Introduction to microbiology essay sample

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This purpose of this experiment was for students to do the colony count methods, estimating the viable cell number of commercial active dried yeasts (ADY).

This experiment allowed the students to perform the plate count technique by serial dilution and two common methods, spread plate and pour plate to determine the colony forming unit (CFU) of yeasts

A ten-fold dilution is used in this experiment, the sample is diluted until it reached the

10-9 dilution. Plating for spread plate started from 10-5, 10-6, 10-7 and 10-8 dilution while for pour plate, it started from 10-6, 10-7, 10-8 and 10-9 dilution.

Having incubated inverted at room temperature (25oC) for 2 days, the viable cells for spread plate and pour plate were calculated.

The CFU/g obtained for spread plate and pour plate were 2. 2 X 1010 CFU/g and 3. 7 X 1010. the CFU/g respectively.

Introduction

Colony count methodology is one of the most accurate methods to determine the calculation of the organisms in a given sample. It enumerates the number of actual live, viable cells in the sample that form colonies on a suitable agar medium. As the optimum medium and conditions varies for one sample to another, the colony count methods provide an estimate of the number of viable cells according to the medium employed, time and

temperature of incubation. Each colony that appears on the agar plate arising either from a clump of cells or from a single cell is referred as a colony forming unit (CFU).

The sample used in this experiment is active dried yeasts (ADY). A serial dilution is performed to suspend the yeasts containing product in water so that the number of microorganisms per ml is small enough to be counted, when the sample is plated. Cells in overcrowded plates may not form colonies but may fuse which lead into erroneous measurements (1). Therefore the sample is diluted and then re-diluted successively with a known volume of diluents until it obtain appropriate colony number which is ideally between 30 and 300 colonies but more recently 25-250 range is recommended.

After that the different dilutions are plated on nutrient media using spread plate and pour plate method to allow the yeast cells to cells to grow and multiply. The number of viable cells in the sample is recorded as colonies forming units/ml or gm, CFU/ml or CFU/gm.

Aerobic Mesophilic Count is one of numerous variations of colony count techniques. As the name suggests, the plates are incubated under aerobic condition and the incubation temperature is in the mesophilic range from 30 °C to 35°C.

As the sample used is yeast, it is incubated in room temperature (25oC) for 2 days.

Literature Review

A serial dilution is an accurate method of making solutions of low molar concentrations. A solution with a molarity greater than that which is required is accurately diluted using a suitable solvent. The dilution used in this experiment is serial " ten-fold" dilutions which reduces the concentration of a solution by a factor of ten that is to one-tenth the original concentration. In the preparation of serial dilution, exactly one ml of original sample is added into 9ml of diluent to get a 1: 10 dilution. 1: 100 dilution is made by adding 1ml of 1: 10 dilution to 9 ml of diluent. The same method applies to get the subsequent dilutions until it reaches the final dilution desired.

(3)

Spread plate is one common method to quantify microorganisms on solid medium. Usually 0. 1 ml of the diluted sample is dispensed on the agar plate but in this experiment 0. 2 ml was used instead to prevent the sample from becoming dry as the incubation period was extended. Using a sterilized device that looks like hockey stick, the sample was spread evenly on the surface of the agar and was allowed to be absorbed into the agar. Plates were inverted and incubated. The colonies of the sample grew only on the surface of agar.

Pour plate is another common method of quantifying microorganisms on solid medium. 1 ml of the diluted sample was dispensed into an empty, sterile petri dish, about 15 ml of molten agar that had been cooled down in water bath to 45°C was added after that. The mixture was gently swirled clockwise, anticlockwise, up and down and left and right. Having allowed the agar to set, the plates were then inverted and incubated. Unlike the colonies

in the spread plate, the colonies here formed throughout the agar, on the surface and within the medium.

The advantages of spread plate technique is that the colonies formed are easily counted as they are all grow on the surface of the agar but this may bring disadvantages too due to the limited space on the plate surface and the cells with low tolerance to oxygen will not grow (4). Pour plate method eliminates such problems because the colonies are well separated and the oxygen is sufficiently supplied to those embedded in the agar allowing them to grow and form colonies. However, this results in difficulty in the counting as the size of the colonies varies between those on surface and in agar. Another disadvantage of pour plate method is that the weak yeast cells will be killed due to the hot molten agar causing the lesser colonies formed compared to spread plate by at least ten fold.

All the plates are incubated inverted to prevent water drops forming on the top of the plate and dripping onto the plates which may cause colonies to be spread quickly resulting in unclear visibility. Being incubated upside down, the water is absorbed back into the place as it condenses (5)

Having incubated in temperature of 250C for 2 days, only plates that contain 25 to 250 colonies are chosen. The number of viable microorganisms in the original sample can be calculated by multiplying the number of colonies in the plate by the dilution factor. The units for the viable cells are CFU/ml or CFU/gm.

