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Mould germination: Data treatment and modelling

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Abstract

The objectives of this study were i/ to examine germination data sets over a range of environmental conditions (water activity, temperature) for eight food spoilage moulds, ii/ to compare the ability of the Gompertz equation and logistic function to fit the experimental plots, iii/ to simulate germination by assessing various distributions of the latent period for germination amongst a population of spores. Data sets (percentage germination, P (%), *versus* time, t) of *Aspergillus carbonarius*, *Aspergillus ochraceus*, *Fusarium verticillioides*, *Fusarium proliferatum*, *Gibberella zeae*, *Mucor racemosus*, *Penicillium chrysogenum* and *Penicillium verrucosum* were analysed. No correlation, or relationship between the mean percentage [mean (P)] and the variance [var (P)] was found. Therefore no transformation of the germination data was required. Experimental data were fitted by using the Gompertz equation $P=A\exp(-\exp[\mu_m e/A(\delta-t)+1])$ and the logistic function $P=P_{max}/(1+\exp(k(\tau-t)))$. Based on the residual mean square error (RMSE), no model performed better than the other one. However, model parameters were generally determined more precisely with the logistic model than with the Gompertz one. The time course of fungal spore germination curves was simulated assuming different distributions of the latent period for germination, lag, amongst a population of spores. The growth rate of germ tubes was calculated by means of the relationship: lag · rate = k. For normal Gaussian distributions, germination curves were symmetrical with respect to the inflection point and should be modelled with the logistic function. Skewed distributions were capable of simulating an asymmetric germination curve that was fitted by the Gompertz model. Future studies should be conducted for assessing whether the distributions assumed in this paper are in accordance with the experimental distributions that are still unknown.

Keywords: Mould; Germination; Lag; Distribution; Model

1. Introduction

Moulds are responsible for significant spoilage and economic losses in the food chain. In addition, some species belonging to the genera, *Aspergillus*, *Fusarium* and *Penicillium* are mycotoxigenic (Magan and Olsen, 2004). The toxins produced are carcinogenic and constitute a risk to animal and human health. Because they cannot simply be destroyed by heat, the development of strategies based on avoiding mould development should be a primary objective.

Predictive mycology aims at forecasting mould development and mycotoxin contamination of raw materials and processed food products (Dantigny, 2004). This is in contrast to predictive microbiology that has focussed on pathogenic food bacteria. However, very few studies addressed the modelling filamentous fungal development. In contrast, many food mycologists are unfamiliar with modelling techniques; while those involved in modelling are developing tools dedicated to bacteria (Dantigny et al., 2005a). Therefore there is a tendency to extend the use of models that are specifically developed for bacteria to moulds.

However, filamentous moulds have certain characteristics which need to be taken into account. Temperature (T) is the main factor for controlling bacterial growth, while the effect of water activity (a_w) on mould growth is more important than T (Holmquist et al., 1983). Subsequently, it was shown that the use of the cardinal model with inflection established to represent the relationship between T and bacterial growth rate (Rosso et al., 1993) could be extended to the relationship between a_w and fungal growth (Rosso and Robinson, 2001; Sautour et al.,

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2001a). In contrast to bacterial growth, moulds form mycelium whose mass, except in the early stages of growth, does not increase exponentially (Koch, 1975). Perhaps because of this difference, the logarithmic transformation that is routinely used for homogenising variance of bacterial growth rate was found inappropriate for stabilising variance in mould growth rate data (Dantigny et al., 2005b). For this purpose it was shown that the square-root transformation was more effective.

Following the first workshop on predictive mycology dedicated to the standardisation of methods for assessing mould germination (Dantigny et al., 2006), this study is concerned with mould germination data treatment and modelling. First, a large number of raw data set (*i.e.*, percentage germination, P (%), *versus* time) were examined for assessing homogeneity of variance. Secondly, the Gompertz equation and the logistic function were compared for their ability to fit the experimental plots. Thirdly, the kinetics of the elongation of the germ tubes were examined for simulating germination curves for eight spoilage moulds, predominantly mycotoxigenic species.

