Patulin and citrinin production by *Penicillium expansum* strains isolated from grapes

Luís Abrunhosa[1], Armando Venâncio[1], Russel Paterson[2] and Zofia Kozakiewicz[2]

[1] Centro de Engenharia Biológica - IBQF, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

[2] CABI Bioscience, UK Centre, Bakeham Lane, Egham TW20 9TY, UK

1. Abstract

Penicillium expansum strains were isolated from grapes from the Douro wine region, and assayed for their capacity of producing patulin and citrinin, which are mycotoxins, on two media. The presence in grapes of this species can cause spoilage during processing and these mycotoxins may be produced. A total of 51 strains were assayed and all strains produce citrinin on YES medium, but only one strain was able to produce it when grown in a grape juice medium (GJM). Patulin was produced by 20% of strains on YES, and 70% produce it on GJM. These data will be presented and discussed.

2. Introduction

Wine is an important agricultural product in the economy of many regions of Portugal. For example, in the last year, 954 thousand hectoliters of Porto Wine were produced in Douro Region (in www.ivp.pt).

Recently some researchers reported the detection of ochratoxin A (OTA), a mycotoxin, in wines. Zimmerli and Dick (1996) reported the presence of OTA at levels ranging from < 3 to 388 ng/l in some wines from the retail market of Switzerland, and Majerus and Otteneder (1996) detected OTA at concentrations up to 7 ng/l on white wines and 200 ng/l on red wines. All the reports, at the present moment, concluded that this mycotoxin is present in wines produced in mediterranean countries. Scott *et al.* (1977) also reported the detection of another mycotoxin in grape juice: patulin was present in juices produced from mouldy grapes but not in fermented juice; citrinin was found in apple juice, but never in grape juice or derived products. They support that patulin is destroyed during the fermentation and by SO_2 and that citrinin, if formed, is unstable in grape juice.

Ochratoxin A, patulin and citrinin are mycotoxins produced by *Aspergillus* spp. and *Penicillium* spp. Mycotoxins, are natural products produced by fungi (yeasts and mushrooms excluded) that evoke a toxic response when introduced in low concentrations to higher vertebrates and others animals by a natural route (Arora *et al.*, 1991). OTA is well-known to be nephrotoxic (associated with the endemic nephropathy of Balkans countries), immunotoxic, carcinogenic and a potent teratogen (Corneli and Maragos, 1998). Citrinin is also nephrotoxic, carcinogenic and mutagenic (Betina, 1989). Patulin is a carcinogen and a teratogen to (Betina, 1989).

3. Material and Methods

The strains of *Penicillium expansum* tested were isolated from grapes of Douro Region and stored at 4°C in slants with Malt Extract Agar (MEA). These strains were inoculated in 9 cm petri dishes with MEA and incubated at 25°C for 7 days. After that, they were inoculated in 9 cm petri dishes with Yeast Extract Sucrose (YES) agar or with a grape juice medium (GJM) agar. After 11 days growth at 25°C, they were analysed by Thin Layer Chromatography (TLC).

The culture analysis by TLC was done by using the agar plug method as described by Paterson and Bridge (1994). We used 20 X 20 cm Merk aluminium plates of silica gel 60 with a layer thickness of 0.2 mm. Plugs were applied as described by Paterson and Bridge (1994) and the TLC pates developed for 17 cm in toluene/ethyl acetate/90% formic acid (5:4:1, v/v/v) (TEF) solvent. As a standard, a solution of three metabolites was used: patulin, griseofulvin and ochratoxin A.

TLC plates were observed at white (normal) light and UV light (366 nm and 254 nm). Spots characteristics (colour and distance travelled) were recorded and, afterwards, plates were sprayed with 3-methyl-2-benzothiazoline hydrazone hydrochloride (MBTH) in water (5 g/l), dried for 15 minutes and heated for 15 minutes at 110°C, for the identification of patulin and citrinin spots.

4. Results

Table 4.1 – Patulin and citrinin production in YES and GJM media by *Penicillium expansum* isolated from Douro region.

Identification code	YES		GJM	
	Patulin	Citrinin	Patulin	Citrinin
DY139	Ø	v	v	Ø
DY141	Ø	v	v	Ø
DY143	Ø	v	v	Ø
DY146	v	v	v	Ø
DY148	v	v	v	Ø
DY149	v	v	v	Ø
DY152	v	v	v	Ø
DY153	v	v	v	Ø
DC160	v	v	v	Ø
DC161	v	v	v	Ø
DC166	Ø	v	v	v
DC170	Ø	v	v	Ø
DY176	Ø	v	v	Ø
DY212	Ø	v	Ø	Ø
DC213	Ø	v	Ø	Ø
DY215	Ø	v	Ø	Ø
DY217	Ø	v	v	Ø
DY218	Ø	v	Ø	Ø
DY221	Ø	v	Ø	Ø
DC226	v	v	v	Ø
DC228	Ø	v	Ø	Ø
DC230	Ø	v	Ø	Ø
DC233	Ø	v	Ø	Ø
DC234	Ø	v	Ø	Ø
DC237	Ø	v	Ø	Ø
DC238	Ø	v	Ø	Ø
DC241	Ø	v	Ø	Ø
DY242	Ø	v	Ø	Ø
DY243	Ø	v	Ø	Ø
DY245	Ø	v	Ø	Ø
DY248	Ø	v	v	Ø
DY250	Ø	v	v	Ø
DY252	Ø	v	v	Ø
DY253	Ø	v	v	Ø
DY255	Ø	v	v	Ø
DY258	Ø	v	v	Ø
DY260	Ø	v	v	Ø
DC263	Ø	v	v	Ø
DC269	Ø	v	v	Ø
DC277	Ø	v	v	Ø
DY283	Ø	v	v	Ø
DY284	Ø	v	v	Ø
DY291	Ø	v	v	Ø
DY293	Ø	v	v	Ø
DY295	Ø	v	v	Ø
DY297	Ø	v	v	Ø
DC299	Ø	v	v	Ø
DC303	Ø	v	v	Ø
DC304	Ø	v	v	Ø
DC305	v	v	v	Ø
DC307	v	v	v	Ø

