

SHORT COMMUNICATION

## Freeze-dried preparations of *Penicillium spinulosum*

IAN BOYD AND KENNETH BULLOCK

**B**Y a freeze-drying process powders have been obtained containing mould spores in even distribution. These are suitable for use in studying the survival of such spores in the dry state under various environmental conditions, including the presence of fungicidal agents. The preparation of such powders by the more difficult and less easily controlled process of spray-drying has been described by Brown & Bullock (1960).

Spore suspensions were prepared by the following technique. Stock cultures of *Penicillium spinulosum* strain 42237 C.M.I. were maintained on 3% malt extract agar slopes incubated at 25° for 21 days and subsequently stored at 5°. Malt extract agar plate cultures were prepared from a stock slope and incubated at 25° for 21 days; spores were then washed off the surface of the cultures with sterile water. Gentle shaking wetted the large clumps of spores which were pipetted off and discarded. The remaining, as yet unwetted, spores were suspended in a further volume of sterile water by vigorous shaking and the suspension was filtered through a No. 3 sintered glass filter. The final spore suspension contained approximately 85% single spores. Viable counts were performed by the roll-tube method using rose bengal malt extract agar (Brown, 1957).

In the drying process a quantity of spore suspension was added to an aqueous 10% kaolin suspension so that the mixture gave a viable count of about  $200 \times 10^3$ /ml. The mixture (50 ml) was rapidly frozen in a 1 litre round-bottomed flask by rotating in a solid carbon dioxide/acetone mixture. The flask was then attached to a condenser cooled to -72° by solid carbon dioxide and the pressure reduced to 0.1 mm Hg. This primary drying was continued until the temperature of the external surface of the flask rose to 15° (4 hr). The resultant flaky powder was triturated lightly in a mortar and finally dried (16 hr) over phosphorous pentoxide (20-23°/1 mm). Even distribution of spores in the final powder was established by making viable counts on ten replicate 100 mg samples and submitting the results to an analysis of variance ( $P = 0.50-0.75$ ) (Boyd, 1965).

Portions of the powder were stored over phosphorus pentoxide at 5°, 25° and 37°. Samples were removed at intervals, suspended in water and viable counts made on suitable dilutions. The results of one experiment (Fig. 1) show that the spores are obviously sensitive to increased temperature. When the powder was stored at 5° the viable count remained constant up to 40 days.

From the School of Pharmacy, Sunderland Technical College, Co. Durham, and the Department of Pharmacy, Manchester University, Manchester, 13.

## MOULD SPORE POWDERS

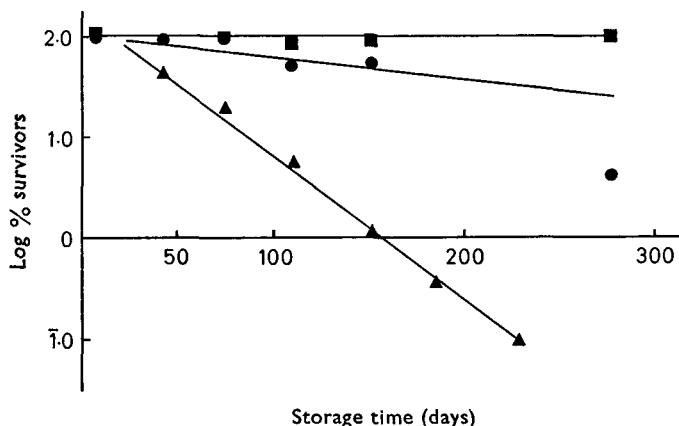


FIG. 1. Effect of temperature of storage on the survival of spores of *P. spinulosum* freeze-dried in kaolin. ■—■ 5°; ●—● 25°; ▲—▲ 37°.

Using the same technique attempts were made to prepare similar spore-containing powders of peptone, malt extract and sodium chloride, but the results were less satisfactory (Table 1). Except for the peptone powder, a smaller percentage of spores survived the drying process and, for some reason at present unknown, replicate counts on these powders during storage showed greater variation than counts on the kaolin powder. The results, however, confirmed that *P. spinulosum* spores die off with increasing rapidity as the storage temperature is increased above 5°.

TABLE 1. THE PERCENTAGE KILL OF SPORES DURING FREEZE-DRYING IN KAOLIN, PEPTONE, MALT EXTRACT AND SODIUM CHLORIDE POWDERS AND THE EFFECT OF STORAGE ON SPORE SURVIVAL, IN THESE POWDERS

Powder	Freeze-drying		Storage			
	% spores killed after		Storage time (days)	% survivors at storage temperatures of		
	Primary drying	Secondary drying		5°	25°	37°
Kaolin .. ..	46	49	76	98	95	19
			148	90	56	1
Peptone .. ..	20	21	65	74	52	8
			177	59	1	0
Malt extract ..	90	92	56	94	45	34
			156	107	3	0.2
Sodium chloride	88	93	56	101	20	6
			156	3	0	0

## References

- Boyd, I. (1965). M.Sc. Thesis, Manchester University.  
 Brown, M. R. W. (1957). M.Sc. Thesis, Manchester University.  
 Brown, M. R. W. & Bullock, K. (1960). *J. Pharm. Pharmac.*, **12**, 124T-126T.