Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 1205 - 1209

Research Article Biotechnology

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

SCREENING OF A PECTINOLYTIC FUNGAL STRAIN; *Aspergillus foetidus* MTCC 10367 FOR THE PRODUCTION OF MULTIPLE ENZYMES OF INDUSTRIAL IMPORTANCE

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ABSTRACT

Aspergillus foetidus MTCC 10367 associated with industrial fruit waste sample from Galla food processing industry, Chittoor District, Andhra Pradesh, was isolated and identified using standard microbiological method (serial dilution-spread plate technique). This fungal isolate was screened for hydrolytic and depolymerising multiple enzymes of industrial importance. Many enzymes such as Pectinases, Cellulases, Proteases, Phytases and Amylases produced by *Aspergillus foetidus* have relevant biotechnological applications in several industrial areas. This study revealed the ability of *Aspergillus foetidus* to produce varieties of hydrolytic enzymes and provides additional information to support future research about industrial potential of *Aspergillus foetidus* MTCC 10367.

KEYWORDS:Screening, Multi-enzyme activity, *Aspergillus foetidus* and Industrial fruit waste.

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INTRODUCTION

Microorganisms produce a variety of enzymes that have been successfully used on industrial scale[1, 2]. Microbial enzymes have found their applications in textile (Amylase, Cellulose, Oxedoreductase); detergents (Protease, Lipase, Cellulase); paper (Xylanase and Lipase); food (Pectinase, Protease, Cellulase) and leather (Protease, Lipase) industries[3].In recent years, the potentials of using fungi as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extra-cellular enzyme activity [4]. Fungi are of interest due to their easy cultivation, and high production of enzymes[5]. This study therefore, is aimed at screening of multiple enzymes namely Pectinases, Cellulases, Proteases, Phytases and Amylases from *Aspergillus foetidus* MTCC 10367 associated with industrial fruit waste.Enzymes cleaving pectic substances are known as pectinolytic enzymes or pectinases[6].Industrial Pectinolytic enzymes are produced by the fungi namely, *Aspergillus sp*, *Rhizopus stolonifer*, *Alternaria mali*, *Fusarium oxysporum*, *Neurospora crassa*[7]. Cellulases are enzymes capable of hydrolyzing cellulose to smaller sugar components[8]. *Aspergillus sp*. and *Trichoderma sp*. are most widely known to produce cellulase[9]. Proteases are complex enzymes which hydrolyze protein molecules, *Aspergillus sp*. are of greater importance due to their higher protease producing ability[10]. Phytases are classified as the family of histidine acid phosphatases, which catalyze the hydrolysis of phytic acid to inorganic phosphate and myoinositol phosphate derivatives[11]. Phytase producing strains include *Aspergillus*, *Penicillium, Mucor* and *Rhizopus*. However, several studies have confirmed *Aspergillus* strains to be the best producers of extracellular phytase[12]. Amylase is employed in processing industries for the hydrolysis of starch into simple sugar constituents[13]. Fungal sources are *Aspergillus* species and *Penicillium brunneum*[14].

MATERIALS AND METHODS

Collection of samples

Industrial fruit waste samples were randomly collected in and around Galla food processing industry, Tenepalli village, Chittoor District, Andhra Pradesh and transported to the laboratory in sterile polythene bags[15]. Samples were pooled to prepare a composite mixture.

Isolation and Screening of Aspergillus foetidus for Multiple Enzymes

One gram of composite sample was homogenized in sterile distilled water and 10-fold serial dilutions were prepared. One ml aliquot's from each dilution was plated on Potato Dextrose Agar (PDA) plate by spread plate method, incubated for 3 to 5 days at 37± 2˚C, containing antibiotic ampicillin (100µg/mL) to restrict the bacterial growth. More than 30 different fungal strains of *Aspergillus, Penicillium, Fusarium* and *Rhizopus* etc. were isolated and identified. A potential pectinolytic fungal strain was identified and characterized as *Aspergillus foetidus* MTCC 10367 by IMTECH, Chandigarh.

Aspergillus foetidus was inoculated in 50 mL Potato Dextrose broth incubated at 28 ± 2°C on a rotary shaker at 150 rpm for 3 days and screened for multiple enzymes such as Cellulases, Proteases, Phytases and Amylases in addition to Pectinases on suitable substrates, using disc plate method[16]. The size of clearing zone formed around the colonies corresponds to the enzyme activity.

Pectinases

The fungal culture was inoculated on Czapek-mineral salt screening agar medium containing 2 $NaNO_3$; 1.0 K_2HPO_4; 0.5 $MgSO_47H_2O$; 0.5 KCl ; 2 Peptone; 20 Agar (g/L) in1000 ml distilled water at pH 6.5 supplemented with 1% citrus pectin as substrate. The petridishes containing screening agar medium were incubated at 37˚C for 24 hours. 1% Cetyl Trimethyl Ammonium Bromide (CTAB) was

added to observe the zone of clearance which indicated pectinase activity.

