THE USE OF ACID, ROSE BENGAL AND STREPTOMYCIN, OR AUREOMYCIN FOR THE DETERMINATION OF YEASTS AND MOULDS IN BUTTER

By

Karl-Friedrich Weiss, B.S.A.

The University of Manitoba

A Major Thesis submitted to the
Faculty of Graduate Studies and Research
The University of Manitoba
in candidacy for the degree of
Master of Science

1960

ACKNOWLEDGMENTS

The writer is pleased to express his gratitude to Dr. J. M. Nesbitt, Chairman, Department of Dairy Science, for his encouragement and guidance during the course of this study. The writer also thanks Dr. N. James and Dr. T. B. M. Payne, Department of Microbiology, for their suggestions.

Sincere thanks are extended to Dr. Anna K. Storgaard, Department of Plant Science, for valuable assistance with the statistical analysis of the experimental data and for other helpful criticisms.

THE USE OF ACID, ROSE BENGAL AND STREPTOMYCIN, OR AUREOMYCIN FOR THE DETERMINATION OF YEASTS AND MOULDS IN BUTTER

By

Karl-Friedrich Weiss, B.S.A.

The University of Manitoba

Abstract of

A Major Thesis submitted to the

Faculty of Graduate Studies and Research

The University of Manitoba

in candidacy for the degree of

Master of Science

1960

In the first section of this experiment, 4 different treatments (2 media - potato dextrose agar and malt agar, in combination with each of 2 bacterial growth inhibitors - acid, and rose bengal and streptomycin) for determining the yeast and mould counts in butter were compared. Sixty-four butter samples obtained at random from commercial creameries were used. Five plates were prepared per treatment.

The analysis of variance of the counts for all samples combined indicated significant differences between media at ($P = 0.05$) and between bacterial inhibitors at ($P = 0.05$). However, the variance for the interaction between media and inhibitors was significant, and when this was used as the error term the variances for media and inhibitors were not significant. Comparison of the interactions indicated that the treatment of malt agar with acid was inferior to all other treatments.

In the second section, 2 treatments (potato dextrose agar with either aureomycin or rose bengal and streptomycin) were compared using the same butter samples as above. Four plates were prepared per treatment. The mean count for the medium containing the aureomycin was significantly higher than that for the medium to which the rose bengal and streptomycin were added, but the pattern was not identical for all samples.

Further, more specific studies are required to determine

why all the treatments used did not support equal numbers of yeasts and moulds and why all cultures did not react to the treatments in the same manner.

TABLE OF CONTENTS

INTRODUCTION

Yeast and mould counts are used as a measure of sanitation in the manufacture of butter. The damaging effects of certain yeasts and moulds which are of tremendous importance, may be greatly reduced through eradicating the source of contamination by means of strict sanitary control, and through frequent checking by routine microbiological examination. Yeast and mould counts provide a reasonably good indication of the conditions under which the butter was manufactured, even though they do not accurately measure either the quality of the raw material or the keeping quality of the finished product. High yeast and mould counts usually mean faulty methods of manufacture, e.g., inefficient pasteurization, unsanitary churns, pipes and fittings, or other contamination of the butter after pasteurization.

Yeast and mould counts, as well as other microbiological determinations, are relative rather than absolute. The oxygen tension, pH, humidity, temperature, and nutrient requirements of the different species of yeasts and moulds are too variable to permit optimum growth for all the different species of yeasts and moulds when a standard procedure is used. Uniform methods are essential, however, or no standard can be set, and the results at different times and from different locations will not be comparable. It is therefore necessary that methods are

used which will provide the optimum conditions for the greatest number of organisms.

One medium may give higher counts of yeasts and moulds than another, but from a practical point of view this does not necessarily mean that such a medium is to be preferred; other factors such as simplicity and uniformity in preparation, as well as cost, must be taken into account. Many different media have been tested in order to find the most suitable one for the determination of yeasts and moulds in butter. The Official Method for the Examination of Dairy Products (1953) prescribes the use of either acidified malt agar or acidified potato dextrose agar.

The acidified media mentioned above were also used by soil microbiologists for the enumeration and isolation of soil fungi (moulds). In soil microbiological work, however, the acid is now gradually being replaced by a mixture of the dye rose bengal and the antibiotic streptomycin as the bacteriostatic agent.

The purpose of this investigation was to assess the relative efficiency of different combinations of basic media (agars) and bacterial inhibitors used for the determination of yeasts and moulds in butter. Media to which a mixture of rose bengal and streptomycin was added

as a bacteriostatic agent were compared with media which were acidified (pH 3.5 ± 0.1) to inhibit bacterial growth. Aureomycin alone was also compared with the rose bengal and streptomycin mixture, using one common basic medium.

