Effects of several factors on fungal spore germination in solid state fermentation of coprah cake

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SUMMARY

Optimization of the culture conditions of *Aspergillus carbonarius* (Asca), *Aspergillus* sp (C11B52) and *Penicillium* sp (V26A25), cultured in coprah cake solid state fermentation (SSF), was undertaken, using experimental matrices. The effects of seven culture parameters (humidity = F1, initial $(NH_4)_2SO_4$, $CaCl_2$ and $MgSO_4.7H_2O$ concentrations = F2, F6, and F7, respectively, initial pH = F3, inoculum size = F4 and temperature = F5) on spore germination time and respiratory activities of the strains were studied. In general, the shortest lag phase was observed under the lowest values of the F1, F2, F3 and F7 parameters. Under these conditions *Aspergillus carbonarius* (Asca) and *Aspergillus* sp (C11B52) showed the highest respiratory activities (6.3% and 10.2%, respectively), while the highest respiratory activity (≈ 5%) of *Penicillium* sp was observed at the lower values of F2, F3, F4 and F5 parameters.

Keywords: *Aspergillus carbonarius, Aspergillus sp., Penicillium sp.*, coprah cake, spore germination, solid state fermentation, Lag phase, germination time, respiratory activity, protein enrichment.

RESUME

Effets de différents facteurs sur la germination de spores de champignons filamenteux cultivés sur tourteau de coprah en milieu solide.

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L'optimisation des conditions de culture d'*Aspergillus carbonarius* (Asca), *Aspergillus* sp (C11B52) et *Penicillium* sp (V26A25), cultivés sur tourteau de coprah en milieu solide, a été réalisée en utilisant des plans d'expérience. Les effets de sept paramètres de culture (humidité = F1, concentrations initiales de $(NH_4)_2SO_4$, $CaCl_2$ et de $MgSO_4.7H_2O$ = F2, F6, et F7 respectivement, pH initial = F3, taille d'inoculum = F4 et température = F5) sur le temps de germination de spores et les activités respiratoires des souches ont été étudiés. Les temps les plus courts pour la germination de spores ont été obtenus pour les valeurs les plus faibles des facteurs F1, F2, F3 et F7. Sous ces conditions expérimentales *Aspergillus carbonarius* et *Aspergillus* sp. ont montré les plus grandes activités respiratoires (6,3% et 10,2% respectivement) alors que l'activité respiratoire la plus élevée (≈ 5%) observée pour *Penicillium* sp. a été obtenue avec les plus faibles valeurs des paramètres F2, F3, F4 et F5.

Mots clés: *Aspergillus carbonarius, Aspergillus sp., Penicillium sp.*, Tourteau de coprah, germination de spores, Phase de latence, temps de germination, activité respiratoire, enrichissement en protéines.

INTRODUCTION

The economic and social significances of fungi, as sources of food and biologically active metabolites, stimulated the interest in their growth physiology. One of the important variables of fungal growth is spore germination time, since it determines the duration of the lag phase, and, consequently, the duration of the fungal fermentation. A prolongead lag phase in fungal fermentation leads to increased cost of production of microbial products. It, is, thus, essential to achieve a shorter spore

germination time, either by selecting a fungal strain of short lag phase or by regulating culture conditions, e.g., tannic acid addition (Gaitis and Marakis, 1994).

A strain of *Aspergillus carbonarius*, with high tanninolytic ability, has been isolated from mouldy carob (Marakis, 1980). Although this fungus physiology has been fairly well studied for microbial protein enrichment and metabolite (enzymes) production in submerged shaker cultures (Marakis, 1980, 1985; Marakis and Diamandoglou, 1990), no study on the growth of this strain of *A. carbonarius* in SSF has been carried out, so far. A comparative study of this strain with *Aspergillus* sp and *Penicilium* sp, previously studied for protein enrichment (Roussos *et al*, 1994) and enzyme production (Roussos *et al*, 1995) in SSF system, is attempted.

This paper describes the effects of seven factors on the spore germination time and respiratory activities of *A.carbonarius, Aspergillus* sp and *Penicillium* sp, cultured in coprah cake SSF.

MATERIALS AND METHODS

MICROORGANISMS

Aspergillus carbonarius (Bainier) Thom, was isolated from mouldy carob beans (Marakis, 1980), while *Aspergillus* sp (C11B25) and *Penicillium* sp (V26A25) were obtained from the microbial collection of the Biotechnology laboratory of ORSTOM (Roussos *et al*, 1995).

MEDIA

A mineral solution of 0.36% Na_2HPO_4, 0.3% KH_2PO_4 and 1% urea, on initial substrate dry matter (IDM), was added to coprah residue, while $(NH_4)_2SO_4$, $CaCl_2$ and $MgSO_4.7H_2O$ solution of specific concentration, as shown in Table 1, were then added to obtain the desired initial humidity and mineral contents.