2. Materials and methods

2.1. Raw data

The raw germination data, percentage germination P (%) *versus* time t (h), were obtained for *Aspergillus carbonarius* (Mitchell, 2006), *Aspergillus ochraceus* (Pardo et al., 2004), *Fusarium verticillioides* and *Fusarium proliferatum* (Marín et al., 1996), *Gibberella zeae* (Beyer et al., 2005), *Mucor racemosus* (Dantigny et al., 2002), *Penicillium chrysogenum* (Dantigny et al., 2005c) and *Penicillium verrucosum* (Pardo et al., 2006).

2.2. Data treatment

In order to investigate homogeneity of variance, for each data set, the correlation coefficient, r, was determined by performing a linear regression of the variance data (Zwietering et al., 1994). The Student's t test was used as described previously (Dantigny et al., 2005b): $t_{stud} = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$, where n is the number of observations.

2.3. Model equations

Two models for fitting the germination data were used:

the Gompertz equation :

$$P = A \cdot \exp\cdot\left(-\exp\left[\frac{\mu_m \cdot e(1)}{A}(\delta - t) + 1\right]\right) \quad (1)$$

where A (%) was the asymptotic P value at $t \to +\infty$, μ_m (% h^{-1}) was the slope term of the tangent line through the inflection point (t_i) as defined further, δ (h) was the t-axis intercept of the tangent through the inflection point and t was the time (h). The inflection point was determined as follows (Dantigny et al., 2003): $t_i = \delta + A/(\mu_m\, e(1))$

the logistic function : $$P = \frac{P_{max}}{1 + \exp[k(\tau - t)]} \quad (2)$$

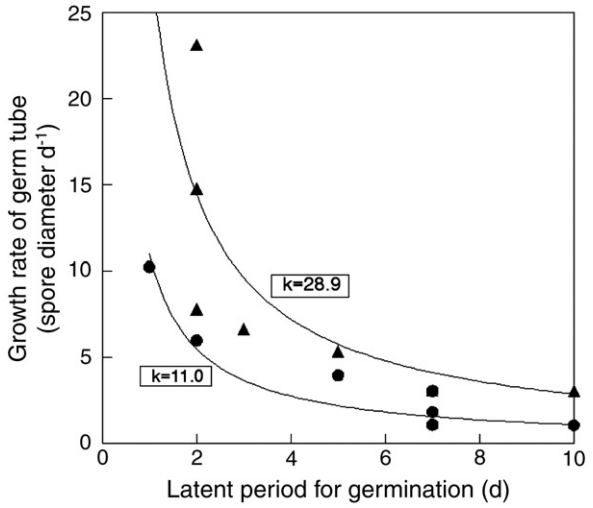

Fig. 1. Relationship between latent period for germination, lag (d) and the growth rate of germ tube, r (spore diameter d^{-1}) for *Aspergillus chevalieri* grown at 25 °C on (▲) nutrient and (●) plain gelatine. Calculations were based on conidia of 5 μm in diameter. Data were taken from Snow (1949).

where P_{max} (%) was the asymptotic P value at $t \to +\infty$, τ (h) was the inflection point where P equals half of P_{max}, t was the time (h) and k (h^{-1}) was related to the slope of the tangent line through the inflection point.

Deriving Eq. (2) with respect to time :

$$\left(\frac{dP}{dt}\right) = \frac{-P_{max}(-k[\exp(k(\tau - t))])}{(1 + \exp(k(\tau - t)))^2} \quad (3)$$

The slope of the tangent line at τ , is :

$$\left(\frac{dP}{dt}\right)_{t=\tau} = \frac{P_{max}k}{4}. \quad (4)$$

The logistic function is symmetric about the point of inflection, unlike the Gompertz function.

2.4. Model fitting

Nonlinear regressions were performed by using StatGraphics Plus version 5.1 (Statistical Graphics Corp., Herndon, VA, USA) and SlideWrite 5.0 (Advanced Graphics Software, Inc., Carlsbad, CA, USA) as described previously (Dantigny, 1998). These softwares were based upon the Levenberg–Marquardt Algorithm. The goodness of fit was evaluated by means of the root mean square error, RMSE, (Ratkowsky, 2004) and extracted from ANOVA tables.

