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Isolation and Screening of Mannanase Producing Fungi

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Abstract

Mannanase have potential to improve the quality of products of various industries including pulp and paper, food and feed, textile, etc. This study was aimed to isolate and screen potential mannanase producers from different regions of Indore. Fifty three fungi were isolated from various soil samples using PDA plates as an isolating medium. The mannanase production was evaluated by cultivating isolated fungi in primary screening medium containing LBG as substrate. Screening of these isolates for hydrolysis of mannan was confirmed by Congo red indicator dye also added in primary screening medium. Based on experimental analysis, we have found that twenty one isolates have exhibited mannanase activity on solid screening medium. Isolates showing a good zone of mannan hydrolysis were further screened out on liquid medium containing LBG as substrate. Fungal strain CP1 gave maximum mannanase production on secondary screening.

Key words: Isolation, Screening, Mannanase, Fungi, LBG

Introduction

Hemicelluloses are types of polysaccharides that are widely found in nature and make up the cell walls of plant cells.[1] The primary building blocks of hemicelluloses, i.e. mannans are produced when mannose is joined by ß-1,4 glycosidic linkages. They are extensively distributed throughout plant seeds and endosperms.[2,3] Hemicellulose, for instance, accounts approximately 25–30% of the weight of dry wood, and its estimated percentage of plant components is one third. The two most important forms of hemicelluloses are hetero-1,4-β-D-mannans and hetero-1,4-β-D-xylans.[4] Enzymes called mannanases are mostly made by microorganisms, yet there have also been reports of mannanases made by plants and animals. Although acidic and neutral mannanases are more prevalent, most microbial mannanases are extracellular and may function in a broad pH and temperature range. Since they target the hydrolysis of complex polysaccharides of plant tissues into simple compounds like manno-oligosaccharides and mannoses, microbial mannanases have gained importance in biotechnology. Mannanases have a

well-established role in the paper and pulp industries. More recently, they have been used in the food and feed technology, coffee extraction, oil drilling, and detergent industries.[5]

Materials and Methods

Locust bean gum (LBG), was obtained from Sigma St. Louis, MO, USA. Mannose, dinitrosalicylic acid, yeast extract, peptone, beef extract, guar gum, Luria broth, dextrose, PDA (Potato Dextrose Agar Media) and pH strips (pH 1-14) were obtained from Himedia laboratories, India.

Isolation

Fungal strains were isolated from decaying wood soil, collected from different regions of Indore. One gram of soil from each sample was dissolved in 10 ml of sterile distilled water. One ml of thoroughly mixed sample was used for serial dilution.[6] The serially diluted samples were plated on potato dextrose agar plate containing 0.05% streptomycin using spread plate technique.[7] Streptomycin antibiotic was used for suppressing the growth of unwanted bacteria. Plates were then incubated at 30°C for 3-5 days. The colonies appearing on the plate were taken on the slant of the same medium for further study.

Primary Screening

Screening of the isolates for mannanase activity shall be performed by the modified method of Arotupin and Olaniyi (2013) using potato dextrose agar plate containing 0.5% LBG (Locust Bean Gum) as a substrate and 0.06% Congo red as an indicator dye. Plates were incubated at 30°C for 5 days. After incubation, fungal strains showing zones of mannan hydrolysis were selected as a mannanase producer.[8]

Secondary Screening

Secondary screening was performed on liquid media containing 1% LBG, 0.1% K_2HPO_4 and 0.05% $MgSO_4.7H_2O$. 50 ml media was taken in each 250 ml Erlenmeyer flask and pH of the medium was adjusted to 6. After autoclaving the media, 10^6 spores were inoculated from 5th day old fungal cultures. Flasks were incubated at 37°C temperature and 120 rpm shaking condition for 5 days. After incubation supernatants were collected by centrifugation at 12,000 rpm for 10 minutes as the crude enzyme source.[9]

Preparation of Substrate and Mannanase Assay

The substrate used routinely for the study of mannanase is galactomannan from locust bean gum (Ceratonia siliqua) with a mannose:galactose ratio of 4:1.[10] 0.5% LBG as a substrate for mannanase assay was prepared by dissolving 0.5-gram LBG in 50 mM citrate buffer pH 5.0. Solution was kept on a magnetic stirrer for 1 hour at 60°C. After this solution was centrifuged at 10000 rpm for 10 min. Clear solution obtained from centrifugation was used for enzyme assay.

0.5 % (w/v) locust bean gum dissolved in a 50 mM citrate buffer at pH 5.0 was used as the substrate mixture. 0.9 ml of the substrate mixture was added to 0.1 ml of the crude enzyme solution and incubated at 50 °C for 10 minutes. Afterwards 1 ml of dinitrosalicylic acid (DNS) was added to each enzyme – substrate mixture and boiled

for10 minutes. The absorbance of the mixture was thereafter measured at 540 nm in a spectrophotometer. The amount of mannose released was determined by the method of Miller (1959).[11] One unit of mannanase was defined as the amount of mannanase that released 1 micro mole of mannose in one millimeter of the reaction mixture under the assay conditions.