The equation

Procedure

A. Serial dilution of active dry yeast (ADY)

i. 10g of or 11g of ADY was weighed and 90 ml or 99 ml of 0. 1% peptone water was added respectively. The yeast slurry was shaken well but gently for 10 minutes.

ii. To prepare a 10-2 dilution, exactly 1 ml of 10-1 was transferred into 9 ml of diluents using pipette and was mixed well in a circular motion. The dilution technique was observed carefully.

iii. Step 2 was repeated 7 more times to obtain a dilution of 10-9.

B. Plating using spread and pour plate method

i. Spread plate: 0. 2 ml of 10-5, 10-6, 10-7 and 10-8 dilution was spread onto duplicate plates of malt agar using a “ hockey stick”. The technique was observed carefully.

ii. Pour plate: 1 ml of 10-6, 10-7, 10-8 and 10-9 dilution was transferred into duplicate Petri dish using a pipette and 15 ml of molten malt agar (app 45°C) and was mixed gently and the agar was allowed to set. The technique was observed carefully.

iii. The plates were incubated inverted at room temperature (25°C) for 2 days.

iv. After incubation, plates that contain between 30-300 or 25-250 colonies were chosen; the colonies in each plate were counted and the number of yeast cells was worked out as CFU/g.

Results and Calculation

Method Dilution Colonies/ plate CFU/g**

Plate 1 Plate 2 Average

(i) Spread plate 10-5 TNTC TNTC TNTC

10-6 TNTC TNTC TNTC

10-7 TNTC TNTC TNTC

10-8 49 39 44 2. 2 X 1010

(ii) Pour plate 10-6 TNTC TNTC TNTC

10-7 TNTC TNTC TNTC

10-8 TNTC TNTC TNTC

10-9 28 46 37 37 X 1010

Note: * TNTC – Too numerous to count (exceeding 250 colonies

* TFTC – Too few to count (below 25 colonies/plate)

** The results is corrected to 1 decimal place

Calculation

(i) CFU/g using spread plate method:

CFU/g = (no. of colonies) X (1/dilution factor) X (1/volume)

= 44 X 1/10-8 X 1/0. 2

= 2. 2 X 1010 CFU/g (Correct to 1 decimal place)

(ii) CFU/g using pour plate method:

CFU/g = (no. of colonies) X (1/dilution factor) X (1/volume)

= 37 1/10-9 X 1/1

= 3. 7 X 1010 CFU/g (Correct to 1 decimal place)

Discussions

The result obtained from the experiment was for spread plate method, 2. 2 X 1010 CFU/g whereas for pour plate was 3. 7 X 1010 CFU/g. Theoretically the CFU for pour plate should be lesser at least by 10 fold as melted agar keep at 45oC was poured into the petri dish containing sample dilution which caused thermal shock for the yeasts cells. Those weak cells will be killed.

Yeasts are " facultative anaerobes" meaning they can survive and grow with or without oxygen. Unlike in the pour plate which embedded colonies in agar to support the growth of some facultative anaerobes cells, the surface of the spread plate agar was exposed to oxygen thus the growth of some cells could not be supported. This is one of the reasons why the result of spread plate obtained was lesser compared to the result of pour plate

Another reason could come from experimental errors occurred either during performing the serial dilution or plating the sample dilution. The accuracy of the serial dilution and plate count method depends on homogeneous dispersal of organisms in each dilution (2).

One of the sources might be due to the improper measurement of diluent and to be diluted volumes. The volume of the diluents transferred to petri dishes should be the exact amount. However, in the practice, it could be lesser or more due to the parallax error while reading the pipette. The difference in the volume might contain thousands of cells. There were also some potential for some volume to be carried over on the outside of pipette (6).

Another possible sources might come from the insufficient mixing of the sample. Dilution might not be fully mixed prior to the removal of the volumes (1). Mixing is important for separating the clumps in the solution of the sample. If the sample were not mixed well, the clumps would still be present in it resulting in low viable count.

In addition, errors could have been made during counting of the colonies from the plates. For pour plates, the colonies formed varied in size, it was harder to differentiate between the colonies on surface and in agar resulting in the difficulty in counting therefore some colonies might be excluded from calculation.

Last but not least, the bacteria from the surrounding could have contaminated the samples. It was either the media or the equipment that was highly contaminated upon transferring from the pipette to the petri dish

or spreading the sample on the agar using the hockey stick. Those bacteria might also be calculated as the colonies thus the CFU counted at the end of experiment were totally wrong.

Conclusion

The experiment's result was not a satisfactory as the CFU for spread plate, 2. 2 X 1010 CFU/g, was more than the CFU for pour plate, 3. 7 X 1010 CFU/g. Theoretically, the CFU of pour plate should be lesser than CFU of spread plate as the cultures were exposed to the 45°C+ melted agar temperatures. The heat brought thermal shock for the yeasts cell hence those weak cells was killed. This indicated that there were errors made during the serial dilution and plate count basically due to pipetting inconsistency, insufficient mixing, inaccuracy in plate counting and contamination.

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