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Short communication
Predicting fungal growth: the effect of water activity on *Penicillium roqueforti*

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Abstract

The effect of water activity on the colony growth of *Penicillium roqueforti* is studied by predictive modelling techniques. Measured colony diameter growth curves are fitted to estimate the growth rate and lag phase of the curves. The colony growth rate was modelled by a quadratic function of transformed water activity (a_w) values, as suggested by Baranyi et al. (Food Microbiol. 10 (1993) 43–59). The lag time was modelled as a function of water activity, by means of the sum of a constant term and a hyperboloid function of a_w raised to the second power. The lag-phase of *Penicillium roqueforti* was found insensitive to the water activity in the range of its higher ($a_w > 0.92$) values. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Penicillia play an important role in ripening mould fermented food. The species of the genus *Penicillium* are exclusively used in the production of mould fermented food in Europe: *P. roqueforti* for blue cheeses e.g. Roquefort, Gorgonzola, Stilton, Gammelost; *P. camemberti* for white cheeses e.g. Brie and Camembert; and *P. nalgiovense* and *P.*

chrysogenum for meat products (salami-type sausages) (Geisen, 1993).

Penicillium species cause food spoilage, too. Penicillia were the most frequent in hard, semi-hard and semi-soft cheeses as associated mycoflora. A total of 91% of the strains isolated from cheese surface by Lund et al. (1995) were penicillia. Similarly, Jesenská (1987) identified *Penicillia* as the most common species (92%) in the surface mycoflora of sausages produced in Slovakia. She has also reported on the proportion of penicillia strains isolated from dried milk powder, where they represented 45% of almost 44 000 isolated strains. Many species of penicillia are able to attack fruits; as much

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as 30% of all fruit decay can be attributed to this genus (Hayes, 1993).

Although *Penicillium roqueforti* is able to produce several mycotoxins, such as PR-toxin, eremofortin, roquefortin C, mycophenolic acid, patulin, penicillic acid and isofumigaclavins, these toxins are unstable or of low concentration. Some strains that are considered as GRAS ('generally as safe') are used, as starters, for cheese production.

Our study was carried out to apply the modelling methods of Baranyi et al. (1993) and Gibson et al. (1994) to the growth data of *Penicillium roqueforti*. The objective was to provide quantitative information on the effect of water activity on mould growth.

A few studies have already been published on the effect of glycerol on the germination and growth of *Penicillium roqueforti* (Gervais et al., 1988; Gervais, 1990). The water activity of a food product, however, is commonly reduced by the addition of NaCl, this is why we chose this latter humectant. Models resulting from this study would allow a food manufacturer to predict the germination and growth of *P. roqueforti* with respect to water activity in roquefort-type cheeses. The results could also be used to prevent the growth of *P. roqueforti* as a spoilage mould in other food products.

2. Materials and methods

2.1. Strain

Penicillium roqueforti PR3 was used throughout the study. The strain, originally from Chr. Hansen A/S (Horsholm, Denmark), is used to produce roquefort-type cheese in Slovakia.

2.2. Media and cultivation

Sabouraud Agar (Fluka, Buchs, Switzerland) was used as a basal medium. Media of different water activity (a_w) were prepared by adding an adequate volume of sterile saturated solution of NaCl. The actual a_w values were determined by Novasina TH 200 (Novasina, Pfäffikon, Switzerland). Thermoconstanter TH 200 was calibrated against all six saturated salt solutions in the range of a_w 0.98 to 0.11. The pH of the medium was adjusted to the values of

4, 5, 6, 7 and 8 with hydrochloric acid or NaOH solutions.

The spores were inoculated by a touch of bacteriological loop. The cultivation was carried out on two parallel petri slides (diameter 170 mm) containing agar with the appropriate a_w and pH. The inoculated petri dishes were incubated at $25 \pm 1^\circ\text{C}$, in plastic boxes. No change of a_w of the media was detected after 1 and 5 days of the experiment.

2.3. Mathematical and statistical methods

The diameters (y , expressed in mm) of the two parallel colonies were measured in vertical and horizontal directions daily, at the same time (t , expressed in days). The average value of the four diameters was used in modelling. The growth function of Baranyi et al. (1993) was applied which was able to model both the limited and the limitless growth, i.e. curves with or without an upper asymptote (Fig. 1).

