Egyptian Journal of Biology, 2013, Vol. 15, pp 48-54 http://dx.doi.org/10.4314/ejb.v15i1.7
© Printed in Egypt. **Egyptian British Biological Society (EBB Soc)**

Diversity of pectinolytic molds on major indian mango cultivars

P Jaiswal *, SN Jha, AK Singh & R Bhardwaj

Agricultural Structures and Environmental Control Division, Central Institute of Post-harvest Engineering & Technology, Ludhiana-141004, India

Abstract

The diversity of pectinolytic fungi on nine major Indian mango cultivars was studied. A total of 71 moulds belonging to 10 genera and 18 species were isolated from fruit surfaces, 49 of which showed pectinase activity. *Aspergillus niger* was the most frequent (30%) followed by *A. fumigatus*, *A. flavus*, *A. alternata*, *Fusarium oxysporium*, *A. roseogriseum* and *Paecilomyces variotti*. *A. niger* isolated from the Banganapalli cultivar from Andhra Pradesh in 2010 showed the highest pectinolytic activity. The majority of the fungi showed wide pH tolerances indicating that they could be important candidates for the production of enzymes using liquid media containing mango peel, a by-product of the mango-processing industry.

Keywords: Pectinase activity; pectinolytic zone; pH, *A. niger*; diversity index.

Introduction

Microorganisms associated with post-harvest spoilage of fruits have drawn the attention of scientists for years, and some have been identified and isolated for exploitation in post-harvest treatment (Wisniewski & Wilson 1992, Wilson *et al*. 1993). Extensive studies have been conducted on the diversity of epiphytic microbes on annuals or perennials with deciduous leaves (De Jager *et al*. 2001, Joshi 2008) and the long-lasting leaves of evergreen trees such as mangoes (De Jager *et al*. 2001). Recently, Jha *et al*. (2010) showed that the mango fruit surface supports a varied climax community.

In general fruits and vegetables offer nearly ideal conditions for the survival and growth of many types of microorganisms as they are rich in nutrients (Barth *et al*. 2009). Due to their high nutrient content and low pH they are vulnerable to fungal decay (Singh & Sharma 2007). The majority of spoilage microorganisms derive their nutrients for growth by degrading plant-cell walls using one or more lytic enzymes (Barth *et al*. 2009). Pectinases are the first cell-wall-degrading enzymes secreted by pathogens, and are important virulence factors (Tomassini *et al*. 2009). This in turn reduces post-harvest life and finally leads to spoilage and an inedible undesirable quality (Lebeda *et al*. 2001). In particular, fungi are known to produce large quantities of such enzymes (cellulases, pectinases and hemicellulases), which play an important role in spoilage (Miedes & Lorences 2004). Plant cell walls are weakened by the activity of pectin-degrading enzymes, thereby exposing other polymers for degradation by hemicellulases and cellulases.

On the basis of the optimal pH for activity, pectinolytic enzymes are divided into acidic and alkaline pectinases. They are of significant importance in the current biotechnological era owing to their extensive applications in several conventional industrial processes. Alkaline pectinases are mainly used in the degumming and retting of fibre crops, the pre-treatment of pectic wastewater from the fruit-juice industry, paper making, oil extraction and coffee / tea fermentation. Acidic pectinases are used mainly in the fruit-juice and wine industries in the extraction, clarification and liquefication processes, the maceration of plant tissue and the isolation of protoplasts by selectively hydrolyzing the polysaccharides of the middle lamella (Kashyap *et al.* 2001).

The current investigation was undertaken to study the diversity of pectinolytic fungi on the surface of mangos, and to work out the optimum pH for pectinolytic enzyme production.

* Author for correspondence: tel +91-161-2313170 email : *pranitajaiswal@gmail.com*

Materials & Methods

The fungal population present on nine mango cultivars (Alphonso, Banganapalli, Chausa, Dashehri, Kesar, Langra, Maldah, Mallika and Neelam) was collected from the orchards of nine Indian states (Andhra Pradesh, Bihar, Gujarat, Karnataka, Maharashtra, Orissa, Punjab, Tamil Nadu and Uttar Pradesh) for two consecutive years (2009-10). They were isolated using the wash-off method for isolation of fungi (Jha *et al.* 2010). The fruit was put aseptically in a beaker with a known quantity of sterilized distilled water and shaken for 45 min in an orbital shaker at 100 rpm (REMI- CIS 24BL). Suitable dilutions of washing water in a beaker were made and plated on Rose Bengal Chloramphenicol and incubated at 28°C for mould proliferation. Pure cultures were obtained by sub-culturing three to four times and maintained on the same media at 4°C for further experimental work.

