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Physiological traits of *Penicillium glabrum* strain LCP 08.5568, a filamentous fungus isolated from bottled aromatised mineral water

L. Nevarez, V. Vasseur *, A. Le Madec, M.A. Le Bras, L. Coroller, I. Leguérinel, G. Barbier

Université Européenne de Bretagne, France
Université de Brest, EA3882 Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, IFR148 ScInBioS, ESMISAB, Technopôle de Brest Iroise, 29280 Plouzané, France

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ABSTRACT

Penicillium glabrum is a ubiquitous fungus distributed world wide. This fungus is a frequent contaminant in the food manufacturing industry. Environmental factors such as temperature, water activity and pH have a great influence on fungal development. In this study, a strain of *P. glabrum* referenced to as LCP 08.5568, has been isolated from a bottle of aromatised mineral water. The effects of temperature, a_w and pH on radial growth rate were assessed on Czapeck Yeast Agar (CYA) medium. Models derived from the cardinal model with inflection [Rosso et al., 1993 An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. J. Theor. Bio. 162, 447–463.] were used to fit the experimental data and determine for each factor, the cardinal parameters (minimum, optimum and maximum). Precise characterisation of the growth conditions for such a fungal contaminant, has an evident interest to understand and to prevent spoilage of food products.

1. Introduction

Filamentous fungi are widely distributed in the environment and responsible for numerous spoilage of food products (Pitt and Hocking, 1997; Samson et al., 2004). In addition to the economic losses associated to their visual appearance, another concern is the possibility of off-flavours and mycotoxins production. The most widespread and frequent mould spoilages of food products are caused by several genera such as *Aspergillus*, *Fusarium* or *Penicillium*. Among this last genus, *Penicillium glabrum* is a ubiquitous and cosmopolitan fungus, frequently encountered in food manufacturing industry, due to its wide presence and its important conidiation (Pitt and Hocking, 1997). This filamentous fungus has been previously isolated in a large variety of products as cheese (Northolt et al., 1980; Hocking and Faedo, 1992), maize (Mislivec and Tuite, 1970), commercially marketed chestnuts (Overy et al., 2003), rice (Kurata et al., 1968), jam (Udagawa et al., 1977) and bottled water (Cabral and Fernandez Pinto, 2002; Ancasi et al., 2006). To our knowledge, this fungal contaminant does not seem to produce any known mycotoxin that could threat the food safety and the consumer health (Pitt and Hocking, 1997). Nevertheless, no precise affirmation can be formulated due to inherent differences which could be observed among several strains of the same species. Despite its large implication in food contamination, to our knowledge, very few studies have been conducted to characterise precisely growth conditions of this species.

Growth of filamentous fungi is influenced by a variety of environmental or intrinsic factors. Temperature and water activity (a_w), for example, are recognised as the most important ones that determine the ability of moulds to grow (Dantigny et al., 2005). Other factors such as the composition and intrinsic factors of the product, especially pH, potentially influence the fungal development.

In order to analyse the physiological traits of a strain of *P. glabrum* isolated from a polyethylene terephthalate (PET) bottled aromatised mineral water, the present study aims at determining the cardinal values of this strain for temperature, a_w and pH. After investigating in solid medium, its mycelial growth response towards different factors: temperature, a_w and pH, the development of this strain was studied by using a predictive mycology approach.

For over 20 years, predictive microbiology was focused mainly on food-pathogenic bacteria (Buchanan, 1993) and despite a similar interest, modelling filamentous fungal growth has not received the same level of attention. Actually, quantification of fungal growth is more complicated because, whereas bacteria reproduce by fission and grow homogeneously through a liquid medium, filamentous fungal growth implicated the development of tree-dimensional ramified hyphae with apical growth (Gibson et al., 1994; Gibson and Hocking, 1997). Taking account of these difficulties, the predictive mycology has been developed in several studies (Dantigny et al., 2005) by adapting

* Corresponding author. Fax: +33 2 98 05 61 01.
E-mail address: Valerie.Vasseur@univ-brest.fr (V. Vasseur).

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different models used for bacterial investigations (Ratkowsky et al., 1983; Davey, 1989; Rosso et al., 1993; Baranyi et al., 1993; Miles et al., 1997). It appears that cardinal models with inflection (CMI) are suitable for modelling the effect of environmental factors on fungal growth (Rosso and Robinson, 2001). This kind of model originally developed for bacteria (Rosso et al., 1993; Rosso et al., 1995) has been successfully used to the effect of a_w on growth of several filamentous fungi such as *P. chrysogenum* or *Aspergillus flavus* (Sautour et al., 2001a).

In the present study, CMI were used to model the effects of temperature, a_w and pH on the radial growth rate of *P. glabrum*. This method allows the estimation of the cardinal values of this filamentous fungus for each tested factor. These results define the eco-physiological requirements of this fungal contaminant and have an evident interest to understand its contamination abilities in food manufacturing industry.