Result and Discussion

The soil samples collected from Indore region were processed in the laboratory and 53 fungal strains were isolated in PDA media. All isolates were processed for primary screening to identify mannanase producing fungi. Out of 53 fungal strains, 21 strains were showing zones of mannan hydrolysis around the colony. Photograph showing the zone of hydrolysis around the colony (Fig 1). Colonies showing a good zone of hydrolysis were selected for secondary screening, which is done on liquid media (Fig 2). In the secondary screening, out of 07 mannanase producers selected from primary screening, CP1 fungal strain showed higher mannanase activity. Soil is an excellent source for isolation of fungi. Several strains of *Aspergillus*, *Trichoderma* and other species of fungi have been reported to be efficient mannanase producers. Sae-Lee, Arotupin and Olaniyi reported that ability of formation of clear zones by these isolates on agar medium containing LBG could be allocated by their genetic make up to secrete active mannanase with high diffusion rate.[8,12]

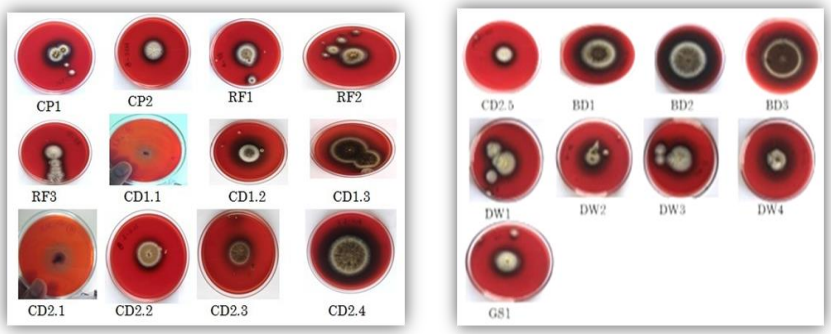

Fig 01- Primary screening of fungi showing zone of mannan hydrolysis around colony

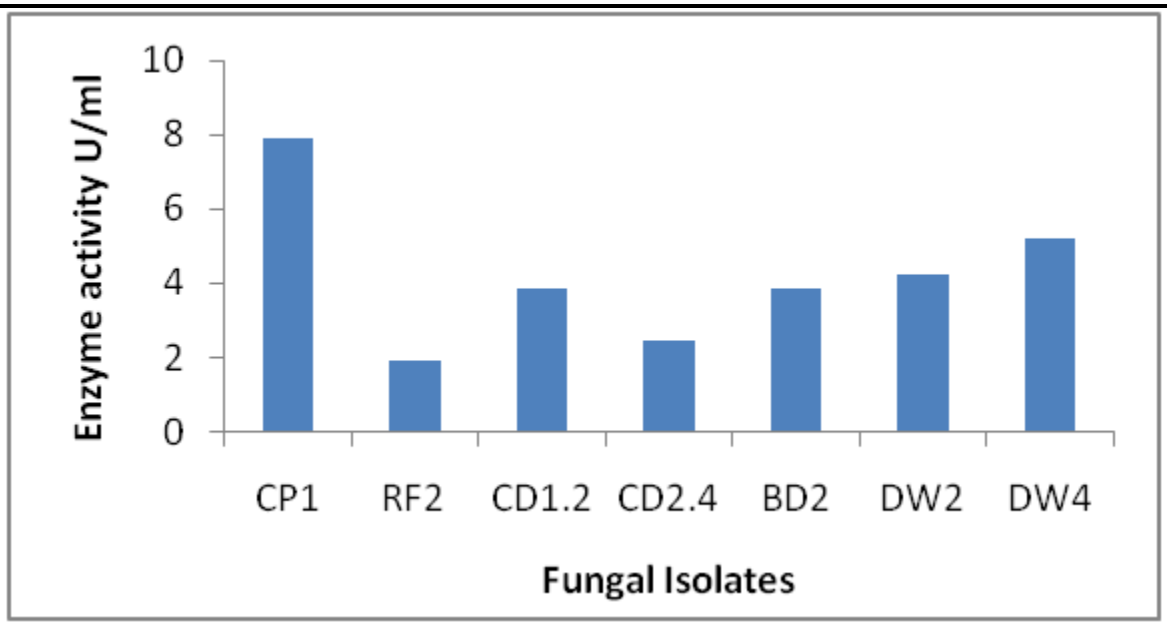

Fig 02- Secondary screening of fungi showing mannanase activity

Conclusion

Fungal strains with the ability to produce mannanase were isolated from various soil samples from different regions. Over 53 fungal isolates, 21 strains were shown in mannanase production in screening medium. Colonies showing higher zones of mannan hydrolysis were selected for secondary screening. Fungi were found to produce mannanase in submerged fermentation using LBG as sole carbon source. Secondary screening shows quantitative estimation of mannanase production. Selected isolates gave a considerable amount of mannanase production in liquid media but strain CP1 gave maximum mannanase production.

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