(legend: v - mycotoxin detected; Ø - mycotoxin not detected)

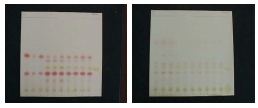

Fig. 4.1 - Metabolic profiles of isolated Y139, Y141, Y143, C146, Y148, Y149, Y152, Y153, C160 and C161 detected by TLC when: a) grown in YES (sprayed with MBTH and photographed at white light); b) grown in GJM (sprayed with MBTH and photographed at white light).

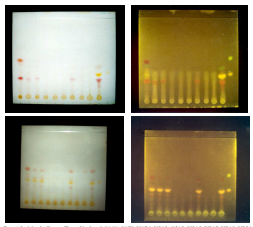

Fig. 4.2 - Metabolic profiles of isolated C166, C170, Y176, Y212, C213, Y215, Y217, Y218, Y221 and C226 detected by TLC when: a) grown in YES (sprayed with MBTH and photographed at white light); b) grown in YES (sprayed with MBTH and photographed at 366 nm); c) grown in GJM (sprayed with MBTH and photographed at white light); d) grown in GJM (sprayed with MBTH and photographed at 366 nm).

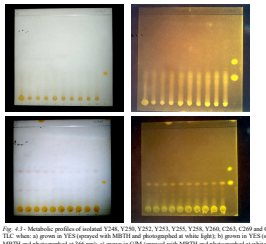

Fig. 4.3 - Metabolic profiles of isolated Y248, Y250, Y252, Y253, Y255, Y258, Y260, C263, C269 and C277 detected by TLC when: a) grown in YES (sprayed with MBTH and photographed at white light); b) grown in YES (sprayed with MBTH and photographed at 366 nm); c) grown in GJM (sprayed with MBTH and photographed at white light); d) grown in GJM (sprayed with MBTH and photographed at 366 nm).

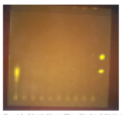

Fig. 4.3 - Metabolic profiles of isolated C166, C170, Y176, Y212, C213, Y215, Y217, Y218, Y221 and C226 detected by TLC when: a) grown in GJM (sprayed with MBTH and photographed at 366 nm).

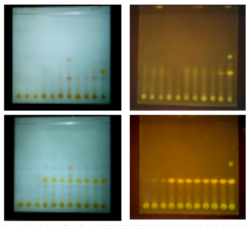

Fig. 4.4 - Metabolic profiles of isolated Y283, Y284, Y291, Y293, Y295, Y297, C299, C303, C304, C305 and C307 detected by TLC when: a) grown in YES (sprayed with MBTH and photographed at white light); b) grown in YES (sprayed with MBTH and photographed at 366 nm); c) grown in GJM (sprayed with MBTH and photographed at white light); d) grown in GJM (sprayed with MBTH and photographed at 366 nm).

5. Discussion and conclusions

We observed that all the 51 strains produce citrinin on YES medium but that only one (strain DC166) was able to produce it when grown in GJM. Patulin was produced by 20% of strains on YES and 70% produce it on GJM. None of our 51 strains was able to produce Ochratoxin A.

We believed that hours results can confirm the ability of *Penicillium expansum* to produce patulin in grapes, as reported Scott *et al* (1977). Also the absence of citrinin from grapes and grape juice reported by these authors was confirmed, since the ability of our strains to produce citrinin was dramatically decreased when they were grown in grape juice media.

Since patulin is destroyed during fermentation and citrinin is not produced in grapes, we conclude that both these mycotoxins are not of concern in wine production.

7. Bibliography

- Arora, D. K., Mukerji, K. G. and Marth, E. H., 1991. Handbook of applied mycology. Volume 3: Foods and Feeds. Marcel Dekker, Inc.. New York. pp.31-68.
- Betina, V., 1989. Bioactive Molecules - Volume 9: Mycotoxins. Chemical, biological and environmental aspects. Elsevier. Amsterdam.
- Corneli, S. and Maragos, C. M., 1998. Capillary electrophoresis with laser-induced fluorescence: Method for the mycotoxin Ochratoxin A. Journal of Agriculture and Food Chemistry, 46, 3162-3165.
- Majerus, P. And Otteneder, H. 1996. Nachweis und Vorkommen von Ochratoxin A in Wein und Traubensaft. Deutsche Lebensmittel-Rundschau, 92, 388-390.
- Paterson. R. R. M. and Bridge, P. D., 1994. IMI Technical Handbooks: nº 1. Biochemical techniques for filamentous fungi. CABInternational. Wallingford.
- Scott, P. M., Fuleki, T. and Harwig, J., 1977. Patulin content of juice and wine produced from moldy grapes. Journal of Agriculture and Food Chemistry, 25, 434-437.
- Zimmerli, B. and Dick, R., 1996. Oc hratoxin A in table wine and grape juice: occurrence and risk assesment. Food Additives and Contaminants, 13, 655-668