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Effect of Temperature, pH and Water Activity on *Penicillium digitatum* Growth

María Luisa Carrillo-Inungaray[1*], Madeleine Hidalgo-Morales[2], Guadalupe del Carmen Rodríguez-Jimenes[2], Miguel Ángel García-Alvarado[2], Mario Ramírez-Lepe[2], Abigail Reyes Munguía[1], Victor Robles-Olvera[2]

[1]Universidad Autónoma de San Luis Potosí, Ciudad Valles, México
[2]Unidad de Investigación y Desarrollo en Alimentos, Instituto Tecnológico de Veracruz, Veracruz, México
Email: [*]maluisa@uaslp.mx

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Abstract

Growth curves fitted *Penicillium digitatum* were used to analyze the effect of temperature, pH and a_w on their growth. To asses observance of independent variable effects on all growth parameters (λ, latency time, μ, growth rate and $y_{\max}$ maximal growth) a slow growth strain of *P. digitatum* was employed. Growth curves were obtained at different conditions of temperature (10˚C - 40˚C), pH (3.0 - 7.0) and water activity (0.800 - 0.990) and growth parameters were calculated by fitting of logistic model. Polynomial models were made by linear regression and all terms (linear, quadratic and interactive effects) were statistically significant ($p < 0.001$). All growth parameters, including maximal growth were affected by environmental conditions; pH effect was more important on maximal growth than that on lag time or growth rate. In some aspects results and modeling behavior of *P. digitatum*, are very similar to modeling of bacterial growth.

Keywords

Fungi Growth Model, *P. Digitatum*, Slow Growth, Food Safety, Food Spoilage

1. Introduction

The consumers claim for foods gently processed with a minimum content of preservatives. In attention to these demands and in order to have safe foods, a high value of one "hard" preservative has been replaced by low values of several "gentle" preservative factors to reach the same preservative effect that in traditionally prepared foods.

[*]Corresponding author.

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To test microbial behavior at these new environmental food conditions, mathematical models developed in predictive microbiology have demonstrated a useful tool to avoid the application of fastidious and expensive microbiological methods. Most models commonly used are polynomial expressions where growth response is a function of environmental growth factors.

Predictive polynomial models of food borne or spoilage bacteria relating growth to temperature, pH, water activity (a_w) and others factors are in literature [1]-[3]. Statistical analysis of these models shows that commonly interactive and quadratic effects are significant; and from data analysis is possible to verify that effects of factor on growth parameters of slow growth strains are less important than those observed on fast growth strains. Knowledge about interactive effects of these factors on fungi growth has not been extensively documented as that on bacterial growth. Furthermore, it is important to analyze the models with the factors of accuracy and bias for the possible discrepancy between observed and predicted values obtained with a model.

Fungi are widely distributed in nature and their prevalence is one of the major causes of food spoilage [4], moreover fungi presence can carry out mycotoxin production [5], therefore their control is necessary in order to have a safe food product [6]. There are some models in literature relating fungi growth to a_w [7] [8] or a_w and temperature [1] [6] [9] [10]. The pH effect on fungi growth has not been widely studied and controversial results have been published. Some authors reported that pH effect was not significant whereas a variable effect was found by others [11] [12]; moreover existence [13] or not existence [6] [11] of pH significant interactive effects has been reported too. Under this controversial find it is evident that more studies about effect of pH and their interactions with other factors are necessary. Otherwise, information about the effects of factors on relatively slow growing fungi is not available. It is important to know behavior of slow growing fungi because they can start to growth during predicted food shelf time if bacterial growth is controlled. Moreover, factors effect is more confused with slow growth strains that fast strains because response to factors may be masked by inherent fast growth rate of fast strains. *Penicillium digitatum*—a deuteromycetus fungus of hiphomycetal class, one of the most common post-harvest pathogens causing green mold rots in citrus fruits [5] [14]—grows relatively slower than other deteriorative molds [15]-[18].

