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Isolation and characterization of lipase for pharmaceutical application

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ABSTRACT

Lipase is a significant modern biocatalyzer and extensively utilized in an assortment of specialized applications. Lipases perform a basic part in assimilation, transport and handling of dietary lipids in most living beings. Lipases are delivered and emitted out by numerous parasites and microscopic organisms, for example, Penicilliumchrysogenum, Pseudomonas aeruginosa, Rhizopusdelamar, Staphylococcus aureus and so forth. For this examination, soil tests were gathered from petroleum treatment facilities and individual settlements were disengaged by surface plating strategies. Biochemical tests, bacterial cytology and motility tests were performed. Techniques were completed for the creation of lipase from the recognized example (Pseudomonas aeruginosa).

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INTRODUCTION

Lipases catalyze the hydrolysis and the union of esters shaped from glycerol and long-chain unsaturated fats. Lipases are broadly disseminated in creatures, plants and microorganisms. Lipases are accessible with a broad scope of properties relying on their source [1]. The tremendous capability of microbial lipases emerges from the realities that they are (1) very steady and dynamic in natural solvents, (2) don't need cofactors, (3) display a severe extent of enantio-and area selectivity, and (4) have a broad scope of substrate explicitness for the change of different uncommon substrates. Over 50 lipases have been recognized, decontaminated and described to date, which start from normal sources, for example, creatures, plants and microorganisms (local or hereditarily designed) [2–4].

Lipase-catalyzed measures have gotten extraordinary consideration, chiefly because of a few points of interest: (1) they are more condition amicable than mass concoction syntheses,(2) they permit assembling of better items, (3) simplicity of recuperation and re-utilization of the lipases, and (4) the chance of utilization in constant tasks. Environmental concerns have supported more broad uses of lipases since lipase-catalyzed responses look like all the more intently the pathways structured ordinarily for the digestion of living things. The lipase segregating capacity incorporates highlights, for example, stereospecificity, selectivity and substrate particularity, which are a lot higher than those of inorganic impetuses. This permits the assembling of high worth included items [3].

Industrially valuable lipases are normally acquired from microorganisms that produce a wide assortment of extracellular lipases. Microbial lipases have been delivered by yeasts, organisms and microorganisms as extracellular,intracellular and cell-bound protein. The extracellular lipases from yeast and microscopic organisms were intriguing

a direct result of the simpler application. Yeast which created extracellular lipase is Candida deformans while microbes are Pseudomonas aeruginosa, P.fragi and so forth [4, 5].

A wide assortment of gram-positive and gram-negative bacterial species are accounted for to deliver lipase yet most generally utilized protein begin from Bacillus and Pseudomonas. Microorganisms in soil happen independently and in totals. To assess the number of microorganisms in a gram of soil(collected from petroleum treatment facility), the dirt is both weakened and blended all together, so the totals are separated with the end goal that a suspension of single cells is accomplished.

The cell suspension was then sequentially weakened so that from certain weakenings, a sensible number of cells (30 to 300) were administered into Petri plates. The examples in Petri plates were then blended in with clean, liquid (liquid)agar medium which was then permitted to cement a technique called pour plating. A few plates were spread plated, where the examples were vaccinated over hardened agar [6].

The plates were brooded for 24 Hrs. at 37 C. The provinces were gotten and moved into inclines. Biochemical tests and motility tests were performed to recognize the sorts of creatures present in the example. When the life forms (Pseudomonas) were distinguished, strategies were completed for creation of lipase from it [7].

MATERIALS AND METHODS

Test assortment

A soil test of profundity 4-5cm profundity with the assistance of sterile spatula was gathered. 1g of the test was suspended in 100ml of sterile refined water. It was unsettled for 30 min on a shaker at 50ºC and was kept up as stock. It was plated in 100μlof supplement agar. The state framing unit was checked following 24 hours. The states were picked and moved into inclines. The kind of life forms present in the example was distinguished. At that point, steps were completed for creation of lipases. The life forms were recognized utilizing gram's recolouring, versatility test and by going through biochemical tests.

Lipase production

The vegetable fat (0.3 ml groundnut oil) was emulsified in the supplement agar by shaking it completely not long before filling the plate. The plates were vaccinated with Pseudomonas aeruginosa culture, and it was hatched for 48hours. After brooding, the agar was overwhelmed with the immersed fluid arrangement of copper sulfate and saved for 10 to 15 minutes.

The abundance reagent was poured off from the plates. The pale blue, green fat globules in the plate demonstrated the nearness of lipase [8–10].

RESULTS AND DISCUSSION

Testing, Identification and Production of Lipase

Test assortment and screening of microorganism

The sequentially weakened soil tests were plated on supplement agar, and bacterial check changed from 1.0x108 to 5.2x108 CFU/g of the dirt example. It was discovered that the dirt examples gathered from petroleum treatment facilities squander tainted locales indicated high bacterial tally [11].

Recognizable proof

Peptone water tubes were arranged, autoclaved and vaccinated with loopful of culture [12]. It was hatched for the time being at 37oC, and biochemical tests were performed. Perceptions have been arranged in Table 1

Table 1: Biochemical Test Results

Parameters	Observations Culture
Bacterial Cytology And Motility	
Gram's Staining	Gram-negative rods
Motility	Motile
Biochemical Tests	
Catalase	+
Oxidase	+
Indole	_
Methyl Red (M.R.M.R.)	_
VogesProskauer(VP)	_
Citrate	+
Urease	_
Nitrate	+
TSI	K/K, gas _
Sugars	
Glucose	Fermentative and Gas _
Lactose	Fermentative and Gas _
Sucrose	Fermentative and Gas _

Gram staining and Craigie's Tube Method was performed to determine cell cytology and motility, respectively.The culture was found to consist of Pseudomonas.The plates showed a fried-egg like an appearance which indicated the presence of Pseudomonas strains. Nutrient agar plates showed

a green pigmentation due to the presence of the bluish-green pigment 'pyocyanin'. Pyocyanin is soluble both in chloroform and water. The distinctive earthy smell was observed from the plates [13–15]. The culture was also found to grow on MacConkey media forming non-lactose fermenting colonies. Indole, MR, V.P. V.P. and H_2S tests gave negative results, whereas catalase and oxidase gave positive results. These observations led us to conclude that Pseudomonas aeruginosa was present in our soil sample.

Production of lipase from Pseudomonas spp.

The particular strain of lipase producing microorganism (Pseudomonas aeruginosa) was identified and greenish-blue globules in the Petri plate, inoculated with Pseudomonas, confirmed the presence of a lipase. The hydrolysis of fat or glycerides resulted in the production of glycerol and fatty acids. When copper sulphate was added, the bluish-green appearance was due to the reaction of copper sulphate with fatty acids.

CONCLUSION

Lipases are currently used in different industrial products and processes, and new areas of application are constantly being added, which include the production of single-cell proteins, cosmetics, pulping, lubricants etc. Pseudomonas aeruginosa isolated from oil-contaminated soil sample was identified as the potent and promising producer of lipase. The lipases produced from Pseudomonas species are thermostable. Thus a promising producer of lipase was isolated from oil-contaminated soil sample and study for commercialization of these can be considered in future.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest for this study.

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