THERMOPHILIC SPORE-FORMING BACTERIA
AEROBIC THERMOPHILIC SPORES

PRINCIPLE

Vegetative cells present in the sample are thermally destroyed, which concurrently activates the thermophilic spores. The aerobic spores are quantitated by a pour plate technique. Acid producing spores are enumerated by use of a differential medium.

SCOPE

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

SPECIAL APPARATUS

A boiling water bath of sufficient size to hold a number of Erlenmeyer flasks.

MEDIA AND REAGENTS

1. Dextrose Tryptone Agar (DTA). Prepare medium according to manufacturer's direction.

   For each specific sample of regular starch, sugar and syrup use 100 mL of regular strength DTA contained in a 250 mL flask. Sterilize.

   For pre-gelled starch, weigh the proper amount of medium into a 500 mL flask or large bottle to equal a 1.5X solution of agar. Add 200 mL of purified water, mix and sterilize.

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.

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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 1).

4. 2% Plain Agar. Prepare agar 2% by weight. Pour mixture in 100 mL volumes in bottles and autoclave for 15 minutes at 121°C.

PROCEDURE

Thermophilic Spore Forming Bacteria-Flat Sours (dextrose fermenting) and Non-Flat Sours (non-dextrose fermenting)

1. For all products (autoclave method)

Aseptically weigh 20 g of sample into a sterile 80 mL water blank and homogenize (Note 2). Pipet 10 mL of the suspension into a flask containing 100 mL of sterile DTA at a temperature of 50-60°C. After the suspension has been added to the DTA, shake the flask to resuspend the starch. Immediately place the flask in a preheated autoclave and heat at 5 lb. pressure (108.4°C) for 10 mins. (Note 3). Exhaust autoclave rapidly.

Immediately remove flask from autoclave, using caution, and cool in a 50-60°C water bath. Gently agitate flask while cooling.

Dispense the entire mixture equally into 5 sterile Petri plates and allow to solidify. Pour a thin layer of 2% plain agar on top of each solidified plate (Note 4). Allow agar to solidify.

Incubate the inverted plates at 53-57°C (Note 5). Remove after 72 hours (Note 6) and count. Flat sour colonies are surrounded by a yellow halo. Total thermophiles are flat sours plus any other colonies present in the agar.

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2. For sugars and syrups only (boiling method)

   Aseptically weigh 20 g of sugar into a sterile 80 mL water blank and homogenize.

   Rapidly bring the solution to a boil and continue boiling for 5 mins. (Note 3). After boiling replace the evaporated water with sterile diluent. Distribute 10 mL of the solution equally across 5 Petri dishes. Pour 15 to 20 mL of sterile DTA which has been cooled to 45-50°C into each Petri dish. Swirl plates and allow to solidify. Pour a thin layer of 2% plain agar on top of each solidified plate and allow to solidify (Note 4). Incubate the inverted plates at 53-57°C (Note 5). Remove after 72 hours (Note 6) and count. Flat sour colonies are surrounded by yellow halos. Total thermophiles are flat sours plus any other colonies present in the agar.

CALCULATION

Flat sour spore count is obtained by adding the typical flat sour colonies from all plates. Multiply by 5 to express in terms of spores per 10 g of sample.

Total aerobic thermophilic spore count is obtained by adding the count for flat sour spores and the count for all other colonies on the plates. Multiply by 5 to express in terms of number of spores per 10 g of sample.

NOTES AND PRECAUTIONS

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

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

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

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3. The flask or bottle caps should be loosened when autoclaving or boiling the sample agar mixture.

4. The thin layer of sterile 2% agar is used to help reduce the possibility of spreaders being formed and may not be necessary depending on past experience.

5. To prevent over drying of the plates during incubation, place a bowl of water inside the incubator chamber.

6. Presumptive counts can be obtained after 48 hours of incubation.

REFERENCE

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