The Scientific Temper
July, 2010; No.1: pp75-78
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Academy of Innovative Research
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SCREENING OF PECTINASE PRODUCING THERMOPHILIC *MUCOR* SP. ISOLATED FROM DECOMPOSTING FRUITS AND VEGETABLES

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ABSTRACT

A thermophilic fungal strain producing pectinases was isolated after primary screening of 12 different isolates on modified Pectin agar medium using different compost and decomposed matter collected from the vegetable and fruit markets of different cities of U.P. Selection of the fungus was done based on the clearance zones and pectinase enzyme production carried out in solid state fermentation. The fungal isolate was initially identified as a *Mucor* sp.

Keywords : Mucor, pectinase, pectin

INTRODUCTION

In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. They are one of the upcoming enzymes of the commercial sector, especially the in juice and food industry (Kashyap *et al.*, 2001), paper and pulp industry (Beg *et al.*, 2001; Viikari *et al.*, 2001). Preparations containing pectin-degrading enzymes used in the food industry are of fungal origin because fungi are potent producers of pectic enzymes and the optimal pH of fungal enzymes is very close to the pH of many fruit juices, which range from pH 3.0 to 5.5 (Ueda *et al.*, 1982). Fungal pectniases used in industrial processes for juice clarification are mainly obtained from mesophilic aspergilli and penicillia (Aguilar and Huirton, 1990) and the range of enzyme sources is being extended through new recombinant and non-recombinant fungal strains. Thermophilic fungi are potential sources of various industrially important thermostable enzymes, such as lipases, xylanases, proteases, amylases and pectinases. These enzymes have numerous applications in the detergent, starch, food, paper and pharmaceutical industries (Maheshwari *et al.*, 2000). Higher cost of the production is perhaps the major constraint in commercialization of new sources of enzymes. Though, using high yielding strains, optimal fermentation conditions and efficient enzyme recovery procedures can reduce the cost. In addition, technical constraint includes supply of cheap and pure raw materials and difficulties in achieving high operational stabilities, particularly to temperature and pH. Therefore, the understanding of various physiological and genetic aspects of pectinase is required for producing thermostable and acid stable strains of pectinolytic fungi. Literature highlighting the optimization, biochemical characterization, genetics and strain improvement studies of pectinases from mesophilic fungi (Bartha *et al.*, 1981; Fanelli *et al.*, 1978; Marciano *et al.*, 1982; Marcus *et al.*, 1986) is available. However, the studies on pectinases from thermophilic fungi are

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I have completed my masters in bioinformatics from Ch. Charan Singh University, Meerut and currently pursuing PhD in Biotechnology from Kumaun University, Nainital (Bheemtal Campus). The present area of research is to isolate and screen the pectinase producing thermophilic fungi from decomposting fruits, vegetables and also from soil. After screening identification of fungi will be done and further the fungi will be used for production of pectinase. The future plan includes purification and characterization of thermostable pectinases. Sequencing of termostable pectinase will also be done so that a comparative analysis will be done among various pectinase sequences available.

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Dr. Mohommad Arif

M.Sc. Zoology (Entomology) Doctor of Philosophy (D.Phil.), Zoology (Entomology), Service Experience : 30 Years. Research Experience : 36 years approx. including .D.Phil., Area of Specialisation : Entomology & Development of transgenic pea using cry1A© genes for insect resistance, Present Post : Scientist 'E' Area of Current Interest Agrotechnology of Jatropha & its transformation for oil enhancement using DGAT genes Special Mention : Visited Antarctica in XI Indian Expedition during 1991-92. Specimens of Springtails, collembolans, / flies Nematodes /Mites/ were collected from East Antarctica.

RESEARCH PAPERS:74, POPULAR ARTICLES:14, CHAPTERS IN BOOK:8, BOOKS/TECHNICAL BULLETIN

1. Arif, Mohommad and Ahmed, Zakwan (2009) Bio-Diesel : Jatropha curcas (A Promising Source)

2.Arif, M. and Kumar, N. (1996). Angora Rabbit farming for wool production, DESIDOC, New Delhi, 1-45.

lacking. Considering the biotechnological importance of thermophilic fungi in the enzyme industry, the present paper reports the isolation of pectin degrading thermophilic fungi from various sources and their screening.

