International Journal of Food Microbiology 143 (2010) 109–117

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Evaluation of *Penicillium expansum* isolates for aggressiveness, growth and patulin accumulation in usual and less common fruit hosts

Fiorella Neri *, Irene Donati, Francesca Veronesi, David Mazzoni, Marta Mari

Criof, Department of Protection and Improvement of Agricultural Food Products, Alma Mater Studiorum, University of Bologna, via Gandolfi, 19 Cadriano Bologna, Italy

ARTICLE INFO

Article history:
Received 10 March 2010
Received in revised form 27 July 2010
Accepted 3 August 2010

Keywords:
Apple
Apricot
Mycelial growth
Aggressiveness in fruit hosts
Patulin
Penicillium expansum
Peach

ABSTRACT

Experiments were carried out *in vivo* and *in vitro* with four isolates of *Penicillium expansum* (I 1, E 11, C 28 and I 12) to evaluate their aggressiveness, growth and patulin accumulation in both usual (pears and apples) and less common hosts (apricots, peaches, strawberries and kiwifruits) of the pathogen. The 75% of isolates showed the ability to cause blue mould in all tested hosts. In particular, C 28 and I 1 were the most and the least aggressive isolates, respectively (52.9 and 10.6% infection and 20.7 and 15.4 mm lesion diameters). 'Candonga' strawberries and 'Pinkcot' apricots showed the largest lesion diameters (29.8 and 25.3 mm), followed by 'Conference' pears, 'Spring Crest' peaches and 'Abate Fetel' pears. With the exception of 'Candonga' strawberries, the formation of colonies and mycelial growth of *P. expansum* isolates on fruit puree agar media (PAMs) was stimulated in comparison to a standard growth medium (malt extract agar, MEA). Two of the most aggressive isolates in our assays (I 12 and C 28) showed the greatest accumulation of patulin both *in vitro* and *in vivo*, while the least aggressive isolate (I 1) produced patulin only in a few growth media and cvs. Patulin concentration on fruit PAMs was higher than patulin detected in infected fruit tissues. Apple PAMs were the more favorable substrates for patulin accumulation *in vitro* (maximum concentration 173.1 and 74.1 μg/mL in 'Pink Lady and 'Golden Delicious' PAMs, respectively) and 'Pink Lady' apples inoculated with the isolate E 11 showed the greatest accumulation of patulin in the whole *in vivo* assay (33.9 μg/mL). However, infected tissue of cv Golden Delicious showed lower average accumulation of patulin (1.7 μg/mL) than that of cv Pink Lady (19.1 μg/mL), and no significant differences in patulin concentrations were found among 'Golden Delicious' apples and tested cvs of pears, kiwifruits and strawberries. Peaches were highly susceptible to patulin accumulation, showing average concentrations of 27.4 and 18.6 μg/mL *in vitro* and *in vivo*, respectively. Apricots were also consistently positive for patulin accumulation, both *in vitro* (average values of 20.1 μg/mL) and *in vivo* (average values of 9.4 μg/mL). Our study showed the potential of some less common hosts of *P. expansum* (in particular peaches and apricots) to support patulin production, indicating that a steady monitoring of patulin contamination should be carried out in fruit substrates other than apples and pears.

1. Introduction

Penicillium expansum Link (agent of blue mould disease) is one of the main causes of spoilage of pears and apples after harvest and is frequently isolated from a wide range of other fruit, including stone fruit, soft fruit and berry fruit (Snowdon, 1990; Sommer et al., 1974). The pathogen penetrates typically through wounds or injuries produced during harvest and handling. Infection may also occur through stem end, open calyx tube and lenticels in pome fruits or it may gain entry through infection sites of other primary fruit pathogens. Over-mature or long-stored fruit are more susceptible to *P. expansum* infection. Blue mould develops even at low temperatures used for fruit storage (−1°–0 °C), although its development is favored by warm environment at retail and consumer sites (Mari et al., 2009). Besides the economic impact caused by fruit infection, current interest for *P. expansum* is the health hazard caused by ability of the pathogen to produce patulin (Moake et al., 2005), a mutagenic and embryo toxic substance produced by most isolates of the pathogen (Sommer et al., 1974; Andersen et al., 2004; Morales et al., 2008a). For this mycotoxin the limits of 50, 25 and 10 μg/kg have been set in Europe for fruit juices and fruit nectar, solid apple products and apple based products for infants and young children, respectively (Anon., 2003), and 50 μg/L is the norm for patulin regulation of apple juice and cider in many countries (Anon., 2004).

