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Pectinase enzyme producing Microorganisms

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Abstract- Pectinase producing microorganisms were isolated from pectin rich sites using selective isolation technique. These studies deal with screening the pectinase enzyme producing microorganisms. Best pectinolytic activity, as indicated by the diameter of clear, hydrolyzed zones on the medium plates containing commercial citrus pectin as sole carbon source. The strains of *Penicillium* spp and *Aspergillus* sps have good prospect for Pectinase production microorganisms based on the zone formation.

Index Terms- pectinase, pectinolytic activity, *Penicillium* spp, *Aspergillus* spp, isolation.

I. Introduction

Biotechnology is application of living organism and their components to industrial products and processes that requires less energy and are based on renewable raw materials (Awan, 1993; Rolin, 1993; Ridley, 2001). Microorganism can live in everywhere, in the air, water and soil, and in the body of human beings and other creatures. Society benefits from microorganisms in many ways. They are necessary for the production of bread, cheese, bear, antibiotics, vaccines, vitamins, enzymes, and another important product. Microorganisms are indispensable components of our ecosystem (Han *et al*., 2005).

Microbial enzymes are routinely used in many environmentally friendly and economic industrial sectors. Environmental pollution is no longer accepted inevitable in technological societies. Over the past century there has been a tremendous increase in awareness of the effects of pollution, and public pressure has influenced both industry and government. There is increasing demand to replace some traditional chemical processes with biotechnological processes involving microorganisms and enzymes such as Pectinase (Bajpai, 1999; Bruhlmann *et al*., 2000).

Pectinase is a well known term for commercial enzyme preparation that break down pectin; a polysaccharide substrate, found in the cell wall of plants. Pectinase (E.C.3.2.1.15) constitutes a complex enzymatic system responsible for the degradation of pectic substances (Farooqahamed, *et al*., 2003). Pectinase is an enzyme that breaks down pectin. Pectic substances are glycosidic macromolecules with high molecular weight. They form the major components of the middle lamella and primary plant cell wall. Pectic substances consists of protopectins, pectinic acids, pectins and pectic acids. The main chain of pectin is partially methyl esterified 1,4 – D-glacturonan. Demethylated pectin is known as pectic acid (pectate) or polygalacturonic acid. This enzyme splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. The two major sources of the enzyme pectinase are plant and microorganism. But for both technical and economic point of view microbial source of pectinase has become increasingly important (Puangsri *et al*., 2005).

Today, the enzymes are commonly used in many industrial applications, and the demand for more stable, highly active and specific enzymes is growing rapidly. It was estimated that in 1995, the world sale of industrial enzymes would be 1.0 billion US dollars, while the world market for industrial enzymes is expected to be in the range between 1.7 and 2.0 billion US dollars by the year 2005 (Godfrey, 1996). According to a recent publications the industrial enzymes have already reached a market of 1.6 billion US Dollars (Demain, 2000). Interestingly, 60% of the total world supply of industrial enzymes is produced in Europe, and the remaining 40% from USA and Japan. Also approximately 75% of the industrial enzymes are hydrolases, with carbohydralases being the second largest group. In the present study, pectinase producing microorganisms were isolated from pectin rich sites.

II. Materials and Methods

Selection of microorganisms

Various parameters were studied during the work. Fungal species have an ability of using any kind of nutrient source for its growth.

Preparation of Raw Materials

Fresh orange and Mosambi peel and pulp waste was collected from nearby fruit shop. The “Starter culture” was prepared using different namely orange peel, orange pulp, mosambi peel and mosambi pulp. Each 25g of fruit waste were mixed with soil in a pot.

Soil samples

The following soil samples were used in this study.

1. Trial 1	:	Orange peel + orange pulp + soil
2. Trial 2	:	Mosambi peel + Mosambi pulp + soil

Every day water could be sprinkled. After two months, the soil degraded with fruit waste, which was used as an inoculum “Precursor”. This can be used for screening the pectinolytic fungal isolation.

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More than thirty different strains of fungi have been isolated from pectin rich sites for these studies. Out of thirty microorganisms, only ten strains were pectinase producing microorganisms.

Isolation of Fungi

The fungi were isolated from pectin rich sites. The isolated fungi were inoculated on Potato Dextrose Agar (PDA) medium. The solid medium contained (g/l): potato extract - 200ml, dextrose - 20g, agar -20g. A supplement of 0.1% ampicillin was added to avoid the contamination. p^H value was adjusted to 5.6 before autoclaving at 121˚C for 15 min. Inoculated plates were incubated at incubated at 30˚C for 5-7 days. Pure cultures were obtained by repeated sub-culturing on PDA plates and maintained at 4˚C on PDA slants.

Sub-cultured and Maintenance of Microorganism

The strains were sub-cultured on Potato Dextrose Agar slants and incubated for 72 h at 30˚C. The sub cultured strains were maintained in a refrigerator at 4˚C and sub cultured at monthly intervals. Sporulated cultures on PDA slants were obtained after 5 days of incubation at 30˚C.

Identification of Fungi

Ten isolates were isolated from the precursor and the isolates were examined and identified at the Centre for Advanced studies in Botany, Madras University, Chennai. The microscopic structures of the isolates were studied using a Trinocular Microscope with Digital System make "Biolex - CX" with the help of Books described in Kenneth and *et al* (1968), Kenneth and *et al* (1965) and Domsch and *et al* (1980).

