

Variability in production of extracellular enzymes by different fungi isolated from rotten pear, peach and grape fruits

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Abstract. Fifteen fungal pathogens, viz. *Penicillium chrysogenum*, *Penicillium expansum*, *Monilinia fructigena*, *Trichothecium roseum*, *Cladosporium herbarum*, *Aspergillus niger*, *Coprinus psychromorbidus*, *Alternaria alternaria*, *Phytophthora palmivora*, *Pythium* sp., *Fusarium solani*, *Alternaria* sp., *Monilinia* sp., *Guignardia bidwellii* and *Rhizopus stolonifer* isolated from rotted fruits were evaluated for the production of different enzyme on solid culture media. All the isolates produced enzymes on the media used, 87.67% of the isolated fungi were found to produce amylases, 80.00% of the fungi produced lipases, 73.33% produced proteases, cellulases by 66.67% and pectinases by 60.00%. The array of enzymes produced differs between different fungi. *Penicillium* sp., *Alternaria alternata*, *Rhizopus stolonifer* and *Alternaria* sp. were found to produce all the tested enzymes on culture media. These pathogens are known to attack almost all the plants, fruits, vegetables and cause many diseases. The differences in the enzymatic production of different fungal pathogens represent their virulence and specificity in causing different fruit rot diseases.

Keywords: Culture media; Extracellular enzymes; Fungal pathogens; Peach; Pear; Grape.

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Introduction

Microorganisms are invaluable sources of natural products for industrial and biomedical development (Turner and Aldridge, 1983). They are common sources of commercial enzymes due to their high production capability, low cost, susceptibility to genetic manipulation, high biotechnological interest, pharmaceutical products and medical therapy (Rao et al., 1998). They are often more stable than enzymes derived from other sources (Sunitha et al., 2013). Fungi in general are well characterized microorganisms due to their capacity to produce different extracellular compounds; some of them are biologically very important causing severe reactions in other organisms (Mustafa and Kaur, 2009). These compounds include different types of enzymes and biologically active molecules. The microorganisms can represent a new source in obtaining enzymes with different potentialities. Enzyme cellulase and protease has wide applicability in various industrial processes like biofuel production, agricultural waste management, triphasic biomethanation and ligand binding studies (Chakraborty et al., 2000; Lu et al., 2004; Dias et al., 2008; Vaithanomsat et al., 2009). Most of the fungi produce enzymes which degrade the plant cell wall and hence result in pathogenesis. The degradative enzymes produced by different fungi are important in food deterioration, breakdown of organic matter and host infection.

Plant cell wall is complex structure of polymers, viz. Pectin, cellulose, hemicellulose, polysaccharides and lipids (Karr and Albersheim, 1970), for its degradation extracellular enzymes like pectinase, cellulase, protease, amylase, etc. are produced by different fungal pathogens and cause different diseases. Not much work seems to be reported on enzyme production by different pathogenic fungi causing rot of fruits. Experiments were therefore carried out to study the production of extracellular enzymes produced by different fungal pathogens for studying their virulence in causing rot of different fruits.

Materials and methods

Isolation of fungal pathogens from fruits

Fifteen fungal pathogens, viz. *Penicillium chrysogenum*, *Penicillium expansum*, *Monilinia fructigena*, *Trichothecium roseum*, *Cladosporium herbarum*, *Aspergillus niger*, *Coprinus psychromorbidus*, *Alternaria alternaria*, *Phytophthora palmivora*, *Pythium* sp., *Fusarium solani*, *Alternaria* sp., *Monilinia* sp., *Guignardia bidwellii* and *Rhizopus stolonifer* were previously isolated from rotted pear, peach and grape fruits, stored on PDA medium at 4 °C in culture collection of Mycology and Plant Pathology Research Lab, Department of Botany, University of Kashmir and were screened for the production of different enzymes.

Detection of enzymes produced by isolated fungi

The production of following enzymes was detected: Amylase, Cellulase, Pectinase, Protease and Lipase. For determining the enzymatic activity the rot causing fungal pathogens were inoculated on the specific culture media for each enzyme to be investigated. These were incubated at 28 ± 2 °C and the enzymatic index was determined after 96 h. Enzyme activity index or enzymatic Index (EI) was expressed by the relationship between average diameter of the degradation halo and average diameter of the colony (Hankin et al., 1971; Hankin and Anagnostakis, 1975)

Determination of amylolytic activity

The ability to degrade starch by the fungal pathogens is an essential criterion to determine amylolytic activity. The medium used was nutrient agar supplemented with 0.2% soluble starch at pH 6 (Hankin and Anagnostakis, 1975). After 96 h of incubation the plates were flooded with iodine solution which visualized the clear halos around the fungal colonies.