Most studies on patulin are focused on apples and their products (Sant'Ana et al., 2008). This is justified by the following reasons: apples are the most susceptible fruit to *P. expansum* infection in many producer countries, contamination by patulin frequently occurs in apple industry and limits for patulin has been established for apple-

* Corresponding author. Criof, Department of Protection and Improvement of Agricultural Food Products, University of Bologna, via Gandolfi, 19, 40057 Cadriano di Granarolo Emilia, Bologna, Italy. Tel.: +39 051 766563; fax: +39 051 765049.
E-mail address: fiorella.neri@unibo.it (F. Neri).

0168-1605/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2010.08.002

based products. The presence of patulin in fruit other than apples has been less investigated (Laidou et al., 2001; Anon., 2002; Morales et al., 2008b; Spadaro et al., 2008), although *P. expansum* has a broad host range and early studies on patulin reported the occurrence of this mycotoxin in a variety of fruit (Buchanan et al., 1974; Frank et al., 1977; Scott et al., 1977). In the study by Buchanan et al. (1974), only plums were, for example, a poor substrate for patulin accumulation, while pears, peaches, apricots and cherries infected by *P. expansum* accumulated concentrations of patulin similar to those reported for apples. The role of patulin in plant pathogenesis is still unknown. In general, secondary metabolites of fungi are not required for their growth under normal conditions, but can presumably confer some selective advantage in certain situations (Bhatnagar et al., 2002). Whether mycotoxins act in pathogenesis as factors of pathogenicity or virulence is a controversial issue. Different results are found in the literature depending on fungal species and type of toxin (Xu and Berrie, 2005; Hof, 2007), and contrasting results have also been reported with regard to the relationship between levels of patulin produced and aggressiveness of *P. expansum* isolates (Sommer et al., 1974; McCallum et al., 2002; Baert et al., 2007).

The aim of this study was to evaluate the aggressiveness of some isolates of *Penicillium expansum* to different fruit hosts and to investigate the influence exhibited by hosts on the isolates' growth and patulin accumulation in *in vivo* and *in vitro* assays.

2. Materials and methods

2.1. Pathogen

Four isolates of *P. expansum* (I 1, E 11, C 28 and I 12) obtained from blue mould decayed pears and belonging to the CRIOF collection were used. A monoconidial culture of each isolate was grown at 20 °C on malt extract agar (MEA; Oxoid, UK) until use. Conidial suspensions of each *P. expansum* isolate were prepared by washing the pathogen colonies with sterile distilled water containing 0.05% v/v Tween 80. Spore concentrations were adjusted to 10^3 conidia/mL, by means of a haemocytometer. In the trials of mycelial growth, *P. expansum* was grown for 2 days on Czapek-dox agar (Oxoid, UK).

2.2. Fruit

Both typical and less common fruit hosts of *P. expansum* were tested. Pears (cv Conference and Abate Fetel), apples (cv Golden Delicious and Pink Lady), apricots (cv Pinkcot), peaches (cv Spring Crest), strawberries (cv Candonga) and kiwifruits (cv Hayward), were purchased from the Emilia-Romagna Apofruit packinghouse. Only undamaged and disease-free fruits were used in the experiments. Physical–chemical characteristics were analyzed on 20 fruit of each cv before inoculation (Table 1). Firmness (Newtons), not determined in strawberries, was measured after removing the skin, on opposite sides of each fruit, using a Chatillon digital penetrometer fitted with an 8 mm probe (11 mm for apples). Soluble solids content (%) was determined using a digital refractometer (Atago Co., Tokyo, Japan) in a portion of filtrate obtained by blending each fruit. The values of pH and titratable acidity (mequiv. 10/mL of pure juice) were determined using an automatic titrator (Crison Instruments, Modena, Italy) by titrating fruit juice (obtained by diluting homogenized flesh with distilled water in a ratio 1:5 and filtering the solution in a vacuum) with 0.1 N NaOH to pH 8.10. Fruit were stored at 0 °C until testing (a maximum of 3 days for stone fruits and strawberries and 1 month for pome fruits and kiwifruits).

