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Modeling red cabbage seed extract effect on *Penicillium corylophilum*: Relationship between germination time, individual and population lag time

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ABSTRACT

The inhibitory effect of a red cabbage seed extract on germination time, individual (single spore) and population lag time of *Penicillium corylophilum* was studied. First, to compare the biological variability of single spore germination and lag times under stressful conditions, data were collected at levels of red cabbage seed extract varying from 0 to 10 mg/g (150 spores observed in each trial of germination, ca 50 spores in each individual lag experiment). Experiments were performed on malt agar at 25 °C, pH 5.2, a_w 0.99. The data, without any transformation, were statistically analyzed; several probability distribution functions were used to fit the cumulated germination times and the individual lag times of spores. In both cases, the best fit was obtained with the Normal distribution. In parallel, lag times at the population level (ca 2000 spores per trial) were collected for the same range of plant extract. Not surprisingly, the difference between individual and population lag times could be explained by a stochastic process. More interestingly, it was shown that under stressful conditions, the population lag time did not correspond to the time required for germination of 95% of spores, but to a much longer time. Finally, it was deduced from the statistical analysis, completed by microscopic observations, that the plant extract affected mainly the hyphal elongation (and then the lag time) and not the germination. Next, secondary models were developed to quantify the effect of red cabbage seed extract on the median of germination times, individual and population lag times. The Minimum Inhibitory Concentrations (MICs) were estimated. It was shown that the red cabbage seed extract MIC for *P. corylophilum* lag time did not depend on the inoculum load. Application of the secondary models allowed us to conclude that under the conditions of our experiment, the addition of 10 mg/g of red cabbage seed extract enabled extension of lag time to two weeks.

1. Introduction

Molds are able to grow within a large range of food products on which they subsequently may cause spoilage (Pitt and Hocking, 2009b). Mold spoilage is a multistage phenomenon that begins with contamination of foods by one or a few spores, in most of the cases, and ends with the formation of visible mycelia after sequenced steps of growth (Dagnas and Membré, 2013; Horner and Anagnostopoulos, 1973).

Aiming at predicting the mold-free shelf life of food products, most studies in the literature were based on combining mycelium growth rate and lag time, with the latter being recognized as the most important contributing factor in the determination of shelf-life (Gougouli et al., 2011; Huchet et al., 2013). In the literature, studies on "lag time" include studies on germination time (Dantigny et al., 2005; Gougouli and Koutsoumanis, 2012; Kalai et al., 2014), individual lag time (Burgain et al., 2013) and/or population lag time (Astoreca et al., 2012; Garcia et al., 2010; Membré and Kubaczka, 2000).

At a single spore level, germination time and individual lag time are linked but not equal; lag time encompasses time for germination plus the beginning of hyphal elongation. That has been demonstrated by Gougouli and Koutsoumanis (2013) in their study on the effect of storage temperature on the kinetic behavior of *Aspergillus niger* and *Penicillium expansum* individual spores. In this latter study, it was also shown that the lag time variability of single spores was mainly due to the germination variability, results which might be explained by the biological complexity of the germination process: spore activation, spore swelling, germ tube formation (d'Enfert, 1997). In Fig. 1, an attempt to summarize the spoilage phenomenon is presented. Spoilage takes places when, after contamination, there is the formation of a visible mycelia. The time to visible growth includes the lag and the beginning of the linear growth (Gougouli et al., 2011); the lag includes germination and exponential growth (Gougouli and Koutsoumanis,

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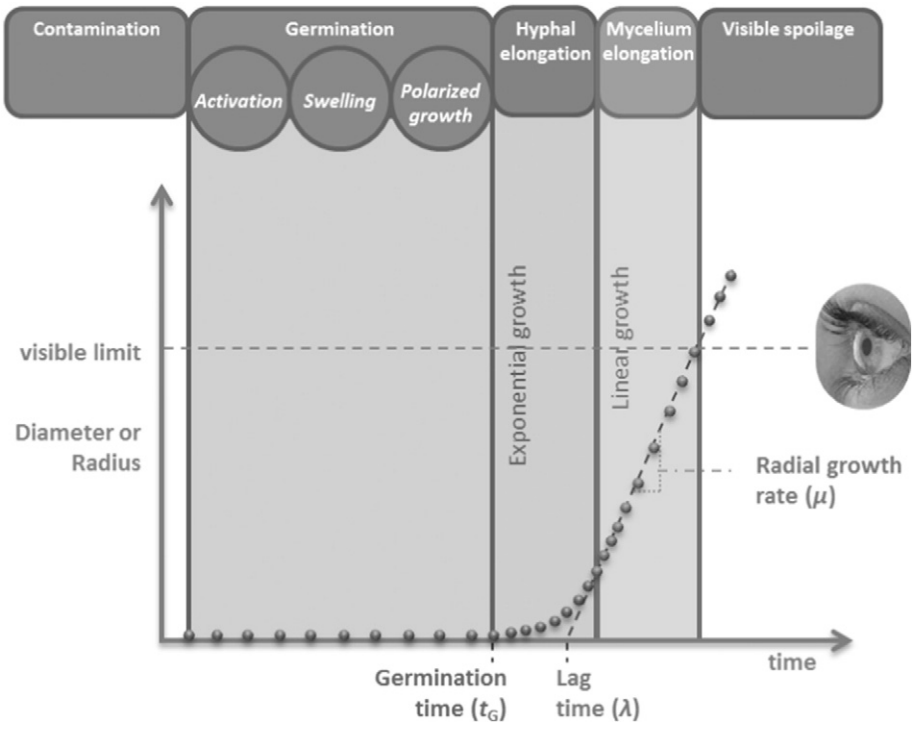