Determination of Cellulolytic activity

The methodology given by Teather and Wood (1982) was used to determine cellulase activity. Medium used was Czapek Dox Agar medium containing sucrose (30 g), NaNO₃ (2 g), K₂HPO₄ (1 g), MgSO₄ (0.05 g), KCl (0.5 g), FeSO₄ (0.01 g) and Agar (20 g) supplemented with 1% carboxy methyl cellulose. After 96 h (4 days) of incubation the plates were flooded with 0.2% aqueous congo red solution and destained with 1M NaCl solution for 15 min. The presence of yellow areas around fungal colony indicates the presence of cellulase activity.

Determination of pectinolytic activity

Methodology given by Hankin et al. (1971) was used to determine pectinolytic activity. Medium used was pectin agar medium containing yeast extract (1 g), Agar (15 g), pectin (5 g) in 1,000 mL distilled water at pH 5. After 96 h of incubation the culture plates were flooded by 1% aqueous solution of hexadecyl trimethyl ammonium bromide. It precipitates intact pectin in the medium and thus clear zones around the colony indicated pectinase activity by the fungi.

Determination of proteolytic activity

Medium used was Frazier's gelatin agar, containing nutrient agar and 0.4 % gelatin (4 g.L⁻¹). After incubation at 30 °C for 96 h the complete degradation of gelatin was seen around the colonies. The plates were treated with ammonium sulphate that enhance clear zones around the colonies.

Determination of lipolytic activity

To detect the lipolytic activity medium described by Sierra (1957) was used. The medium contains peptone (10 g), NaCl (5 g), CaCl 2H₂O (0.1 g), agar (20 g) at pH 6, tween 20 was sterilized separately and added 1 mL per 100 mL of the medium that serves as lipid source. After incubation for 96 h the presence of halos were observed.

Results

In the present study enzymatic index of the fifteen isolated fungal pathogens, viz. *Penicillium chrysogenum*, *Penicillium expansum*, *Monilinia fructigena*, *Trichothecium roseum*, *Cladosporium herbarum*, *Aspergillus niger*, *Coprinus psychromorbidus*, *Alternaria alternaria*, *Phytophthora palmivora*, *Pythium* sp., *Fusarium solani*, *Alternaria* sp., *Monilinia* sp., *Guignardia bidwellii* and *Rhizopus stolonifer* is given in Table 1.

It was revealed from the results that *P. chrysogenum*, *A. alternata*, *R. stolonifer* and *Alternaria* sp. were found to produce all the tested enzymes in culture media. Among the isolated fungi, 86.67% of the isolated fungi were found to produce amylases, 80.00% of the fungi produced lipases, 73.33% produced proteases, cellulases by 66.67% and pectinases by 60.00% (Figure 1, Table 1).

All the fungi produced extracellular enzymes on the solid culture media which is interpreted by clear zones around microbial colonies. The term enzyme production here refers to synthesis of the enzyme by the fungus as well as its activity in the medium after it is produced. Maximum cellulase activity was found in *T. roseum* followed by *P. expansum*, *A. alternata*, *F. solani*, *Alternaria* sp. and *P. chrysogenum* than rest of the fungal pathogens. Cellulase activity was found absent in *M. fructigena*, *C. herbarum*, *A. niger*, *Monilinia* sp. and *G. bidwellii*. Maximum amylase activity was observed in *A. niger*, followed by *T. roseum*, *P. expansum*, *Monilinia* sp. and *P. palmivora* while *C. herbarum* and *F. solani* doesn't show any amylase activity.

Maximum pectinase activity was observed in *A. niger* followed by *C. herbarum*, *M. fructigena*, *Alternaria* sp. and *F. solani*. Pectinases were found absent in *T. roseum*, *C. psychromorbidus*, *P. palmivora*, *Pythium* sp. and *Monilinia* sp. Protease activity was reported maximum in *C. herbarum*, *A. niger*, *P. chrysogenum*, *T. roseum*, *C. psychromorbidus* and *Monilinia* sp. Lipase activity was observed

maximum in *A. niger*, *T. roseum*, *P. palmivora*, *P. expansum*, *Monilinia* sp., *Alternaria* sp. and *P. chrysogenum*. Enzyme Protease was found absent in *P.*

expansum, *P. palmivora*, *Pythium* sp. and *G. bidwellii* likewise enzyme lipase was absent in *M. fructigena*, *Cladosporium* sp. and *C. psychromorbidus*.