2. Materials and methods

2.1. Isolation and identification of the mould

Visible pellets were observed in a sealed PET bottle of aromatised mineral water. Three samples of 100 mL were shaken and filtered through sterile membrane porosity 0.45 µm (Millipore, Guyancourt, France). Visible hyphae were then transferred on Potato Dextrose Agar medium (PDA, Difco Laboratories, Detroit, MI, USA) and incubated for 7 days at 25 °C. A loopfull taken from a visible colony was examined under a microscope for morphological visualisation. Microscopic evaluation of the filamentous fungi isolated, indicated morphology similar to the description given by Pitt and Hocking for the genus *Penicillium* (phialides bearing chains of conidies) (Pitt and Hocking, 1997; Samson et al., 2004). The phialides were attached to the stipe directly, so the species produces monoverticillate penicilli and was classified in the subgenus *Aspergilloïdes*. Identification of the mould was further completed with inoculation of different media incubated at different temperatures following the reference method (Pitt, 1988). Observations were made on the morphology and diameters of the colonies and this filamentous fungus was characterised as *P. glabrum* (Wehmer) Westling. This strain was registered as LMSA 1.01.421 in "Souchothèque de Bretagne" (University of Brest, France / www.ifremer.fr/souchotheque) and LCP 08.5568 in the fungal collection of Laboratory of cryptogamy, Museun Nationnal d'Histoire Naturelle (Paris, France / www.mnhn.fr).

2.2. Media preparation and culture conditions

The effect of each factor tested experimentally on the growth of this strain of *P. glabrum*, was studied in solid cultures using inoculum consisted in conidia harvested from 7 days-old grown in PDA medium at 25 °C, 0.99 a_w and pH 5.5. Conidia were suspended in 1 mL of sterile water with 0.01% Tween 80 (Sigma-Aldrich, Saint Louis, MO, USA). One drop of inoculum containing 10^4 spores/mL, was applied with thin pipette, on two points equidistant from the center and the edge of Petri dish that contained the Czapeck Yeast Agar medium (CYA).

2.2.1. Temperature investigations

Standard CYA medium was used and contained 3% sucrose, 0.5% yeast extract, 0.1% K_2HPO_4, 1.5% agar and 1% Czapek concentrate (5% KCl, 30% $NaNO_3$, 5% $MgSO_4{\cdot}7H_2O$, 0.01% $FeSO_4{\cdot}7H_2O$ and 0.01% $CuSO_4{\cdot}7H_2O$). pH and a_w were respectively measured at 6.8 and 0.99. After inoculation of 12 replicates (6 plates), for each condition tested, media were then incubated for 7 days at temperatures in the range 5–45 °C.

Table 1
Volumes of sterile H_3PO_4 5 M, H_3PO_4 2 M and NaOH 1 M, added to 200 mL of CYA medium to adjust the pH

pH values	H_3PO_4 5 M (mL)	H_3PO_4 2 M (mL)	NaOH 1 M (mL)
0.5	16.0	–	–
1.0	5.4	–	–
2.0	1.10	–	–
3.0	–[a]	1.65	–
4.0	–	1.10	–
5.0	–	0.60	–
6.0	–	0.35	–
7.0	–	0.05	–
8.0	–	–	0.65
9.0	–	–	1.55
10.0	–	–	3.35
11.0	–	–	5.10

[a] No addition of solute.

2.2.2. Water activity investigations

CYA media were adjusted to various a_w from 0.79 to 0.99 by substituting a part of water by glycerol (w/w) according to the relation of Langmuir (Lerici et al., 1996): M (water (g)/glycerol (g)) = 0.236 a_w / (1 − 0.99 a_w). Inoculations were realised, as described previously except that inoculum was only applied in one point per plate. Triplicate plates were inoculated for most a_w tested (0.79, 0.81, 0.83, 0.85, 0.87, 0.89, 0.91, 0.92, 0.93, 0.94) and for highest values (0.95, 0.96, 0.97, 0.98 and 0.99), 8 replicated plates were realised. The different media were incubated at 25 °C for 7 days. During the experiments, a_w of each medium was stabilised by placing Petri dishes in 1.5 L closed boxes with a glycerol–water solution of the same a_w as the medium (Sautour et al., 2001b). Stability of the different media was also controlled by assessing a_w with FA-st/1 (CBX Scientific Instruments, Romans, France).

2.2.3. pH investigations

Cultures of *P. glabrum* strain LCP 08.5568 were realised in different CYA media with pH adjusted to each experimental condition. Precise volumes of sterile H_3PO_4 5 M, H_3PO_4 2 M and NaOH 1 M, were added respectively for pH 0.5–2.0, pH 3.0–7.0 and for pH 8.0–11.0 (Table 1). The adjusted media from pH 0.5 to 11.0 were inoculated as previously described using 8 replicates (4 plates) for each conditions tested. The different media were then incubated at 25 °C for 7 days. The pH values of each medium used, was also measured after 7 days of culture in order to confirm their stability.

2.3. Growth rate calculation

Each factor was studied individually at 5 levels of temperature, 12 levels of a_w and 11 levels of pH containing for each level 12, 3 or 8 and 8 replicates respectively. The radius of the colony (mm) was measured in two directions at right angle and the mean was plotted against time (d). The radial growth rate µ (mm d^{-1}) was defined as the slope of the straight line.

2.4. Model equations

The relationship between the growth rate (μ) and the 3 environmental factors tested (temperature, a_w and pH) were assessed using the equations described below. The equations are based on the cardinal model with inflection (CMI) approach. For temperature the CMI originally developed by Rosso et al. (1993) was used

$$\mu(T) = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)\right]}. \quad (1)$$