J.Sci.Soc.Thailand, 21(1995) 47-50

SHORT REPORT

COST EFFECTIVE AND CONVENIENT VERSION OF THE DROP PLATE METHOD

KAMNIRD SUPANWONG AND KALTIMA PICHAI

Ethanol Fermentation Research Unit, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, THAILAND.

(Received October 21, 1994)

ABSTRACT

Determination of colony forming units (CFU) by the Drop Plate method usually requires 3 agar plates and 3 calibrated pipettes. Here, a modification of this method is described using 1 agar plate to accommodate 6 drops, one from each dilution. Accuracy of the method is maintained even when 1 calibrated pipette is used to make all 6 drops.

INTRODUCTION

The Drop Plate method[1] has been improved to achieve very high accuracy[2] by improvement of the dilution method and use of 0.1% peptone solution as a diluent. However, the method still requires 3 agar plates and 3 calibrated pipettes and is not convenient when working with unfamiliar samples since one has to determine appropriate dilutions by performing total counts using a bacterial counting chamber or using 6 agar plates to determine CFU at all 6 dilutions.

Using the Drop Plate method, we observed very low variability in the numbers of colonies in each drop. It was possible to count the colonies in one drop and still retain the accuracy of the method. Thus the number of plates could be reduced from 3 to 1, accommodating 6 drops from 6 dilutions. This makes the Drop Plate method more cost effective and convenient, since one does not have to estimate the proper dilutions. Success in performing accurate viable counts is guaranteed by this new version.

Normally, one calibrated pipette is used to take a sample from each dilution i.e., 6 calibrated pipettes for one sample. It would be ideal if the Drop Plate method could be performed by using 1 agar plate and 1 calibrated pipette to take samples from all 6 dilutions starting from the highest dilution.

TABLE 1. Colony forming units of *Saccharomyces cerevisiae* TISTR 5168 measured by the Drop Plate method: Standard version (STD) and new version (NV).

Time (hour)	CFU/ml x 10^5								
	1st Experiment			2nd Experiment			3rd Experiment		
	STD	NV	D	STD	NV	D	STD	NV	D
0	10.00	11.00	-1.00	3.78	3.40	0.38	4.14	4.20	-0.06
3	17.30	15.00	2.30	19.50	19.00	0.50	18.70	19.00	-0.30
6	80.00	85.00	-5.00	42.10	46.00	-3.90	41.30	42.50	-1.2
12	136.00	135.00	1.00	76.20	77.50	-1.30	74.40	75.00	-0.60
24	160.00	115.00	45.00	143.00	130.00	13.00	153.00	140.00	13.00
48	143.00	130.00	13.00	167.00	160.00	7.00	137.00	135.00	2.00
72	140.00	135.00	5.00	116.00	105.00	11.00	142.00	140.00	2.00
96	122.00	110.00	12.00	105.00	100.00	5.00	130.00	125.00	5.00
	Mean		9.0375	Mean		3.96	Mean		2.48
	SD		15.7746	SD		6.0455	SD		4.7009
	t-value		1.6204	t-value		1.8527	t-value		1.4921
	P-value		>0.05	P-value		>0.05	P-value		>0.05

