STAPHYLOCOCCUS AUREUS (SPREAD PLATE METHOD)

PRINCIPLE

Coagulase Positive Staphylococci are quantitated in samples by the use of selective and differential media. The group is confirmed by testing presumptive colonies for coagulation. Caution must be exercised when applying the method since isolates may be pathogenic.

SCOPE

The method is applicable to starches, sugars, syrups and most co-products of the corn wet milling industry.

SPECIAL APPARATUS

Sterile spreading rods.

MEDIA AND REAGENTS

1. Use one of the following:

   a. Baird-Parker Agar Base. Prepare medium according to manufacturer's directions. Sterilize by autoclaving at 121°C for 15 mins. Cool to 45-50°C, add prewarmed (45-50°C) Egg Yolk Tellurite Enrichment (EYTE) to the agar base, 50 mL EYTE per 950 mL agar base. Mix completely and dispense in 15 mL volumes into Petri dishes and solidify.

   b. Vogel-Johnson Agar. Prepare medium according to manufacturer's directions. Sterilize by autoclaving at 121°C for 15 mins. Cool to 45-50°C, add Potassium Tellurite Solution 1% . Mix completely and dispense in 15 mL volumes into Petri dishes and solidify.

   c. Mannitol Salt Agar. Prepare medium according to the manufacturer's directions. Sterilize by autoclaving at 121°C for 15

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mins. Cool to 45-50°C, then dispense in 15 mL volume in Petri dishes and solidify.

2. Rabbit Coagulase Plasma EDTA. Rehydrate according to manufacturer's directions with sterile water. Dispense 0.5 mL into 10 × 75 mm tubes.

3. Butterfield's Phosphate Diluent

   Stock solution: Dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of purified water, adjust to pH 7.2 with about 175 mL of 1*N* NaOH solution and dilute to 1 L volume. Store under refrigeration.

   Diluent: Dilute 1.25 mL of stock solution of 1 L volume with purified water. Prepare dilution blanks using this solution.

4. Dilution Blanks. Fill dilution bottles to 80 mL or desired volume with Butterfield's Phosphate Diluent (Note 1).

PROCEDURE

Aseptically weigh 20 g of sample into a sterile 80 mL water blank and homogenize (Note 2). This is the primary dilution and represents a sample dilution factor of 5. The number of dilutions depends on the individual sample and may be determined by past experience.

Pipet 0.5 mL (Note 3) of each sample dilution onto the surface of duplicate, media of choice. Spread the inoculum evenly onto the surface of each plate with separate sterile spreading rods. Allow the surface of the plates to dry thoroughly. Invert the plates and incubate at 35-37°C for 46-50 hrs.

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After incubation count those colonies which conform to the morphological description in the table below.

Selective Medium	Colony Characteristics Morphology	Gram Stain
Vogel-Johnson Agar Medium	Black Surrounded by Yellow Zone	Positive cocci (in clusters)
Mannitol Salt Agar Medium	Yellow Colonies with Yellow Zone	Positive cocci (in clusters)
Baird-Parker Agar Medium	Black, Shiny, surrounded by clear zones	Positive cocci (in clusters)

Record the results and calculate the number of presumptive colonies per gram of sample. To confirm the colonies select at least two colonies of each morphological type counted. Inoculate the presumptive colonies into individual tubes which contain 0.5 mL coagulase plasma. Incubate at 35-37°C, examining periodically over 6 hrs. subsequently at suitable intervals for up to 24 hrs. for any degree of coagulation. All tubes showing any degree of coagulation are considered positive for the presence of Coagulase Positive Staphylococci (Note 4).

CALCULATION

1. Presumptive (+) Coagulase *Staphylococci* count per gram of sample =

$$\text{Average number of presumptive (+) colonies} \times \frac{\text{sample dilution factor}}{\text{mL of inoculum spread plated}}$$

2. $$\%\ \text{confirmed} = \frac{\text{number of Coagulase (+) colonies confirmed by plasma} \times 100}{\text{total presumptive (+) count}}$$

3. Coagulase (+) *Staphylococci* count per gram of sample =

(% confirmed)(presumptive (+) count per gram of sample)

NOTES AND PRECAUTIONS

1. When sterilizing dilution blanks, a portion of the diluent may be lost. If this occurs, the sterilized blanks are brought to the proper volume with the

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sterile diluent.

2. When running counts on pregelatinized starches, no more than 5 g of sample per 95 mL of diluent may be used.

3. If high counts are anticipated, pipet 0.1 mL instead of 0.5 mL onto the plate.

4. The coagulase test must be performed with a positive control.

REFERENCE

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.