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

Laurent Nevarez, Valérie Vasseur, A. Le Madec, M. A. Le Bras, Louis Coroller, Ivan Leguérinel, Georges Barbier

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.

[*] Corresponding author. Fax : +33 2 98 05 61 01

E-mail address: laurent.nevarez@univ-brest.fr

Abstract

Penicillium glabrum is an 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.

Keywords: *Penicillium glabrum*, predictive mycology, food spoilage, fungal growth, temperature, water activity, pH, cardinal values, mineral water

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 an 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 heath (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 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 has 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 *Penicillium 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

136 0,01% Tween 80 (Sigma-Aldrich, Saint Louis, MO, USA). One drop of inoculum containing 10^4
137 spores /ml, was applied with thin pipette, on two points equidistant from the center and the edge of
138 Petri dish that contained the Czapeck Yeast Agar medium (CYA).
139
140 Temperature investigations: standard CYA medium was used and contained 3 % sucrose, 0.5
141 % yeast extract, 0.1 % K_2HPO_4, 1.5 % agar and 1 % Czapek concentrate (5 % KCl, 30 % $NaNO_3$, 5
142 % $MgSO_4$, 7 H_2O, 0.01 % $FeSO_4$. 7 H_2O and 0.01 % $CuSO_4$. 7 H_2O). pH and a_w were respectively
143 measured at 6.8 and 0.99. After inoculation of 12 replicates (6 plates), for each condition tested,
144 media were then incubated for 7 days at temperatures in the ranges 5-45 °C.
145 Water activity investigations: CYA media were adjusted to various a_w from 0.79 to 0.99 by
146 substituting a part of water by glycerol (w/w) according to the relation of Langmuir (Lerici et al.,
147 1996): M (water(g) / glycerol (g)) = 0.236 a_w / (1-0.99 a_w). Inoculations were realised, as described
148 previously except that inoculum was only applied in one point per plate. Triplicate plates were
149 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
150 highest values (0.95, 0.96, 0.97, 0.98 and 0.99), 8 replicated plates were realised. The different
151 media were incubated at 25 °C for 7 days. During the experiments, a_w of each medium was
152 stabilised by placing Petri dishes in 1,5 l closed boxes with a glycerol-water solution of the same a_w
153 as the medium (Sautour et al., 2001b). Stability of the different media was also controlled by
154 assessing a_w with FA-st/1 (CBX Scientific Instruments, Romans, France).
155 pH investigations: cultures of *P. glabrum* strain LCP 08.5568 were realised in different CYA
156 media with pH adjusted to each experimental condition. Precise volumes of sterile H_3PO_4 5M,
157 H_3PO_4 2M and NaOH 1M, were added respectively for pH 0.5-2.0, pH 3.0-7.0 and for pH 8.0-11.0
158 (Table 1). The adjusted media from pH 0.5 to 11.0 were inoculated as previously described using 8
159 replicates (4 plates) for each conditions tested. The different media were then incubated at 25 °C for
160 7 days. The pH values of each medium used, was also measured after 7 days of culture in order to
161 confirm their stability.
162

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

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

The CMI modified by Sautour et al. (2001a) was used for a_w

$$\mu(a_w) = \frac{\mu_{opt}(a_w - 1)(a_w - a_{w\,min})^2}{(a_{w\,opt} - a_{w\,min})\left[(a_{w\,opt} - a_{w\,min})(a_w - a_{w\,opt}) - (a_{w\,opt} - 1)(a_{w\,opt} + a_{w\,min} - 2a_w)\right]} \quad \textbf{(2)}$$

For pH the CMI described by Rosso et al. (1995) was used

$$\mu(pH) = \frac{\mu_{opt}(pH - pH_{min})(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_{opt})^2} \quad \textbf{(3)}$$

2.5. Model fitting and determination of cardinal conditions

Before fitting, a square-root transformation was performed to homogenise the variance of the experimental growth rate (Dantigny and Bensoussan, 2008). Cardinal values were determined by iterative calculation based on minimising the sum of squares of the residual values (SSR) with NLINFIT function of MATLAB R2008A (The Math-works). 95 % confidence intervals were obtained by using traditional methods based on a linear approximation with NLPARCI function in MATLAB. For each factor modeled the Root Mean Square Error (RMSE) was calculated in order to measure the goodness of fit of each model. According to Ratkowsky (2004), this criterion should be preferred to the regression coefficient r^2 for non-linear models.

