Biotechnology Letters Vol 11 No 7 503-508 (1989)
Received as revised 8th June

STUDIES OF THE SURVIVAL OF FUSARIUM OXYSPORUM CONIDIA PRODUCED BY SUBMERGED CULTURE

A. DURAND, C. VERGOIGNAN, and S. ALMANZA
Plate-forme de Prédéveloppement en Biotechnologie
INRA, 17 rue Sully 21034 DIJON France

SUMMARY : To study the survival of conidia of Fusarium oxysporum produced by submerged culture on malt extract, a harvesting process and different packaging and storage conditions have been tested. Conidia dried with talc and stored at +4°C preserve their viability after about 4.5 months.

INTRODUCTION

Fusarium wilt diseases are the most important pathological problem in various soilless cultures in greenhouses such as tomatoes, muskmelons, carnations, etc (Y. Couteaudier, 1981). To attempt to eradicate these diseases, the growers use soil treatments with methylbromide or steam but the efficiency is very low. Another possibility is to use resistant varieties when they exist, but very often the pathogenic Fusarium quickly adapts its pathogenicity to the new plants (Alabouvette, 1987).

Alternatively, many workers have demonstrated that some soils are resistant to this desease (Rouxel et al., 1979 ; Alabouvette et al, 1980) due to the existence of antagonistic microflora in these soils and competition phenomenon for nutrients. Strains isolated from a suppressive soil and introduced in infested soils after a treatment by steam showed the establishment of the suppressiveness in these soils (Rouxel et al., 1979 ; Cook,1985). Based on these results, the incorporation and multiplication of antagonistic microorganism could be a useful strategy, but to achieve effective biological control, it is necessary :

- to produce the selected microorganism at a low price,

- to perfect harvesting, packaging and conservation processes,in order to be sure that the antagonist survives, multiplies and inhibits pathogens in the soil.

Based on previous results (Tello-Marquina et al, 1980 ; Tello-Marquina et al, 1984 ; De la Broise, 1988), we describe the scaling-up of a process producing a non-pathogenic Fusarium and the behaviour of conidia during conservation under different conditions.

MATERIALS AND METHODS

Biological material

The strain used has been isolated from a wilt disease resistant soil in the south of France. The workers of La Station de Recherche sur la Flore Pathogène dans le sol in Dijon have selected a non-pathogenic strain named : Fusarium oxysporum F.O. 47. This strain is maintained on malt-extract agar (soluble malt-extract,10 g/l, agar, 15 g/l) at 25°C.

Culture conditions

The production of conidia has been achieved in a 400 L reactor (Biolafitte). The medium used was a malt-extract solution at 50 g/l. The pH was regulated at pH 5.0 with H_2SO_4 (4N) and NaOH (4N), the temperature was maintained at 25°C and the aeration rate was 2 v.v.m.. Aseptic inoculation was performed from a 50 L reactor containing the same medium, inoculated with flasks (1.5 L) incubated on shaker for 3 days.

After 72 hours, we obtained about $6.25 \pm 0.94 \times 10^8$ conidia per ml of the broth. At the end of the cultivation, the broth was filtered in a filter press under about 0.5 bar pressure (porosity of plates was 5.7 µm). The residual cake was subjected to different treatments and storage conditions as shown in Fig 1. At the different steps of the process, we measured the conidia number and the viability as described below during a period of 7.5 months.

Conidia numeration and viability

Conidia formation was followed by numeration with a Malassez hemacytometer. For the viability measurements, a small quantity of the congelated or dried cake is homogeneized and diluted with sterile water solution of Tween 80 (1%). One milliliter of appropriate dilutions is settled in Petri dishes before adding malt-extract agar (malt-extract 10 g/l, agar 15 g/l) and incubated for 3 days at 25°C.

For each numeration, 6 dilutions were made on 3 different samples and for each dilution,5 Petri dishes were incubated. The results are expressed as colony forming unit (cfu) per gram of cake.