Table 1. Relation between code numbers (limits of factor values) and real values of the factors examined.

Factors	Limits of factor values	
	-1	1
F1 = Initial humidity (% IDM)	60	70
F2 = $(NH_4)_2SO_4$ (%IDM)	5	10
F3 = Initial pH	6	7
F4 = Inoculum size (spores/g IDM)	$2x10^6$	$2x10^7$
F5 = Temperature (°C)	25	30
F6 = $CaCl_2$ (% IDM)	0,3	0,6
F7 = $MgSO_4.7H_2O$ (% IDM)	0,3	0,6

CULTURE CONDITIONS

The values of the seven factors studied are presented in Table 1. The optimization of the culture conditions (Table 2) was approached by using experimental matrices (De Meo *et al*, 1985). Solid state fermentationwas carried out according to the procedure of Raimbault and Alazard (1980).

SPORE GERMINATION DETERMINATION

Spores were considered to be germinated when their germ tube lengths were one and a half times the size of the spore diam (Frossard and Oertli, 1982).

MEASUREMENT OF CO_2

Carbon dioxide in the dry output air flow was continuously measured by using a gas chromatograph, as per the method of Saucedo-Castañeda *et al* (1993).

PROTEIN EVALUATION

Proteins were estimated by total nitrogen titration, as per the total carbonization method (CHN), using a LECO SP 428 apparatus (USA), or by Kjeldhal using Kjeltabs CTQ catalyso, Thomson-Capper Ltd (Prolabo) France. The quantity of ammonium nitrogen was determined colorimetricaly using the Alliance Evolution II automatic apparatus (France) and the value was deducted from the total nitrogen. Results obtained were multiplied by a factor of 5.3 for the unfermented coprah cake and by 6.25 for the fermented products (Rham, 1982).

Table 2. Experimental matrice with code numbers of the factors examined.

Expt.	Limits						
	F1	F2	F3	F4	F5	F6	F7
1	-1	-1	-1	1	1	1	-1
2	1	-1	-1	-1	-1	1	1
3	-1	1	-1	-1	1	-1	1
4	1	1	-1	1	-1	-1	-1
5	-1	-1	1	1	-1	-1	1
6	1	-1	1	-1	1	-1	-1
7	-1	1	1	-1	-1	1	-1
8	1	1	1	1	1	1	1

RESULTS AND DISCUSSION

Spore germination time and respiratory activities were found to depend on fungal strain and culture conditions. All the strain developed well enough on coprah cake in SSF, though with different respirometric profiles. This was not only because of their physiological differences (Roussos *et al*, 1994), but also due to the culture conditions.

A. carbonarius showed the shortest lag phase (2.5 h) and the maximum respiratory activity (6.3% CO_2) after 18 h incubation in experiment No. 1, while the longest

(15 h) lag phase and the lower respiratory activity (3.75% CO_2) was observed in experiment No. 7 (Table 3). Roussos *et al* (1994) also reported a lag phase of 3 h and a respiratory activity of 7%, for the same strain and substrate. On the basis of the above data, and those presented in Fig. 1, the optimization of *A.carbonarius* growth could be achieved if a) substrate initial humidity, inoculum size and incubation temperature values are about 70%, $2x10^7$ and 30°C, respectively, b) nitrogen source and pH are less than 5% and 6, respectively and c) concentrations of $CaCl_2$ and $MgSO_4.7H_20$ are lower than 0.3%. This microorganism has shown similar nutritional or physical requirements, in shaker submerged culture system (Marakis and Diamantoglou, 1990) as well as in solid state fermentation of carob pods (Lambraki *et al*, 1994), thereby indicating that this strain requires same culture conditions, independent of the cultivation system.

Table 3. Growth parameters of *A. carbonarius, Aspergillus* sp. and *Penicillium* sp. uner different culture conditions

Microorganisms	Expt No	Shortest germination Time (h)	Maximum CO_2 production (%)	CO_2 production rate (h^{-1})	Optimum temperature (°C)
A. carbonarius	1	2,5	6,3	0,37	30
Aspergillus sp.	1	15	10,2	0,34	30
Penicillium sp.	1,8	10	?	0,14	25
	2,5	?	4,8	0,14	?

Aspergillus sp. (C11B52) showed growth requirements, similar to *A. carbonarius* (Fig. 1), but the shortest lag phase (15 h) of this strain was 6 fold longer than that of *A. carbonarius*, as observed in experiment No. 1, while the longest lag period was 38 h in experiment No. 7. A temperature of 30°C was found to be optimum for both of the *Aspergillus* strains (Table 3). This result is similar to that reported by Roussos *et al* (1995) in their studies on isolation of fungal populations at different temperatures. They found that *Aspergilli* species were predominant at 30°C (= 75% of the total population), while the population of *Aspergillus* species was 25%, when the isolation temperaturewas 25°C. On the other hand, the maximum CO_2 production (%) in case of *Aspergillus* sp., was 1.7 fold higher than that of *A. carbonarius* (Table 3), though both the strains showed similar CO_2 production rates (0.34 h^{-1} and 0.37 h^{-1} for *Aspergillus* sp. and *A. carbonarius*, respectively).

