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PAPER

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Specifications for identity and purity of certain food additives

Emulsifiers, enzyme preparations,
flavouring agents,
food colours,
thickening agents, miscellaneous food additives

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APPENDIX A

DETERMINATION OF ANTIBIOTIC ACTIVITY

Scope

This procedure is designed for the determination of antibiotic activity in enzyme preparations derived from microbial sources.

Principle

The assay is based on the measurement of inhibition of bacterial growth under specific circumstances.

Culture plates

Six organisms are tested: Staphylococcus aureus ATCC 6538; Escherichia coli ATCC 11229; Bacillus cereus ATCC 2; Bacillus circulans ATCC 4516; Streptococcus pyrogenes ATCC 12344; and Serratia marcescens ATCC 14041. Make a test plate of each organism by preparing a 1:10 dilution of a 24 hour Trypticase Soya Broth culture in Tryticase Agar (TSA) (for Streptococcus pyrogenes ATCC 12344 a 1: 20 dilution).

Pour 15 ml of plain TSA into a Petri dish and allow the medium to harden. Overlay with 10 ml of seeded TSA and allow to solidify. Place a paper disk (prepared according to disk preparation below) of the tested enzyme on each of the 6 inoculated plates.

Disk preparation

Make a 10% solution of the enzyme by adding 1 g of enzyme to 9 ml of sterile, distilled water.

Mix thoroughly with a Vortex mixer to obtain a homogeneous suspension. Autoclave suitably paper disks (for instance, S&S Analytical Filter Papers No. 740-E, 12.7 mm in diameter), then saturate them with the enzyme by application of 0.1 ml (about 3 drops) of a 10% solution of the enzyme to the disk surface. Prepare 6 disks (1 for each of the 6 organisms) for each enzyme: place one disk on the surface of the 6 inoculated agar plates.

Incubation

Keep the 6 plates in the refrigerator overnight to obtain proper diffusion. Incubate the plates at 37° for 24 hours. Examine the plates for any for any inhibition zones that may have been caused by the enzyme preparation.

Interpretation

A visually clear zone around a disk (total diameter: 16 mm) indicates the presence of antibacterial components in the enzyme preparation. If an enzyme preparation shows obvious antibacterial activity against 3 (or more) organisms it is concluded that antibiotic agents are present.