ENUMERATION OF MICRO-ORGANISMS BY A COLONY COUNT TECHNIQUE

Principle

The number of viable micro-organisms in liquid or dry samples is determined by plating a specified quantity of sample or sample dilution on Plate Count Agar (PCA) dish. Dishes are incubated at 30°C for 72h under aerobic conditions and the number of microbial colonies is counted.

Field of application

Method is applicable for enumeration of viable bacteria, yeasts, and molds from liquid and dried enzyme semifinal- and final products.

Equipment

Autoclave	
Water bath	45 °C ± 0.5°C
Incubator	30 °C ± 1 °C
Vortex	
Sterile Petri dishes:	Ø 80 mm to 100 mm
Sterile dilution bottles	100 ml
Sterile dilution tubes	10 ml
Sterile pipettes	1 ml and 10 ml
Glass flasks	250 ml
Colony counter	

Reagents

All solutions are prepared in deionized water, Milli-Q or equivalent.

1. Plate Count Agar (PCA)

Weigh 23.5 g of Plate Count Agar (e.g. Difco 0479) and dissolve into 1000 ml of water. Boil to ensure complete dilution of medium components. Divide 200 ml aliquots to 250 ml glass flasks. Sterilize in autoclave for 15 min at 121 °C. Sterile PCA agar is melted in a microwave oven or water bath and tempered to 45°C before use.

2. Dilution fluid (0.9 % w/v NaCl)

Dilute 9.0 g NaCl (e.g. Merck 6404) into 1000 ml of water. Divide 90 ml aliquots to appropriate glass flasks. Sterilize in autoclave for 15 min at 121 °C.

Samples

1. Dry samples

Aseptically weigh 10.0 g of sample to 90 ml of dilution fluid. Vortex until the mixture is homogenous (= 10^{-1} dilution). Prepare dilution series from the 10^{-1} dilution by pipetting 1 ml of 10^{-1} dilution to 9 ml of dilution fluid (= 10^{-2} dilution) and 1 ml of 10^{-1} dilution to 99 ml of dilution fluid (= 10^{-3} dilution). Vortex all the samples carefully. The dilution series can be continued further by following the dilution principle described above.

2. Liquid samples

Aseptically pipet 10.0 ml of sample to 90 ml of dilution fluid. Vortex until the mixture is homogenous (= 10^{-1} dilution). Prepare dilution series from the 10^{-1} dilution by pipetting 1 ml of 10^{-1} dilution to 9 ml of dilution fluid (= 10^{-2} dilution) and 1 ml of 10^{-1} dilution to 99 ml of dilution fluid (= 10^{-3} dilution). Vortex all the samples carefully. The dilution series can be continued further by following the dilution principle described above.

Procedure

1.0 ml of all samples including the original sample mix or undiluted enzyme sample and all the required dilutions are pipetted aseptically on empty Petri dishes as duplicates. 15-20 ml of tempered 45°C PCA-agar is poured to each of the plates. Samples are mixed to PCA-agar by carefully swaying the plates. The procedure from preparing the first sample dilution to pouring the PCA-agar should not take more than 15 minutes. Dishes are placed on an even surface and the agar is let to solidify. Dishes are moved to a 30°C ± 1 °C incubator and incubated upside down for 72h.

Results

Number of microbial colonies is counted from dishes that contain less than 300 colonies. Colonies are counted from both of the duplicate dishes from two dilutions.

The number of micro-organisms/ml or /g is calculated accordingly:

$$N = \frac{\Sigma C}{[n_1 + (0{,}1 \times n_2)]\, d}$$

ΣC = combined number of colonies (a´2 dishes from 2 dilutions)
n_1 = number of dishes from the first dilution
n_2 = number of dishes from the second dilution
d = dilution factor of the first dilution

The number of colonies from the second dilution should be at least 15.
Results are reported with the accuracy of two significant digits/ml or /g.

Example:

First dilution 10^{-2}; colonies on dishes 168 + 215 = 383
Second dilution 10^{-3}; colonies on dishes 14 + 25 = 39

$$N = \frac{383 + 39}{[2 + (0{,}1 \times 2)] \times 10^{-2}} = \frac{422}{0{,}022} = 19\,182 = 1{,}9 \times 10^4 \text{ cfu/ml or /g}$$

cfu = colony forming unit

If no colonies are detected in any of the plates result is reported as $< d^{-1}$ cfu/ml (liquid product) or $< d^{-1}$ cfu/g (dry product) (d = dilution factor of the smallest dilution).

References

International Standard ISO 4833, 2nd edition 1991-03-01. Microbiology - General Guidance for the enumeration of micro-organisms – Colony count technique at 30 °C.