The novelty of this work is to identify the important factors to consider building a predictive model; since it is possible that a rapid growth rate in microorganisms could mask the significant effects of environmental factors and their interactions. Therefore, the objective of this work was to analyze the effect of growth factors and their interactions on slow growing fungi by modeling the *Penicillium digitatum* growth in temperature, a_w and pH growth ranges reported for this fungus.

2. Materials and Methods

2.1. Experimental Design

Growth kinetics of *Penicillium digitatum* were obtained at conditions of water activity (0.800, 0.895 and 0.990) and pH (3.0, 5.0 and 7.0) established by a centered sides design: $2^k + 2k + 1$ [19]; inoculated media were incubate at 10˚C, 20˚C, 30˚C, and 40˚C. Factors limit values were elected considering minimum and maximum reported values for *P. digitatum* growth. All experiments were performed in duplicated.

2.2. Media Preparation

Saboraud dextrose agar (Bioxon) was prepared following manufacturer's instructions. Norrish [20] and Ross [21] equations were employed to calculate sucrose (Hycel) needed to reach the desired a_w; pH was adjusted by addition of citric acid (Baker) 10% (p/v). Sterilized and acidified agar was aseptically distributed in petri dishes.

2.3. Preparation of Inoculum

Penicillium digitatum was isolated from spoiled citrus fruits and identified according to Samson *et al.* [22]. The strain was cultured in Saboraud dextrose agar slants and incubated at 25˚C for 7 days. A spore suspension was prepared by overlaying sporulated cultures with 10 mL of sterile distilled water. The spore suspension was used immediately.

2.4. Inoculation

The spore suspension (approximately 10^3 spores) was centrally inoculated in petri dishes by pouring 2 μL as was indicated by Fustier *et al*. [23] to give a circular inoculum of 2.5 mm of diameter. The inoculated plates were incubated for 20 days in hermetically closed plastic containers to avoid dehydration.

2.5. Fit of Fungi Growth Curves

The colony size increase was followed by measuring colony diameter every day and this was plotted versus incubation time. Growth parameters were calculated by fitting of logistic equation (Equation (1)):

$$y = \frac{y_{\max}}{1 + \left(\frac{y_{\max}}{y_0} - 1\right) e^{-\mu_{\max} A_n(t)}} \tag{1}$$

where $A_n(t) = \begin{cases} 0, & (t \le \lambda) \\ t - \lambda, & (t \ge \lambda) \end{cases}$, $y_{\max}$ is maximum growth; y_0, initial inoculums; $\mu_{\max}$, growth rate and $A_n(t)$ is a discontinuity in the time.

2.6. Model Development

Polynomial models relating natural logarithm of *P. digitatum* growth parameters (growth rate, lag time and maximum growth) to temperature, a_w and pH were built by linear regression. Natural logarithm of growth parameters was choose with the purpose of diminish variance and avoid negative lag phases.

2.7. Performance Analysis of Models

Models were analyzed by the accuracy (AF) (Equation (2)) and bias factors (BF) (Equation (3)), introduced by Ross [24], modified by Baranyi *et al*. [25] and as were used by López *et al*. [26]. These factors were calculated as follow:

$$AF = \exp\left(\sqrt{\frac{\sum_{k=1}^{n} (LnP - LnO)^2}{n}}\right) \tag{2}$$

$$BF = \exp\left(\frac{\sum_{k=1}^{n} (LnP - LnO)}{n}\right) \tag{3}$$

were P and O are predicted and observed values, respectively, and n is the experimental points number. A bias factor value greater than 1 indicate that, on average, predicted are greater than observed values; otherwise, an accuracy factor of 1 indicate a good concordance between predicted and observed values.

3. Results and Discussion

Below are presented the results of the effect of temperature, pH and water activity on *Penicillium digitatum*-growth.

3.1. Results of *Penicillium digitatum* Growth

P. digitatum growth was observed in 23 of 36 experimental conditions. Not growth was reported when lag time was prolonged after an incubation time of 20 days and this occurred at extremes experimental conditions of temperature, a_w or pH. Except for lag time, each growth phase was well described by growth curves and their shape was concordant with experimental conditions. Generally, when two factors remain constant, effect of third factor gave curves sufficiently separates to observe effect levels of such factor. Consequently, calculated growth parameters (**Table 1**) were coherent and, as it was expected, minimal and maximum growth were observed at extreme and central conditions, respectively.

