

1 Extraction efficiency of kojic acid from ammonia-treated or non-treated agar medium with various methanol aqueous solvents

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Kojic acid is one of the major secondary metabolites produced by fungal species of *Aspergillus section Flavi*. In this study, we compared the extraction efficiency of kojic acid from agar media using the five solvents of different mixing ratio of methanol/water (100 %, 75 %, 50 %, 25 % and 0 % of methanol), at two (low or high) kojic acid levels. The effect of ammonia exposure on the extraction efficiency was also examined. In all conditions tested, stable extraction was achieved by 100% methanol and 100 % water, showing extraction efficiency of 75-86 % with low standard deviations. The ammonia exposure did not affect the extraction efficiency when using above two solvents, indicating that 100 % methanol or 100 % water is suitable rather than methanol/water mixture.

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2 Whole agar dish culture extraction method to assess the survival of aflatoxigenic fungi in soil samples

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In this study, we investigated a method of extracting the whole agar dish culture to assess the existence and survival of producing fungi of aflatoxins (AFs) in field soil samples. In a control experiment using a known aflatoxigenic *Aspergillus flavus* strain with dichlorvos (DV) -coated agar or DV-free agar, no significant difference on the recovery of colony numbers was observed while DV clearly inhibited AFs accumulation. Therefore, a method of extracting the whole dish culture of DV-free agar followed by aflatoxin B₁ (AFB₁) analysis was applied to assess the survival of

aflatoxigenic fungi in soil samples during storage. Soil samples stored at different temperature (4 °C, -20 °C, and -80 °C) and duration (3-12 months) were cultured and analyzed for the accumulation of AFB₁ in triplicate. Each soil sample suspension was cultured on DV-free agar for 7 days at 25 °C, and the whole dish culture was extracted by methanol and the amount of AFB₁ was measured. AFB₁ amounts were drastically decreased after the fourth month of storage at all temperatures tested. After the fifth month, AFB₁ amounts of samples stored at 4 °C were significantly lower than those stored at -20 °C or -80 °C, indicating that temperatures below -20 °C may be suitable for longer storage of soil samples. This whole dish culture extraction method is easy to handle and will be applicable to estimate the population of aflatoxigenic fungi among various soil samples from different origin or conditions.

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