# FREEZE DRYING AND RECOVERY OF *Aspergillus flavus* NRRL 6513 STRAIN

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Research or applied studies using live organisms requires their constant supply in appropriate conditions. All preservation techniques are aimed at storage of the organism for several weeks or months to many years with minimal loss of its viability or other key properties during storage. Various methods are available for the preservation of filamentous fungi based on their type and degree of sporulation. Freeze drying is employed for the successful preservation of spore forming varieties of fungal cultures.

*Aspergillus flavus* NRRL 6513 culture obtained from various sources and maintained in the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy which gave an average aflatoxin level of 63.77 ppm was used for the freeze drying and recovery. It was periodically subcultured in potato dextrose agar at 15 days interval to maintain its viability (Shotwell *et al*., 1966).

Lyophilisation was carried out as per ATCC Technical bulletin. Ten ml of 20 per cent solution of autoclaved skim milk was prepared in test tubes and stored under refrigeration until use. About two ml of the chilled skim milk solution was introduced into the test tube containing freshly subcultured fungus. The surface of the culture was gently scraped with a pipette. The suspension obtained was transferred back into the test tube containing the remaining skim milk and mixed thoroughly. A concentration of at least 100 spores per ml of skim milk solution was ensured before freeze drying. Two milliliters of the skim milk suspension was dispensed into each vials meant for freeze-drying and were kept under refrigeration until further processing. The vials were kept at $^{-}$50°C for four days followed by lyophilisation for 24 hours at 300mm torr at $^{-}$80°C. This was followed by vacuum packing at $^{-}50^{0}$C. All the vacuum packed vials were stored at $^{-}$50°C for one month (Simione and Brown, 1991).

One vacuum sealed vial was randomly selected after one month of storage and was opened under sterile condition. Few drops of distilled water were added to the vial and the entire contents were transferred to a test tube containing five ml of distilled water. This was kept at room temperature for four hours until the contents got rehydrated. Few drops from the test tube were transferred to test tube containing potato dextrose agar. The test tubes were kept at room temperature for seven days to obtain sufficient fungal growth.

Aflatoxin was produced on maize as per modified standard procedure (Shotwell *et al*., 1966). Maize was procured from the local market. It was washed thoroughly using tap water and all impurities were removed. The maize was again washed with tap water at least five times and then with distilled water for three times. After washing, maize was evenly spread on a paper and kept for air dry. One hundred grams of the dried maize was placed in a 250ml conical flask and 15ml of distilled

water was added to it and allowed to stand for two hours. Later the conical flask with maize was autoclaved at 125$^0$C, 15lbs for 15 minutes. After autoclaving, the conical flask with maize, it was kept for cooling for one hour. One loop of *Aspergillus flavus* culture from potato dextrose agar was inoculated into maize aseptically and the flask was shaken to mix the culture properly. The inoculated flasks containing maize was kept in a slanting position and was periodically shaken. After obtaining full growth, the maize was autoclaved again and kept for drying at 60$^0$C in hot air oven overnight. Later it was ground into powder using a mixer grinder.

Fungal subculture in potato dextrose agar resulted in velvety green uniform culture (Fig. 1). The maize culture turned completely green in 10 days (Fig. 2). Aflatoxin concentration of the representative sample of ground moldy maize powder was quantified by thin layer chromatography (TLC) at Animal Feed Analytical and Quantity Control Laboratory, TANUVAS, Namakkal. The amount of aflatoxin B1 estimated in the maize powder made from recovered culture was estimated to be 75.86 ppm. Humber (1997) observed that whatever may be the method of preservation, the viability of preserved sample should be checked few days after its preparation. Nakasone *et al.* (2004) reported lyophilisation as preservation method of choice for many spore forming fungi that produce large numbers of spores of 10-µm or less in diameter. The amount of aflatoxins B1 produced by the recovered culture of *Aspergillus flavus* NRRL 6513 strain was slightly more compared to the levels of aflatoxins B1 produced using its stock culture. This better yield proved lyophilisation as an effective method of preservation of *Aspergillus flavus* NRRL 6513 strain. The higher yield obtained could be due to the better environmental conditions obtained during the growth of the recovered culture. Similar higher levels of aflatoxin production in higher environmental temperature were observed by Lin *et al* (1980).

**Summary**

Study was undertaken to assess the effectiveness of lyophilisation in the preservation and recovery of Aspergillus flavus NRRL 6513 strain. The stock culture with known level of Aflatoxin B1 production ability was periodically subcultured in potato dextrose agar at 15 days interval to maintain its viability. Skim milk solution was used for the preservation of stock culture. The fungal culture was lyophilised and vacuum packed. The lyophilised fungus was recovered and aflatoxins B1 level was checked again in maize culture. The post lyophilisation level of Aflatoxin B1 was comparable to the amount produced by stock culture.

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