Table 1. Growth parameters of *Penicillium digitatum* at different experimental conditions calculated by logistic model.

Condition			Growth parameter		
T	a_w	pH	(h)	($mm \cdot h^{-1}$)	y_{max} (mm)
10	0.990	3	106.74	0.009	8.49
10	0.990	5	99.732	0.017	7.27
10	0.990	7	292.95	0.023	5.88
20	0.800	5	221.22	0.017	6.12
20	0.800	7	147.97	0.017	13.79
20	0.895	3	112.91	0.009	8.04
20	0.895	5	48.409	0.015	11.17
20	0.895	7	65.246	0.017	14.58
20	0.990	3	6.7422	0.018	26.37
20	0.990	5	5.8246	0.032	21.23
20	0.990	7	15.819	0.041	16.17
30	0.800	5	55.023	0.022	9.28
30	0.800	7	34.029	0.021	19.66
30	0.895	3	33.939	0.012	10.83
30	0.895	5	13.454	0.019	14.11
30	0.895	7	16.765	0.020	17.39
30	0.990	3	2.2646	0.022	29.69
30	0.990	5	1.8088	0.038	22.49
30	0.990	7	4.5418	0.047	16.12
40	0.800	7	41.609	0.016	10.17
40	0.990	3	4.0443	0.017	12.13
40	0.990	5	2.9867	0.028	8.64
40	0.990	7	6.9338	0.033	5.83

3.2. Results of Factors Effect on Lag Time

Lag time was measured in most cases where growth was observed, and it was as long as 293 h at extreme conditions. Not correlation was found between lag time and growth rate but generally slowest growth rates match with long latency times and small maximal growth. Temperature increases of 10˚C caused not proportional reduction of latency times, at pH 3 and a_w 0.990, lag times are 16 times longer at 10˚C than at 20˚C, and 3 times longer at 20˚C than 30˚C, but around 0.5 times at 30˚C than at 40˚C. In most conditions, at same temperature and a_w values, lag times are longer at extreme pH (3 and 7) than at central value (pH 5). Repeatedly, lag times are two or three times longer at pH 7 than a pH 5; whereas those obtained at pH 3 are bigger but very close to those calculated at pH 5 (except for 0.895 a_w). Otherwise, lag times are progressively shorter as a_w values are increased.

3.3. Results of Factors Effect on Growth Rate

In all tested conditions, growth rates were generally smaller than those reported for other deteriorative fungi [9] [11] [15] even 10 times smaller than other *P. digitatum* strains reported by Plaza *et al*. [19] and Dantigny *et al*. [27]. These differences between growth rates observed and those reported in literature can be related primarily to differences between strains nature.

Growth rate of *P. digitatum* showed an optimum temperature value between 20˚C and 30˚C, similar to these reported by Plaza *et al*. [18]. It was noted that *P. digitatum* tolerance to experimental extreme temperatures (10˚C and 40˚C) increased when a_w values were increased. Growth rates obtained at pH 5 and 7 were very similar and higher than those observed at pH 3. These results are concordant with that published by Neumeyer's team [28] who established that *Pseudomonas* growth rate is unaffected by pH in the range pH 5.4 to 8.6. Similar results have been reported for fungi growth [5] [11] [12], in general, when pH interval studied is around of optimal pH range for microorganism growth, not significant lineal or interactive effects were found regardless of acids nature (acetic, lactic, phosphoric, citric or hydrochloric acids) used to adjust pH. Otherwise, significant effects were reported when pH range studied is far of optimal region for growth when potassium citrate [13] was used to buffering media in a pH range of 2.8 to 3.3. With this arguments and bearing in mind the parabolic classic shape obtained when pH is plotted versus growth rate, it is logic to find significant effects when all pH growth range is analyzed. Besides pH interval studied or nature of acid used to adjust pH, differences between effects could be due also to inherent acid tolerance and optimal growth pH of each microorganism.

