5410° E) Tamilnadu, India. The samples were immediately transferred to squeeze glass bottles and stored at 4°C. Chemicals were purchased from Himedia, Mumbai, India.

Screening of marine actinomycetes

One gram of the marine sediment sample was taken and mixed with 100 ml of sterile distilled water and kept in a shaker (SI 600/600R) acinterlab, USA for 10 minutes and the supernatant was serially diluted. The actinomycetes isolation agar media was sterilized after solidification. 0.5 ml of the sample was aseptically transferred to the sterile petriplates [7]. Those were stored at37 °C for 10 days and examined for colony formation. Actinomycetes strains were purified by multiple streaking techniques used for lipase production.

Screening and identification of lipase producing actinomycetes

The tributryin agar plates were prepared and the test organisms were streaked on the agar surface. Those plates were incubated for 5 days at 37 °C. After incubation period, the lipase producing actinomycetes were screened by the formation of the clear zone. The highest zone forming actinomycetes was selected for production of lipase [8].

Substrate selection

Coconut cake and groundnut cake were used as substrates. There are reports on different oil cakes have been used as substrate [9]. They were procured from a local market in Coimbatore, Tamilnadu, India and were dried at room temperature to reduce the moisture content and ground to the desired size.

Media preparation

10g of desired groundnut cake was mixed separately with nutrient media and sterilized. The positive strain actinomycetes were inoculated into the respective waste media. The flasks were kept in a shaker (SI-600/600R) acinterlab, USA at 180 rpm and incubated for seven days at 37°C. The supernatant from the cultural broth was collected through centrifugation at 6000 rpm for 15 min at 4°C. The supernatant was used as crude enzyme source to estimate lipase production. The enzyme extract was partially purified by ammonium sulphate fractionation (70% saturation) and dialyzed for further applications [10].

Optimization of different parameters for lipase production

Influence of different p^H, temperature, carbon and nitrogen sources

Sterile production medium was prepared for different parameters such as p^H, temperature, carbon sources and nitrogen sources. Each

flask was incubated at different pH, (5, 6, 7, 8 and 9), and temperature (30°C, 37°C and 47°C), carbon sources (fructose, sucrose, and dextrose) and nitrogen sources (urea, ammonium nitrate and peptone) and additive sources (tween 20). They were similar to earlier reports [11-13].

RESULT AND DISCUSSION

Screening of lipase producing actinomycetes

The positive actinomycetes strain was confirmed by the production of clear zones around the colonies in tributryin agar plates. Among 8 lipase producing actinomycetes Mac -5 showed the highest zone formation than the other microbes and it was selected as a best strain for lipase production. The actinomycetes strain was identified by powdered white, mycelia formation and was confirmed as rod shaped gram positive bacteria. The thermal stability of the actinomycetes was checked and recorded at 60-80°C. Selected marine actinomycetes were highly thermo stable in nature, so it was used for the production of lipase and the enzyme extract was used as a good detergent additive which can withstand higher temperatures.

Effect of initial pH on Lipase production

The pH of the fermentation medium greatly affected the extracellular lipase production of actinomycetes selected strain. Table-1 shows that the extracellular lipase production was maximum in acidic conditions at pH 6.0. Similar results were reported, where lipase production from *Penicilllium simplicissimum* was maximum in acidic conditions using babassu oil cake as a substrate [14].

Effect of temperature on lipase production

Temperature is one of the major factors that strongly influence the activity of any microbial enzymes microorganisms are very sensitive to temperature changes [15]. In this present study maximum extra cellular lipase production was observed at 30°C and it was more or less constant (Table-2). However, beyond 60°C the lipase activity drastically dropped down indicating that higher temperature is suitable for lipase production from actinomycetes. This study is comparable with the study carried out while working on lipase production from streptomyces spp [16]. Where lipase activity was maximum, the optimum temperature for extra cellular lipase production from *Bacillus sonorensis* selected strain was 70 ºC indicating that the strain is a thermophilic microorganism and the further experiments were carried out at this temperature.