RESULTS AND DISCUSSION

The effects of the filtration, congelation and drying

After 72 h cultivation, the numeration with a Malassez hemocytometer was : $6.25 \pm 0.94 \times 10^8$ conidia/ml broth.
Just after the filtration, by the suspension dilution method and incubation in Petri dishes we obtained $5.44 \pm 0.49 \times 10^9$ c.f.u/g wet cake. Taking into account the total weight of the cake obtained by filtration of 20 liters of the broth (2480 g) we have 1.35×10^{13} c.f.u/20 l or about 6.75×10^8 c.f.u/ml broth. We conclude that no losses occur during the filtration and that 100 % of the conidia produced can germinate.
As shown in Figure 1, different treatments are made on this cake. The difference between the two freezing modes is that for the slow freezing we froze a thick layer (about 3 cm) and for the fast freezing, the cake had only 3 mm thickness. Just after freezing, the values obtained are summarized in the Table 1.

Table 1 : Influence of the freezing mode on the viability of the conidia

Freezing mode	c.f.u/g wet cake	viability loss (%)
slow	$4.15 \pm 1.0 \times 10^9$	24
fast	$4.95 \pm 0.6 \times 10^9$	9

We can observe that a fast freezing at -18°C seems better for maintaining the viability of conidia.
The frozen cakes were stored at -18°C and periodically we have measured the viability.
Based on previous results obtained in our laboratory (De la Broise, 1988) another part of the cake coming from the same culture was treated as follows :
1 kg of wet cake (27.5 % dry-matter) was diluted with 1100 ml of water and well homogeneized with 3.3 kg of talc in a

kneading-trough.This mixture was then dried at 20°C for 3 days.Taking into account the dilution in the talc, we obtained just after the drying 1.39 ± 0.23x10^9 c.f.u/g mixture. The residual water percentage was about 6%, so on the basis of dry matter we had obtained 1.48 ±0.24x10^9 c.f.u/g dry mixture for a theoretical value of 1.50x10^9 c.f.u/g dry mixture. The germination rate (100 %) was not affected by the addition of talc and the drying at 25°C.

Effect of conservation modes on the viability of conidia

As mentioned on Figure 1, different storage conditions have been studied to follow the conidia viability when :

- they are stored at -18°C, the cake humidity being 72.5%
- they are stored at +4°C or +20°C after drying with talc (residual humidity about 6 %)

The conidia viability was measured every week at the beginning of the storage, and after, every month for about 6 months. For the conidia stored at -18°C, we observe during the first month a fast decrease of the viability. After this period, taking into account the plating technique accuracy, we observe a stabilization, the conidia remaining constant for about 3-4 months for both the slow and the fast frozen conidia. After 4.5 months we observed a further fall of the viability (Figure 2).

For the dried cakes, the evolution during the storage is very different. We observe no change during about 4.5 months for the dried cake stored at +4°C. On the other hand,the dried cake stored at +20°C shows a fast decrease of viability with a short stabilization between 3 and 8 weeks (Figure 3).

The behaviour of the different samples in relation to the storage conditions can be explained by the fact that different kinds of conidia (mainly microconidia and chlamydospores) exist at the end of the culture and the evolution of the percentage of the different kinds can differ during storage.

In conclusion, after drying with talc, no losses occur during the drying process and the conidia viability remains constant for 4.5 months when the dried cake is stored at 4°C. Moreover, for field spreading, the process is very easy, only a water suspension of the dried cake being necessary.