3.4. Results of Factors Effect on Maximal Growth

Maximal growth was observed at 30˚C, pH 3 and 0.990 a_w values and this growth parameter increased as pH or a_w values were augmented. These results are interesting because maximal growth is commonly limited by nutrient availability or toxic metabolites accumulation, and its maximal value is finally reached earlier or later according to lag time and growth rate. In consequence, frequently this growth parameter does not show correlation with environmental factors, in fact, it is common to find in literature only lag time and growth rate models.

3.5. Results of Analysis and Performance of Developed Models

Since *P. digitatum* is a citrus spoilage fungus, a predictive model of *P. digitatum* growth on citrus products should be expected. In this sense, data for building model should be obtained from *P. digitatum* growth on a medium of a citrus similar composition, However, when models are created of this way, levels of independent variable, as pH or a_w, are restricted by food nature (because its stability can be affected); consequently, effects of independent variables cannot be quantified. Therefore, to measure the effect of studied variables, laboratory culture media are the best option, although the model applicability obtained by this way is limited and validation tests are necessary before model use. Because the objective of this paper was to analyze independent variables effect, this last strategy to develop models was used in this work.

Coefficients of polynomial models developed are presented in **Table 2**. Statistical analyses of model terms demonstrate that all effects were significant ($p < 0.0001$). Lag phase, growth rate and maximum growth were mainly affected by temperature, whereas the effect of a_w and pH are smaller. In all cases, a_w and pH interaction exerts smaller effects than those produced by temperature. Models can predict correctly growth rate and maximal growth but a more important discrepancy is observed for lag time, these observations were confirmed by accuracy and bias factors (**Table 3**). Accuracy factor for growth rate model was the closest to 1, whereas this factor indicate not good relation between observed and predicted lag time values. Otherwise, the bias factor of three models was 1, which indicates that on average, none model underestimates or overestimates growth parameters.

Table 2. Coefficients of regression of polynomial models for each *P. digitatum* growth parameter.

Polynomial model:

$$y = \beta_0 + \beta_1 \times T + \beta_2 \times a_w + \beta_3 \times \text{pH} + \beta_4 \times T \times a_w + \beta_5 \times T \times \text{pH} + \beta_6 \times a_w \times \text{pH} + \beta_7 \times T^2 + \beta_8 \times a_w^2 + \beta_9 \times \text{pH}^2$$

Parameter	$\ln y_{max}$	$\ln \mu$	$\ln \lambda$
β_0	−22.235	31.681	24.508
β_1	0.461	0.189	−0.631
β_2	19.903	−89.292	19.397
β_3	2.833	−0.030	−4.791
β_4	−0.188	−0.044	0.117
β_5	−0.003	−0.002	−0.004
β_6	−2.854	0.744	3.687
β_7	−0.005	−0.002	0.008
β_8	2.613	50.122	−33.136
β_9	−0.007	−0.045	0.143

Table 3. Accuracy and bias factors of polynomial models of *P. digitatum* growth.

Model	Accuracy factor	Bias factor
μ	1.28	1.00
λ	2.43	1.00
y_{max}	1.51	1.00

Predicted curves are generally described between replicates and the variability observed is product of variability propagation of each predicted growth parameter. As was established previously, the more important discordance between observed and predicted values was obtained with latency model, results of this nature are commonly observed in lag time models for bacterial growth models. This discordance is originated by high variability of observed lag time. A more important variability has been previously reported for lag time than for growth rate [29] and it has been explained because growth rate is one implicit characteristic of the microorganism, while lag time is more affected by not controlled variables [30] [31] such as time and environmental history.

4. Conclusion

These results suggest that implicit growth rate is an important factor to take into account when a strain is elected to build a predictive model; it is possible that a fast growth rate could mask significant effects of environmental factors (such pH) as well as their interactions. These results showed that pH exerts a significant effect, furthermore, interactive and quadratic effects are too significant—at least when citric acid is used to adjust pH, and they are regularly underrated when fungi growth models are developed. Since the most important effect of pH is observed on maximal growth, it is important to verify nonexistence of factor effects before omitting the modeling on this growth parameter. Nevertheless, further studies on effect of undissociated acid concentration and nature of acid and solute employed to adjust pH and a_w, are necessary.