Isolates were characterized based on their hyphal and spore characteristics (Samson *et al.* 2004). Identification of filamentous fungi was further confirmed at the National Type Culture Collection Center (NTCC) at the Indian Agricultural Research Institute, New Delhi, again based on morphological features.

The isolates were screened for their pectinolytic potential based on their ability to produce a halo on the pectin solid media (Hankins medium). The isolated cultures were inoculated aseptically on the PDA plates and incubated at 28 ± 2 °C for 4 days to prepare mother agar plates. Agar discs from the leading edges of actively growing mould colonies on mother culture plates were cut using a sterile laboratory cork borer (8 mm diameter) and transferred onto Hankins medium. The plates then were incubated at 28 ± 2 °C. Plates were flooded with 1% solution of hexadecyltrimethylammoniumbromide precipitant after three days of incubation (Molina *et al.* 2001). A clear zone around colonies within 15 min indicated that a culture had pectinolytic activity.

The screened isolates were inoculated under aseptic conditions in 250 ml Erlenmeyer flasks containing potato dextrose broth (PDB) and incubated at 28 ± 2 °C with shaking on a rotary incubator shaker at 100 rpm for 5 days. The cell-free broth was recovered following centrifugation at 10,000 rpm for 10 min, and the supernatant was used as crude extract. The polygalacturonase activity in the crude extract was determined by a colorimetric method using polygalacturonic acid as the substrate. The reducing sugars released were measured using the dinitrosalicylate (DNS) method (Miller 1959). Under standard assay conditions, one unit of enzyme activity was defined as the amount that liberates one μmol of galacturonic acid per minute.

All readings were taken in triplicate. The frequency of occurrence (PF) of pectinolytic mould on the mango surface was calculated as the percentage of the total number of microbes isolated. The occurrence of a specific mould was calculated as the percentage of the total frequency of all pectinolytic fungi. Species richness was the number of species per sample. Simpson's Index (D) was calculated by applying the formula: $D = \sum (n/N)^2$, Where n = number of individuals of a particular species, N = number of individuals of all species. Simpson's Index of Diversity (1-D) was then calculated.

Results

A total of 71 taxa of pectinolytic fungi belonging to 10 genera and 18 species were isolated from the surface of nine Indian mango cultivars (data not shown).

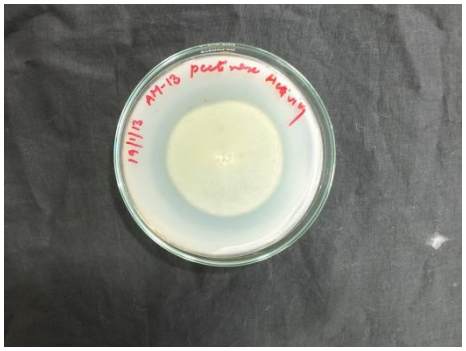

Figure 1: Pectinolytic zone produced around a fungal colony.

Of these 71 isolates, 49 showed pectinolytic activity as evidenced by the pectinolytic zone around their colonies (Fig. 1). The fungi of the mango fruit surface were dominated by pectinolytic moulds (Table 1), except for the Banganapalli cultivar in 2009. The mean frequency of occurrence was 100% for two cultivars, indicating that all the fungi isolated from these cultivars had pectinolytic activity: other cultivars also had very higher frequencies of occurrence. All the *Aspergillus* species isolated from the various mango cultivars exhibited pectinolytic activity (*A. niger, A. fumigatus, A. flavus*) except *A. terreus*. The other major pectinolytic isolates were *Alternaria alternata* and *Fusarium oxysporium*.