3. Results and discussion

3.1. Effect of temperature

The experimental growth results obtained in different conditions of temperature after 7 days of culture in CYA medium, were used to model the growth of this strain according to equation 1 of the CMI (Fig. 1). The minimal, optimal and maximal temperatures were estimated to 6.6, 24.3 and 33.8 °C respectively (Table 2). A good quality of fit was obtained as suggested by the low RMSE value of 0.077.

The optimal temperature around 24 °C for this strain of *P. glabrum*, is in accordance with literature data for this species that describes also an optimum around 25° C (Pitt and Hocking, 1997; Sinigaglia et al., 1998). Similar results were also reported in studies related to *P. chrysogenum* (Gonzalez et al., 1988), *P. expansum* (Lahlali et al., 2005), *P. digitatum* and *P. italicum* (Plaza et al., 2003). Meanwhile, optimal temperature varied slightly from 20 °C for *P. polonicum* (Nunez et al., 2000) to 30 °C for *P. citrinum* (Gonzalez et al., 1988; Montani et al., 1988). The range of

temperatures from 20 to 30 °C is frequently encountered in food manufacturing industries and may be also reached in non-refrigerated storage of some products as bottles of aromatised mineral water.

The maximal temperature condition for this filamentous fungus was close to 34 °C which is in accordance with some data reporting the absence of growth above 37 °C (Pitt and Hocking, 1997) but differs from others reporting a fungal growth up to 40 °C (Sinigaglia et al., 1998). Results obtained for this strain of *P. glabrum* also showed the minimal temperature condition of 7 ° C which may differ from literature data, reporting a slight development of microcolonies up to 4 mm after several days at 5.0 °C (Pitt, 1988).

3.2. Effect of water activity

As reported previously (Sautour et al., 2001a), a gradual increase in the radial growth rate was exhibited at sub optimal water activities. In contrast a sharp decrease in the growth rate was observed was noticed between the optimum and 1 (Fig 2). The minimal and the optimal a_w were estimated to 0.820 and 0.983 respectively (Table 2). A good quality of fit was obtained as suggested by the low RMSE value of 0.078.

The minimal a_w for this stain 0.82 was less than the minimal value 0.88 a_w reported previously in another study for this species (Sinigaglia et al., 1998). Filamentous fungi are among the organisms capable of growing below 0.90 (Pitt and Hocking, 1997) and most *Penicillium* species presented a minimal a_w between 0.82 and 0.86 (Northolt et al., 1995). Similar a_w conditions are tolerated by some xerophilic *Penicillium* species as *P. chrysogenum* growing above 0.78-0.81 (Hocking and Pitt, 1979; Sautour et al., 2001b) or *P. roqueforti* growing from 0.82 (Gock et al., 2003). The minimal a_w for growth obtained in our study was lower than results obtained from *P. hordei*, *P.aurantiogriseum* (Marin et al., 1998) and *P. olsonii* (Lopez-Diaz et al., 2002). Several other *Penicillium* species showed minimal a_w around 0.90 as *P. expansum* (Lahlali et al., 2005), *P. verrucosum* (Cairns-Fuller et al., 2005) or *P. italicum* and *P. digitatum* (Lahlali et al., 2006).

The estimated optimal a_w condition was 0.98 which is in accordance with literature data on this species, reporting also the same value (Sinigaglia et al., 1998). Most *Penicillium* species also showed similar response to medium a_w and optimal conditions around 0.97-0.98 (Hocking and Pitt, 1979). For example, the optimal a_w for growth was estimated to 0.98 for *P. chrysogenum* using the same CMI than that described by eq (2) in this study (Sautour et al., 2001a).

3.3. Effect of pH

Radial growth rate was almost constant in the pH range 2.0-7.0 (Fig. 3). Experimental data were fitted by the model eq (3) rather satisfactorily, as suggested by the low RMSE value, 0.089 (Table 2). The optimal and the maximal pH values were 5.5 and 11.2 respectively but the minimal pH was estimated in the negative range at -2.1. Application of another model (Zwietering et al., 1992), gave with even a higher RMSE, aberrant minimal pH when applied to the same data (results not shown).

These results obtained showed the difficulty to model the growth response of this strain under very acidic conditions. Future studies should be directed to find a convenient model that fits correctly the pH growth response of this filamentous fungus. Nevertheless, the experimental data obtained gave some precious information as no fungal growth was observed at pH 0.5 which indicate that the minimal pH conditions seemed to be between 0.5 and 1.0. It differs from previous description of this species reporting a minimal pH value of 2.0 (Sinigaglia et al., 1998).

