International Journal of Food Microbiology 165 (2013) 111–120

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Genetic structure and natural variation associated with host of origin in *Penicillium expansum* strains causing blue mould

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ARTICLE INFO

Article history:
Received 20 December 2012
Received in revised form 10 April 2013
Accepted 18 April 2013
Available online 1 May 2013

Keywords:
Penicillium expansum
Patulin
High Resolution Melting
Host specificity
Pathogenicity
Virulence

ABSTRACT

Blue mould, caused by *Penicillium expansum*, is one of the most economically damaging postharvest diseases of pome fruits, although it may affect a wider host range, including sweet cherries and table grapes. Several reports on the role of mycotoxins in plant pathogenesis have been published, but few focussed on the influence of mycotoxins on the variation in host preference amongst producing fungi. In the present study the influence of the host on *P. expansum* pathogenicity/virulence was investigated, focussing mainly on the relationship with patulin production. Three *P. expansum* strain groups, originating from apples, sweet cherries, and table grapes (7 strains per host) were grown on their hosts of isolation and on artificial media derived from them. Strains within each *P. expansum* group proved to be more aggressive and produced more patulin than the other two groups under evaluation when grown on the host from which they originated. Table grape strains were the most aggressive (81% disease incidence) and strongest patulin producers (up to 554 μg/g). The difference in aggressiveness amongst strains was appreciable only in the presence of a living host, suggesting that the complex pathogen–host interaction significantly influenced the ability of *P. expansum* to cause the disease. Incidence/severity of the disease and patulin production proved to be positively correlated, supporting the role of patulin as virulence/pathogenicity factor. The existence of genetic variation amongst isolates was confirmed by the High Resolution Melting method that was set up herein, which permitted discrimination of *P. expansum* from other species (*P. chrysogenum* and *P. crustosum*) and, within the same species, amongst the host of origin. Host effect on toxin production appeared to be exerted at a transcriptional level.

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1. Introduction

Blue mould is considered one of the most important postharvest diseases of pome fruit worldwide (Pianzzola et al., 2004), although it can cause serious decay amongst a wider range of harvested commodities such as stone fruit, soft fruit and berry fruit (Neri et al., 2010). The disease may be caused by various *Penicillium* species, of which *Penicillium expansum* is the most aggressive and commonly encountered. Amongst other species, *P. solitum*, *P. crustosum*, and *P. chrysogenum* have been reported (Pitt et al., 1991; Sanderson and Spotts, 1995). Blue mould, being a soft rot, severely affects the quality properties of the infected fruit making it unmarketable, with consequent economic losses for retailers. Furthermore, species in genus *Penicillium* are well known producers of pharmaceutically active compounds but also dangerous mycotoxins (Frisvad and Samson, 2004). For example, *P. expansum* has been shown to produce several toxic compounds including patulin, which has mutagenic, immunotoxic, and neurotoxic properties (Castoria et al., 2012), so that its content in apple-derived products has been regulated by the European Commission (2006). Since patulin is extremely stable at acidic pH and resistant to pasteurization, it is critical for the fruit processing industry to detect and minimize *P. expansum* rots in fruits destined to juice production. However, the effectiveness of the control is often reduced by several pathogen characteristics such as: i) the development of resistance to the few fungicides permitted in the postharvest phase; ii) the penetration through wounds (produced during picking and handling operations), natural openings (stem end, open calyx tube, lenticels, etc.) or infection sites of other primary pathogens; iii) the ability to grow both at refrigeration temperatures used for storage (−1, 0 °C) and in warmer environments at retail and consumer sites, particularly on over-mature/long-stored fruits (Mari et al., 2009). Most studies on patulin are focussed on apples and derived products, since apple fruits are reported as the most susceptible and the only ones for which regulatory limits have been imposed (Sant'Ana et al., 2008). However, patulin production at considerable levels has been reported even on other fruit hosts (Larsen et al., 1998; Neri et al., 2010; Piemontese et al., 2005; Reddy et al., 2010). Therefore, it is conceivable that in the future, patulin contamination of fresh fruits other than apples and their derived products might become a bigger issue for the food industry.

The role of mycotoxins in plant pathogenesis is still not completely understood. In general, secondary metabolites produced by fungi are

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http://dx.doi.org/10.1016/j.ijfoodmicro.2013.04.024

Table 1
Penicillium spp. strains, host of isolation, species, patulin production (on PDA) and SNP-cluster. In bold the selected *P. expansum* strains and the accession numbers of partial β-tubulin sequences deposited in GenBank are reported.