Mango Cultivar	**Frequency (%)**		
	2009	**2010**	**mean**
Alphonso, Karnataka	100	50	75
Alphonso, Maharashtra	57	100	79
Banganapalli, AndhraPradesh	0	100	50
Banganapalli, Odisha	100	75	88
Chausa, Punjab	50	100	75
Chausa, Uttar Pradesh	75	100	88
Dashehri, Punjab	50	100	75
Dashehri, Uttar Pradesh	100	100	100
Kesar, Gujarat	67	100	83
Kesar, Maharashtra	100	100	100
Langra, Uttar Pradesh	67	100	83
Maldah, Bihar	50	100	75
Mallika, Odisha	20	20	35
Neelam, Tamil Nadu	67	100	83

Table 1: Frequency of occurrence of pectinolytic fungi isolated from major mango cultivars

Overall frequencies showed the predominance of *Aspergillus niger* (Table 2), followed by *Aspergillus fumigatus* and *Aspergillus flavus*, which are all pectinolytic: others were less frequent. The species composition did not vary much among cultivars, but the maximum species richness was found on mangoes of the Alphonso cultivar from Maharashtra for both years (Table 3) with six species of fungus belonging to *Aspergillus*, *Alternaria*, *Fusarium* and *Paecilomyces*. Several cultivars only had *Aspergillus niger* and two only had *Aspergillus fumigatus* (Appendix).

The mould isolates showing the highest pectinolytic activity (where the lytic zone was greater than 8 mm) were further screened for optimizing enzymatic activity with respect to pH (Table 3). Pectinase-producing isolates showed different optima, with *Aspergillus niger* (M15) at pH 6 and *Aspergillus fumigatus* (M17) and *Aspergillus niger* (M48) at pH 7. Three isolates (*Alternaria alternata* M09, *Acremonium roseogriseum* M14 and *Aspergillus flavus* M18) showed maximum pectinase activity at pH 8.

Mould isolate	Frequency
Acremonium roseogriseum	1
Alternaria alternata	5
Aspergillus fumigatus	13
Aspergillus flavus	9
Aspergillus niger	16
Fusarium oxysporium	4
Paecilomyces variotii	1
Total	49

Table 2: Frequency of occurrence of pectinolytic fungi on a range of mango cultivars

Code	Isolates	Enzyme units per ml			
		pH 5	pH 6	pH 7	pH 8
M01	*Aspergillus niger*	43.4	48.4	48.2	46.1
M09	*Alternaria alternata*	47.5	46.5	47.5	62.9
M11	*Paecilomyces variotii*	44.9	54.4	57.2	53.7
M14	*Acremonium roseogriseum*	68.9	72.4	67.4	77.4
M15	*Aspergillus niger*	89.0	95.0	74.8	81.7
M17	*Aspergillus fumigatus*	84.5	84.5	86.7	75.5
M18	*Aspergillus flavus*	44.2	41.5	45.1	46.3
M48	*Aspergillus niger*	52.0	50.6	70.5	54.8

Table 3: Effect of pH on the pectinolytic activity of moulds isolated from different mango cultivars.

Discussion

Mango (*Mangifera indica* L.) is a universally important popular delicious nutritionally rich fruit. Being rich in pectin content (Tandon & Garg 1999), mango peel offers a suitable growth medium for pectinolytic microorganisms. In the current study the genus *Aspergillus* was found to be a common inhabitant of the mango surface, isolated from almost all mango cultivars. *Aspergillus* species are highly aerobic and are common contaminants of fruit, vegetables and other substrates from which they extract their nutrients: some are involved in food spoilage (Pelczar *et al.* 2008).

Three (*niger, fumigatus, flavus*) of the four different species of *Aspergillus* isolated from mango cultivars exhibited pectinolytic activity, and the same three are considered to be major fruit spoilage fungi (Okereke *et al.* 2010, Gautam *et al.* 2011). *Aspergillus niger* in particular is reported to cause post-harvest spoilage of most fruits including mango (Gautam *et al.* 2011). Besides *Aspergillus, Alternaria alternata* and *Fusarium oxysporium* were the other major pectinolytic moulds isolated from the mango surface, also reported to cause spoilage of many fruits and vegetables (Pocasangre *et al.* 2000, Okereke *et al.* 2010).

The mango fruit surface was dominated by moulds with pectinolytic activity. Mango peel is considered an important source of pectin (Tandon & Garg 1999) and hence can provide a suitable substrate for such microorganisms. Microbes with pectinolytric activity will have a better chance of survival on the mango surface, hence the higher frequency of occurrence of pectinolytic molds. *Aspergillus niger* had highest frequency of occurrence followed by *Aspergillus fumigatus* and *Aspergillus flavus*. *Aspergillus niger* is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments (Frazier &

Westhoff 2008). Artificial infection studies have shown that mango fruit is susceptible to infection at all stage of ripeness (Palejwal *et al.* 1987).

The composition of pectinolytic fungi did not vary much among the cultivars. The majority of the isolated fungi are common contaminants of fruit (Tournas & Katsoudas 2005). However, their diversity varied from cultivar to cultivar and from place to place, highest on Alphonso in Maharashtra. This might be due to the prevailing climatic conditions that affect the microbial population on plant surface (Thompson *et al.* 1993, Jha *et al.* 2010). Atmospheric pollutants, both gaseous and particulate, the use of agrochemicals and naturally occurring epiphytes (Andrews 1992) also affect the microbial community.

Pectinolytic enzymes are of significant importance in the juice, food, paper and pulp industries (Kashyap *et al*. 2001) based on their pH requirement for optimal activity. The molds isolated from mango surface showed fairly high pectinolytic activity with variable pH optima. The highest enzyme activity was recorded in *Aspergillus niger* at pH 6. This is the most commonly used fungus for the industrial production of pectinolytic enzymes (Naidu & Panda 1998). In general fungi are the major sources of acidic pectinases (Favela-Torres *et al*. 2006), suitable for fruit juice extraction and clarification, and the majority of commercial preparations of pectinase are obtained from fungi (Aguiler & Huitron 1987). However, such enzymes are not suitable for vegetable purees and other preparations which need almost neutral pH (Jayani *et al.* 2001). The other pectinolytic moulds screened here showed pH optima of neutral to alkaline pH, and hence have the potential to be utilized for these processes. Most isolates had wide pH tolerance, and hence these fungi from mango could prove important candidates for the production of polygalacturonase by submerged fermentation using liquid media containing mango peel, a by-product of the mango processing industry, and for other biotechnological processes.

Acknowledgements

The authors thank the Director CIPHET and Head (AS & EC Division), CIPHET, Ludhiana for infrastructure and facilities, and for providing financial assistance to carry out research activities. We gratefully acknowledge members of a subproject **(**Development of nondestructive systems for evaluation of microbial and physico-chemical quality parameters of mango'' Code number ''C1030'') of the National Agricultural Innovation Project, Indian Council of Agricultural Research, for making available the different mango cultivars.

References

Aguilar G & Huitron C (1987) Stimulation of the production of extracellular pectinolytic activities of *Aspergillus* sp. by galacturonic acid and glucose addition. *Enzyme & Microbial Technology* 9: 690-696

Andrews JH (1992) Biological control in the phyllosphere. *Annual Review of Phytopathology* 30: 603–635

Barth M, Hankinson TR, Zhuang H & Breidt F (2009) Microbiological spoilage of fruits and vegetables. pp. 135-183 in Sperber WH & Doyle MP (eds) *Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety*. Springer Science+Business Media, LLC.

De Jager ES, Wehner FC & Korsten L (2001) Microbial ecology on the mango phylloplane. *Microbial Ecology* 42: 201-207

Favela-Torres E, Aguiler CN, Contraras-Equivel JC & Viniegra-Gonzalez G (2005) Pectinase. pp. 265-287 in Pandey A, Webb C, Soccol CR & Larroche C (eds) *Enzyme technology*. Asiatech Publishers Inc., New Delhi, India.

Frazier CW & Westhoff CD. 2008. *Food microbiology*. 4th ed. Tata McGraw Hill Publishing Company Limited: New Delhi.