Fig. 1. Schematic representation of the biological processes involved in mold spoilage with the associated kinetic parameters: After contamination, the spore may germinate; then, the newly formed germ tube grows to form a mycelium. Spoilage happens when this mycelium reaches a visible limit. Adapted from Gougouli and Koutsoumanis (2013).

2013); last the germination includes activation, swelling and polarized growth (d'Enfert, 1997).

In terms of quantification towards shelf-life prediction, germination time has been extensively modeled with development of primary and secondary models (Dantigny et al., 2013; Deschuyffeleer et al., 2013), but the documentation on individual lag time is still missing (Gougouli and Koutsoumanis, 2013).

Nevertheless, so far, most of the mold growth studies have been conducted with inocula originated from a high number of spores (Dagnas et al., 2014; Gougouli and Koutsoumanis, 2010; Huchet et al., 2013; Marín et al., 2009; Morales et al., 2008; Sautour et al., 2003). In these cases, the population lag time is dominated by the shortest lag times observed at the individual level. This phenomenon of lag distribution has been also called "stochastic effect"(Baranyi, 2002). It is important to keep in mind that the population lag time does not represent the actual lag time of a mycelium on a food product, since in reality the contamination occurs with a limited number of spores. Thus, it is recognized that research studies in the field of predictive mycology should move towards investigating kinetics of individual spores, rather than populations'.

A link between germination and population lag time has been observed also. For example, Dantigny et al. (2002) have reported that the population lag time corresponds to the time required for germination of all spores. This result was established in non-stressful environmental conditions, so it would be worth investigating whether it could be generalized to other conditions.

From the food manufacturer perspective, the extension of shelf life of numerous products which are susceptible to spoilage by molds is considered of great importance. For decades, food industries have prevented mold spoilage by chemical preservatives such as sorbates and benzoates (Guynot et al., 2005; Hunter and Segel, 1973). However, there is a current trend to replace these inhibitors by clean-label solutions due to consumer demand for healthier foods, without chemicals. Some of the alternative methods utilized so far for the inhibition or retardation of mold growth include biopreservation (Dalié et al., 2010; Lind et al., 2005), essential oils (Gomez-Sanchez et al., 2011; Kuorwel et al., 2011; Manso et al., 2013; Sivakumar and Bautista-Baños, 2014), and plant extracts (da Cruz Cabral et al., 2013; Negi, 2012). More recently it was shown that a water-soluble extract of red cabbage seeds (*Brassica oleracea* var. *capitata* f. *rubra*) exhibited antifungal properties against *A. niger*, *Penicillium corylophilum* and *Eurotium repens* and could be applied to extend shelf-life of bakery products (Gordien et al., 2013). However for such a compound, the minimum concentration that inhibits mold growth requires further study.

In summary, to progress towards accurate predictions of mold-free shelf life, there is still a need for studies focused on the link between germination time, individual lag time and population lag time. In particular, the variability of response observed when the mold spores are stressed has not been yet fully elucidated. Likewise, among the clean-label solutions, the possibility of using plant extract to delay the mold growth has not yet been fully explored. In that context, the objective of this study was to work on the biological spore variability and specifically on the relationship between germination and lag time, under various concentrations of red cabbage seed extract. *P. corylophilum*, a mold that has been frequently associated with bakery product spoilage (Pitt and Hocking, 2009a), was chosen for conducting this piece of research.

2. Material and methods

2.1. Fungal strain

The fungal strain used in this study was isolated from a French bakery product. It was identified using phenotypic and genotypic methods by the Laboratory of Biodiversity and Microbial Ecology (LUBEM, Brest, France) as *P. corylophilum* UBOCC-A-112081. It was provided by the Culture Collection of "Université de Bretagne Occidentale" (UBOCC, Plouzané, France) and was subcultured bimonthly on Malt Extract Agar (MEA, AES Chemunex, Bruz, France).