2.5. Simulations

2.5.1. Determination of the latent period for germination

Germination curves were simulated on the basis of the length of the germinated tubes as follows:

For t<lag L (spore diameter) = 0 (5)

For $t \geq$lag L (spore diameter) = (t−lag)rate (6)

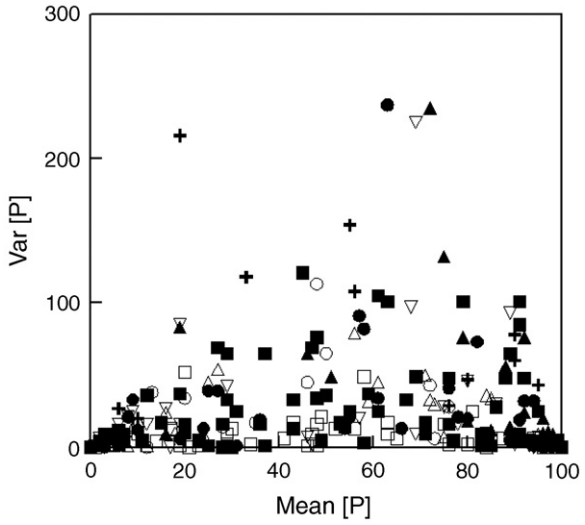

Fig. 2. Variance (var) of P, plotted against the mean of P for: (■) *Aspergillus carbonarius*, (△) *Aspergillus ochraceus*, (●) *Fusarium verticillioides*, (▽) *Fusarium proliferatum*, (▲) *Gibberella zeae*, (✚) *Mucor racemosus*, (□) *Penicillium chrysogenum* and (○) *Penicillium verrucosum*.

where rate (spore diameter h^{-1}) or (spore diameter d^{-1}), the rate of extension of the germ tube, lag (h) or (d), the lag for germination. A spore was considered to have germinated when the length of the germ tube equalled the spore diameter, $L=1$.

For assessing the effect of the distribution of the lag amongst the population, 99 spores were considered. The first spore was attributed the cumulative probability of 0.01, the second spore 0.02 and so on. The centiles of the lag distribution, obtained through using Microsoft Excel® 2000, were attributed to the population of spores. Normal distribution of the lag, $N(\eta, \sigma)$ where η is the mean and σ is the standard deviation, was assumed. For $N(10, 1)$, the following lag times, 6.67 h, 10 h and 11.28 h were attributed to the first, the 50th and the 90th spore respectively. For this distribution a cumulative probability of 0.95 is obtained approximately for: $\text{lag}=\eta+2\sigma$. Therefore the lag of the 95th spore was 12 h. It was assumed that all spores were observed.

2.5.2. Determination of the growth rate of germ tube

In order to determine the rate of extension of the germ tube, it was assumed that:

$$\text{rate}\cdot\text{lag}=k. \quad (7)$$

This empirical law was based upon the observation that the average rate of germ tube elongation decreased with increasing latent period for germination, (Snow, 1949). It was assumed that this observation also holds true for a single spore. The average growth rate of germ tube was plotted against the latent period for germination of *Aspergillus chevalieri* grown on nutrient and plain gelatine at 25 °C at controlled humidities (Fig. 1). The growth rate of germ tubes increased with a decrease in the latent period for germination (*e.g.*, at high humidities). The average k values were 11.5 and 27.2 on plain gelatine and nutrient gelatine respectively. The value $k=10$ was chosen arbitrarily unless stated otherwise.

3. Results

3.1. Homogeneity of variance

For each data set, an analysis of variance of the raw germination data was carried out. For none of the fifty analysed data sets, a correlation between the variance and the mean was found. Therefore there was no need to transform the raw germination data to homogenise variance.

If there is no linear correlation, this does not necessarily mean that there is no other correlation (Zwietering et al., 1994). The variance data are shown on Fig. 2. In more than 95% cases, the variance was less than or equal to 100. For these values, visual inspection did not show any relationship between the variance and the mean. In four cases, the variance was greater than or equal to 200. However, these peaks were obtained at different mean [P] values depending on the moulds (*i.e.*, 20% for *M. racemosus*, in the range 60–80% for *F. verticillioides*, *F. proliferatum* and *G. zeae*).

When the variance is greater, (say var [P]>300), the data set should be examined for reproducibility. Each data set described results of replicated and in some cases repeated experiments (for *G. zeae* macroconidia). Macroconidia are the spores of the non-sexual stage (anamorph) of the fungus. The anamorph of *G. zeae* is called *Fusarium graminearum*.