MATERIALS AND METHODS

Isolation of thermophilic fungi

Thermophilic fungi were isolated from different compost and decomposed matter collected from the vegetable and fruit markets of Meerut, Modinagar and Ghaziabad cities of U.P. (India). Isolation medium of following composition, gL-1 (Pectin - 10.0; Sucrose - 10.0; Tryptone - 3.0; Yeast extract - 2.0; KCl - 0.5; $MgSO_4$. $7H_2O$ - 0.5; $MnSO_4.5H_2O$ - 0.01; $(NH_4)_2SO_4$ - 2.0) supplemented with mineral salt solution of composition g/100 mL ($CuSO_4.5H_2O$ - 0.04; $FeSO_4$ - 0.08; Na_2MoO_4 - 0.08; $ZnSO_4$ - 0.8; $Na_2B_4O_7$ - 0.004, $MnSO_4$ - 0.008), 1 mL; distilled water to make 1L solution; pH 5.5-6.0 was used (Singh and Sandhu, 1986). To the above medium, ampicillin (100 mg/ mL) was added to restrict bacterial growth. The inoculated plates were incubated at 50ºC for 5-7 days. The cultures were further purified by sub culturing on YPSS (Yeast soluble starch agar) medium having composition, gL-1 (Starch - 15; Yeast extract - 0.4; K_2HPO_4 - 0.23; KH_2PO_4 - 0.2; $MgSO_4.7H_2O$ - 0.05; Citric acid - 0.052; pH - 5.5-6.0).

Screening of soil fungal isolates for pectinolytic activity

Preliminary screening of isolates for pectinase production was carried out by disc plate method (Acuna-Argulles, 1995). A total of twelve isolates were assayed for pectinase activity using pectin containing agar medium. Culture plates with pectin containing agar medium were inoculated with each isolate and incubated for 3-5 days at 50°C. Isolates were replicated 2 to 3 times

Figure 1: Selected *Mucor* sp. (7 days old culture grown at 50°C)

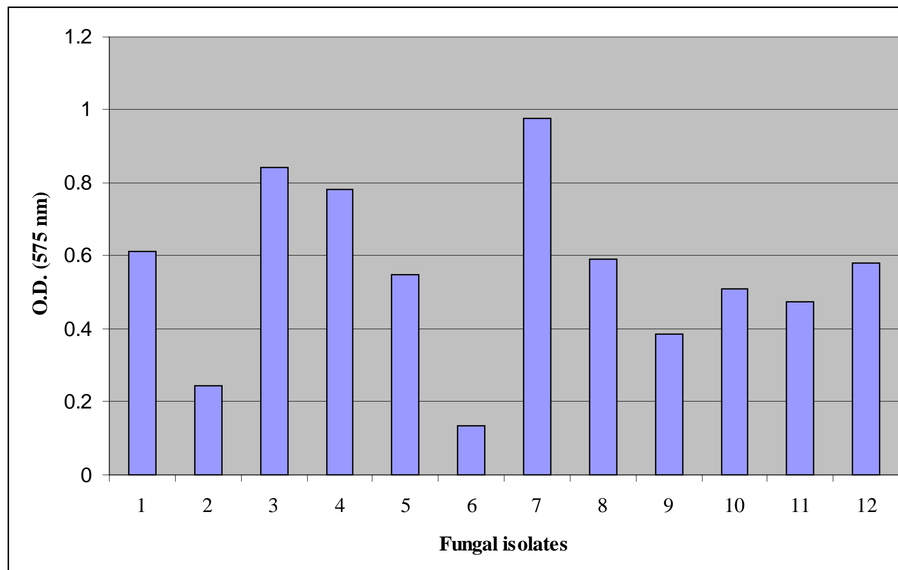

Figure 2: Semiquantitative analysis of thermophilic fungal isolates for pectinase production.

and tests were performed twice. After incubation, plates were stained with aqueous 0.05% ruthenium red solution for 1hr and rinsed with deionised water. Cultures expressing pectinase activity exhibited a clear zone around the margins of the colony. Isolates without a clear zone usually exhibited a ring of intense staining around the colony. The cultures showing larger zones will be further screened by individually plating on YPSS medium containing pectin in place of starch and incubated at 50ºC.

Selection and Identification of Fungus

Selection of the fungus was done based on the clearance zones formed around colonies. Identification of the genus was based on morphological and biochemical characteristics (Phutela *et al.*, 2005).

RESULTS

Isolation and Screening of isolates

Thermophilic fungal strains isolated from various sources and sites of different cities of U.P. were purified and their cultural and morphological characteristics were examined (Cooney and Emerson, 1964). Fungal cultures were further screened by modified yeast soluble starch agar plate method and the zone of clearance was calculated. Isolate 7 and Isolate 3 cultures had a zone of clearance above 3 mm.

High pectinase producing strains were further screened semi quantitatively by plate assay method. Results in Fig. 2 shows that the maximal pectinolytic activity was observed in isolate 7 (O.D. value - 0.978) closely followed by isolated 3 (0.843) and isolate 4 (0.784). Minimum enzyme activity was observed in isolate 6 (0.136).

Selection and Identification

On the basis of morphological and biochemical characteristics the isolate was identified as a *Mucor* sp. (Fig. 1).

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