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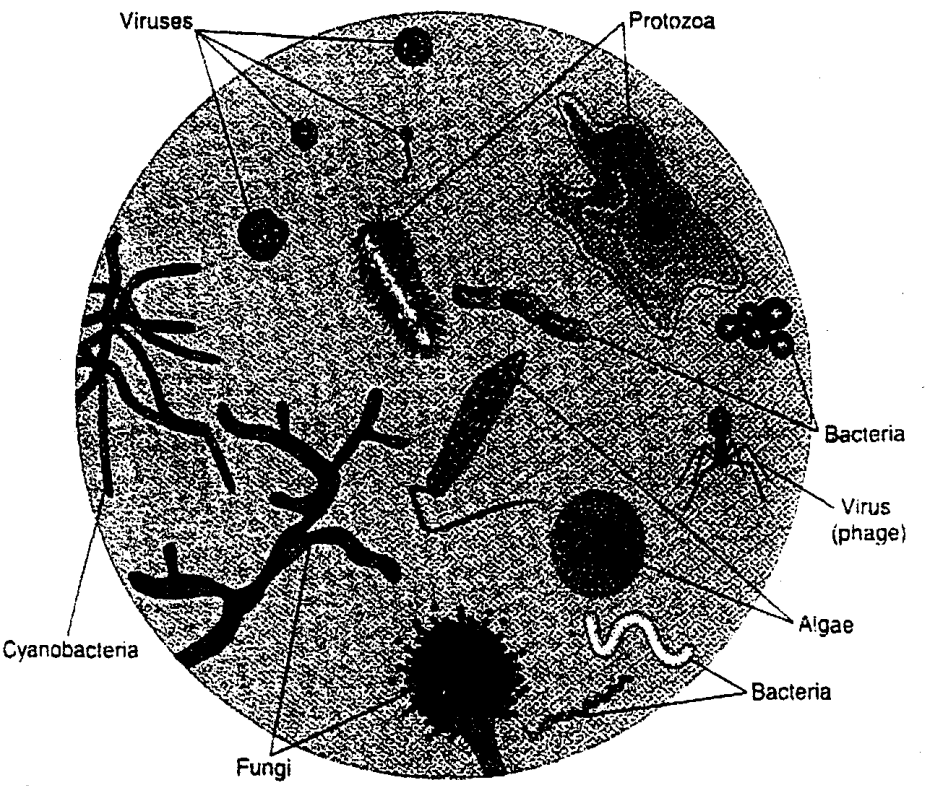

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PRELIMINARY ASSESSMENT OF PATULIN AND CITRININ FROM *PENICILLIUM EXPANSUM* STRAINS SUBMITTED TO DIFFERENT PRESERVATION TECHNIQUES

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During a study of mould contamination of grapes and the assessment of the mycotoxin production capability of the isolates, 51 *Penicillium expansum* strains were isolated and screened for secondary metabolite production. These strains fall into 4 categories according to the ability to produce patulin or citrinin or both mycotoxins in relation to the culture media used for growth and assessment (Abrunhosa *et al.*, 2001).

When grown in culture filamentous fungi exhibit a high tendency toward spontaneous change. Preservation procedures aim at maximizing both the longevity and the stability of stock cultures. In this study 10 of the isolated strains, representative of the 4 classes obtained, were preserved using 4 different methods (subculture every 2 months and maintenance at 4°C, preservation under mineral oil, drying on silica gel and freeze drying) in order to assess the influence of the preservation technique used on the ability to produce patulin and citrinin. The preserved cultures were revived at defined time periods (0.5, 2-3 and 6 months), using Malt Extract Agar (MEA) –Blakeslee's formulation–, Yeast Extract Sucrose agar (YES) and Grape Medium agar (GM). After 7 days at 25 °C, the cultures were transferred to YES and GM for mycotoxin detection using thin layer chromatography (Singh *et al.*, 1991).

All the strains tested are citrinin producers. Citrinin is always detected on YES agar independent of the preservation method. At time zero (control) only one strain produced citrinin on GM corroborating previous studies. However, it was observed that four other strains started producing detectable levels of the mycotoxin when grown in GM after a period of time of preservation. In regard to patulin detection, strains Y146, Y149, C161, C166 and C170 were patulin positive prior to preservation (control). This pattern was maintained throughout the experiment in a quite consistent manner, regardless of the preservation technique or culture media used. Strain Y139 was originally patulin negative in YES medium whereas in GM patulin was always detected. In YES medium the tendency is towards patulin production with time. In the case of strains Y248, C230, C237 and Y291, patulin was not detected in either culture media before preservation. However, the tendency is once more towards patulin detection with time. Patulin was never detected for strain C230 preserved by subculture and maintenance at 4 °C. Testing with a patulin gene probe (Paterson *et al.*, 2000) is under consideration, in case after 1 year preservation results remain negative, to determine whether the original inoculum used suffered alteration and the gene is no longer present or if it is not being expressed. Considering the overall results obtained, variability in the profiles of the mycotoxins tested seems to be more strain specific than dependent on the preservation technique used.

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