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EFFECT OF STORAGE CONDITIONS OF SPORES OF *PENICILLIUM DIGITATUM* ON ITS PARAMETERS OF GROWTH

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ABSTRACT

The objective of this work was determine the effect of temperature (0 and -9°C) and time (0, 7, 14 and 21 days) of storage, on the parameters of growth, (lag time and growth rate) of one spore suspension of *Penicillium digitatum*. Experimental

conditions were established by one factorial full design. The growth was realized in dextrose Sabouraud agar and the diameter of the colony was measured daily. The parameters of growth were calculated by fit of the growth curves with the Baranyi equation using the DMFit software. The results showed that the lag time first increased with the duration of storage in low temperature and then decreased as the duration of storage ($p < .05$), while the growth rate only was influenced by the time of storage and not by the temperature.

Keywords

Penicillium digitatum, spore, lag time, growth rate.

RESUMEN

El objetivo de este trabajo fue determinar el efecto el efecto de la temperatura (0 y -9°C) y tiempo (0, 7, 14 y 21 días) de almacenamiento, sobre los parámetros del crecimiento (tiempo de latencia y velocidad de crecimiento) de una suspensión de esporas de *Penicillium digitatum*. Las condiciones experimentales fueron establecidas por un diseño factorial completo. El crecimiento fue realizado en agar dextrosa Sabouraud y el diámetro de las colonias fue medido diariamente. Los parámetros de crecimiento fueron calculados por el ajuste de las curvas del crecimiento con la ecuación de Baranyi usando el programa DMFit. Los resultados demostraron que el tiempo de latencia inicialmente aumenta con la duración del almacenamiento en baja temperatura y posteriormente disminuye conforme aumenta el tiempo de almacenamiento ($p < .05$), mientras que la velocidad de

crecimiento solamente fue influenciada por el tiempo y no por la temperatura de almacenamiento.

PALABRAS CLAVE

Penicillium digitatum, esporas, tiempo de latencia, velocidad de crecimiento.

INTRODUCTION

Food microbiologists know that spores are one low activity metabolic form resistant to adverse factors such as low temperature, for this some researchers realize one reactivation step by avoiding lag time prolonged while another esteem that such effect not exist and realize the kinetics directly of the refrigerated spore suspension.

Some researchers affirm that the damaged cells, one time that recover them generate the same subpopulations that those cells not damaged (Baranyi and Roberts, 1993), other researchers have confirmed that temperature historic, yet without verify cell damage, affect the behavior of cells microbial to inoculate them in culture media (McKellar and Knigth, 2000). Some works have been realized in bacteria (Jackson and Wodbine, 1963) in which have observed one increased in the lag time with the stress duration but they studied only one stress temperature. Bréand *et al*. (1997) observed that for a fixed stress temperature, the lag time first increased with the stress duration and then decreased as the stress duration increased. There is little information about the effect of stress temperature on lag time of bacteria, and less on lag time and growth rate of filamentous fungi.

Most of studies of fungi growth require one spore suspension to inoculate the culture media or foods. Some investigators prepare such suspension culturing the strains on potato-dextrose agar slants for 10 days at 25°C and harvest the spores with 10 mL of 0.1% Tween 80 and used it the same day (López-Malo *et al*., 1998), or prepared of one culture of 6 days at 20°C and harvesting the spore suspension in deionized water containing 0.2 % Tween 20 (Chardonnet *et al*., 2002) while another do it of one culture of 5 days to 25°C (Stiles Battey *et al*., 2001).

From the food predictive microbiology viewpoint, the way in that growth rate data are obtained is important since the study of fungal growth is realized trough the growth kinetic based in compilation of growth data and then to generate mathematical models capable to predict the growth parameters of molds in different conditions. Thus the aim of this work was to study the response of *Penicillium digitatum* when the spore suspension employed for calculate its parameters was storage in stress conditions.

MATERIALS AND METHODS

Spores suspension

In this study was used *Penicillium digitatum* isolated of citrus fruits and identified accorded to Samson *et al*. (1995/d) and was grown on dextrose Sabouraud agar for 7 days at 25°C, the time necessary to obtain cultures with sufficient spores, which were harvested with 10 mL of 0.1% Tween 80 (Merck) solution sterilized by membrane (0.45 □m) filtration. The spore suspension was adjusted with the same solution to give a final spore concentration of 10^6 spore/mL. The suspension was

distributed in three portions and were treated according to full factorial design: one of them was employed immediately after its preparation and was used as reference, the other two portions were storage to 0 and -9°C for 7, 14 and 21 days.

Inoculation

Plates of dextrose Sabouraud agar with water activity 0.990 and pH 5.0 were inoculated directly with 2 □L of each one portions and too were revitalized in agar dextrose Sabouraud slants to 25°C for 7 days and prepared the spore suspension again, proceeding after to inoculate the culture media. All experiments were carried out with at least three separate replicate Petri plates per treatment and collocated in plastic box and were incubated to 30°C for 30 days.

Growth measurement

The plates inoculated were observed every day to observe growth and each 24 hours the diameter of colonies was measured with one ruler. The diameter of colonies was plotted against time and was obtained growth curves of each condition which were adjusted with the Baranyi equation using the software DMFit, obtaining thus the parameter growth of mold in each condition.

