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Identifying Molds on Food Products

Isolating, identifying and counting molds from foods, such as almonds, is often complicated by overgrowth of some colonies in the petri plate by rapidly growing spreading molds. These molds such as Rhizopus and Botrytis species produce woolly or cottony colonies that fill the plate and obscure the majority of the colonies that are slower growing and which form low colonies with only a small amount of aerial growth. These smaller colonies such as Alternaria, Aspergillus, Cladosporium and Penicillium species are frequently the molds of interest since they cause spoilage and can produce mycotoxins such as aflatoxin, citrinin, etc.

We have developed a medium that was designed to inhibit the rapidly growing molds yet still let them form small colonies and at the same time allow development of the remaining mold flora. This medium consists of a nutrient basal medium at a pH that allows optimum growth plus added inhibitors. Chlortetracycline is added to inhibit bacterial growth. Rose bengal and dichloran inhibit the proliferation of the spreading molds while influencing the remaining molds only slightly when added at the appropriate concentrations.

Sterilized agar is poured into petri plates and allowed to solidify. Then the inoculum is added to the surface (0.1 ml) and spread evenly. After 4-5 days at 25°C (77°F) the plates can be examined and individual colonies selected for isolation.

The medium formulation is:

Glucose		10.0 g
Peptone		5.0 g
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	0.5 g
Potassium phosphate, monobasic	KH_2PO_4	1.0 g
Agar		15.0 g
Distilled water		1 l

Final pH 5.6

Rose bengal (certified) is made up in water 0.5 g/20 ml water and 1 ml of this stock solution is added per liter of medium (25 ppm rose bengal final concentration).

Dichloran (2,6-dichloro-4-nitroaniline) is made up in ethyl alcohol 0.02 g/10 ml and 1 ml added per liter for a final concentration of 2 ppm. (Refrigerate to prevent alcohol loss from stock solution). The medium is sterilized 15 minutes at 15 PSIG and a chlortetracycline added to the medium cooled to 50°C. Chlortetracycline stock solution (keep in refrigerator) is prepared by dissolving 0.1 g/100 ml water and filter sterilizing. This sterile solution is added 1 ml/100 ml of medium for a final concentration of 10 ppm.

Sources of the specialized reagents are:

Peptone - Difco, Detroit, or
BBL Microbiology Systems, Cockeysville, MD

Rose Bengal - Eastman Organic Chemicals, Rochester, NY

Tetracycline, hydrochloride - Calbiochem-Behring, La Jolla, CA

Dichloran - Aldrich Chemical Co., Milwaukee, WI

Reference:

King, A. Douglas, Jr., Ailsa D. Hocking, and John I. Pitt. Dichloran-Rose Bengal Medium for Enumeration and Isolation of Molds from Foods. Appl. & Environ. Microbiol. 37:959-964, 1979.

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