Detection and Determination of *Bacillus cereus* in Foods, and Beverages.

Food poisoning caused by *Bacillus cereus* may occur when foods are prepared and held without adequate refrigeration for several hours before serving. *B. cereus* is an aerobic spore forming bacterium that is commonly found in soil, on vegetables, and in many raw and processed foods. Consumption of foods that contain >10 ^{6} *B. cereus*/g may result in food poisoning. Foods incriminated in past outbreaks include cooked meat and vegetables, boiled or fried rice, vanilla sauce, custards, soups, and raw vegetable sprouts. Two types of illness have been attributed to the consumption of foods contaminated with *B. cereus*. The first and better known is characterized by abdominal pain and diarrhea; it has an incubation period of 4-16 h and symptoms that last for 12-24 h. The second, which is characterized by an acute attack of nausea and vomiting, occurs within 1-5 h after consumption of contaminated food; diarrhea is not a common feature in this type of illness.

Examination of Foods for *B. cereus*

A. Sampling

If the quantity of food to be examined is large, take representative samples of 50 g each from different parts of the suspect food because contamination may be unevenly distributed.

B. Transporting and storage of samples

Transport and examine samples promptly without freezing, if possible. If samples must be shipped to the laboratory, pack them in insulated

shipping containers with enough gel-type refrigerant to maintain them at 6°C or below. Upon receipt in the laboratory, store the samples at 4°C and analyze as soon as possible. If analysis cannot be started within 4 days after collection, freeze samples rapidly and store at -20°C until examined. Thaw at room temperature and proceed with analysis as usual. Dehydrated foods may be stored at room temperature and shipped without refrigeration.

Culture media

- Mannitol-egg yolk-polymyxin (MYP) agar

Procedure

Preparation of food homogenate

Prepare as directed in lab 2

Dilution

Prepare decimal dilutions by pouring 1ml in 9 ml of dilution water.

Plate Count Techniques

This procedure is suitable for the examination of foods expected to contain more than 1000 *B. cereus* per gram.

Inoculate duplicate MYP agar plates with the homogenate and each dilution of homogenate by spreading 0.1 ml evenly on to each plate in duplicate with sterile bent glass streaking rods (hockey sticks). Incubate plates 24 hours at 30˚C.

Counting Colonies

The number of eosin pink colonies surrounded by lecithinase zone are counted. If reactions are not clear, incubate plates for added 24 hours before counting. Plates must ideally have 15-150 colonies.

Five or more colonies of presumptive *B.cereus* are picked from plates and transferred to nutrient agar slants for confirmation .

Calculations:

As per lab

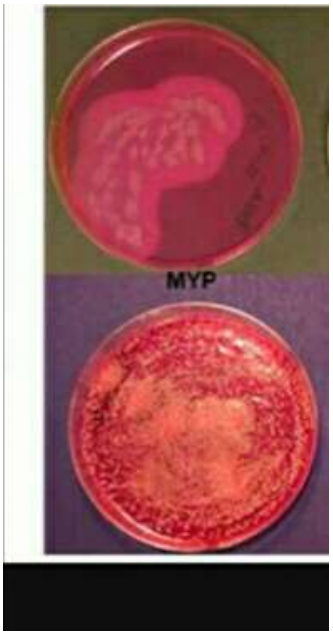
Reporting:

After confirmation, the number of *B.cereus* colonies is multiplied by the reciprocal of the dilution that the countable plate represents (It should be noted that the dilution factor is 10-fold higher than the sample dilution since only 0.1 ml was plated) and report as *B.cereus*/gram.

Expression of Results:

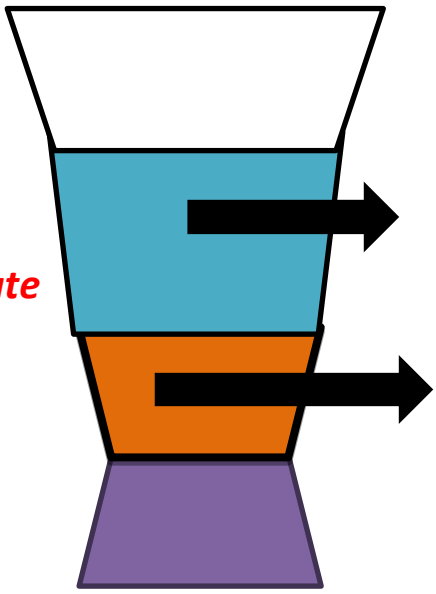
Bacillus cereus= Present/Absent

Figure 1.3 *B. cereus* on MYP agar



The pictures indicate colonies on myp(Mannitol egg yolk polymyxine agar) agar .certain number of eosin pink colonies surrounded by lecithinase zone are counted

Enumeration of B.cereus



1-food homogenate

450ml of sterile distilled water

or buffered peptone water

50g sample

1;10

2 dilution

1ml 1ml 1ml 1ml

9ml

Peptone water

1;100 ***1;1000*** ***1;10000*** ***1;100000*** ***1;1000000***

3-plating

0.1ml on myp

OR KG(kim goepfert)

4-Incubate plates 24 hours at 30 ċ

5 staining with gram stain

6-biochemical test a-nitrate red b-starch hyd c-gelatine d-acid from manitol e-litmus milk ƒ – motilityt