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Preparation of fungal conidia impacts their susceptibility to inactivation by ethanol vapours

Thien Dao, Philippe Dantigny *

Laboratoire de Génie des Procédés Microbiologiques et Alimentaires, Université de Bourgogne ENSBANA, 1 Esplanade Erasme, 21000 Dijon, France

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ABSTRACT

A common protocol employed for the preparation of conidia employs flooding a fungal colony grown on semi-solid media under optimum conditions with an aqueous solution. In contrast, conidia produced in a natural environment are usually not hydrated when disseminated in air and can be produced under water stress. In order to simulate the latter conditions, cultures were grown at different water activities and conidia were dry-harvested on the lid by turning the dishes upside-down then gently tapping the bottom of the box. This study aimed at assessing the effect of the preparation of fungal conidia on their inactivation by ethanol vapours. Firstly ethanol vapours (either 0.30 or 0.45 kPa) were applied to conidia obtained from the standardised protocol and to dry-harvested conidia for some species of *Penicillium*. While all dry-harvested conidia remained viable after 24 h of treatment, about 1.0, 3.5 and 2.5 $\log_{10}$ reductions were observed for hydrated conidia of *Penicillium chrysogenum*, *Penicillium digitatum* and *Penicillium italicum* respectively. Secondly ethanol vapours (0.67 kPa) were applied to dry-harvested conidia obtained from cultures grown at 0.99 a_w and at reduced water activities. For all species, the susceptibility to ethanol vapours of conidia obtained at 0.99 a_w was significantly greater than that of conidia obtained at reduced water activities. Conidia produced in a natural environment under non-optimal conditions would be much more resistant to ethanol vapours than those produced in the laboratory. This phenomenon may be due to a reduced intracellular water activity of dry-harvested conidia.

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1. Introduction

Moulds can develop at the field level, but also during storage of raw products, subsequent transport and sale, causing considerable economic losses annually for food producers and consumers alike. Fungal spores can be disseminated widely in the air and they are mainly responsible for these losses (Richard-Molard et al., 1985). After germination of the spores, moulds spread rapidly among the fruits, cereals, and food. Postharvest losses are 5–10% when postharvest fungicides are used (Cappellini and Ceponis, 1984). Without fungicides, losses of 50% or higher have occurred in some years (Lurie et al., 1995). For example, in a 1993 test to assess the decay potential of stone fruit, an average of 52.8% (range 15–100%) of the fruit decayed during the ripening of eight collections that had not been treated with postharvest fungicides (Margosan et al., 1997). Both green mould and blue mould, also known as *Penicillium* rots, are caused by *Penicillium digitatum* and *Penicillium italicum*, respectively. They are one of the most common postharvest diseases, especially on citrus fruits. They cause up to 90% of citrus fruit losses in transit, storage and after sales (Agrios, 2005).

For a long time, ethanol has been used as a fungicide treatment. Ethanol is used commercially in many products, such as perfumes, food, paints, alcoholic beverages and additives. Ethanol is a small molecule produced either by chemical synthesis or by microbial fermentation. Recently, there has been substantial interest in non-biological control agents as well as biological control agents to replace the existing chemical applications. Non-biological control involves chemicals that are Generally Regarded As Safe "GRAS" product such as ethanol (Karabulut et al., 2004; Romanazzi et al., 2007) as alternative treatments.

The inhibitory effect can be obtained by adding ethanol directly or by using ethanol vapours. The use of ethanol vapours at a dose rate of 2 ml/kg to limit the development of *Botrytis cinerea* on table grapes was also reported (Chervin et al., 2005). The complete inhibition of the growth of *Penicillium notatum*, corresponding to the minimum inhibitory concentration (MIC) of ethanol in vapour phase, was observed at a concentration of 8.6 mmol/L air (i.e., 0.21 kPa) (Tunc et al., 2007). The inhibitory effects of ethanol vapour on the germination of *Penicillium glaucum* and *Sterigmatocystis nigra* were early demonstrated by Lesage (1895, 1897). The MIC for the germination of *Penicillium chrysogenum* conidia by ethanol vapours (0.13 kPa) generated by an ethanol solution was estimated to 4.3% (wt/wt) (Dantigny et al., 2005). Ethanol vapour at 0.16% (v/v)

* Corresponding author. Tel.: +33 3 8039 6671.
E-mail address: phdant@u-bourgogne.fr (P. Dantigny).

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Table 1
Composition of solutions to adjust the ethanol vapour pressure to 0.30, 0.45 and 0.67 kPa, and experimental conditions.

Component	Ethanol pressure (kPa)		
	0.30	0.45	0.67
Ethanol (g)	8.60	13.5	20
Water (g)	51.8	54.3	80
Glycerol (g)	39.6	32.2	0
Temperature (°C)	20	20	25

(0.16 kPa) was also effective in retarding the apparition of green rot by *P. digitatum* and blue rot by *P. italicum* on oranges (Yuen et al., 1995). But, at these concentrations, the inhibition due to ethanol was clearly reversible (Dantigny et al., 2005). Therefore, it would be highly desirable to inactivate fungal conidia by means of higher ethanol concentrations. Exposing both peaches and oranges to ethanol vapours (3.6–7.5 kPa) generated by liquid solutions in the range 70–100% proved effective at inhibiting fungal growth (Lihandra, 2007).

More recently, it was shown that conidia of *P. chrysogenum*, *P. digitatum* and *P. italicum* were inactivated by ethanol vapours generated by 10% ethanol solutions (Dao et al., 2008). In that study the standardised protocol was used. Spore suspensions were obtained by flooding the mycelium grown at optimal conditions. However, the physiological state of fungal spores at the time they are treated greatly influences germination and inhibition by subsequent treatments (Dantigny and Nanguy, 2009). For example, extending for 48 h the hydration time of conidia of *Penicillium aurantiaugriseum* and *Penicillium roqueforti* produced at 0.88 a_w, prior to heat treatment, resulted in further heat inactivation (Blaszyk et al., 1998). The same authors have also stated that conidia produced at 0.88 a_w were significantly more sensitive to both sodium benzoate and potassium sorbate than were conidia produced at 0.99 a_w. The aims of this study were to assess the effects of i/ hydration of the conidia and ii/ the water activity during sporogenesis, on the inactivation of conidia of *P. chrysogenum*, *P. digitatum* and *P. italicum* by ethanol vapours.

2. Materials and methods

2.1. Moulds

P. chrysogenum was isolated from a spoiled pastry product. *P. digitatum* and *P. italicum* were isolated from a spoiled satsuma. The moulds were identified based on the descriptions of Samson et al. (1995). The moulds were maintained on potato dextrose agar medium (PDA; bioMérieux, Marcy l'Etoile, France) at room temperature (18 to 25 °C).

2.2. Medium

The PDA medium (0.85, 0.90, 0.95 and 0.99 a_w) was used for production of conidia and at 0.99 a_w for viability assessment. Water activity was adjusted by substituting a part of the water with an equal weight of glycerol (Gervais et al., 1988). The initial pH for all experiments was 5.7 ± 0.1. All plates were incubated at 25 °C. All trials were performed in triplicate.

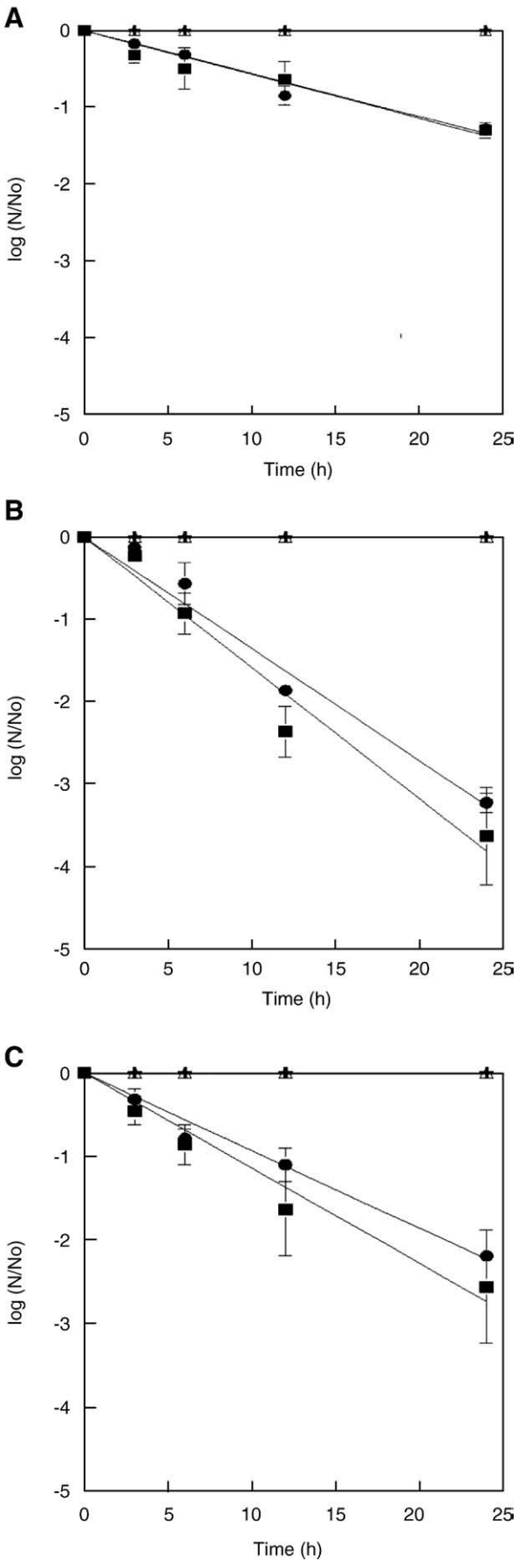

Fig. 1. Effect of ethanol vapour pressure on inactivation of dry-harvested conidia at 0.30 (△), 0.45 kPa (x) and on hydrated conidia at 0.30 (●), 0.45 kPa (■). *Penicillium chrysogenum* (A), *Penicillium digitatum* (B) and *Penicillium italicum* (C). Error bars represented standard deviations.

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