Isolate name	Host	Identification	Patulin production (μg/cm²)	SNP-cluster	Accession n.
Pex1	Apple	*Penicillium crustosum*	0	6	
Pex2	Cherry	*Penicillium expansum*	4.23	1	
Pex3	Cherry	*Penicillium expansum*	4.62	1	
Pex4	**Apple**	**Penicillium expansum**	11.37	2	KC342827
Pex5	**Apple**	**Penicillium expansum**	2.45	2	KC342828
Pex6	**Apple**	**Penicillium expansum**	13.11	2	KC342829
Pex7	**Apple**	**Penicillium expansum**	6.26	2	KC342830
Pex8	Apple	*Penicillium expansum*	8.30	1	
Pex9	Cherry	*Penicillium expansum*	12.47	1	
Pex10	Pear	*Penicillium crustosum*	0	4	
Pex11	Cherry	*Penicillium chrysogenum*	0	3	
Pex12	Almond	*Penicillium crustosum*	0	5	
Pex13	Cherry	*Penicillium expansum*	5.09	1	
Pex14	Cherry	*Penicillium expansum*	7.19	1	
Pex15	**Cherry**	**Penicillium expansum**	6.44	1	KC342831
Pex16	Cherry	*Penicillium expansum*	6.40	1	
Pex17	Cherry	*Penicillium expansum*	4.53	1	
Pex18	**Cherry**	**Penicillium expansum**	7.53	1	KC342832
Pex19	**Cherry**	**Penicillium expansum**	12.96	1	KC342833
Pex20	Cherry	*Penicillium expansum*	0.60	1	
Pex21	Almond	*Penicillium chrysogenum*	0	3	
Pex22	Cherry	*Penicillium expansum*	5.76	1	
Pex23	Cherry	*Penicillium expansum*	6.98	1	
Pex24	Cherry	*Penicillium expansum*	5.18	1	
Pex25	Cherry	*Penicillium expansum*	6.41	1	
Pex26	Cherry	*Penicillium expansum*	6.63	1	
Pex27	Cherry	*Penicillium chrysogenum*	0	3	
Pex28	Apricot	*Penicillium chrysogenum*	0	3	
Pex29	Apricot	*Penicillium chrysogenum*	0	3	
Pex30	Almond	*Penicillium crustosum*	0	1	
Pex31	**Cherry**	**Penicillium expansum**	10.18	1	KC342834
Pex32	Cherry	*Penicillium expansum*	4.38	1	
Pex33	**Cherry**	**Penicillium expansum**	8.34	1	KC342835
Pex34	Cherry	*Penicillium expansum*	6.06	1	
Pex35	Cherry	*Penicillium expansum*	6.07	1	
Pex36	**Cherry**	**Penicillium expansum**	7.60	1	KC342836
Pex37	Cherry	*Penicillium expansum*	6.06	1	
Pex38	Cherry	*Penicillium expansum*	5.61	1	
Pex39	Cherry	*Penicillium expansum*	6.34	1	
Pex40	Cherry	*Penicillium expansum*	7.39	1	
Pex41	**Cherry**	**Penicillium expansum**	11.30	1	KC342837
Pex42	Cherry	*Penicillium expansum*	7.82	1	
Pex43	Cherry	*Penicillium expansum*	8.17	1	
Pex44	Cherry	*Penicillium crustosum*	0	4	
Pex45	Apple	*Penicillium expansum*	6.30	1	
Pex46	Cherry	*Penicillium expansum*	3.32	1	
Pex47	**Apple**	**Penicillium expansum**	7.71	1	KC342838
Pex48	Grape	*Penicillium expansum*	10.25	1	
Pex49	Grape	*Penicillium expansum*	9.11	1	
Pex50	Grape	*Penicillium expansum*	9.79	1	
Pex51	Grape	*Penicillium expansum*	0.00	1	
Pex52	Grape	*Penicillium expansum*	7.26	1	
Pex53	Grape	*Penicillium expansum*	6.87	1	
Pex54	Grape	*Penicillium expansum*	21.22	1	
Pex55	Grape	*Penicillium expansum*	13.47	1	
Pex56	Grape	*Penicillium expansum*	8.66	1	
Pex57	Grape	*Penicillium expansum*	6.34	1	
Pex58	Grape	*Penicillium expansum*	6.82	1	
Pex59	Raspberry	*Penicillium crustosum*	0	5	
Pex60	**Grape**	**Penicillium expansum**	21.56	1	KC342839
Pex61	**Grape**	**Penicillium expansum**	28.48	1	KC342840
Pex62	Grape	*Penicillium expansum*	26,17	1	
Pex63	**Grape**	**Penicillium expansum**	27,67	1	KC342841
Pex64	Grape	*Penicillium expansum*	12.20	1	
Pex65	Grape	*Penicillium expansum*	15.58	1	
Pex66	Grape	*Penicillium expansum*	19.96	1	
Pex67	**Grape**	**Penicillium expansum**	24.94	1	KC342842
Pex68	Grape	*Penicillium expansum*	10.10	1	
Pex69	Grape	*Penicillium expansum*	12.93	1	
Pex70	Grape	*Penicillium expansum*	15.91	1	
Pex71	Grape	*Penicillium expansum*	16.84	1	
Pex72	**Grape**	**Penicillium expansum**	18.45	1	KC342843
Pex73	Grape	*Penicillium expansum*	0.76	1	
Pex74	Grape	*Penicillium expansum*	0.75	1	

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