For *P. verrucosum* at 0.85 a_w, variance as high as 600 was found at mean [P] equalled to 32 and 51.6%. Each individual experiment (*i.e.*, run 1, run 2 and run 3) was examined independently and fitted with the Gompertz and the logistic model (see Table 1). Runs 1 and 2 were characterised by significantly smaller average lag times for germination, δ (h), than that obtained for run 3. Similarly, the germination time, τ (h), was greater for run 3 than those estimated for the other runs. Therefore, run 3 plots should not be pooled with the other plots.

Table 1
Parameter values obtained by fitting *Penicillium verrucosum* individual germination plots at 0.85 a_w with the Gompertz and the logistic models

Run	Model					
	Gompertz			Logistic		
	Parameter estimate [95% confidence interval]					
	A (%)	μ_m (% h^{-1})	δ (h)	P_{max} (%)	k (h^{-1})	τ (h)
1	91.3 [86.1, 96.5]	1.31 [1.09, 1.54]	88.5 [82.4, 94.6]	87.5 [82.6, 92.5]	0.061 [0.045, 0.077]	123 [119, 127]
2	93.5 [80.8, 106.2]	1.05 [0.75, 1.36]	79.8 [67.1, 92.5]	87.6 [78.1, 97.1]	0.049 [0.029, 0.069]	123 [115, 130]
3	143.4 [20.3, 266.4]	1.14 [0.93, 1.35]	125 [113, 137]	99.5 [60.9, 138.5]	0.050 [0.026, 0.074]	169 [148, 189]

3.2. Comparison of the models

The models were first compared on the basis of root mean square error (RMSE) (Table 2). Two software packages were used for fitting the experimental data. Indeed, in some cases, StatGraphics and SlideWrite failed to converge using the logistic and the Gompertz model respectively. Overall the Gompertz model scored 26/24 over the logistic model. In addition, in 9 cases out of 50 the RMSE values obtained for the different models differed by less than 10%. A more detailed analysis showed that it was similar for the *Aspergilli*, *G. zeae*, *M. racemosus* and *P. verrucosum*. The Gompertz model performed better than the logistic model for *P. chrysogenum* and *F. verticillioides*. Conversely, the logistic model could be preferred for fitting *F. proliferatum* plots, suggesting that the choice of a model did not depend upon the mould.

The majority of the data sets examined the influence of water activity on germination. For *A. ochraceus*, *F. verticillioides* and *P. verrucosum*, the logistic model performed better than the Gompertz model at water activities close to the optimum. However, this observation was not confirmed with the *F. proliferatum* data. For *A. carbonarius* the logistic model performed better than the Gompertz at a_w closest to the optimum at 25 °C, 30 °C and 40 °C, but not at 35 °C.

Table 2
RMSE values for the Gompertz and the logistic models fitted to germination data with StatGraphics[a] and SlideWrite[b] softwares