From the modeling of the pH response, the optimal pH condition of 5.5 and the large tolerance observed for this filamentous fungus towards a large range of pH conditions, were in accordance with literature describing optimal growth rate of many filamentous fungi around pH 5.0 (Pitt and Hocking, 1997) and in the pH range 3.0 to 8.0 (Wheeler et al., 1991). As reported in literature, sensibility of this strain of *P. glabrum* towards alkaline conditions appeared higher than

acidic ones. The pH response observed for this strain could be compared with other pH studies on several *Penicillium* species conducted in solid medium (Wheeler et al., 1991). From these results, *P. citreonigrum* seemed to present a similar response than *P. glabrum* and its optimum was defined at pH 4.4-6.3. The results obtained in our study were also similar to those observed for *P. jensenii* (Sacks et al., 1986) as this filamentous fungi seemed not very sensitive to pH range from 3.5 to 7.1 but showed an important fungal growth decrease just below at pH 3.3. *P. roqueforti* also showed a large tolerance to several pH values tested from 4.5 to 7.5 (Gock et al., 2003). In a large range of values, the medium pH seems to have a very low influence on the growth of this fungus as reported also for several *Penicillium* species between pH 4.0-10.0 (Thompson et al., 1993). The tolerance observed here for *P. glabrum* towards a large acid pH range may explain the presence of this species on a large variety of food products of different pH. The pH sensibility increase in the alkaline range until the estimated maximal pH value of 11.18. This value seemed coherent with the results previously obtained on different *Penicillium* species (Wheeler et al., 1991).

Considering the good fit of the temperature and a_w models (RMSE of 0.077 and 0.078 respectively) and the estimated cardinal values, the method of CMI developed by Rosso *et al.*, seemed well adapted to analyse the effect of both factors on the growth of this strain of *P. glabrum.* The robustness of the approach of Rosso *et al.* of has been reported in a study on the effects of temperature and a_w on *Aspergillus carbonarius* growth (Tassou et al., 2007). Analysis of the results obtained with other predictive mycology methods, showed that Rosso *et al.* approach was the most adapted to model the growth of this filamentous fungus in different conditions. This method has been successfully used, for example, in *P. chrysogenum, Aspergillus flavus, A. parasiticus, A. oryzae* to model the effect of a_w on fungal growth (Rosso and Robinson, 2001; Sautour et al., 2001a). This method has also the advantage to define fungal growth rate (μ), by 4 parameters with concrete physiological meaning: optimal growth (μ_{opt}) and minimal, optimal and maximal conditions for each factor tested. Thus application and fitting of these models allowed to calculate

297 these parameters for each factor tested. For this reason, the use of CMI method has been well
298 adapted to provide physiological characteristics of this strain of *P. glabrum* for temperature and a_w.
299 Nevertheless some difficulties were shown to fit the experimental data with the CMI in very acidic
300 conditions. Cardinal models are versatile tools that can adapt to the different shapes of the curves μ
301 vs temperature and μ *vs* a_w. There are no reason that could prevent the CMI from fitting data pH *vs*
302 pH with a good accuracy. The lack of fit that was demonstrated under acidic pH may be due to no
303 data were available between pH 0.5 and 1, but this should be verified.
304
305 The different results obtained in this study provide useful background to improve
306 characterisation of the strain of *P. glabrum* isolated from PET bottled aromatised mineral water.
307 The microbiological quality of bottled mineral water is of great interest but has not been very
308 largely investigated. In addition to indigenous bacteria that do not induce any risk to public heath,
309 mineral water may sometimes contain contaminants as bacteria or filamentous fungi. Some authors
310 described that the fungal foreign bodies visible in the mineral water samples, were made up of
311 pellets with a diameter of 3 to 20 mm (Fujikawa et al., 1997). The most frequent fungal genera
312 isolated from mineral water were *Penicillium* followed by *Cladosporium*, *Trichoderma*,
313 *Aspergillus*, *Alternaria* and *Acremonium* (Fujikawa et al., 1997; Liceaga-Gesualdo et al., 2001;
314 Hageskal et al., 2006). Among the genus *Penicillium*, *P. citrinum* and *P. glabrum* were the 2 most
315 isolated species (Cabral and Fernandez Pinto, 2002). Although filamentous fungi in water usually
316 do not generate public health problems, nevertheless some of the fungi isolated from bottled
317 mineral water as *Alternaria alternata* and *P. citrinum* have some toxigenic potential which could
318 determine some health risk (Cabral and Fernandez Pinto, 2002).
319 The contamination of these products may be explained by microbial presence from the
320 surrounding environment when filling and capping bottles of mineral water (Fujikawa et al., 1997).
321 This last hypothesis was supplied by the fact that many filamentous fungi as some *Penicillium*
322 species disperse a large number of spores in the environment.

