Handbook of Culture Media for Food Microbiology, J.E.L. Corry et al. (Eds.)

Aspergillus flavus and *parasiticus* agar (AFPA)

This monograph has been reviewed by members of the IUMS-ICFMH Working Party on Culture Media and given 'Proposed' status.

Description and history

This is a selective medium for the enumeration in foods of the mycotoxin producing fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Bothast and Fennell (1974) developed *Aspergillus* differential medium (ADM), containing 1.0% yeast extract, 1.5% tryptone and 0.5% ferric chloride, recommending incubation at 28°C for 3 days. Hamsa and Ayres (1977) incorporated streptomycin and dichloran into ADM and recommended incubation at 28°C for 5 days. Both media relied upon the formation of a bright orange-yellow reverse pigment by *Aspergillus flavus* and related species. Pitt et al. (1983) further refined the formulation, producing *Aspergillus flavus* and *parasiticus* agar (AFPA), which gives sufficient colour development to enable recognition of *Aspergillus flavus* (or *Aspergillus parasiticus*) colonies within 42–48 h at 30°C.

Composition (grams)

Yeast extract	20.0
Peptone (bacteriological)	10.0
Iron (III) ammonium citrate	0.5
Dichloran (2,6-dichloro-4-nitroaniline)	0.002
Chloramphenicol	0.1
Agar	15.0
Distilled or deionised water	1000.0

Preparation

Dissolve yeast extract, peptone, iron (III) ammonium citrate and agar in the water by heating. Add 1.0 ml of a 0.2% (w/v) ethanolic solution of dichloran and 10 ml of a 1% ethanolic solution of chloramphenicol. Adjust to pH 6.2 ± 0.2 and sterilize by autoclaving at 121°C for 15 min. Cool to 50°C and dispense 15 ml amounts into sterile 9 cm diameter Petri dishes. Dry and use immediately or store at 4 ± 2°C in the dark for up to 4 weeks before using.

Physical properties

Appearance	Amber, clear.
pH	6.2 ± 0.2

Shelf life

Ready to use medium	4 weeks at 4 ± 2°C.

Inoculation method for samples

Surface spread 0.1 or 0.2 ml of diluted sample per 9 cm diameter plate.

Incubation

At 30°C for 42–48 h.

Reading of results and interpretation

Aspergillus flavus and *Aspergillus parasiticus* produce an orange-yellow (chrome yellow) reverse colony pigmentation. *Aspergillus niger* sometimes produces colonies with a light yellow reverse, but is readily distinguished from *Aspergillus flavus* after further 24–48 h incubation by the production of black conidial heads. *Aspergillus ochraceus* produces an orange-yellow reverse, but only forms colonies after prolonged incubation.

Quality assessment

Use a stab inoculation procedure. Examine reverse of colonies after 42–48 h incubation at 30°C for typical pigmentation (see above). If doubt exists over *Aspergillus niger*, continue incubation for a further 24–48 h. Prolonged incubation is not recommended.

Test strains	CSIRO[1]	A-NRRL[2]
Aspergillus flavus	3084	3251
Aspergillus parasiticus	2744	2999
Aspergillus niger	2522	3361

[1] Commonweatlh Scientific and Industrial Research Organisation, PO Box 52, North Ride, New South Wales, 2231, Australia.
[2] USDA Northern Utilization Research and Development Division, Peoria, Illinois, USA.

References

Bothast, R.J. and Fennell, D.I. (1974) A medium for rapid identification and enumeration of *Aspergillus flavus* and related organisms. Mycologia 66, 365–369.

Hamsa, T.A. and Ayres, J.C. (1977) A differential medium for the isolation of *Aspergillus flavus* from cottonseed. J. Food Sci. 42, 449–453.

Pitt, J.I., Hocking, A.D. and Glenn, D.R. (1983) An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J. Appl. Bacteriol. 54, 109–114.