Mould	*Aspergillus carbonarius*[a]								
Factor	Water activity/temperature (°C)	0.90/25	0.90/30	0.90/35	0.90/40	0.93/25			
Model	Gompertz	**8.16**	11.93	**0.09**	**60.34**	16.34			
	Logistic	9.47	**7.25**	2.38	64.36	**7.79**			
Factor	Water activity/temperature (°C)	0.95/15	0.95/20	0.95/25	0.95/30	0.95/35	0.95/40		
Model	Gompertz	**20.80**	33.00	24.10	11.05	73.19	**112.8**		
	Logistic	25.17	**26.55**	**16.45**	**4.65**	**36.79**	131.1		
Factor	Water activity/temperature (°C)	0.987/15	0.987/20	0.987/25	0.987/30	0.987/40			
Model	Gompertz	**68.13**	**42.95**	45.09	**83.96**	43.48			
	Logistic	90.03	64.55	**33.37**	90.12	**25.15**			
Mould	*Aspergillus ochraceus*[b]								
Factor	Water activity	0.85	0.90	0.95	0.99				
Model	Gompertz	**25.65**	**23.58**	35.06	12.46				
	Logistic	30.45	24.52	**26.42**	**6.94**				
Mould	*Fusarium verticillioides*[b]								
Factor	Water activity	0.88	0.90	0.92	0.94	0.96	0.98		
Model	Gompertz	**45.66**	**9.52**	**6.55**	**16.82**	**18.45**	23.20		
	Logistic	50.98	17.76	20.34	21.36	25.74	**19.19**		
Mould	*Fusarium proliferatum*[a]								
Factor	Water activity	0.88	0.90	0.92	0.94	0.96	0.98		
Model	Gompertz	25.37	27.61	55.91	30.80	**13.38**	5.63		
	Logistic	**17.40**	**24.34**	**42.33**	**21.34**	18.32	**5.14**		
Mould	*Gibberella zeae*[a]								
Factor	Kind of spores	Macroconidia 1	Macroconidia 2	Macroconidia 3	Ascospores				
Model	Gompertz	**45.03**	27.69	**14.25**	**35.29**				
	Logistic	46.11	**25.97**	21.64	44.07				
Mould	*Mucor racemosus*[b]								
Factor	Temperature (°C)	15	25						
Model	Gompertz	**90.68**	47.71						
	Logistic	91.06	**44.20**						
Mould	*Penicillium chrysogenum*[b]								
Factor	Ethanol (%, wt/wt)	0	0.5	1	1.5	2	2.5	3	3.5
Model	Gompertz	**9.89**	18.97	37.07	7.86	**4.09**	**10.20**	**4.15**	**9.54**
	Logistic	19.30	**12.43**	**25.14**	**5.98**	7.33	18.50	7.18	9.34
Mould	*Penicillium verrucosum*[b]								
Factor	Water activity	0.85	0.90	0.95	0.99				
Model	Gompertz	**18.97***	**37.30**	55.99	26.70				
	Logistic	25.25*	44.06	**39.57**	**12.99**				

Boldface data indicate the least value. *Average of two individual experiments (run 1 and run 2).

Table 3
Parameter estimates of the Gompertz model for the germination data of *P. chrysogenum*

Ethanol %, wt/wt	Estimates for each parameter											
	A (%)			μ_m (% h^{-1})			δ (h)			t_i [a] (h)		P (t_i) [b] (%)
	Value	95% CI [c]	t	Value	95% CI	t	Value	95% CI	t	Value	95% CI	
0	101.8	98.5–105.1	63.0	29.0	26.1–31.9	20.5	8.2	8.0–8.4	85.7	9.5	9.1–9.9	37.7
0.5	104.1	98.8–109.3	40.7	28.0	24.2–31.9	14.9	10.3	10.1–10.5	97.0	11.7	11.2–12.1	38.4
1.0	103.3	95.8–110.7	29.0	29.9	23.3–36.5	9.5	10.5	10.1–10.8	65.6	11.8	11.1–12.4	38.0
1.5	101.0	97.6–104.4	61.3	30.0	26.1–34.0	15.7	10.6	10.5–10.8	113.6	11.8	11.4–12.2	37.2
2.0	104.6	101.2–108.0	63.6	20.2	18.8–21.7	28.4	10.8	10.6–10.9	152.3	12.7	12.4–13.0	38.4
2.5	110.3	103.9–116.7	36.0	19.8	17.8–21.8	20.7	13.0	12.8–13.3	106.1	15.0	14.4–15.7	40.4
3.0	106.9	103.0–110.8	56.6	15.6	14.6–16.6	32.0	16.0	15.8–16.2	160.7	18.5	18.1–19.0	39.3
3.5	64.5	62.7–66.4	70.6	4.7	4.3–5.1	23.4	19.2	18.7–19.8	69.9	24.2	23.1–25.4	23.7

[a] t_i, inflection point for the Gompertz model.
[b] $P(t_i)$, percentage of germination at the inflection point for $t=t_i$.
[c] CI, confidence interval.

At a temperature close to the optimum, *e.g.* 30 °C, the logistic model was characterised by a lower RMSE than the Gompertz for *A. carbonarius*. The difference between the RMSE values obtained at 0.987 a_w was not significant. Conversely, at 15 °C, the Gompertz model appeared more suitable than the logistic one. With the notable exception of the ethanol free medium, the Gompertz model performed better than the logistic one at the highest ethanol concentrations.

A model should also provide consistent estimates of the model parameters. According to this goal, the Gompertz model that provided erroneous estimations of A could be criticised. In fact, for *P. chrysogenum* in the range of 2–3% ethanol, the 95% confidence intervals for A were greater than 100%, Table 3. These values were unrealistic because A represents the maximum percentage of germination. This parameter is of great interest for assessing germinability of moulds but the most important parameters to be estimated are probably the lag time δ and the germination time, τ.