REFERENCES

- Alabouvette, C., Rouxel, F. and Louvet, J. (1980) Ann. Phytopathol., 12, 11-19
- Alabouvette, C. (1987) In "Integrated and biological control in protected crops" Ed. R. Cavallaro. Proceedings of a meeting CEE. Heraklion, 1985.
- Cook, R.J. (1985) Phytopathology, 75, 1, 25-29
- Couteaudier, Y. and Alabouvette, C. (1981) Acta Horticulturae, 126, 153-158
- De la Broise, D. (1988) "Croissance et sporulation de Fusarium oxysporum en milieu liquide et solide". Thesis. Université de Compiègne (France)
- Rouxel, F. Alabouvette, C. and Louvet, J. (1979) Ann. Phytopathol., 9, 199-207
- Tello-Marquina, J.C. and Alabouvette, C. (1984) Agronomie, 4, 885-890
- Tello-Marquina, J.C., Alabouvette, C. and Louvet, J. (1980) Phytopathol., 12, 227-233

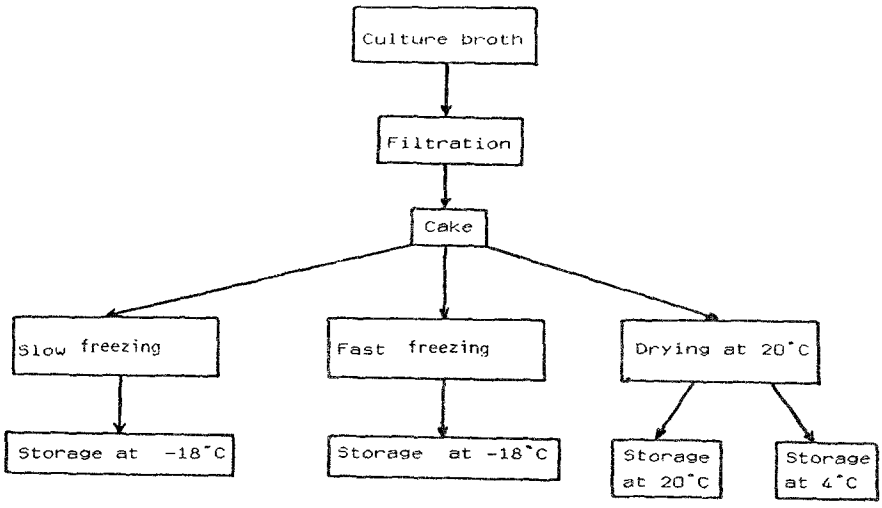

Figure 1 : Experimental procedure used for the study of conidia viability

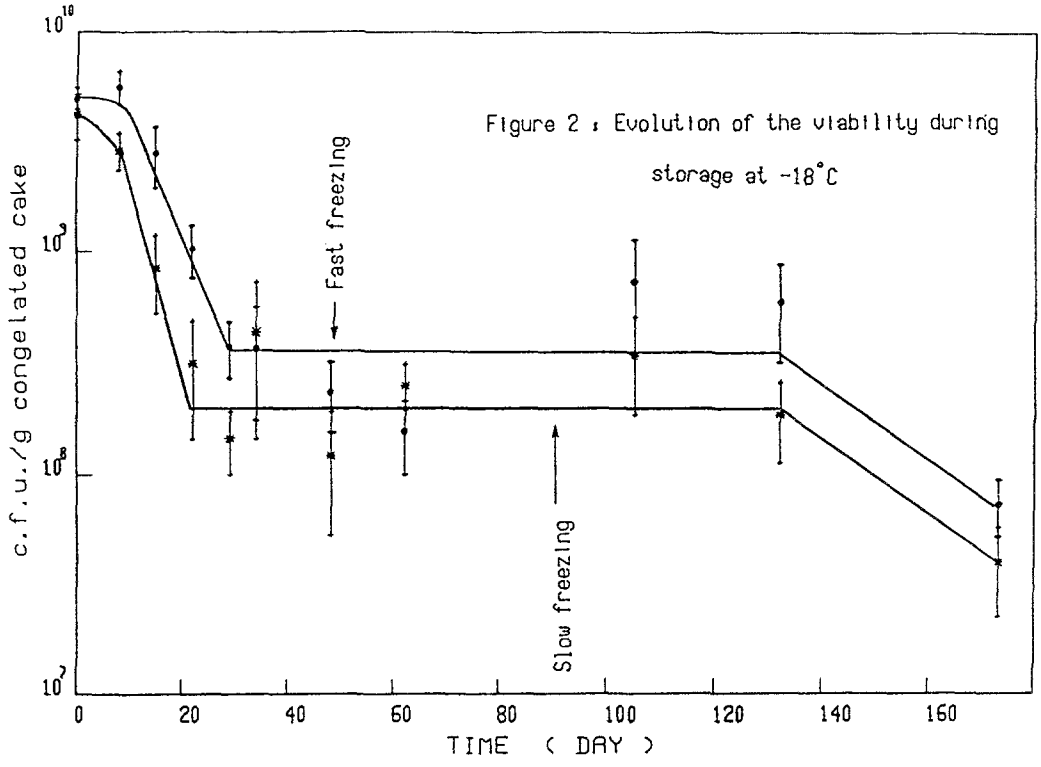

Figure 2 : Evolution of the viability during storage at -18°C

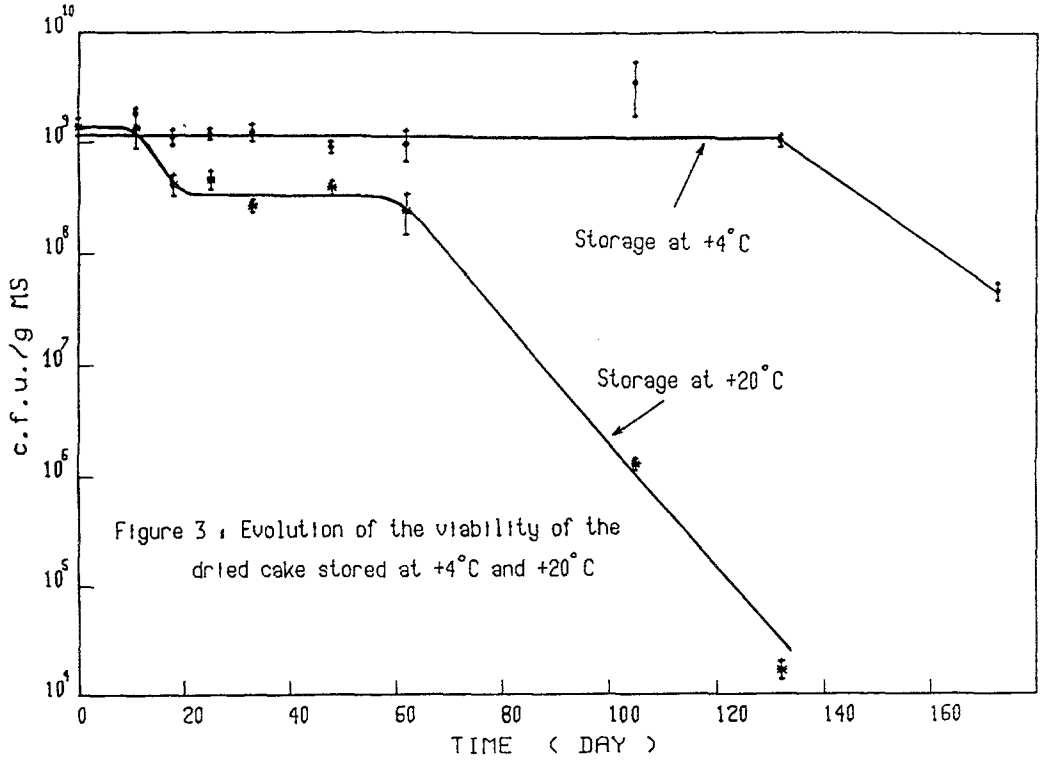

Figure 3 : Evolution of the viability of the dried cake stored at +4°C and +20°C