The maximum colony growth rate (g), and lag-phase (λ), estimated by the above curve fitting, were modelled as a function of water activity and pH values. A useful transformation of water activity was applied, as introduced by Gibson et al. (1994):

$$b_w = \sqrt{1 - a_w}$$

The natural logarithm of the colony growth rates was modelled by a two-variate quadratic function (model 1):

$$\ln g = C_0 + C_1 b_w + C_2 b_w^2 + C_3 \text{pH} + C_4 \text{pH}^2 + C_5 b_w \text{pH} \quad (1)$$

The coefficients $C_0 \dots C_5$ were estimated by linear regression.

The special case, when the coefficients of the pH-terms are set to be zero, will be called model 2. The question, whether the pH-terms can be omitted from the model, was analyzed by an F -test comparing the respective residual mean squares of model 1 and model 2 (see Table 1, and the plots on model 2, in Fig. 2 and Fig. 3).

The square root of the estimated lag parameters, $L = \sqrt{\lambda}$, can be seen plotted against the respective a_w values in Fig. 4. The variance-damping square root transformation is frequently used in regression analy-

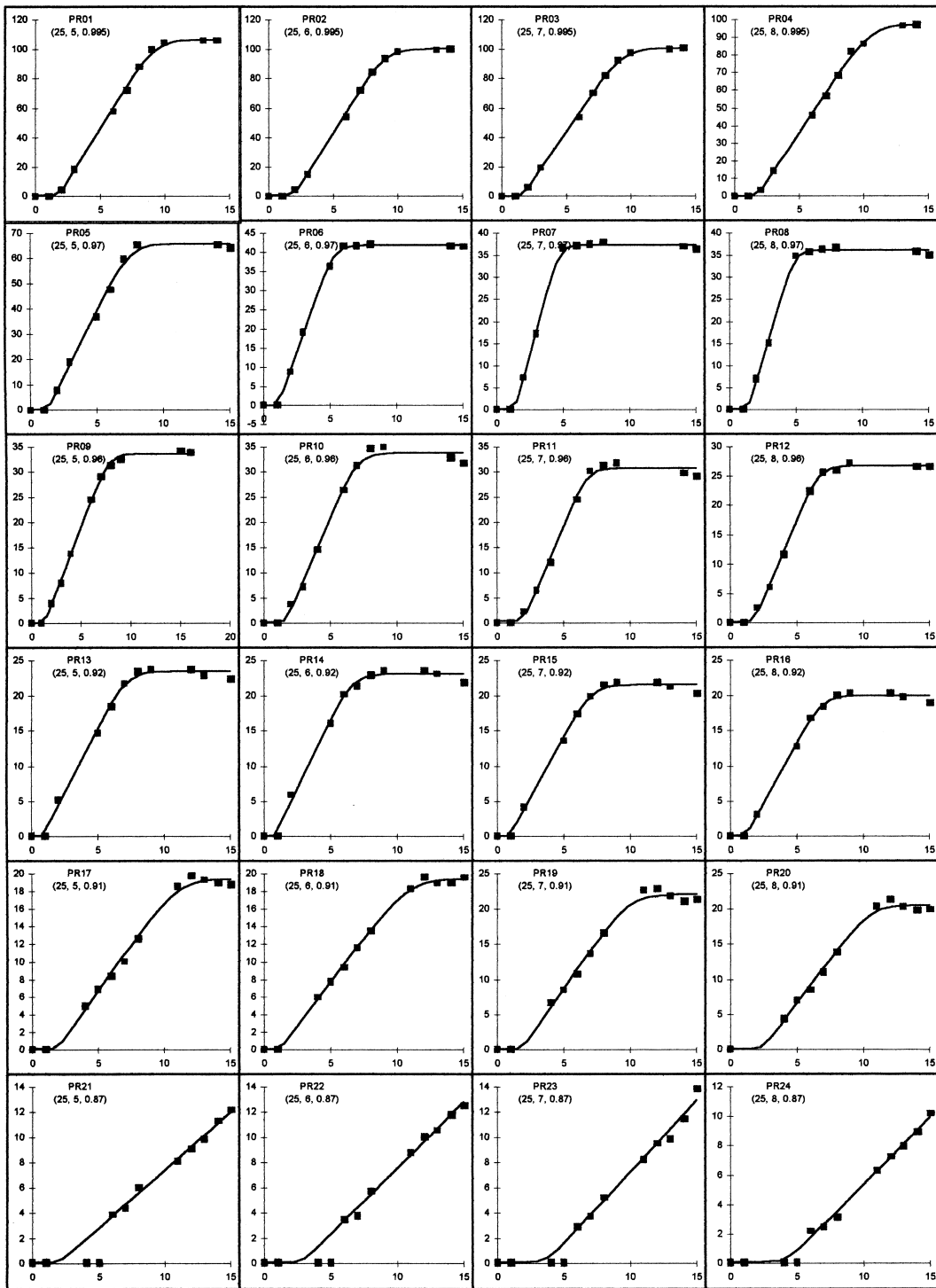


Fig. 1. Growth curves of *Penicillium roqueforti* PR3 under four pH values (5, 6, 7 and 8, varying horizontally in the plot) and six different a_w values of water activity (0.995, 0.97, 0.96, 0.92, 0.91 and 0.87, varying vertically).

sis and, empirically, we found it appropriate for our modelling purposes, too. The L values were similar for $a_w > 0.92$, but increased dramatically for lower a_w . This is why we assumed the model

$$L = L_0 + \left(\frac{a}{a_w - a_w(\min)} \right)^n \quad (2)$$

to describe the effect of a_w on the square root of the lag time, where a , L_0 , $a_w(\min)$ and n are model parameters (positive numbers). From the structure of the model, it can be seen that, as a_w decreases and approaches $a_w(\min)$, the lag will be bigger and bigger, converging to infinity.

3. Results and discussion

