Patulin and citrinin production by
Penicillium expansum strains isolated from grapes

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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.

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).

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 analyses by TLC was done by using the agar plug method as described by Paterson and Bridge (1994). We used 20 x 20 cm Mark alumimium 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 plates developed for 17 cm in toluene/ethyl acetate/20% formic acid (4:1:1, 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 hydroxide chloride (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.

We observed that all the 51 strains produce citrinin on YES medium but that only one strain DC106 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 these 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, and the ability of our strains to produce patulin 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.

References: