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Review

Significance of the physiological state of fungal spores

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ABSTRACT

In predictive mycology, most of the studies have been concerned with the influence of some environmental factors on fungal growth and production of mycotoxins, at steady-state. However, fluctuating conditions, interactions between organisms, and the physiological state of the organisms may also exert a profound influence on fungal responses in food and in the environment. In the laboratory, fungal spores are widely used as a biological material. They are produced under optimal conditions then, partially re-hydrated for obtaining standardized spore suspensions. In real conditions, spores are produced under suboptimal conditions and can be submitted to various stresses prior to their germination. It was illustrated how the sporulation/post-sporulation conditions, the re-hydration and the age of the spores affected greatly their physiological state and consequently their resistance to heat, inhibitors and their germinability. It was hypothesised that the observed responses to environmental factors during inactivation and germination could be correlated to the intracellular water activity of the spores.

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1. Introduction

Predictive mycology aims at predicting fungal development and production of mycotoxins in food and raw products (Dantigny et al., 2003). Most of the studies have been concerned with fungal growth. Germination and inactivation of fungal spores have been also examined, but to a lesser extent. It is readily understandable that fungal spores should be produced in the laboratory for assessing germination and inactivation. But, spores are also widely used as an inoculum for studying fungal growth. To date, an alternative strategy consisting of using agar plugs covered with mycelium cut from the growing margins of colonies grown on Petri dishes has been developed (Fariña et al., 1997; Marín et al., 1998; Ramos et al., 1998; Lee and Magan, 2000; Abellana et al., 2001).

The standardized protocol for producing spore suspensions can be summarized as follows. Fresh spores are obtained after mycelium has been grown on various semi-synthetic media (0.99 a_w) such as Potato Dextrose Agar (PDA) or Malt Extrat Agar (MEA), (Dantigny et al., 2006). However, media characterised by lower a_w are required for growing xerophilic fungi such as *Wallemia sebi*. Then, spore suspensions are obtained by flooding the mycelium with an aqueous solution usually set at a_w of the subsequent experiments. It has been shown

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that harvesting spores in isotonic conditions resulted in better viability due to the compatible solutes, such as sugar polyols, remain in the spores (Ypsilos and Magan, 2004). Spore suspensions are also usually filtered so that the large mycelium fragments are removed and standardized to a desired concentration.

In order to produce spores within the minimum period of time, the environmental conditions are set at the optimum for growth in terms of water activity and temperature. Additional objectives can be to increase the number of produced spores (Eicher and Ludwig, 2002) or to avoid erratic germination (Pitt and Christian, 1968). In the laboratory, spores are produced under optimum conditions, but such conditions are very unlikely in the environment.

It has been shown that nutritional and environmental conditions prevailing during spore formation may exert a profound influence on their viability (Darby and Mandels, 1955), heat resistance (Conner and Beuchat, 1987a,b; Beuchat, 1988), resistance to preservatives, germination time (Blaszyk et al., 1998) and chemistry (Jackson and Schisler, 1992).

It is clear that spores obtained in the laboratory do not have the same physiological state than those disseminated in the environment. Firstly, all the factors including the physiological state that may affect fungal responses are reported. Secondly, the influence of the age of the spores, the sporulation/post-sporulation conditions and the rehydration that may lead to different physiological states are illustrated. Thirdly, the reasons why, a_w for sporulation and the rehydration impact on the germination and the inactivation of fungal spores are discussed.

2. Factors that affect fungal responses

2.1. Environmental factors

Most studies in predictive mycology have been concerned with the effect of environmental factors, principally water activity, temperature, pH, modified atmospheres on fungal growth and production of mycotoxins, (Fig. 1). Comparatively, other studies on the effect of preservatives, inhibitors and media are scarce. However, the latter factor is of paramount importance for assessing the influence of the other factors on mycotoxin production. In fact the production of mycotoxins greatly depends upon the composition of the media (for example Northolt et al., 1976). In addition there have been indications that the composition of the substrate may have a profound influence on the minimum a_w for mycotoxin production (Northolt, 1979). Accordingly, the effect of environmental factors on production of mycotoxins has been assessed on grains in many studies. For example, barley grains and corn were used for studying the effect of a_w and temperature on ochratoxin production by *Aspergillus ochraceus* (Ramos et al., 1998) and the effect of modified atmospheres and a_w on fumonisin B_1 production by *Fusarium verticillioides* and *F. proliferatum* (Samapundo, 2007a,b), respectively.

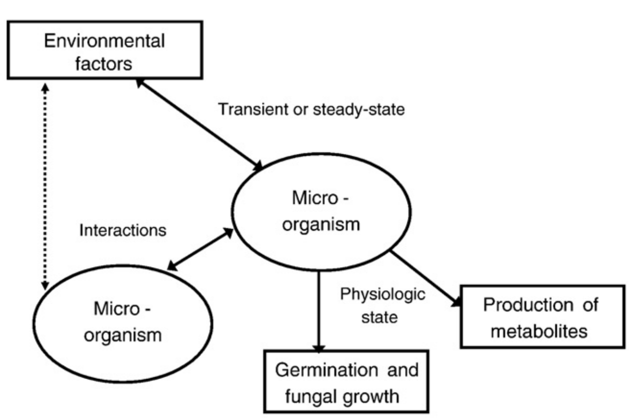

Fig. 1. Factors that affect fungal responses in food and in the environment.

Because some moulds belonging to the three main genera *Aspergillus*, *Fusarium* and *Penicillium* are responsible for mycotoxin production, it is necessary to stress at this stage the effect of environmental factors on growth and the production of mycotoxins. Mycotoxins are secondary metabolites whose production is not or partially associated with growth. The biosynthetic pathways responsible for mycotoxin production are regulated generally at the protein synthesis level depending upon the environmental conditions. They are also coordinated to the main metabolic pathways responsible for growth and production of primary metabolites. It has been demonstrated that optimum conditions for OTA production by *Aspergillus carbonarius* are very different from those for growth (Mitchell et al., 2004). It was also reported that the optimal temperature for growth of *Fusarium* differed from that for fumonisin production (Samapundo et al., 2005), thus suggesting that the metabolic pathways responsible for mycotoxin production and for growth depended in a different way from the environmental conditions.

2.2. Transients and steady-state

Regulation of the metabolic pathways is a strategy used by microorganisms to adapt to frequent changes in their environments. Unfortunately, very little information on the modelling of fungal growth or germination under fluctuating conditions is available. In the literature, most of the studies have been dedicated to the effect of steady-state environmental factors. The lack of experimental devices allowing automatic monitoring of growth and germination, in addition to the use of solid media may explain this shortage of experiments carried out under transient conditions.

Steady-state is a very poor assumption in the environment where non constant conditions prevailed. For example the effects of fluctuating moisture and temperature conditions on growth and viability of fungi in building materials were investigated (Pasanen et al., 2000). It has been stated that moisture conditions on the surface were critical for development of fungal growth in a material, because fungi grow on the surface but the medium can also serve as a reservoir of water. Therefore, it appeared that mass transfer phenomena should be taken into account for explaining the experimental results. Fluctuating conditions have been also examined in the fields of plant pathology. The effect of temperature on the length of the incubation period of rose powdery mildew was studied (Xu, 1999). It was shown that models derived from constant temperature experiments give accurate predictions when they are used to predict the development under fluctuating temperatures. However, it should be underlined that fungal responses to changes in the environmental conditions may also depend on the nature of these changes, either abrupt (shift) changes or gradual (linear) changes.

2.3. Interactions

Two kinds of interactions may be considered, i)interactions between fungi and the environment and ii)interactions between fungi and other microorganisms. Due to utilisation of substrates of the medium and production of metabolites, fungal growth results in changes in the environmental conditions. By considering a pure culture whose mycelium is developing on an ever "new" medium, this phenomenon can be ignored. But, due to competition for the substrate and inhibition by products, interspecific interactions should be taken into account. Interactions between different species are relevant in the domain of ecophysiology. The impact of environment and interspecific interactions between spoilage fungi and *Aspergillus ochaceus* on growth and ochratoxin production was evaluated in maize grain (Lee and Magan, 2000). It was shown that the Index of Dominance (Magan and Lacey, 1984) was dependent on the prevailing