These parameters should be estimated with the greatest accuracy. The greater the t-value, and the smaller the confidence interval, the more accurate the estimates are. With the notable exception of *P. verrucosum* at 0.90 a_w, the germination time was characterised by greater t-values (as much as twice) than those obtained for the lag time (Tables 3 and 4). In contrast, the other parameters were estimated with a similar accuracy. The average t-value for P_{max}, 62.0 was greater than the average t-value for A, 52.6. But, the average t-value for k, 19.1, was less than the average t-value for μ_m, 23.6. Based upon the two latter criteria, model parameters were estimated with a greater accuracy with the logistic model than with the Gompertz one.

The inflection point obtained by the Gompertz equation was estimated according to $t_i=\delta+A/(\mu_m\ e(1))$ (Table 3), and compared to the inflection point obtained by the logistic function, τ, Table 4. In three cases, for 1.5, 2 and 3.5%, wt/wt ethanol the 95% confidence intervals did not overlap, thus demonstrating that t_i was less than τ. The percentage of germination, $P(t_i)$ and $P(\tau)$ when all spores were viable (*i.e.*, ethanol in the range 0–3%, wt/wt) was equal to about 40% (Table 3) and 50% (Table 4) at the inflection points, for the Gompertz model and for the logistic function respectively. The fact that the inflection point for the Gompertz model is not located at 50% germination explained why t_i was less than τ.

3.3. Simulation of germination kinetics

For distribution N (10, 1), the average lag time for germination was equal to 10 h. For $k=10$, the average rate of elongation of the germ tubes (*i.e.*, the rate of elongation of the

Table 4
Parameter estimates of the logistic model for the germination data of *P. chrysogenum*

Ethanol %, wt/wt	Estimates for each parameter										
	P_{max} (%)			k (h^{-1})			τ (h)			P (τ) [a] (%)
	Value	95% CI [b]	t	Value	95% CI	t	Value	95% CI	t	
0	99.0	95.0–103.0	51.1	1.15	0.97–1.34	12.9	10.1	9.9–10.2	131.8	49.5
0.5	100.2	97.0–103.3	65.0	1.23	1.09–1.38	17.4	12.2	12.1–12.3	226.9	50.1
1.0	100.2	94.8–105.6	38.4	1.35	1.09–1.61	10.7	12.2	12.0–12.4	149.9	50.1
1.5	98.9	96.8–101.0	97.8	1.33	1.20–1.46	21.0	12.4	12.3–12.4	299.3	49.5
2.0	98.9	95.6–102.2	62.5	0.89	0.80–0.99	19.1	13.3	13.2–13.5	164.2	49.5
2.5	101.5	96.3–106.7	41.2	0.87	0.72–1.01	12.3	15.7	15.4–15.9	131.6	50.8
3.0	99.2	96.0–102.3	65.1	0.67	0.60–0.74	19.8	19.3	19.1–19.5	197.1	49.6
3.5	63.1	61.4–64.8	74.6	0.30	0.27–0.33	20.3	26.3	25.9–26.7	134.1	31.6

[a] $P(\tau)$, percentage of germination at the inflection point for $t=\tau$.
[b] CI, confidence interval.

50th spore) was equal to 1 spore diameter h^{-1}. Let us consider the 50th spore, about 1 h was required for the germ tube length to equal that of the spore diameter. Therefore, the germination time of the 50th spore was 11 h. At that time, all the spores were characterised by shorter lag phases for germination and faster rate for elongation for those already germinated. Thus the germination time was 11 h for $k=10$ (see Fig. 3). For $k=30$, the growth rates of germ tubes were 3 fold those obtained for $k=10$. Accordingly, the germination time was only 10.3 h but the shape of the germination curve was not significantly different from that obtained for $k=10$ (not shown).

For distribution N (20, 1), 2 h was required for having the germ tube of the 50th spore equal to the spore diameter. In fact, the average rate of extension for a lag equal to 20 h was about half of that obtained for 10 h. Thus the germination time was 22 h (see Fig. 3).

The distributions N (10, 1) and N (10, 2) were compared. No difference was noticed in the germination time for the average latent period for germination was equal to 10 h in both cases. However, the slope of the germination curves was related to the standard deviation. The slope of the germination curve was decreased with an increase in the standard deviation.