Table 1
Physical–chemical characteristics of fruit before inoculation (means ± standard deviations).

Fruit	Firmness (N)	SSC (%)	pH	Total acidity (meq/100 mL)
'Abate Fetel' pear	49.71 ± 6.03	14.97 ± 0.06	4.40 ± 0.02	2.90 ± 0.15
'Conference' pear	67.57 ± 7.93	13.50 ± 0.06	4.55 ± 0.02	2.00 ± 0.06
'Golden Delicious' apple	64.97 ± 6.83	16.83 ± 0.06	3.36 ± 0.08	7.94 ± 0.00
'Pink Lady' apple	76.43 ± 5.99	14.43 ± 0.06	3.45 ± 0.09	7.61 ± 0.26
'Pinkcot' apricot	10.23 ± 6.36	10.77 ± 0.06	2.76 ± 0.02	33.92 ± 0.00
'Spring Crest' peach	47.56 ± 7.41	10.53 ± 0.09	2.96 ± 0.01	15.79 ± 0.01
'Candonga' strawberry	n.d.	9.63 ± 0.25	3.43 ± 0.05	12.31 ± 0.05
'Hayward' kiwifruit	70.78 ± 13.13	6.5 ± 0.00	3.36 ± 0.02	19.48 ± 0.05

n.d. = not determined.

2.3. Aggressiveness of P. expansum isolates

Batches of fruit were wounded with a sterile nail (one wound per fruit in the equatorial zone; 2 × 2 × 2 mm) and dipped for 1 min in a conidial suspension (10^3 conidia/mL) of each *P. expansum* isolate. Three replicates of 20 fruit were used for each isolate and cv. The percentage of infected wounds and the diameter of the lesions (mm) were recorded after 7 days of incubation at 20 °C. Fruit with no infection were not counted for lesion size measurements.

2.4. Measurements of pH in fruit wounded site and in Czapek-dox liquid medium

The pH of mesocarp of each fruit used for aggressiveness evaluation was measured by placing the pH electrode InLab 427 (Mettler Toledo) connected to a SG2-SevenGo pH meter (Mettler Toledo) at approximately 15 mm depth through the wound site. The pH of healthy tissue was measured in non-inoculated fruit (controls) kept for 7 days at 20 °C. Three replicates of 20 fruit were used for each isolate, cv and treatment.

Conidial suspensions of each isolate were inoculated in 3 tubes containing 10 mL of Czapek-dox liquid medium (CLM, Oxoid, UK) to achieve the final concentration of 10^3 conidia/mL and incubated at 20 °C for 14 days. The pH of the medium was measured daily starting after 3 days of incubation using the instrument described above.

2.5. Effect of fruit based media on P. expansum isolates development

To assess only the effect of constitutive characteristics of the host, conidial suspensions and mycelial disks of each *P. expansum* isolate were cultured *in vitro* on growth media derived from boiled fruit purees. Within a few days of harvesting, fruit samples of the same batches used for *in vivo* experiments (with the peel but without the core or the stone) were mixed until a fine puree was obtained, and stored at −24 °C until needed. Puree agar media (PAMs) from each cv were obtained by adding aliquots of 300 mL sterile agar solution (9 g agar technical, Oxoid, in distilled water) to 600 g of fruit purees previously boiled for 45 min into 1 L bottle (Baert et al., 2007). Colony-forming units (CFU) and mycelial growth of *P. expansum* isolates cultured on fruit PAMs were compared with growth on MEA (control). Six replicate dish cultures were used for each isolate, treatment and assay. Aliquots of 100 μL of conidial suspension (10^3 conidia/mL) of each *P. expansum* isolate were spread onto Petri dishes containing 20 mL of each fruit PAM and onto dishes of MEA. The CFU were counted 3 days after incubation at 20 °C. To test rates of mycelial growth, a mycelial disc (6 mm diameter) was taken from the periphery of an actively growing agar culture and placed at the centre of a Petri dish containing MEA or PAM. After 7 days of incubation at 20 °C, the diameter of the colonies was recorded.

2.6. Determination of patulin in vitro and in vivo

In vitro and *in vivo* experiments were carried out to determine the effect of different growth media and fruit hosts on patulin accumulation.