The growth data modelled in this work includes growth curves for *Penicillium roqueforti* PR3 at six a_w values and four pH values (Fig. 1). The colony diameter 'growth curves' were typical of microbial growth with a lag-phase, followed by linear phase and an upper asymptote.

The F -test applied to the two quadratic functions (model 1 and 2) describing the observed growth rates (see Table 1) shows that model 2, with b_w as the only variate, explains the variance of the growth rate satisfactorily, because disregarding the effect of pH in the range of 5–8, does not change the goodness of fit significantly. Therefore, it is sufficient to consider the colony growth rates of *P. roqueforti* as a function of water activity. This simplified model is demonstrated in Fig. 2 and Fig. 3. The optimum a_w value 0.998 for growth, and the respective colony growth rate of 13.4 mm/day, were calculated using the procedure of Gibson et al. (1994).

The modelling of the lag phase was based on Eq. (2). The straightforward application of that model was not suitable for practical fitting purposes because the estimation of the minimum water activity value,

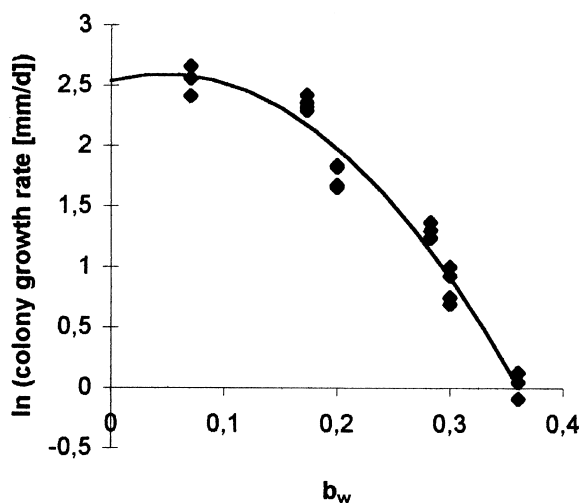


Fig. 2. Fitting, by a parabola, the natural logarithm of the colony growth rate (g) of *Penicillium roqueforti* PR3 against the transformed water activity values, $b_w = \sqrt{1 - a_w}$. Diamond: observed 'ln g ' values as obtained from the growth curves of Fig. 1. Continuous line: fitted parabola.

$a_w(\min)$, and the curvature parameter, n , was numerically unstable (high standard errors obtained for these parameters). Therefore, $a_w(\min)$ was determined experimentally on bread crusts placed over the salt solutions with the several standard a_w values at 25°C. The obtained minimum water activity was $a_w(\min) = 0.84$. Magnan and Lacey (1984) found a similar $a_w(\min) = 0.83$ at 25°C. The curvature parameter, n , was fixed empirically as $n = 2$, so there remained only two parameters to fit, L_0 and a , when regressing the lag-observations to a_w (Table 2 and Fig. 4).