The germination plots generated for normal distributions were fitted by the Gompertz and the logistic models (see Fig. 3). The RMSE obtained with the logistic function were less than those obtained for the Gompertz model. In addition, the estimates of the asymptotic germination percentage, A (%), were greater than 100% with the Gompertz model. Similar observations were made when fitting the germination curves of *P. chrysogenum* in the range of 2–3% ethanol. These results demonstrated that the shape of the germination curve is symmetrical with respect to the inflection point for normal distributions of the lag time for germination and the growth rate of the germ tubes amongst the population of spores.

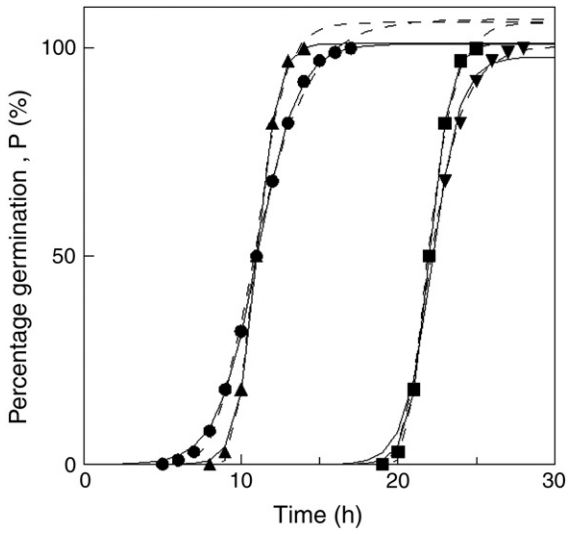

Fig. 3. Germination plots generated assuming normal distributions of the lag time for germination, lag (h), (▲) N (10, 1); (●) N (10, 2); (■) N (20, 1) and a skewed distribution (▼) N (20, 1) for the faster spores (from the 1st to the 50th) and N (20, 2) for the slower spores (from the 50th to the 99th). The rate of germ tube elongation, rate (spore diameter h^{-1}) was calculated according to: rate = 10/lag. Logistic (—) and Gompertz (– – – –) fitting.

In order to simulate a non-normal distribution, it was assumed that the faster spores (*i.e.*, from the 1st to the 50th) and the slower spores (*i.e.*, from the 50th to the 99th) were characterised by the distributions N (20, 1) and N (20, 2) respectively. Overall, the distribution of the latent period for germination amongst the population of spores was not symmetrical with respect to the mean value. In such a case, the germination curve differed from the one obtained for the normal distribution N (20, 1) only after the germination time (22 h). With respect to that point, the germination curve was not symmetric and a better fit was achieved by using the Gompertz model (RMSE = 3.5) as compared to the logistic model (RMSE = 15.5).

4. Discussion

The germination curve can be described by primary kinetic models such as the Gompertz and the logistic equations. Prior to fitting the experimental data, percentage germination (P) *versus* time, the relationship between the variance, var [P] and the mean was examined. Before the appearance of the germ tubes, the percentage of germination was equal to 0%, whereas in most cases all spores were viable (*i.e.*, the maximum percentage of germination was 100%). For these values, the variance was nil, thus maybe explaining why some authors have assumed a maximum variance at 50% germination. This assumption was implicit while using the following transformation $\sqrt{P+50}$ (Pardo et al., 2005a,b) or explicit while using the logit transformation $\mathrm{Ln}[P/(100-P)]$ (Huang et al., 2001; Perryman et al., 2002; Kalolewski et al., 2004). In predictive microbiology, the logit transformation was used for modelling the growth/no growth interface that involved the use of probability models, where the response variable was typically binomial (Ratkowsky, 2002). When kinetic models are used such as in this study, the logit transformation should be avoided. Otherwise, the experimental data $P=0\%$ and $P=100\%$ are not taken into account.

In none of the 50 data sets that were examined, a relationship between the mean and the variance was exhibited. Therefore, there was no need for transforming mould germination data. However, examination of the variance may be useful prior to pooling data that were replicated or repeated. It was shown for *P. verrucosum* at 0.85 a_w that a high variance (greater than or equal to 600) was synonymous for experiments characterised by different lag times for germination, δ and germination time, τ.