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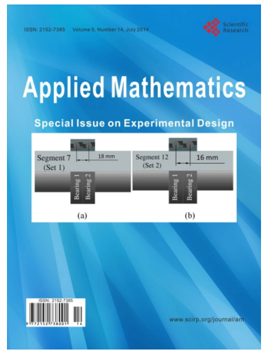
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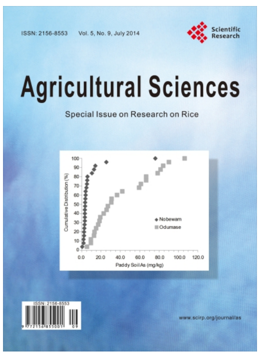
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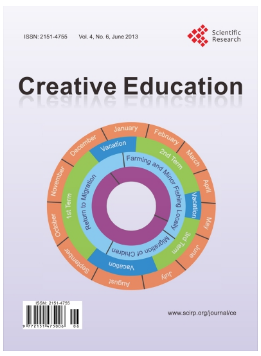
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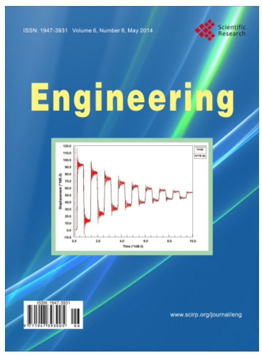
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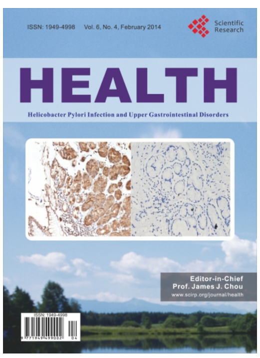
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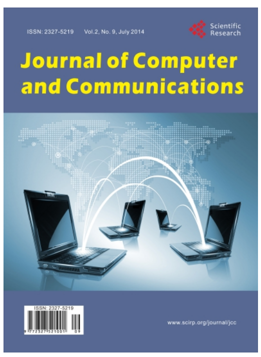
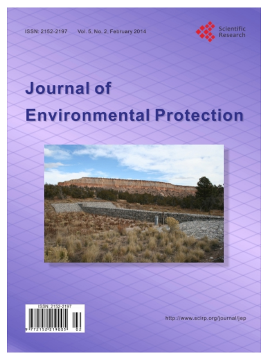

Journal of Computer
and Communications


Journal of Cancer Therapy
Special Issue on Bone Tumor Research


Journal of
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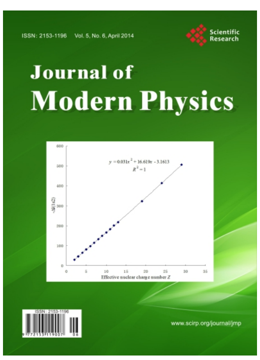
Journal of
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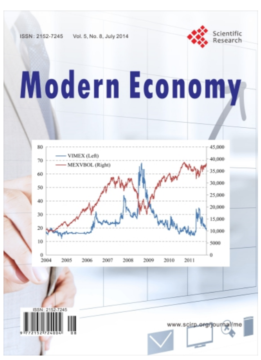
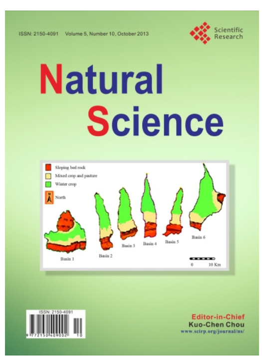

Modern Economy


Natural
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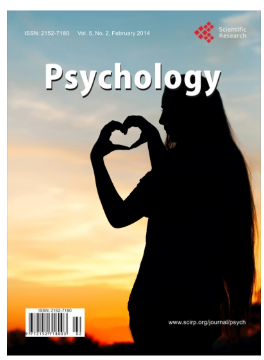
Psychology