THERMOPHILIC SPORE-FORMING BACTERIA
ANAEROBIC THERMOPHILIC SPORES
H_2S PRODUCING

PRINCIPLE

Vegetative cells present in the sample are thermally destroyed, which concurrently activates the thermophilic spores. The thermophilic anaerobic H_2S-producing spores are quantitated by the presence of sulfide production in a selective and differential medium.

SCOPE

The method is applicable to starches, sugars, syrups and coproducts of the corn wet milling industry.

MEDIA AND REAGENTS

1. Sulfite Agar. Prepare medium according to the manufacturer's direction. Add 10 mL of a 5% ferric citrate solution to each liter of SA. Dispense 15 mL of SA into each tube (6 tubes per sample) (Note 1). Sterilize the medium at 121°C for 15 minutes.

2. 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 to 1 L volume with purified water. Prepare dilution blanks using this solution.

3. Dilution Blanks. Fill dilution bottles to 80 mL or desired volume with Butterfield's Phosphate Diluent (Note 2).

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PROCEDURE

Aseptically weigh 20 g of sample into a suitable sterile 80 mL water blank and homogenize (Note 3).

Evenly distribute 20 mL of the suspension among the 6 SA tubes (Note 4). The tubes should be swirled gently several times.

Place in a boiling water bath and boil for 15 minutes (Note 5). Gently rotate the tubes frequently for the first 5 minutes to ensure even distribution.

Cool the tubes rapidly in cold water, solidifying the agar, and incubate at 53-57°C for 70-74 hours. Count all colonies that are black in color.

CALCULATION

(Total number black colonies) 2.5 = sulfide spoilage spores per 10 g of sample

NOTES AND PRECAUTIONS

1. Use 20 × 150 mm culture tubes.

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

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

4. The use of wide tip pipets is recommended for regular starch suspensions. Keep suspension under constant agitation during the pipetting operation.

   If medium is not prepared the day of the test, then the SA tubes must be exhausted. To exhaust the SA tubes, place them in an autoclave in flowing steam for 20 minutes.

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5. For sugars and syrups, boil for 5 minutes.

REFERENCE

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