Cellulases

Carboxy Methyl Cellulose (CMC) (1% w/v) was used as substrate in screening agar medium. The petriplates were incubated at 37˚C for 24 hrs. Plates were flooded with 1% Congo red solution for 15 minutes then de-stained with 1M NaCl solution for 15 minutes. Clear zones around the colonies indicated cellulase activity.

Proteases

To test the presence of protease activity (1% w/v) casein was used as substrate in screening agar medium. Enzyme activity was indicated by the formation of a clear zone around colonies after precipitation with 1 M HCl solution. [17]

Phytases

The presence of Phytases activity was detected on screening agar medium containing (1% w/v) Calcium phytate as substrate. Zone of hydrolysis for Calcium phytate was recognized by a clear halo around fungal colonies.

Amylases

Amylase activity was detected on screening agar medium containing (1% w/v) starch as substrate. Plates were incubated at 37°C for 24 hours. Clear zones of hydrolysis around the fungal colonies were observed by staining with 50mM Iodine.

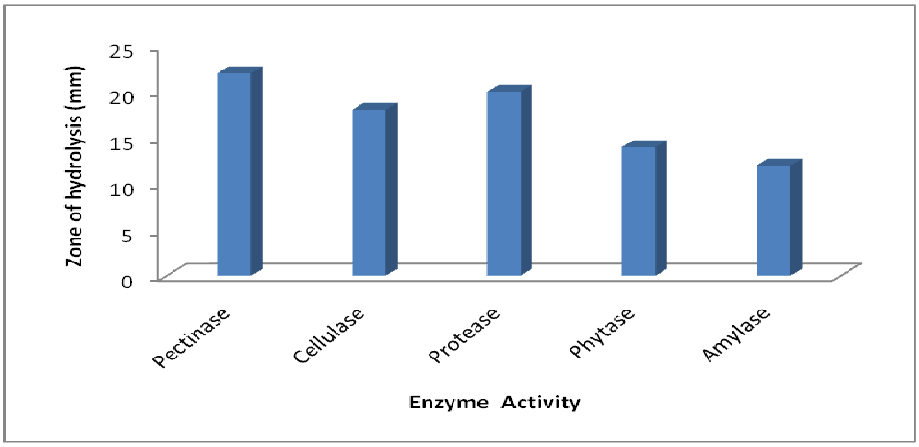
RESULTS

Aspergillus foetidus MTCC 10367 associated with industrial fruit waste was screened for multiple enzyme activity on various substrates (Citrus Pectin, Carboxy Methyl Cellulose (CMC), Casein, Calcium phytate and Starch) amongst which the enzyme activity was found to be maximum for Pectinase and minimum for Amylase.

Table
Showing Zone of Hydrolysis

Enzyme	Substrate	Zone of hydrolysis (mm)
Pectinase	Citrus Pectin	22
Cellulase	Carboxy Methyl Cellulose (CMC)	18
Protease	Casein	20
Phytase	Calcium phytate	14
Amylase	Starch	12

Graph 1
Showing Multiple Enzyme Activity



DISCUSSION

Fungi as a result of their competitive saprophytic ability expressed by fast mycelial growth, spores production, presence of efficient and extensive systems of powerful enzymes are capable to utilize complex chemical substances as energy sources. This ability of fungi makes them potentially important in nature[18].In the screening of *Aspergillus foetidus* MTCC 10367 for the production of multiple hydrolytic enzymes, the method of radial diffusion in solid media indicated the activity of various enzymes in qualitative form[19]. Although, extra-cellular enzymes may be produced in liquid or solid media, the use of solid media however permits a fast screening of fungi, allowing detection of specific enzymes[3].The ability of *Aspergillus foetidus* MTCC 10367 to utilize starch has been attributed to the fact that starch is the most abundant carbon source in the environment serving as major reserve carbohydrate[20].Fungi are a rich source of new bio-catalysts in nature that have been endowed with vast potential to produce arrays of enzymes[4].These have great potentials of being used as biotechnological source of industrially relevant enzymes [21].

CONCLUSION

Aspergillus foetidus MTCC 10367 showed clear zone of hydrolysis with respect to the enzymes such as - Pectinases, Cellulases, Proteases, Phytases and Amylases. This ascertains the multi-enzyme activity. This study confirms the potentiality of this novel strain for multi-enzyme production. Further investigations in this regard will help us understand the characteristics of this strain for potential biotechnological and industrial applications.

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