Gautam AK, Sharma S, Awasthi S & Bhadauria R (2011) Diversity, pathogenicity and toxicology of *A. niger*: an important spoilage fungi. *Research Journal of Microbiology* 6: 270-280

Jayani RS, Saxena S & Gupta R (2005) Microbial pectinolytic enzymes: a review. *Process Biochemistry* 40(9): 2931–2944

Jha SN, Jaiswal P, Narsaiah K, Bhardwaj R, Sharma R & Basedia A (2010) Postharvest micro-flora of major cultivars of Indian mangoes. *Scientia Horticulturae* 125 (4): 617–621

Joshi SR (2008) Influence of roadside pollution on the phylloplane microbial community of *Alnus nepalensis* (Betulaceae). Revista de Biología Tropical 56(3): 1521-1529

Kashyap DR, Vohr, PK, Chopra S & Tewari R (2001) Application of pectinases in commercial sector: a review. *Bioresource Technology* 77: 215-227

Lebeda A, Luhová L, Sedláková D & Janková D (2001) The role of enzymes in plant–fungal pathogens interactions. *Journal of Plant Disease Protection* 108: 89-111

Miedes E & Lorences EP (2004) Apple (*Malus domestica*) and tomato (*Solanum lycopersicum*) fruits cell-wall hemicelluloses and xyloglucan degradation during penicillium expansum infection. *Journal of Agricultural & Food Chemistry* 52: 7957–7963

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry* 31: 426-428

Molina R, Pilz D, Smith J, Dunham S, Dreisbach T, O'Dell T & Castellano M (2001) Conservation and management of forest fungi in the pacific NorthWestern United States: an integrated ecosystem approach. pp. 19-63 in Moore D, Nauta MN, Evans SE & Rotheroe M (eds) *Fungal conservation: issues and solutions*. Cambridge UK: Cambridge University Press

Naidu GSN & Panda T (1998) Production of pectolytic enzymes - a review. *Bioprocess Engineering* 19: 355-361

Okereke VC, Godwin-Egein MI & Arinze AE (2010) Assessment of postharvest rot of mango at different stages of market in Port Harcourt, Nigeria. *International Journal of Current Research* 11: 6-10

Palejwal VA, Patki CK, Bhatt SV & Modi VV (1987) Post-harvest spoilage of mangoes by *Aspergillus niger*. *International Journal of Food Microbiology* 5: 111-116

Pelczar MJ, Chan ECS & Krieg NR (2008) *Microbiology. 5th ed*. Tata McGraw Hill Publishing Company Limited, New Delhi, India

Pocasangre L, Sikora RA, Vilich V & Schuster RP (2000) Survey of banana endophytic fungi from Central America and screening for biological control of *Radopholus similis*. *Acta Horticulturae* 531: 283–289

Samson RA, Hoekstra ES & Frisvad JC (2004) *Introduction to food and airborne fungi*. 7th ed. Utrecht, The Netherlands

Singh D & Sharma RR (2007) Postharvest diseases of fruit and vegetables and their management. In Prasad D (ed.) *Sustainable Pest Management*. Daya Publishing House:New Delhi, India

Tandon DK & Garg N. (1999) Mango waste: a potential source of pectin, fiber and starch. *Indian Journal of Environmental Protection* 19: 924-27

Thompson IP, Bailey MJ, Fenlon JS, Fermor TR, Llilley AK, Lynch JM, McCormack PJ, McQuilken MP, Purdy KJ, Rainey PB & Whipps JM (1993) Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (*Beta vulgaris*). *Plant & Soil* 150: 177–191

Tomassini A, Sella L, Raiola A, D'Ovidio R & Favaron F (2009) Characterization and expression of *Fusarium graminearum* endopolygalacturonases *in vitro* and during wheat infection. *Plant Pathology* 58: 556-564

Tournas VH & Katsoudas E. 2005. Mold and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology* 105: 11-17

Wilson CL, Wisniewski ME, Droby S & Chalutz E (1993) A selection strategy for microbial antagonists to control post-harvest diseases of fruits and vegetables. *Scientia Horticulturae* 53: 183-189

Wisniewski ME & Wilson CL (1992) Biological control of post-harvest diseases of fruits and vegetables. *Scientia Horticulturae* 27: 94-98

الملخص العربي

تنوع عفن البيكتينوليتيك على أصناف المانجو الرئيسة فى الهند

جايسوال ب. - جها س. ن. - سينج أ. ك. - بهاردواج ر.

