Research Article

Isolation and Production of Amylase from *Aspergillus niger* Using Jackfruit Seed as Substrate

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ABSTRACT

Amylase producing fungal strains was isolated form coconut retting waste water samples collected from Kanyakumari District, Tamil Nadu. The amylase production was screened on starch agar plates and confirmed by the presence of clear zone. Among the four positive strains, one potential isolate was selected for further studies and it was identified as *Aspergillus niger* based on cultural and morphological characteristics. Amylase enzyme was produced by submerged fermentation using jackfruit seed as a substrate. Enzyme activities and protein content of both crude and purified enzyme suspension were measured. The fungi produced good quantity of enzyme when jackfruit seed as substrate.

Keywords: Amylase, Jackfruit seed, *Aspergillus*, Fermentation.

INTRODUCTION

Enzymes are among the primary products obtained for human needs through plants, animals and microorganisms. Currently, the use of enzymes in industrial area is increasing due to increase in their use in food, beverages, textile, leather and paper industries.[1,2] Amylase is a group of enzyme, that catalysis the hydrolysis of starch to give diverse products including dextrins, and progressively smaller polymers such as glucose, maltose and maltotriose units.[3]

They are important enzymes employed in the starch processing industries[4] and which alone covers approximately 30% of the enzyme market. They have opened new frontiers of many commercial biotechnological processes, including renewable energy, pharmaceuticals, saccharification or liquefaction of starch, detergent industries, warp sizing of textiles, fibers, paper industries, foodstuffs, baking, clarification of haze formed in beer or fruit juices and for pretreatment of animal feed to improve digestibility.[5,6]

The enzyme can be obtained from several sources, such as plants, animals and microorganisms. Several microorganisms can be obtained for enzyme production especially fungi have gained much attention because of the availability and high productivity.[7]

Filamentous fungi are important organisms for production of useful enzymes and biologically active secondary metabolites.[8] *Aspergillus* species are particularly interesting for industrial enzymes due to their easy cultivation and high production of extracellular enzymes of large industrial potential.[9] The enzyme used in many of industrial processes such as food, baking, brewing, detergent, textile and papers, also in clinical, medical and analytical chemistry.[2] The present study aimed to isolate amylase producing fungi from the environment.

MATERIALS AND METHODS

Sample Collection

Water samples were collected from coconut retting site of Kanyakumari District, Tamil Nadu, India.

The sample was collected into a sterile sampling container and immediately transported to the laboratory for further study.

Isolation of Fungi

The collected sample was serially diluted with sterile distilled water and about 100 µl of diluted sample was inoculated on to Potato Dextrose Agar plates, evenly spread with the help of L-rod. The plates were incubated at 25-28 °C for 3-5 d.

Screening of Amylase Production

Starch agar medium plates were prepared and inoculated with single spore inoculums of fungal isolates. The plates were incubated at 25-28 °C for 3 d, after the plates were flooded with iodine solution and observed for clear zone around the fungal colony.

Identification of Fungi

Cultural characteristics of the fungi were studied by observing the fungal growth on potato dextrose agar and the morphology was studied by Lacto Phenol Cotton Blue (LPCB) staining method.

Production of Amylase

Production of amylase was carried out by submerged fermentation with 50 ml of production medium containing (g/l) 0.8 g NaCl, 0.8 g KCl, 0.1 g $CaCl_2$, 2.0g

Na_2HPO_4, 0.2g $MgSO_4$, 0.1 g $FeSO_4$, 8.0g Glucose, 2.0 g NH_4Cl, 10.0 g jack fruit seed as substrate (pH 6.2). The medium was inoculated with 48 h old fungal culture and incubated at 28°C for 5-7 d.

Crude Enzyme Extraction

The medium was filtered through Cheesecloth and centrifuged at 8000 rpm for 10 min. The supernatant was filtered through Whatmann No1 filter paper and the filtrate was used as crude enzyme suspension.

