CHAPTER 1

Determination of Total Plate Count

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Introduction:

Total Plate Count (TPC) is the enumeration of aerobic, mesophillic organisms that grow in aerobic conditions under moderate temperatures of 20-45°C or TPC provides an estimate of the total number of aerobic microorganisms in foods including fish and fishery products. This method is used to determine the level of aerobic bacteria, yeast, molds and fungi. This count includes all pathogens and non-pathogens and is used to determine the hygienic status and quality of food. TPC is also called as Aerobic plate count (APC) or total viable count (TVC). This method is useful in determining spoilage/ deterioration of the perishable commodities like seafoods. This is an indicator of the sanitary conditions under which the food has been processed and/or produced and also of the level of Good Manufacturing Practices (GMPs) adopted during processing. It is enumerated by serial dilution of food homogenate and plating on general purpose media such as Plate count agar (PCA) or other non-selective agar medium and further incubation as per the required time temperature of the method used. All the colonies developing on the medium are counted and bacterial load is expressed as colony forming units (CFU) per gram of sample.

Equipment and materials

1. Phosphate buffer or Physiological saline (0.85%) or 0.1% Peptone water
2. Biosafety cabinet/Laminar air flow
3. Petri dishes, glass or plastic (at least 15 × 90 mm)
4. Micropipettes of 1, 5, and 10 ml
5. Dilution bottles borosilicate-resistant glass, with rubber stoppers or plastic screw caps
6. Pipet and petri dish containers, adequate for protection
7. Circulating water bath, for tempering agar, thermostatically controlled
8. Incubator
9. Colony counter, dark-field, Quebec, or equivalent, with suitable light source
10. Plate count agar
11. Refrigerator, to cool and maintain samples at 0-5°C, if needed
12. Freezer, to maintain frozen samples from -15 to -20°C

Procedure

About 25 g of fish muscle is weighed aseptically and homogenised with 225 ml physiological saline in a homogeniser / blender. Serial decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} and others as appropriate depending on the type of sample are prepared with 9 ml physiological saline. 0.1 ml of inoculum from each of the serial dilution is poured onto Plate Count Agar (PCA) plates and spread using sterile glass spreaders for spread plate technique. But, in the case of pour plate technique, 1 ml of inoculum is placed into the petriplates and the sterile cooled ($50^{O}C$) media is poured over it and mixed by gently swirling in clockwise and anticlockwise direction. The plates are incubated at 37 $\pm 1^{0}C$ for 48 h. All the colonies developed on the agar plates are then counted and counts per gram of sample calculated. It is advisable to choose dilutions which give colonies between 30 – 300 ranges. Plates having crowded or spreading colonies which cannot be counted shall be discarded.

Calculation

Pour Plate Technique

Aerobic Plate Count (cfu/g) = (Number of colonies x Dilution) / Weight of sample

Spread Plate Technique

Aerobic Plate Count (CFU / g of sample) = (Number of colonies x Dilution x 10) / Weight of sample

Phosphate Buffer

Solution Stock

solution

Potassium dihydrogen orthophosphate (KH_2PO_4) -

34.0 g Distilled water - 1000.0 ml

Working solution

Dilute 1.25 ml of stock buffer solution to 1000 ml with distilled water. Adjust the pH to 7.2 before use. Sterilize at 121ºC for 15 minutes.

Media composition

Nutrient Agar (NA)

Peptone	-	5.0 g
NaCl	-	5.0 g
Beef Extract	-	1.5 g
Yeast Extract	-	1.5 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.4 ± 0.8

Sterilize at 121^{o}C for 15 minutes.

Plate Count Agar (PCA) or Standard Methods Agar

Tryptone	-	5.0 g
Yeast Extract	-	2.5 g
Dextrose	-	1.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.0 ± 0.2

Sterilize at 121^{o}C for 15 minutes.

Soyabean Casein Digest Agar (Trypticase Soy Agar, TSA)

Tryptone	-	17.0 g
Soya peptone	-	3.0 g
NaCl	-	5.0 g
Dipotassium phosphate	-	2.5g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.3 ± 0.2

Sterilize at 121ºC for 15 minutes.

