14. ISOLATION OF PATHOGENS FROM SEAFOOD

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Introduction

Microbiology is the study of microorganisms like microscopic or barely visible single-celled life-forms such as bacteria, archaea, protozoans. Enumeration in microbiology is an estimation or determination of number of bacterial cells in a given sample. Enumeration of sea food has gained importance due to increased attention being paid to quality aspects of final product. The International Commission on Microbiological Specifications for Foods (ICMSF) established in 1962 to the need for internationally acceptable and authoritative decisions on microbiological limits for foods appropriate with public health safety, and particularly for foods in international commerce.

Methods to enumerate microbes can be divided into two categories.

a) Total cell counts include dead and inactive cells.
b) Viable methods only count cells that are metabolically active,

Direct Microscopic count/ Total cell count

Direct microscopic counts measures number of cells in a population of a given sample under a microscope. This can be possible for liquid samples using special slides known as counting chambers, consisting of a ruled slide and a cover slip. It is constructed in such a manner that the cover slip, slide, and ruled lines delimit a known volume. The number of bacteria in a small known volume is directly counted microscopically and the number of bacteria in the larger original sample is determined by extrapolation. Bacteria can be counted easily and accurately with the petroff-Hausser counting chamber. This is a special slide accurately ruled into squares that are 1/400 mm2 in area; a glass cover slip rests 1/50 mm above the slide, so that the volume over a square is 1/20,000 mm3 i.e. 1/20, 000, 000 cm3. If for example, an average of five bacteria is present in each ruled square, there is 5 x 20,000,000 or 108, bacteria per milliliter.

Advantages:

a) It is quick way of estimating microbial cell number
b) Morphology of the bacteria can be observed as they counted.

Limitations:

a) Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted
b) Difficulty in to count small cells
c) Precision is difficult to achieve
d) Require a phase- contrast microscope if sample is not stained.

Standard Plate Count (Viable Counts): Any cell which has a capacity to divide and form a population or colony is defined as a viable cell. Viable count is also called as plate count or colony count. A viable cell count is usually done by diluting the original sample, plating aliquots of the dilutions on to an appropriate culture medium, then incubating the plates under suitable conditions for the colonies to be grown. Colonies are counted and, from a particular dilution used, the original number of viable cells can be calculated. For accurate determination of the total number of viable cells, it is critical that each colony comes from only one cell, so chains and clumps of cells must be broken apart. However, since one is never sure that all such groups have been broken apart, the total number of viable cells is usually reported as colony-forming units (CFUs) rather than cell numbers. This method of enumeration is relatively easy to perform but major disadvantage is the time necessary for dilutions, plantings and incubation

Enumeration on E. coli by MPN method

Preparation of the Medium

Prepare the medium (Mac Conkey or Lactose broth) in single and double strength concentration. Dispense the double strength medium and single strength medium either 5 ml or 10 ml (5 tubes for solid/ semi solid samples and 10 tubes for water and ice) in each tube and put durham tube in inverted position without air bubbles. Sterilize the medium by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

MPN Testing of samples

1. Take 5 tubes of double strength and 10 tubes of single strength.
2. Add 10 ml of the samples to 5 tubes containing 10 ml double strength medium.
3. Add 1 ml of sample to 5 tubes containing 10 ml double strength strength medium and 0.1 ml water to remaining 5 tubes containing 10 ml double strength medium.
4. Incubate all the tubes at 37°C for 24 hrs.
5. Observe at 24 hrs, If no tubes shows positive for growth and gas production, re-incubate up to 48 hrs.
6. Note the number of tubes for positives from each sets and compare the number of tubes giving positive
7. reaction to the 5tubes MPN standard chart and record it.
8. The result is the total number of bacteria present in the sample as MPN values.
9. For example: 5–4–3 (5 × 10 ml positive, 4 × 1 ml positive, 3 × 0.1 ml positive) = the MPN value is280. So sample contains an estimated 280 coliforms per 100 gram

StepII– (For confirmed total coliforms)

Requirements :- BGLB 2% broth.

Inoculate one loopful of culture from the +ive tubes of step I to BGLB 2% broth. Incubate at 370C for 24 hrs. Note growth and gas production. Results are noted as +ives if there are growth and gas production. Compare with 3 tube MPN table.

Step III – (For faecal coliforms and *E.coli*)

From the +ive tubes of StepII, inoculate one loopful each to EC broth and Tryptone broth. (indole medium). Incubate at 44.50 °C for 24 hrs.

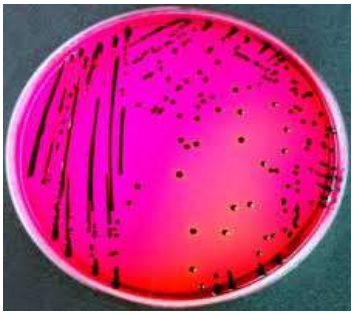
EC broth:-Growth and gas production.

Tryptone broth:-Test for indole produces by adding 4 drops of Kovac's indole reagent. A pink or red color at the top layer indicates a +ive test for indole.

Coliforms bacteria which products gas in EC broth and indole in tryptone broth of 44.50 °C are*E.coli*.

A loopful of sample from each tube showing positive test *(color change with gas)* is streaked onto two selective medium like Eosin Methylene Blue agar or Endo's medium. One plate each is incubated at 37°C and another at 44.5± 0.2°C for 24 hours. High temperature incubation (44.5 ±0.2) is for detection of thermo tolerant *E.coli.*

Colony character of seafood pathogen



Salmonella in XLD Pink colony with or without black spot

Staphlococcus aureus in BP Black colonywith white margin surrounded by opaque zone

Vibrio cholerae in TCBS smooth yellow and slightly flattened with opaque center

V. Parahaemolyticus in TCBS appears as round, opaque, green

Vibrio mimicus on TCBS Agar appears as small 2-3 mm, smooth green colonies

Listeria Monocytogens on PALCAM Greyish green colour colony with black zone

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