Enzyme Assay

Enzyme assay was carried out by DNS method[10]. 0.5 ml enzyme was reacted with 0.5 ml of substrate (1% starch in 100mM Tris buffer) under standard reaction conditions and the reaction was stopped by adding DNS reagent. The amount of maltose released was determined by comparing the absorbance reading of the test enzyme at 540 nm with the standard graph plotted by reacting the known concentration of maltose ranging from 0.05mg/ml to 0.5mg/ml.

Protein Estimation

Protein content of the enzyme was determined by Lowry's method[11] using bovine serum albumin (BSA) as standard. Enzyme activity is expressed as specific activity, which is equivalent to U/mg protein.

Enzyme Characterization

The crude enzyme suspension was purified by ammonium sulfate (40%) precipitation. The precipitate was collected by centrifuging at 8,000 rpm for 20 min, and resuspended in 100 mM Tris buffer pH 6.2. It was dialyzed against the same buffer and freeze-dried. The concentrated sample was passed through a Sephadex G-50 column and eluted with the same buffer at the rate of 15 ml/h. The collected fractions were subjected to assay of enzyme activity and protein concentration.

RESULTS AND DISCUSSION

Isolation and Screening of Fungi Isolates

Fourteen fungal colonies were isolated from the collected water samples, the entire isolates were screened for amylolytic activity on starch agar plates. In this present investigation, four fungal isolates showed positive for amylase production on starch agar and one isolate was selected for further study based on the size of clear zone on starch agar.

Identification of Fungi

The selected fungal isolate was identified by studying cultural and morphological characteristics include colour, texture, pigmentation and colony morphology (Table 1) and was identified as *Aspergillus niger*.

Enzyme Activity and Protein Content

Enzyme activity and protein content of the amylase was assayed in both crude and purified suspension from these results specific activity was measured. The enzyme activity, protein content and specific activity of crude enzyme suspension was 1267 (U/ml), 25375 (mg/ml) and 20.02 (U/mg) respectively and the purified suspension showed 480 (U/ml), 27613 (mg/ml) and 57.52 (U/mg) (Table 2).

Table 1: Cultural and Morphological Characteristics of *A. niger*

S. No.	Characteristics	Results
1	Growth colour on beginning	Dirty white
2	Growth colour on middle	Black
3	Growth colour on later	Black
4	Appearance	Cottony
5	Texture	Smooth Velvety
6	Conidiophores	Present
7	Approx. conidiophores	300-400µm
8	Vesicles	Present
9	Shape of vesicles	ovoid
10	Type of vesicles	Biseriate
11	Phialides	Present
12	Spore type	Conidiospores
13	Arising of Conidia	Central axis
14	Conidia arrangements	Chains

Table 2: Enzyme Activity and Protein Content of Amylase

Enzyme Suspension	Enzyme Activity (U/ml)	Total Protein (mg/ml)	Specific Activity (U/mg)
Crude	1267	25375	20.02
Purified	480	27613	57.52

The results of the present study proved that, the fungal strains capable to produce superior quantity of amylase enzyme. Also, the use of jackfruit seed as substrate for submerged fermentation. Jackfruit seeds were found to be rich in carbohydrates, proteins and minerals.[12] There are many of the low cost substrates including agriculture wastes are used for the production of industrial important enzymes from microorganisms. Shailima Vardhini[13] used different zero value substrates such as wheat bran, potato peel and banana peels for the production of amylase enzyme by *A.niger*. Higher yield of α-amylase production from *A.niger* was achieved when *Ipomoea batatas* was used as substrate.[14] *A.niger* produced high yield of amylase enzyme than *Penicillium* and *Chrysosporium* species when rice and rice bran are used as substrates.

CONCLUSION

It can be concluded that, amylase enzyme can be produced for industrial purposes from *A.niger* grown in the medium containing jackfruit seed as a substrate. Also, the fungi *A.niger* produced amylase enzyme with excellent enzymatic activity and concentration. Hence this potent fungal strain can also be used in large scale industry for amylase production.

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