It was one of the objectives of this study to determine which model fitted experimental data of time courses of fungal spore germination better. For this purpose, two models are currently being used, the Gompertz model (Marín et al., 1996; Plaza et al., 2003; Pardo et al., 2005a,b) and the logistic model (Dantigny et al., 2002, 2005c). Based on the RMSE, the goodness of fit of both the models was similar. It was also impossible to determine for which mould and for which experimental conditions a particular model should be preferred. Therefore other criteria were also evaluated such as the accuracy of the parameter estimates.

The germination time was estimated with a greater accuracy by the logistic model than by the Gompertz equation. Assuming that the widely used definition of the germination time (*i.e.*, the observed time required for 50% of viable spores to germinate) recommended during the last workshop on predictive mycology (Dantigny et al., 2006) is accepted, the logistic model should be privileged because it provides this parameter value without any additional calculation. Care should be taken while using the Gompertz equation because in some cases erroneous estimations of the maximum percentage of germination (*i.e.*, greater than 100%) were obtained.

In many cases, tools that have been developed for bacteria were applied to fungi without taking mould characteristics into account. For example, the Gompertz equation was used to fit the colony diameter of fungi (Valík et al., 1999; Gibson et al., 1994; Membré and Kubaczka, 2000) whereas a simple linear correlation with breakpoint proved effective in estimating the radial growth rate (Dantigny, 2004). The Gompertz function, which is asymmetric about the point of inflection, was used for fitting bacterial growth curves. This can be explained by the decrease of the growth rate with time due to the limitation of substrate and/or inhibition by products.

At present, there is no evidence that the fungal spore germination curves should be asymmetric. The germination curves that have been published appear rather symmetric, especially under optimal conditions. It was shown through simulations that the use of the Gompertz model to fit symmetric plots led to an over-estimation of the maximum percentage of germination.

Germination curves reflect the distribution of the lag time for germination amongst spores. The S-shape of the germination curve is characterised at the beginning by a concave acceleration phase and a convex slowing down phase at the end. Such a shape can be obtained only if the distribution is tailed both sides such as in the normal distribution. Other distributions, such as the beta distribution failed to simulate the S-shape of the germination curve whatever the parameters. It was shown that normal distributions were synonymous with germination curves that are symmetrical with respect to the inflection point. Conversely, asymmetric curves can be obtained when the latent period for germination is not normally distributed.

For simulating the germination curve, the latent period for germination and the growth rate of the germ tube of individual spores should be defined. A relationship between the rate and the lag for germination was determined from average data. It is uncertain whether this relationship also holds true for each spore. For stating that a spore has germinated (*i.e.*, the length of the germ tube is equal to or greater than the spore diameter) very early measurements of the length of the germ tube are required. The rate of elongation of the germ tube had a significant effect on the germination time but not on the shape of the germination curve. The germination curve is very much dependent on the distribution of the lag time for germination amongst the spores. At present, experimental distributions remain unknown, but the assumption of normal distribution was in agreement with some germination curves obtained experimentally. More studies should be conducted to determine whether other distributions than the normal one could be exhibited.

5. Perspectives

Most of the experiments that concern growth of bacteria and moulds were carried out using a large inoculum. However, in most cases, products are contaminated by a low initial level of organisms. In some cases this level may be as low as a single spore. Therefore, for improving the prediction of the development of micro-organisms, the distribution of kinetic parameters such as the lag time should be determined.

Recently a project named "BACANOVA" (2006) aiming at developing and validating novel mathematical techniques to improve the prediction of bacterial lag time was initiated by J. Baranyi. The biological parts of this project were dedicated to direct and indirect measurements of individual lag times of cells/spores.

In the meantime, some studies were concerned with the effect of water activity and temperature on the distribution of the growth rates and lag phases durations of individual spores of *Aspergillus flavus* and *F. verticillioides* on yellow dent corn meal (Samapundo, 2006). The author suggested that lag phase durations could be used as indirect measurements of the germination time.

The present study pointed out the lack of direct measurements for the lag time for germination and for the rate of germ tube extension of individual spores. For this purpose, specific experimental devices allowing the observation of the same spore throughout the germination process are already available (Sautour et al., 2001b). In a first step, experiments should be carried out for determining the distribution of the latent period for germination amongst spores. In a second step, the influence of the environmental factors on these distributions could be assessed.

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