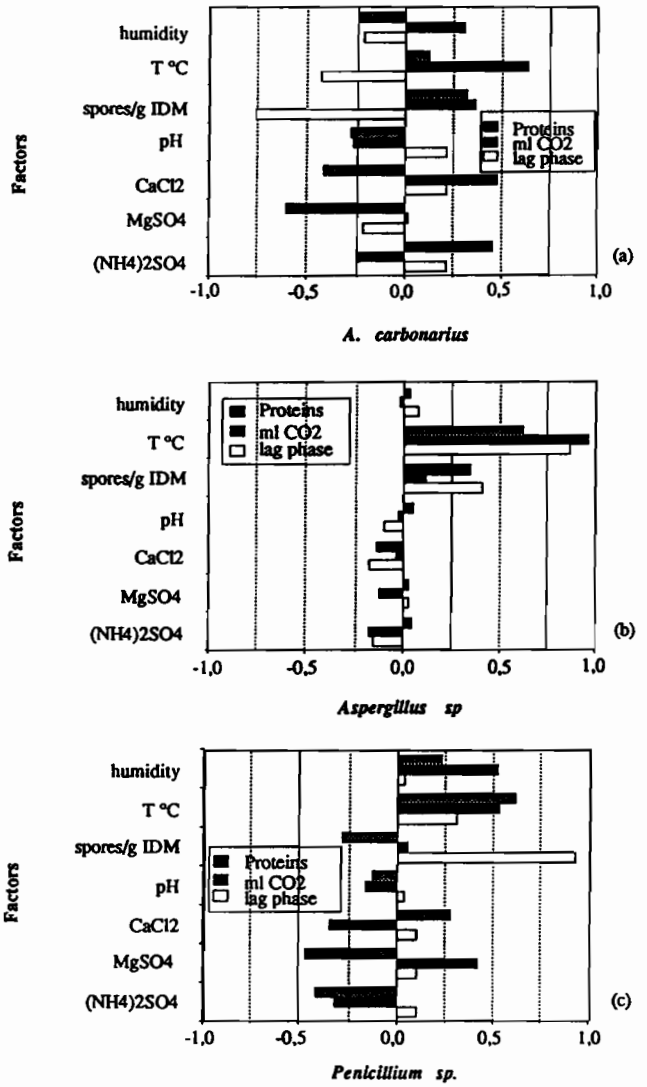

Fig. 1. Protein content, CO_2 production (ml/g IDW), and lag phases of *A. carbonarius, Aspergillus* sp. and *Penicillium* sp. as affected by several culture parameters.

As the extent of CO_2 production indirectly reflects biomass development, the above data indicate that *Aspergillus* sp. C11B52 is most suited for biomass production as well as protein enrichment of agro-industrial byproducts/residues/wastes, while *A. carbonarius* can be of economical benefits in the production of fungal metabolites, such as enzymes, due to its shorter lag phase. Optimization of cultural conditions for each of these two strains for the above exploitation in most efficient manner could be carried out by studing other physico-chemical parameters or by further studing the presently optimized factors at closer range.

Penicillium sp. showed an intermediate (between the other two strains studied) germination time of 10-18 h, but, a comparatively low (4.8%) CO_2 production (Fig. 1). Obviously, this strain is not suitable for fermentation of the above types. Nevertheless, it is interesting to notice that the best and worst growth results for this microorganism were also recorded at experiments Nos. 1 and 7, respectively.

The fact that *Aspergillus* sp. and *Penicillium* sp. were the strains which showed maximum and minimum CO_2 production (%), respectively, is contrary to the report of Roussos *et al* (1994), who cultivated these microorganisms in the same medium. It appears that mineral concentration and aeration flow rate account for these difference in these two kinds of experiments.

Protein values were calculated from the elementary nitrogen composition, from which the amount of ammonium nitrogen was substracted. Titration for the different samples, after 45 h of SSF, revealed that *Aspergillus* sp. cultures had a higher protein content. This was expected as this microorganism presented the highest CO_2 production (Angokai, 1993). *Penicillium* sp. also showed a high protein content, but is characterized by long phase and for this reason it is not economically beneficial.

CONCLUSIONS

Spore germination time and respiratory activity depend on the strain and culture conditions. The culture conditions used in experiment No. 1 proved the most beneficial for the growth parameters of all the fungal strains studied in coprah fermentation. Among these, *Aspergillus* sp. C11B52 is a high-biomass producer, while *A.carbonarius* completes a very quick metabolic cycle.

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