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Research Article

MYCOFLORAL POPULATION OF PUDUKKOTTAI SOIL AND SCREENING OF PECTINASE ENZYME FROM SOIL FUNGI

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ABSTRACT

In the recent research investigation suggested that the population of fungi from Pudukkottai district and screening of pectinase enzyme from soil fungi were performed. The population dynamics of soil fungi such as *Alternaria* sp, *Aspergillus awamori*, *A.flavus*, *A.fumigatus, A.nidulans, A.niger, A.oryzae, A.terreus, Chaetomium* sp, *Fusarium solani, Fusarium* sp, *Penicillium citrinum, P.chrysogenum* and *Trichoderma viride* was isolated and identified from Pudukkottai town bustand and Malaiyur soil. The fungi identified by using standard manual. On the other hand the studies on the primary screening of pectinase enzyme from soil fungi were determined. The screening of pectinse enzymes with the zone of measurement was 15, 12, 10, 10, 15, 15, 20, 10, 15, 17, 10, 14, 15, 5 and 20 mm with respective fungi were analyzed. According to the screening of fungi for enzyme production ratio was increased. The maximum zone of measurement was 45 mm with *Aspergillus niger* recorded and minimum zone of measurement was 12 mm recorded respectively. Alternatively different substrate materials are produced by agricultural waste and fruit processing industry which possess considerable disposal problem and ultimately leads to pollution.

INTRODUCTION

Pectinases are group of enzymes that attack pectin and depolymerize it by hydrolysis and transelimination as well as by desertification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin. These enzymes act on pectin, a class of complex polysaccharides found in the cell wall of higher plants and cementing material for the cellulose network. Pectinases accounts for 10% of global industrial enzymes produced and their market is increasing day by day (Ceci and Loranzo, 1998). Pectinases are classified according to their mode of secretion as extracellular and intracellular pectinases.

Fungi can produce both intracellular as well as extracellular enzymes. All fungi are hetrotrophic, and rely on carbon compounds synthesized by other living organisms. Small molecules like mono disaccharides fatty acids and amino acids can easily pass through but for breaking down of larger complex compounds like pectin, fungi secrete extra cellular enzymes. It is well known that as compared to intracellular enzymes, the extra cellular enzymes are easier to be extracted. Intracellular enzymes require more time and costly chemicals for extraction.

Pectolytic enzymes are added before fermentation of white wine musts, which are made from pressed juice without any skin contact in order to hasten clarification. Another application of pectolytic enzymes during wine making was associated with the technology of thermovinification. During heating the grape mash for few hours large amounts of pectin are released from the grape, this does not occur in traditional processing. It is therefore necessary to add a pectolytic preparation of the heated mash, so that the juice viscosity is reduced. An additional benefit from the process is that the extraction of anthocyanins was enhanced, probably due to a breakdown in cell structure by the enzyme, which allows the pigments to escape more readily and thus helps in color enhancement (Tucker and Woods, 1991).

MATERIALS AND METHODS

Isolation of fungi from soil sample

The fungi were isolated by serial dilution technique on Potato Dextrose Agar (PDA) medium. In this technique, a sample suspension was prepared by adding 1.0 g sample to 10 ml distilled water and mixed well for 15 min and vortexed. Each suspension was serially diluted 10^{-1} to 10^{-6}. 0.1 ml of 10^{-3} and 10^{-4} dilution samples was pipette out onto plates with PDA media, spread with a glass rod and incubated at 28°C for 72

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hrs. After incubation, the plates were observed and each colony that appeared on the plate was considered as colony forming unit (cfu) (Waksman, 1927; Nazir, 2007).

Identification of fungi

The fungal isolates were identified by morphological examination and its characteristics. Morphological characteristics were examined under microscope (Onion *et al*., 1981).

Screening of Pectinase Activity

Pectinase activity was detected by growing fungi in a petriplate on mineral salt agar medium ($NaNO_3$- 2.0 g, KCl- 0.5 g, $MgSO_4.7H_2O$- 0.5 g, K_2HPO_4- 1.0 g, $FeSO_4.7H_2O$- 0.01 g, Citrus pectin -10.0g, Agar- 20.0g, pH- 6.8-7.0, Distilled water- 1000 ml). The inoculated plates were incubated for about 72 hours at room temperature. After the colonies reached around 3 to 4 mm, potassium iodide solution was added to detect the clear zone.

RESULT AND DISCUSSION

In the present study, 22 fungi from Pudukkottai bust stand and 37 fungi from Malaiyur area were isolated. The isolated fungi were identified on the basis of cultural, microscopic and morphological characteristics (Table 1). Earlier work reported that for maximum growth of fungi, potato dextrose agar was most favorable (Maheshwari, 2000).