RESULTS AND DISCUSSION

Table 1 show the parameters of growth obtained in each studied condition and the analyses of the results showed one significant effect ($p < 0.05$) of storage time, and activation of spores on the lag time (Fig. 1) was observed that in accordance with

increase the time storage in stress condition the effect decreased. Similar results were obtained for Bréand *et al*. (1999) for lag time in bacterial culture, although not explain the phenomena. Effect of temperature was not significant for lag time.

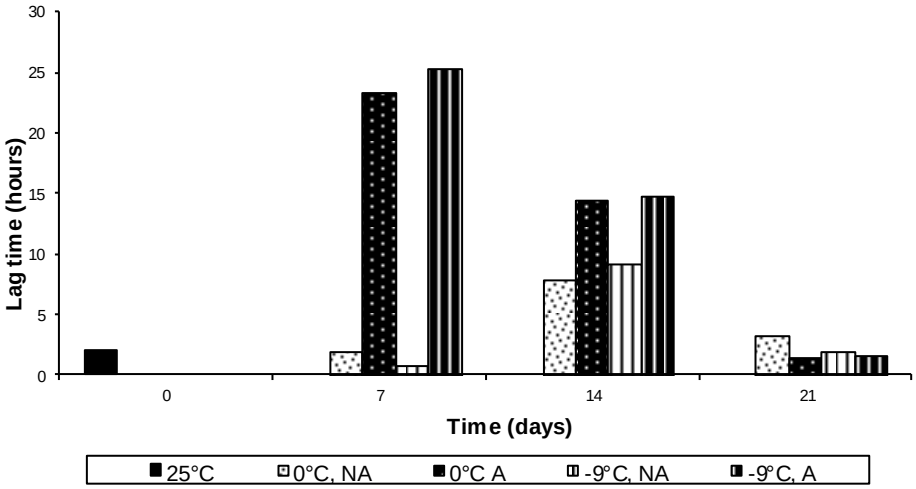
Table 1. Parameters growth – lag time (λ) and growth rate (μ) of *P*. *digitatum* storage to 0° and -9°C, not activated (NA) and activated (A) in dextrose Sabouraud agar.

Tabla 1. Parámetros de crecimiento –tiempo de latencia (λ) y velocidad de crecimiento (μ) de *P*. *digitatum* almacenado a 0° y -9°C, no activada (NA) y activada (A) en agar dextrosa Sabouraud.

Time (days)	25°C		0°C NA		0°C A		-9°C NA		-9°C A	
	λ	μ	λ	μ	λ	μ	λ	μ	λ	μ
0	2.06	0.1395								
7			1.88	0.127	23.34	0.135	0.693	0.132	25.31	0.138
14			7.86	0.129	14.36	0.132	9.16	0.127	14.73	0.134
21			3.18	0.130	1.42	0.130	1.88	0.133	1.54	0.135

Figure 1. Effect of temperature, time and activation on the lag time of *P*. *digitatum* in dextrose Sabouraud agar.

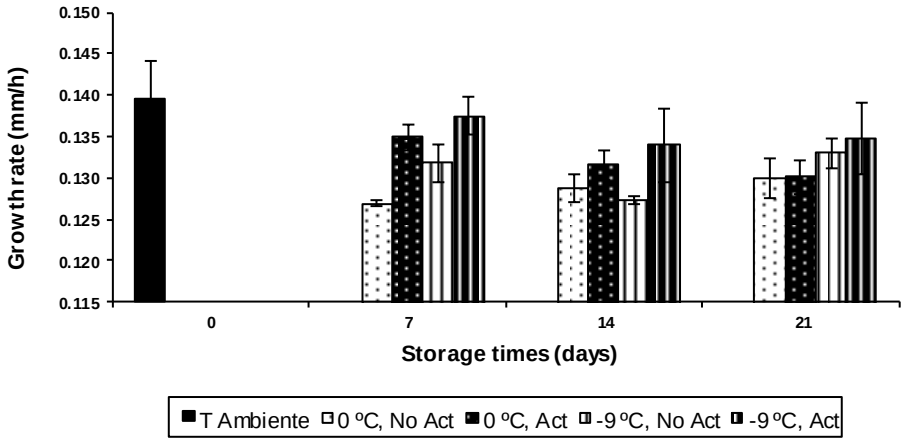
Figura 1. Efecto de la temperatura, tiempo y activación sobre el tiempo de latencia de *P*. *digitatum* en agar dextrosa Sabouraud.



Respect to growth rate, was observed only effect of time (Fig 2), temperature and activation not were significant. However such effect, although is statistically significant, in practice is not, since to make round the growth rate is equal in all conditions. This results are coherent with the concepts and definitions of lag time and growth, due to that is waited that the low metabolism present in the spores and induced by storage conditions, affect only to lag time and that one time that start the development, the growth rate permanence invariable (Baranyi and Roberts, 1993).

Figure 2. Effect of temperature, time and activation on the growth rate of *P*. *digitatum* in dextrose Sabouraud agar.

Figura 2. Efecto de la temperatura, tiempo y activación sobre la velocidad de crecimiento de *P*. *digitatum* en agar dextrosa Sabouraud.



This results doing suppose that despite the low activity inherent to the spores, these put in march one physiological mechanism by adapt to stress, and is manifested when the spores present lag time similar to those spores obtained recently.

CONCLUSIONS

The results shown that stress to low temperature has effect on lag time of *P. digitatum*, which spores were storage in low temperature, however, such effect decreased when the duration of stress was increased, while the growth rate is affected only by effect the time of storage and not by the temperature.

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