Rules for enumerating counts

- The suitable colony counting range is **25-250**/30-300. 15-150 for yeast and molds.
- Always choose replicate plates containing colonies.
- count all colony forming units (cfu) including those of pinpoint size.
- Record the volume and dilution used for plating, and average number of colonies counted.

  Example:

Dilutions	10^{-1}	10^{-2}	10^{-3}
Colonies counted	TNTC	182	45
	TNTC	165	39

TNTC- Too Numerous to count

Since only 10^{-2} dilution shows colonies between 30 and 300, only this pair of plates is considered and average counts determined.

Average number of colonies x dilution

factor CFU/ml =

Volume of sample plated

Average number of colonies in 10^{-2} = 80 + 70 ÷ 2 = 75

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Volume of sample plated:

1 ml CPU/ml = (75 x 10^2)

= 7.5 x 10^3

Result reported as = 7.5 x 10^3 CFU/ML

2. If there are 2 consequetive dilutions showing between 30-300 colonies then the count in each of the dilutions computed. If the count of one dilution is not more than double the other, then the average of both dilutions taken and results reported. *Example:*

   Counts in 10^{-2} dilution : 280 and 290 colonies. CFU/ML = 2.85 x 10^4

   10^{-3} dilution: 40 and 44 colonies. CFU/ML = 4.30 x 10^4

   Since 4.30 is not more than double of 2.85, the average is

   considered (2.85 + 4.30) ÷ 2 = 3.47)

   Result is report as : 3.57 x 10^4 CFU/ML

3. If there are 2 consequetive dilutions showing between 30-300 colonies, and if the count of one dilution is more than double than the other then the lower value is reported. Example:

   Counts in 10^{-2} dilution: 280 and 290 colonies. CFU/ML = 2.85 x 10^4

   10^{-3} dilution: 80 and 70 colonies. CFU/ML = 7.50 x 10^4

   Since 7.50 is more than double of 2.85, the lower value is

   considered. Result is reported as : 2.85 x 10^4 CFU /ML

4. If there are no colonies in any of the dilutions, then the counts is reported as less than one times the lowest dilution plated.

   Example:

   10^{-1} dilution: No

   colonies 10^{-2}

   dilution: No

   colonies

   Results reported as: **Estimated count** < 1x10^1

   CFU/ML or Est. < 1x10^1 CFU/ML

5. If all the dilutions show more than 300 colonies, then the dilution where the colonies are countable is considered. Then all the colonies in that dilution counted and the results computed as **Estimated count.**

   Example:

10^{-1} dilution : Too numerous colonies to count

10^{-2} dilution: Too numerous to count

10^{-3} dilution : 450 and 460 colonies (Average: 455 colonie) Results reported as : **Estimated count** 4.55 x 10^5 CFU/ML

6. If there are too numerous or too many colonies making the counting difficult, then results can be expressed by following one of the following ways.

   Count all the colonies in 13 square centimeters using a colony counter and multiply the counts by 5. This number should be multiplied by the dilution factor used for plating to report as estimated count.

   OR

   Count all colonies in in 5 square centimeters using a colony counter and multiply by 13. This number should be multiplied by the dilution factor used to report the Estimated count. **OR**

   If the number of colonies is more than 100 per sq. cm, then count all colonies in one sq.cm and multiply it by 64. This number should be multiplied by the dilution factor to report the Estimated count.

   (*Multiplying by 5 or 13 depends on the area of the petridish used. The standard petridish of diameter 9 cm has an area of approximately 65 sq. cm)*

References

- IS 5402 : 2012 (Indian Standard MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS — HORIZONTAL METHOD FOR THE ENUMERATION OF MICRO- ORGANISMS — COLONY-COUNT TECHNIQUE AT 30°C)/ISO 4833 : 2003 ('MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS — HORIZONTAL METHOD FOR THE ENUMERATION OF MICROORGANISMS — COLONY- COUNT TECHNIQUE AT 30 °C')
- ISO 6887-3 : 2003 Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products
- Refer: http://ecoursesonline.iasri.res.in/mod/page/view.php?id=88202