BACILLUS CEREUS COUNT
PRESUMPTIVE SPREAD PLATE METHOD

PRINCIPLE

The presumptive *Bacillus cereus* count is obtained by a surface plating technique using a selective differential medium. Caution must be exercised when applying the method since *Bacillus cereus* is an enteropathogenic toxin-forming bacterium.

SCOPE

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

SPECIAL APPARATUS

Sterile Cell Spreaders.

MEDIA AND REAGENTS

1. MYP (Mannitol Yolk Polymyxin) Agar Base

Beef Extract (Difco)	1.0 g
Peptone (Difco)	10.0 g
D-Mannitol (Difco)	10.0 g
Sodium Chloride	10.0 g
Phenol Red	25 mL of a 0.1% Solution
Agar	15.0 g
Distilled Water	900 mL

Mix ingredients. Adjust pH, if necessary, to 7.0-7.2 with 1*N* NaOH or HC1. Sterilize at 121°C under 15 pounds of pressure for 15 min in a steam autoclave. Cool medium to 48-50°C and add 50 mL of 50% Sterile Egg Yolk Emulsion (Difco) to each 1,000 mL of base medium.

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2. Polymyxin B. Sulfate (Pfizer Inc. or equivalent), 0.1%

   Dissolve 50 mg of polymyxin B. sulfate in 50 mL of distilled water. Filter this solution through a 0.45 micron membrane filter. Add 10 mL of this 0.1% polymyxin solution to each 1,000 mL of cooled (48-50°C) base medium.

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 at 4°C.

   Diluent: Dilute 1.25 mL of stock solution to 1 L volume with purified water.

4. Sterile Dilution Water Blanks

   Fill water 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. Twenty mL of the primary dilution can be aseptically transferred to another 80 mL water blank, and the sample is diluted by a factor of 25. 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, dry MYP Agar plates (100 × 15 mm). Spread the inoculum evenly onto the surface of each plate with separate sterile cell spreaders. Allow the surface of the plates to dry thoroughly. Invert the plates and incubate at 30 to 32°C for 46 to 50 hrs.

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Examine the plates for colonies that are pink to violet in color and which are surrounded by a cloudy precipitate zone. Count these as presumptive (+) *Bacillus cereus* colonies (Note 4).

CALCULATION

Presumptive (+) *Bacillus cereus* =
count per gram of sample

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

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 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. To confirm presumptive (+) colonies as positive *Bacillus cereus* the appropriate morphological and biochemical tests must be performed on representative colonies by an experienced laboratory.

REFERENCES

1. FDA Bacteriological Analytical Manual (BAM) 8th Edition, www.cfsan.fda.gov/~ebam/bam-toc.html.

2. Association of Official Analytical Chemistry, www.aoac.org.