Table 1 Isolation and identification of soil fungi from Pudukkottai district

S.No	Name of the fungi	Total number of colonies	
		Pudukkottai (bus stand)	Malaiyur
1	*Alterneria* sp	2	3
2	*Aspergillus* sp	2	1
3	*Aspergillus awamori*	1	2
4	*A.flavus*	2	-
5	*A.fumigatus*	1	2
6	*A.nidulans*	2	-
7	*A.niger*	-	7
8	*A.oryzae*	-	3
9.	*A.terreus*	2	-
10	*Chaetomium* sp	2	-
11	*Fusarium solani*	3	8
12	*Fusarium* sp	2	4
13	*Penicillium citrinum*	1	-
14	*P.chrysogenum*	-	3
15	*T.viride*	2	4
Total number of colonies		22	37

Pectinases can be produced by both submerged and solid state fermentation (SSF). Submerged fermentation is cultivation of microorganisms on liquid broth. It requires high volumes of water, continuous agitation and generates lot of effluents. SSF incorporates microbial growth and product formation on or within particles of a solid substrate (Mudgett, 1986) under aerobic conditions, in the absence or near absence of free water, and does not generally require aseptic conditions for enzyme production (Pandey *et al*., 1999).

In the present study, the maximum pectinase enzyme screened by *A.niger* and *T.viride* and followed other fungal species (Table 2). Many species of fungi are capable of degrading pectin by producing pectic enzymes. The fungal isolates of fruit pulp wastes were also found to produce pectinases. Production of pectin enzymes by fungi such as *Alternaria, Cladosporium, Colletotrichum, Mucor, Penicillium*, and *Trichoderma* was confirmed by Isshiki *et al*., 1997 and Kapat *et al*., 1998. Commercial pectinases are often produced from fungal sources in liquid broths. *Aspergillus* and *Trichoderma* are widely used for the enzyme production. Pectinase production has been reported in solid state cultures employing agricultural by products like *Cassava fibrous* waste (Budiatmen and Lonsane, 1987), Wheat bran (Ghildyal, *et al*., 1981). Apple pomace (Hours, *et al*., 1988) and Citrus wastes (Garzon and Hours, 1992) as substrates and these substrates are found to be the best substrates for the SSF process (Archana and Satyanarayanan, 1997).

Table 2 Studies on the primary screening of pectinase enzyme from soil of pudukkottai district

S.No	Name o f the fungi	Zone of measurement (mm)
1	*Alterneria* sp	15
2	*Aspergillus* sp	12
3	*Aspergillus awamori*	10
4	*A.flavus*	10
5	*A.fumigatus*	15
6	*A.nidulans*	15
7	*A.niger*	20
8	*A.oryzae*	10
9.	*A.terreus*	15
10	*Chaetomium* sp	17
11	*Fusarium solani*	10
12	*Fusarium* sp	14
13	*Penicillium citrinum*	15
14	*P.chrysogenum*	5
15	*T.viride*	20

Aspergillus niger, a filamentous fungus produces several pectinolytic enzymes and are currently used in fruit juice and wine industries (Acuna Arguelles *et al*., 1995). SSF cultures showed higher pectinolytic activities than those obtained by SmF. In SSF, exopectinase activity was maximum, after 72 hours while in SmF, it was delayed further. Pectin lyase production by SmF peaked after 4 days. The comparative ratios of productivities (SSF/SmF) obtained for endo and exopectinase and pectate lyase were 6.51 and 29 respectively, overall the SSF technique is more productive than SmF (Hours, *et al*., 1988) and the results confirmed the findings of earlier study on pectinase production using the strain *Aspergillus niger* (Trejo-Hernandez*, et al*., 1991).

Assay method for the detection and measurement of pectinolytic activity ranged from pure qualitative methods (Hirdebrand., 1971) for demonstrating the presence of enzymes activity to quantitative methods (Colmer*, et al*., 1988, Conway*, et al*., 1988), which determine the activity in terms of actual linkages hydrolyzed.

The most common method for determining pectin esterase activity is the titrimetric method of estimation of carboxyl groups formed in pectin by the enzyme (Cole and Wood, 1961, Tolboys and Busch, 1970). Pectin esterase can also be assayed by measuring zone formation liberated from pectin during the reaction (Zhao, *et al*., 1996).

In order to optimize enzyme production, parameters affecting the enzyme synthesis have to be standardized. Although, optimum conditions may vary for each organism and enzyme

certain factors have been established as the most significant in influencing overall enzyme yield (Fogarty and Kelly, 1983). The critical factor for fungal growth on solid surface is moisture. The control of moisture level within a relatively narrow range was essential for optimizing solid state fermentation. Many microorganisms can grow in solid substrate but only filamentous fungi can grow in the absence of free water. A properly moistened substrate would have a surface film of water to facilitate dissolution and mass transfer of nutrients and oxygen, but antiparticle channels would be left free to permit oxygen diffusion and heat dissipation (Tengerdy, 1985).

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