In our study, the strain of *P. glabrum* isolated from aromatised mineral water, seemed to have very low nutritional requirements as it can develop in visible pellets in such a poor nutritive environment with slight carbohydrate concentrations, various salts and limited oxygen concentration as only a small fraction of air is enclosed in tight sealed bottles. In literature, it was also shown that sometimes, fungal contaminants could use as nutriments, organic compounds releases during storage, from PET (Criado *et al.*, 2005), a beverage bottling material used for conditioning a large variety of commercialised water as the one which is studied here. This aromatised bottled mineral water presented a very high a_w, a pH at 7.0 and the storage of this product was often made at room temperature (around 18-25 °C). The characteristics of this aromatised mineral water may be favourable for the growth of this strain of *P. glabrum* by extrapolating its physiological requirements obtained in solid medium. Several authors have previously reported the presence of this species in commercialised water (Cabral and Fernandez Pinto, 2002; Ancasi et al., 2006). The contamination of this product by this filamentous fungus was also explained by its ubiquitous presence in the environment and its large conidiation in the atmosphere. Moreover, the physiological characteristics of this strain of *P. glabrum* seemed to present important similarities with the temperature, a_w and pH requirements of another frequent fungal contaminant of water products such as *P. citrinum* (Hocking and Pitt, 1979; Gonzalez et al., 1988; Montani et al., 1988; Wheeler et al., 1991; Comerio et al., 1998).

Precise characterisation of growth conditions of this strain of *P. glabrum* has an evident interest to understand its contamination abilities in food manufacturing industry. The influence of temperature, a_w and pH on fungal growth could be taken into account to maintain good conditions on stored product. Nevertheless these results should be considered carefully as fungal contamination of different products could also concern several other species than *P. glabrum* and may interact in a competitive or associative way.

Acknowledgements

This entire work was realised with the financial support of the Brittany region.

References

Ancasi, E.G., Carrillo, L., Benitez Ahrendts, M.R., 2006. Moulds and yeasts in bottled water and soft drinks. Rev. Argent Microbiol. 38**,** 93-96.

Baranyi, J., Roberts, T.A., McClure, P., 1993. A non-autonomous differential equation to model bacterial growth. Food Microbiol 10**,** 43-59.

Buchanan, R.L., 1993. Predictive food microbiology. Trends Food Sci Tech 4**,** 6-11.

Cabral, D., Fernandez Pinto, V.E., 2002. Fungal spoilage of bottled mineral water. Int. J. Food Microbiol. 72**,** 73-76.

Cairns-Fuller, V., Aldred, D., Magan, N., 2005. Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. J. Appl. Microbiol. 99**,** 1215-1221.

Comerio, R., Fernandez, P., V, Vaamonde, G., 1998. Influence of water activity on *Penicillium citrinum* growth and kinetics of citrinin accumulation in wheat. Int. J. Food Microbiol. 42**,** 219-223.

Criado, M.V., Fernandez, P., V, Badessari, A., Cabral, D., 2005. Conditions that regulate the growth of moulds inoculated into bottled mineral water. Int. J Food Microbiol. 99**,** 343-349.

Dantigny, P., Bensoussan, M., 2008. The logarithmic transformation should be avoided for stabilising the variance of mould growth rate. Int J Food Microbiol 121**,** 225-228.

Dantigny, P., Guilmart, A., Bensoussan, M., 2005. Basis of predictive mycology. Int. J. Food Microbiol. 100**,** 187-196.

Davey, K.R., 1989. A predictive model for combined temperature and water activity on microbial growth during the growth phase. J Appl Bacteriol. 67**,** 483-488.

Fujikawa, H., Wauke, T., Kusunoki, J., Takahashi, Y., Ohta, K., Itoh, T., 1997. Contamination of microbial foreign bodies in bottled mineral water in Tokyo, Japan. J Appl Microbiol 82**,** 287-291.