Preparation of Inoculum

50ml of PDB in an Erlenmeyer flask of 250 ml capacity was inoculated with fungal spores maintained on PDA slants and incubated at 30˚C for 4 days, under stationary conditions for development of fungal spores. After 4 days, the content of the flask was decanted off carefully. 50 ml of sterilized 0.9% isotonic NaCl solution (saline solution) was poured on the fungal spores in the flask and shaken vigorously to facilitate the release of spores into the saline solution. This spore suspension was used as inoculums for the further use. After 4 days of incubation, the number of spores as counted by Haemocytometer was found to be 5 x 10^{-7} spores/ml.

Screening of fungal isolates for pectinolytic activity

Plate assay Method

Thirty isolates were cultivated on modified Czapek-Dox agar medium. They contained(g/l): $NaNo_3$-3.0g, K_2HPO_4-1.0g, $MgSo_4.H_2O$-0.50g, Kcl-0.50g, $FeSo_4$-0.01g, Sucrose-30g, agar-15.0g with 1.5% as the sole carbon pectin was added. Agar medium was amended with 0.1% of ampicillin to restrict bacterial growth. p^H value was adjusted to 5.6 before autoclaving at 121˚C for 15 min. 2% of fungal spore suspension was centrally inoculated and plates were incubated at incubated at 28±2˚C for 3-5 days. Pectin utilization was detected by flooding the culture plates with freshly prepared Iodine-Potassium iodide solution (Iodine-1.0g,Potassium iodide-5.0g in 330ml distilled water) (Hankin, *et al*.,1971).This solution gives color to the medium containing pectin resulting in a translucent halo region where pectin is degraded, which indicated the pectinolytic activity. From ten pectinase producing isolates, three was identified for best pectinase producing isolates based on the zone diameter of the clearing zone.

III. RESULTS

Pectinolytic activity

Out of Thirty isolates, ten isolates were pectinase enzyme producing isolates. Ten isolates showed plate assay method of which three isolates was producing best result.

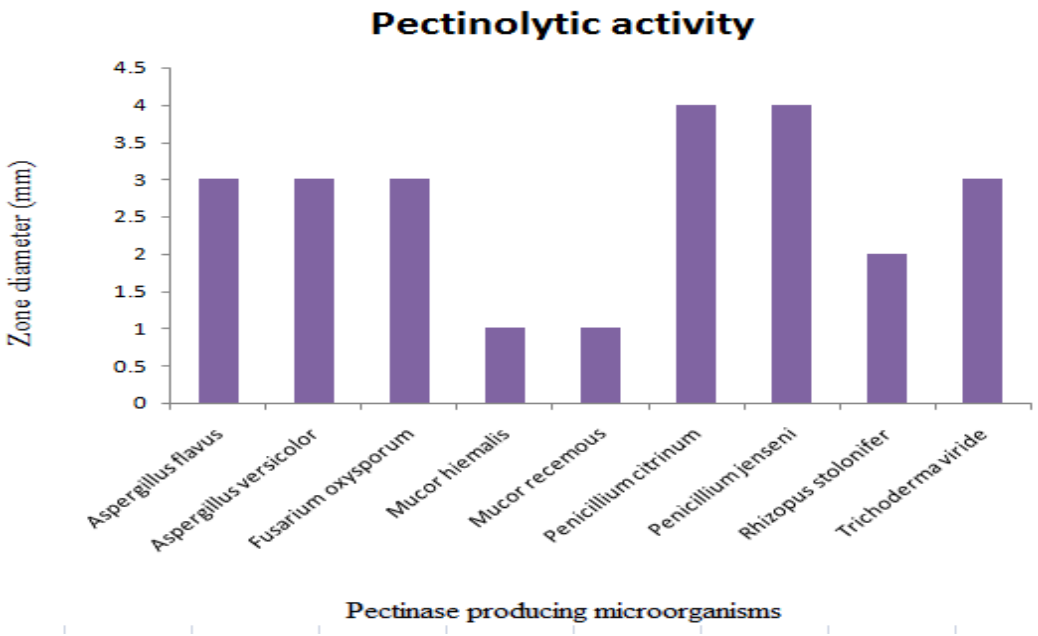
Table 1: Pectinase producing isolates

S.No.	Microorganisms	Zone Diameter (mm)
01.	*Aspergillus niger*	4
02.	*Aspergillus versicolor*	3
03.	*Aspergillus flavus*	3
04.	*Fusarium oxysporum*	3
05.	*Rhizopus stolonifer*	2
06.	*Mucor racemous*	1
07.	*Mucor hiemalis*	1
08.	*Penicillium jenseni*	4
09.	*Penicillium citrinum*	4
10.	*Trichoderma viride*	3

Fig 1: Maximal Pectinolytic activity producing microorganisms

Aspergillus niger *Penicillium jenseni* *Penicillium citrinum*

Fig 2: Pectinolytic activity showed in different isolated strains



IV. Discussion

In this study, 10 fungal strains possessing pectinolytic activity were identified. Based on plate assay method, maximal pectinolytic activity shown by was given by *Aspergillus niger*, *Penicillium jenseni* and closely followed by *Penicillium citrinum* . The plate assay approach that was used in this study has been employed for isolating and screening many enzyme producers(Hankin , *et al*.,1971) .Cellulosic component of urban refuse appear to be good sources of fungi with plant cell wall hydrolyzing activity (Nwodo-Chinedu, *et al*., 2005).

V. Conclusion

It can be summarized that of the three isolates gave better results when compared other isolates. Further studies, we can be followed optimization and fermentation studies. It can be used for various industrial applications including extraction and clarification of juices, processing of fibers, bleaching of paper, removal of pectic waste and maceration of tea leaves.

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