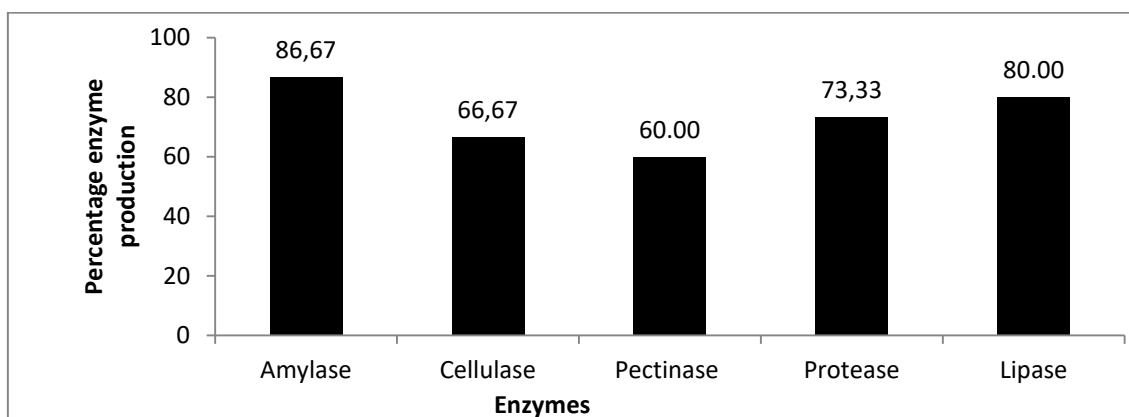


Figure 1. Percentage of isolated fungi showing different enzyme production on solid culture media.

Table 1. Enzymatic index of different rot fungi isolated from fruits.

Fungal pathogen	Enzymatic Index (EI)				
	Amylase	Cellulase	Pectinase	Protease	Lipase
<i>Penicillium chrysogenum</i>	1.26	1.33	1.34	1.68	1.39
<i>Penicillium expansum</i>	1.62	1.82	0.00	0.00	1.79
<i>Monilinia fructigena</i>	1.47	0.00	1.88	1.28	0.00
<i>Trichothecium roseum</i>	1.95	2.03	0.00	1.65	2.17
<i>Cladosporium herbarum</i>	0.00	0.00	1.96	3.06	0.00
<i>Aspergillus niger</i>	3.70	0.00	2.56	2.38	2.42
<i>Coprinus psychromorbidus</i>	1.36	1.27	0.00	1.63	0.00
<i>Alternaria alternaria</i>	1.31	1.43	1.16	1.41	1.28
<i>Phytophthora palmivora</i>	1.51	1.28	0.00	0.00	2.08
<i>Pythium</i> sp.	1.13	1.30	0.00	0.00	1.31
<i>Fusarium solani</i>	0.00	1.40	1.45	1.29	1.26
<i>Alternaria</i> sp.	1.22	1.35	1.74	1.29	1.42
<i>Monilinia</i> sp.	1.62	0.00	0.00	1.52	1.59
<i>Guignardia bidwellii</i>	1.20	0.00	1.24	0.00	1.11
<i>Rhizopus stolonifer</i>	1.28	1.22	1.45	1.23	1.17

Discussion

It was revealed during the present study that all the fungi produced extracellular enzymes on the solid culture media. The array of enzymes produced differs between different fungi. *P. chrysogenum*, *A. alternata*, *R. stolonifer*

and *Alternaria* sp. were found to produce all the tested enzymes, viz. amylase, cellulase, pectinase, protease and lipase on culture media. The differences in the enzymatic production of different fungal pathogens represent their virulence and specificity in causing fruit rot diseases. Plant cell wall is complex structure of

polymers, *viz.* Pectin, cellulose, hemicellulose, polysaccharides and lipids (Karr and Albersheim, 1970), is degraded by the enzymes like pectinase, cellulase, protease, amylase and lipase produced by different virulent plant pathogens (fungal as well as bacterial). These enzymes change the chemistry of cell wall and cause its degradation (Albersheim et al., 1969). Microbial pectinases are important enzymes involved in phytopathologic process and in decomposition of plant dead material. Degredation of the host tissue begins with the production of pectinolytic enzymes which are main enzymes involved in plant attack (Hoondal et al., 2002). Extracellular proteases secreted by fungi especially yeasts have been investigated for industrial use due to organisms fast growth and ability to grow under adverse conditions (Poza et al., 2001). Pectinase and cellulases produced by the rot causing fungal pathogens are essential enzymes in initiating the process of degradation of cell wall (Ahmad et al., 2006) further spread of the pathogen is caused by the enzyme protease and lipase, which are important enzymes in pathogenesis (Hameed et al., 1994). The variation in the enzyme production by different rot fungi suggests there specificity for the rot causing capability and pathogenesis.

Sunitha et al. (2013) reported that cellulases, amylases and pectinases are major enzymes involved in plant polysaccharide degradation along with protease. The production of extracellular cellulase and pectinase by the fungal pathogens indicates that the fungus is well equipped for penetration, decomposition of the host cells (Sieber-Canavesi et al., 1991). Ahmad et al. (2006) reported the enzymatic activities, *viz.* pectinase, cellulase, protease and lipase of fungal pathogens isolated from corn stalk rot disease and reported that the enzyme production differs for different pathogens and depends on the virulence and specificity of the pathogen in causing the disease.

Conflict of interests

The authors declare that there are no conflicts of interest.

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