Gibson, A.M., Baranyi, J., Pitt, J.I., Eyles, M.J., Roberts, T.A., 1994. Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. Int J Food Microbiol 23**,** 419-431.

Gibson, A.M., Hocking, A.D., 1997. Advances in the predictive modelling of fungal growth in food. Trends Food Sci Tech 353-358.

Gock, M.A., Hocking, A.D., Pitt, J.I., Poulos, P.G., 2003. Influence of temperature, water activity

384 and pH on growth of some xerophilic fungi. Int. J. Food Microbiol. 81**,** 11-19.

385 Gonzalez, H.H., Resnik, S.L., Vaamonde, G., 1988. Influence of temperature on growth rate and lag
386 phase of fungi isolated from Argentine corn. Int. J. Food Microbiol. 6**,** 179-183.

387 Hageskal, G., Knutsen, A.K., Gaustad, P., de Hoog, G.S., Skaar, I., 2006. Diversity and significance
388 of mold species in Norwegian drinking water. Appl Environ. Microbiol 72**,** 7586-7593.

389 Hocking, A.D., Faedo, M., 1992. Fungi causing thread mould spoilage of vacuum packaged
390 Cheddar cheese during maturation. Int. J. Food Microbiol. 16**,** 123-130.

391 Hocking, A.D., Pitt, J.I., 1979. Water relations of some *Penicillium* species at 25 deg C. T Brit
392 mycol Soc 73**,** 141-145.

393 Kurata, H., Sakabe, F., Udagawa, S., Ichinoe, M., Suzuki, M., 1968. [A mycological examination
394 for the presence of mycotoxin-producers on the 1954-1967's stored rice grains]. Eisei
395 Shikenjo Hokoku 86**,** 183-188.

396 Lahlali, R., Serrhini, M.N., Friel, D., Jijakli, M.H., 2006. In vitro effects of water activity,
397 temperature and solutes on the growth rate of *P. italicum* Wehmer and *P. digitatum* Sacc. J.
398 Appl. Microbiol. 101**,** 628-636.

399 Lahlali, R., Serrhini, M.N., Jijakli, M.H., 2005. Studying and modelling the combined effect of
400 temperature and water activity on the growth rate of P. expansum. Int. J. Food Microbiol. 103**,**
401 315-322.

402 Lerici, C.R., Nicoli, M.C., Manzocco, L., 1996. Influenza dell'attività dell'acqua sulla tensione di
403 vapore dell'etanolo in sistemi modello alimentar. Industrie Alimentari 35**,** 13-17.

404 Liceaga-Gesualdo, A., Li-Chan, E.C.Y., Skura, B.J., 2001. Antimicrobial effect of lactoferrin digest
405 on spores of a *Penicillium sp*. isolated from bottled water. Food Res Int 34**,** 501-506.

406 Lopez-Diaz, T.M., Gonzalez, C.J., Moreno, B., Otero, A., 2002. Effect of temperature, water
407 activity, pH and some antimicrobials on the growth of *Penicillium olsonii* isolated from the
408 surface of Spanish fermented meat sausage. Food Microbiol 19**,** 1-7.

409 Marin, S., Sanchis, V., Saenz, R., Ramos, A.J., Vinas, I., Magan, N., 1998. Ecological determinants
410 for germination and growth of some *Aspergillus* and *Penicillium spp.* from maize grain. J.
411 Appl. Microbiol. 84**,** 25-36.

412 Miles, D.W., Ross, T., Olley, J., McMeekin, T.A., 1997. Development and evaluation of a
413 predictive model for the effect of temperature and water activity on the growth rate of *Vibrio*
414 *parahaemolyticus*. Int J Food Microbiol 38**,** 133-142.

415 Mislivec, P.B., Tuite, J., 1970. Species of *Penicillium* occurring in freshly-harvested and in stored
416 dent corn kernels. Mycologia. 62**,** 67-74.

417 Montani, M., Vaamonde, G., Resnik, S.L., Buera, P., 1988. Temperature influence on *Penicillium*
418 *citrinum* thom growth and citrinin accumulation kinetics. Int. J. Food Microbiol. 7**,** 115-122.

419 Northolt, M.D., Frisvad, J.C., Samson, R.A., 1995. Occurence of food-borne fungi and factors for
420 growth. In: Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. (Eds.), Introduction to
421 food borne fungi Centraalbureau voor Schimmelcultures, Baarn, pp. 243-250.