قسم التركيبات الزراعية والمكافحة البيئية - المعهد المركزى للتكنولوجيا والهندسة - ليدايانا-141004 - الهند

تم دراسة تنوع فطريات البيكتينوليتيك على تسع أصناف رئيسة من المانجو فى الهند. تم عزل عدد 71 عفن ينتمون لـ 10 أجناس و 18 نوع من على قشرة الفاكهة، ووضح أن عدد 49 من العفن لها نشاط لإنزيم البيكتينيز. كان النوع أسبيرجيليس نيجر هو أكثر الأنواع انتشاراً بنسبة 30% يليه اسبيرجيليس فيميجاتيس 26.53%، أسبيرجيليس فلافيس 18.37%، اسبيرجيليس ألتيرناتا 10.20%، فيساريم أوكسيسبوريم 8.16%، اسبيرجيليس روسيوجريسيوم 2.04%، بايسلوميسيس فاريوتى 2.04%. تم عزل اسبيرجيليس نيجر من الصنف بانجانابالى والصنف أندهرا براديش فى عام 2010م وأوضحا نشاطاً عالياً للبيكتنوليتيك. أوضحت تحمل أغلب أنواع العفن المعزولة مدى واسعاً للإس الهيدروجينى مما يوضح إمكانية وأهمية استخدامها فى انتاج الإنزيمات باستخدام الأوساط المائية والتى تحتوى على قشر المانجو والذى يتبقى بعد الاستفادة من ثمار المانجو أثناء عمليات الصناعة.

Appendix: Diversity of pectinolytic molds on different mango cultivars.

Mango Cultivars	Year	Mold Isolate	Code	Species Richness	Simpson's index (D)
Alphonso, Karnataka	2009	*A. niger*	M01	2	0.56
		A. flavus	M02		
	2010	*A. fumigatus*	M03	3	0.33
		A. niger	M04		
		F. oxysporium	M05		
Alphonso, Maharashtra	2009	*A. fumigatus*	M06	4	0.31
		A. flavus	M07		
		A. niger	M08		
		A. alternata	M09		
	2010	*F. oxysporium*	M10	4	0.31
		P. variotii	M11		
		A. flavus	M12		
		A. niger	M13		
Banganapalli, AndhraPradesh	2010	*A.roseogriseum*	M14	2	0.50
		A. niger	M15		
Banganapalli, Odisha	2009	*A. niger*	M16	1	1.0
	2010	*A. fumigatus*	M17	3	0.33
		A. flavus	M18		
		A. alternata	M19		
Chausa, Punjab	2009	*A. fumigatus*	M20	1	1.0
	2010	*A. fumigatus*	M21	1	1.0
Chausa, Uttar Pradesh	2009	*A flavus*	M22	3	0.33
		A. fumigatus	M23		
		F oxysporium	M24		
	2010	*A. flavus*	M25	2	0.50
		A.fumigatus	M26		
Dashehri, Punjab	2009	*A. fumigatus*	M27	2	0.50
		A. niger	M28		
	2010	*A.fumigatus*	M29	3	0.33
		A. niger	M30		
		A. alternata	M31		
Dashehri, Uttar Pradesh	2009	*A.fumigatus*	M32	1	1.0
	2010	*A. alternata*	M33	1	1.0
Kesar, Gujarat	2009	*A.fumigatus*	M34	2	0.50
		A. flavus	M35		
	2010	*A. flavus*	M36	1	1.0
Kesar, Maharashtra	2009	*A. niger*	M37	1	1.0
	2010	*A. niger*	M38	1	1.0
Langra, Uttar Pradesh	2009	*A. alternata*	M39	2	0.50
		A. niger	M40		
	2010	*A. niger*	M41	1	1.0
Maldah, Bihar	2009	*A. fumigatus*	M42	1	1.0
	2010	*A. fumigatus*	M43	1	1.0
Mallika, Odisha	2009	*A. niger*	M44	1	1.0
	2010	*A. niger*	M45	2	0.50
		F. oxysporium	M46		
Neelam, TamilNadu	2009	*A. flavus*	M47	2	0.56
		A. niger	M48		
	2010	*A. niger*	M49	1	1.0