Our finding, that the lag of *P. roqueforti* (therefore the time to its visible growth, too) is relatively stable for $a_w > 0.92$ but dramatically increasing for $a_w < 0.92$ values, can be an advantage for the application of *P. roqueforti* as a starter culture in the ripening of blue-veined cheeses. The final a_w values of

Table 1
Coefficients and statistical parameters for the growth models of *Penicillium roqueforti* PR3

Model	C_0	C_1	C_2	C_3	C_4	C_5	SS (df)	$F = (SS_2/df_2) / (SS_1/df_1)$
(1)	1.173	2.498	-30.510	1.121	-0.040	-0.644	0.577 (18)	
(2)	2.534	2.498	-26.324				0.637 (21)	0.946

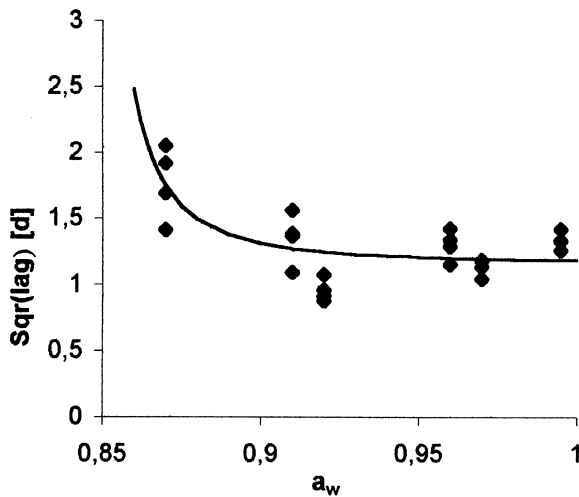


Fig. 3. Resultant model for the colony growth rate (g) of *Penicillium roqueforti* PR3 as a function of water activity. Diamond: observed 'g' values as obtained from the growth curves of Fig. 1. Continuous line: obtained by transforming the fitted parabola of Fig. 2 back to the appropriate scale.

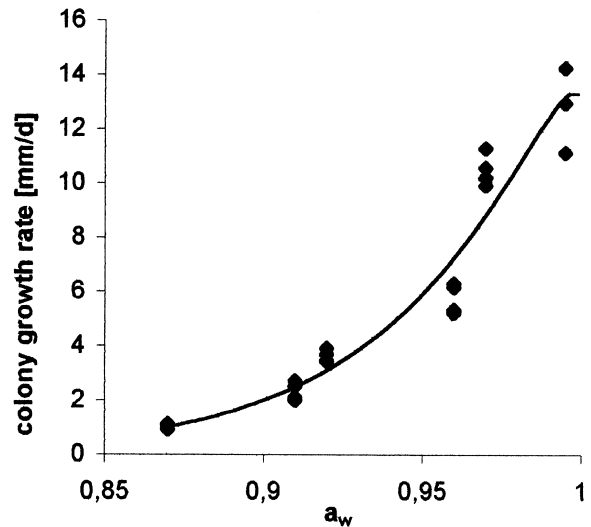


Fig. 4. Square root of the lag, λ , of *Penicillium roqueforti* modelled as a function of water activity. The pH values varied between 5 and 8 for the observed values. Diamond: observed $L = \sqrt{\lambda}$ values as obtained from the growth curves of Fig. 1. Continuous line: fitted curve as described by Eq. (2).

roquefort-type cheeses, that are in the range of 0.91–0.94 (Fernández-Salguero et al., 1986; Valík and Görner, 1994), allow *P. roqueforti* to germinate as quickly as possible and grow during the whole phase of cheese processing including ripening.

Predictive microbiology studies the behaviour of micro-organisms under different physical, physico-chemical or chemical conditions such as temperature, water activity, pH, or antimicrobial compounds. It can help the identification of critical points of the production and distribution process, and the optimisation of production and distribution chains (Zwietering et al., 1990). The modelling approach introduced in this paper is in accord to these objectives and, we feel, can contribute to improve microbial safety and/or shelf life of food products.

Table 2
Results of the nonlinear regression fitting the lag

Parameter	Estimate	S.E.	<i>t</i> -value	<i>P</i> -value
<i>a</i>	0.031	0.003	9.14	0.00001
<i>L</i> ₀	1.14	0.06	20.16	0.00001
Analysis of variance	SS	MS	<i>F</i> -value	<i>P</i> -value
Regression	0.926	0.926	20.87	0.00015
Residuals	0.977	0.044		

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