Effect of carbon on lipase production

In SSF, it is necessary to supply ready sugar for better growth and lipase production and results on the effect of supplementary carbon sources on lipase enzyme production is provided in (Table-3). Among the tested carbon sources, maximum (3.44 U/ml) amount of enzyme production was recorded in sucrose supplemented medium when compared to other carbon sources. However in this study, the variation found in lipase production by supplying different carbon sources is limited, because the maximum lipase producing carbon source is sucrose. The results were correlated with the reports of [17] they have stated that the lipase production by Bacillus coagulants under SSF using melon wastes was highly influenced by sucrose. Similarly, this result is in agreement with the lipase production by *Pseudomonas aeruginosa* PseA with maximum production in sucrose supplied medium [18].

Effect of nitrogen on lipase production

Nitrogen source is one another important factor in any fermentation process for successful production of biomolecules. Experiment on the effects of nitrogen sources on lipase production under solid state fermentation revealed that among the tested nitrogen sources, maximum (3.45 U/ml) amount of enzyme production was recorded in the medium containing ammonium nitrate. There was a report that lipase production by *Rhizophus homothallicus* under SSF was highly influenced by ammonium nitrate supplied medium [19].

Effect of additive sources on lipase production

Lipase is thermostable when olive oil used as carbon sources [20]. Addition of small amount tween 20 and olive oil increased lipase production from (3.47U/ml) [21]. An increase of 1.8 times is reported with 2% olive oil.

Table 1: It shows the effect of pH on different substrate for Lipase activity

S. No.	Substrates	pH	Specific Activity U/mL
1	Coconut Cake	5	3.05
		6	3.12
		7	3.15
		8	3.1
		9	3
2	Groundnut Cake	5	3.2
		6	3.48
		7	3.3
		8	3.25
		9	3.35

Table 2: It shows the effect of Temperature °C on different substrate for Lipase activity

S. No.	Substrate	Temperature °C	Specific Activity U/mL
1.	Coconut cake	30	2.74
		37	2.76
		47	3.00
		30	3.47
2.	Groundnut cake	37	3.45
		47	3.46

Table 3: Effect of Carbon sources on different substrate for Lipase activity

S. No.	Substrate	Carbon sources	Specific Activity U/mL
1	Coconut cake	Sucrose	2.57
		Fructose	2.8
		Dextrose	2.3
2	Groundnut Cake	Sucrose	3.25
		Fructose	3.44
		Dextrose	3.16

Table 4: Effect of Nitrogen sources on different substrate for Lipase activity

S. No.	Substrate	Nitrogen Sources	Specific Activity U/mL
1.	Coconut Cake	Urea	2.7
		Peptone	2.25
		Ammonium Nitrate	2.54
2.	Groundnut cake	Urea	3.25
		Peptone	3.17
		Ammonium Nitrate	3.45

Table 5: Effect of additive sources on different substrate for Lipase Activity

S. No.	Substrate	Additive Sources	Specific Activity U/mL
1.	Coconut Cake	Tween 20	2.82
2.	Groundnut Cake	Tween 20	3.47

This study correlates [22] that lipase production by groundnut oil cake under SSF was highly influenced by ammonium nitrate supplied medium. Similarly [23] reports indicate that the lipase production was high in ammonium nitrate added medium. In this study, we observed that groundnut cake has shown to give a maximum yield compared to coconut cake. The above results was found to be similar to in reports [24].

CONCLUSION

India is an agricultural country and the utilization of agro industrial wastes for the production of enzymes as substrate showed an ecofriendly management of wastes them by reducing the effects of pollution in an environment. The enzyme production cost is very less. Concluded that an agro wastes can be better utilized for the production of novel enzymes like lipase by using marine thermophilic actinomycetes.

CONFLICT OF INTERESTS

Declared None.

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