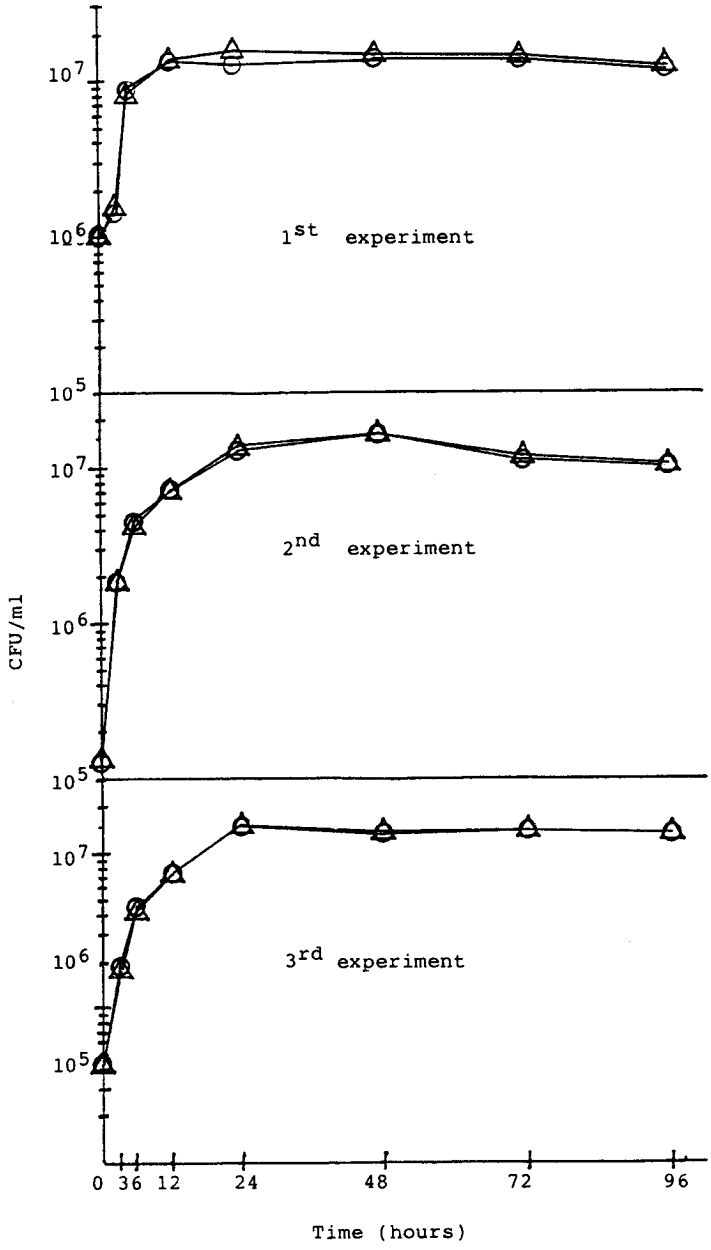

Fig.1. Growth of *Saccharomyces cerevisiae* TISTR 5168; Δ = standard version and O = new version.

MATERIALS AND METHODS

Growth of *Saccharomyces cerevisiae* TISTR 5168 grown in YPG broth (Yeast extract 1%, Peptone 1% and Glucose 2%) incubated at 30°C were measured at 0, 3, 6, 12, 24, 48, 72 and 96 hours by determining CFU by standard Drop Plate method using 3 agar plates and 3 calibrated pipettes and new version using 1 agar plate and 1 calibrated pipette. The experiment was repeated three times.

RESULTS AND DISCUSSION

Table 1 shows that the CFU of *Saccharomyces cerevisiae* TISTR 5168 measured by the standard and new version of the Drop Plate method were not different at the 0.05 significance level in all experiments.

Figure 1 also shows that the growth curves measured by each method were very similar.

The differences between CFU by the standard Drop Plate method and modified version using 1 pipette were statistically insignificant. Thus the Drop Plate method can be performed by using 1 agar plate and 1 calibrated pipette with the same accuracy as the standard method using 3 agar plates and 3 calibrated pipettes. One agar plate can be used for 2 samples if the samples need not be diluted more than 10^{-5} or omitting the first dilution, as it can accommodate up to 10 drops.

This new version is both convenient and economical and will work well with any bacteria or yeasts forming discrete colonies. So it should be very useful in research work that deals with pure culture. The study of one species in the presence of others is also possible provided that there is a selective medium for it. Enumeration of certain species in samples containing mixed cultures such as soil, water or food is also possible if selective media are used.

In our further investigations, we will study the possibility of applying the Drop Plate method in plaque assay and in the enumeration of bacteria in samples containing mixed cultures such as food.

ACKNOWLEDGMENTS

We thank Dr.A. Jatisatienr for statistical analysis and Dr.S.Elliott for correction of English language.

REFERENCES

1. Miles,A.A. & Misra, S.S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg. Camb.* **38**, 732-749.
2. Supanwong, K.(1972). The effect on growth of *Staphylococcus aureus* in milk by certain bacteria and fatty acids. M.Sc.Thesis. University of Strathclyde, Glasgow, Scotland.