Northolt, M.D., van Egmond, H.P., Soentoro, P., Deijll, E., 1980. Fungal growth and the presence of sterigmatocystin in hard cheese. J. Assoc. Off Anal. Chem. 63**,** 115-119.

Nunez, F., Diaz, M.C., Rodriguez, M., Aranda, E., Martin, A., Asensio, M.A., 2000. Effects of substrate, water activity, and temperature on growth and verrucosidin production by *Penicillium polonicum* isolated from dry-cured ham. J. Food Prot. 63**,** 231-236.

Overy, D.P., Seifert, K.A., Savard, M.A., Frisvad, J.C., 2003. Spoilage fungi and their mycotoxins in commercially marketed chestnuts. Int J Food Microbiol 69-77.

Pitt, J.I., 1988. A laboratory guide to commun *Penicillium* species, Second edition. Food Science Australia.

Pitt, J.I., Hocking, A.D., 1997. Fungi and food spoilage, Second edition. Blackie academic and professional.

Plaza, P., Usall, J., Teixido, N., Vinas, I., 2003. Effect of water activity and temperature on germination and growth of *Penicillium digitatum, P. italicum* and *Geotrichum candidum*. J. Appl. Microbiol. 94**,** 549-554.

Ratkowsky, D.A., 2004. Model fitting and uncertainty. In: McKellar, R.C., Lu, X. (Eds.), Modeling microbial responses in food CRC Press, Boca Raton, Florida, USA, pp. 151-196.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N., Chandler, R.E., 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J Bacteriol. 154**,** 1222-1226.

Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient Model To Describe the Combined Effects of Temperature and pH on Microbial Growth. Appl Environ. Microbiol 61**,** 610-616.

Rosso, L., Lobry, J.R., Flandrois, J.P., 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. J Theor. Biol. 162**,** 447-463.

Rosso, L., Robinson, T.P., 2001. A cardinal model to describe the effect of water activity on the growth of moulds. Int J Food Microbiol 63**,** 265-273.

Sacks, L.E., King, A.D., Jr., Schade, J.E., 1986. A note of pH gradient plates for fungal growth studies. J. Appl. Bacteriol. 61**,** 235-238.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., 2004. Introduction to food and airborne fungi, seventh edition. Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.

Sautour, M., Dantigny, P., Divies, C., Bensoussan, M., 2001a. A temperature-type model for describing the relationship between fungal growth and water activity. Int J Food Microbiol 67**,** 63-9.

Sautour, M., Rouget, A., Dantigny, P., Divies, C., Bensoussan, M., 2001b. Prediction of conidial germination of *Penicillium chrysogenum* as influenced by temperature, water activity and pH. Lett Appl Microbiol 32**,** 131-4.

Sinigaglia, M., Corbo, M.R., Ciccarone, C., 1998. Influence of temperature, pH and water activity on "in vitro" inhibition of *Penicillium glabrum* (Wehmer) Westling by yeasts. Microbiol. Res. 153**,** 137-143.

461 Tassou, C.C., Panagou, E.Z., Natskoulis, P., Magan, N., 2007. Modelling the effect of temperature
462 and water activity on the growth of two ochratoxigenic strains of *Aspergillus carbonarius*
463 from Greek wine grapes. J Appl Microbiol 103**,** 2267-2276.

464 Thompson, D.P., Metevia, L., Vessel, T., 1993. Influence of pH alone and in combination with
465 phenolic antioxidants on growth and germination of mycotoxigenic species of *fusarium* and
466 *penicillium*. J Food prot 56**,** 134-138.

467 Udagawa, S.I., Kobatake, M., Kurata, H., 1977. [Re--estimation of preservation effectiveness of
468 potassium sorbate (food additive) in jams and marmalade (author's transl)]. Eisei Shikenjo
469 Hokoku 88-92.

470 Wheeler, K.A., Hurdman, B.F., Pitt, J.I., 1991. Influence of pH on the growth of some toxigenic
471 species of *Aspergillus, Penicillium* and *Fusarium*. Int. J. Food Microbiol. 12**,** 141-149.

472 Zwietering, M.H., Wijtzes, T., de Witt, J.C., van't Riet, K., 1992. A decision support system for
473 prediction of the microbial spoilage in foods. J Food prot 55**,** 973-979.
474
475