REVIEW OF LITERATURE

The Conventional Method

In Canada a tentative method for the determination of yeasts and moulds in creamery butter was suggested by Hood and White (1928). At that time the acceptance of the use of laboratory methods for determining the yeast and mould count of pasteurized butter made necessary the adoption of a standard procedure for routine analysis.

The American Dairy Science Association, through its Committee on Bacteriological Methods, suggested the adoption of uniform procedures for the detection of micro-organisms in butter. For the determination of yeasts and moulds in butter the acid-plate method was suggested (1930).

White and Hood (1931) undertook a study of methods for determining numbers of yeasts and moulds in butter and of the effects of different pH levels on the growth of yeasts and moulds. The authors found that yeast counts in butter were equally satisfactory when a medium of pH 3.5 or pH 4.2 was used. They also stated that a pH of 3.5 had no detrimental effect on the growth of butter yeasts, and no apparent differences in size of colonies or of rapidity of growth could be observed.

The Committee on Bacteriological Methods of the American Dairy Science Association (1933), published a revised report on the methods of analysing dairy products including microbiological analysis of butter. In addition to minor changes in the general procedure, they suggested the use of three new media.

1. Potato dextrose agar.
2. Bacto dehydrated malt agar.
3. Bacto dehydrated wort agar.

Parfitt (1933), during the same year, compared several media to determine their effect upon the yeast and mould count in butter. His conclusions were that whey agar prepared by rennet coagulation produced the lowest yeast and mould count, that the difference between dehydrated agars prepared by the Digestive Ferments Company (whey, malt and wort agars) was slight, and that in order to obtain comparative yeast and mould counts either dehydrated whey, malt or wort agars should be used.

A more thorough investigation of the relative merits of several media for the enumeration of yeasts and moulds in butter was undertaken by Shadwick (1938). The six media he compared were:

1. Freshly prepared potato dextrose agar.

2. Dehydrated potato dextrose agar.
3. Dehydrated peptonized milk agar.
4. Dehydrated malt agar.
5. Dehydrated whey agar.
6. Dehydrated wort agar.

The procedure he followed was based on the methods suggested by the Committee on Bacterial Methods of the American Dairy Science Association (1933). From the results he obtained, he observed the following:

> "It is not possible to note any definite tendencies for any one of the culture media used to give consistently higher counts although it should be noted that in practically all instances the mould counts were appreciably lower than the yeast counts".

The results appeared to indicate a tendency for higher counts among the duplicate plates poured with the potato dextrose agars, which apparently was not so consistently exhibited by other media. The author further stated that in a study of comparative methods and media for determining the yeast and mould counts in butter it was extremely hazardous for one worker to attempt to draw definite conclusions from the results obtained because there were conflicting data, and also because the personal equation might unduly prejudice the interpretation of results.

The Use of Dyes and Antibiotics as Bacteriostatic or Bacteriocidal Agents

The bacteriostatic or bacteriocidal actions of many dye-stuffs (biological stains) have been known for many years. The use of rose bengal for the isolation of soil fungi was originally suggested by Smith and Dawson (1944). They compared media containing rose bengal at a concentration of 1:15,000 with media to which 0.1% and 0.3% boric acid respectively had been added. The counts on the media containing the dye were 100% to 200% higher than those from the media containing boric acid. Rose bengal at a concentration of 1:10,000 was too toxic and reduced the counts of fungi somewhat.

Littman (1947) observed that the growth of many pathogenic fungi was retarded or completely inhibited on strongly acidified dextrose agar. He suggested, therefore, the use of a medium composed of 1% dextrose, 1% peptone, 1.5% oxgall, 2% agar, 1/100,000 crystal violet, and 30 units of streptomycin per ml of agar. Crystal violet and streptomycin when used together in oxgall agar inhibited the growth of both Gram-positive and Gram-negative bacteria from heavy inocula of feces, sputum and other grossly contaminated specimens. Moulds and yeasts grew as non-spreading, well-separated colonies which were easy to isolate in pure

culture.

Martin (1950) noted that although the medium suggested by Littman was excellent for the determination of total numbers of fungi, the growth of some forms was retarded so greatly and the colour of all was so intense that a considerable portion of the colonies had to be transferred to other media before identification could be made. The substitution of 1/30,000 rose bengal for the crystal violet improved the medium in this respect. Martin also noted that several common soil fungi would not grow on a medium sufficiently acid to prevent growth of all bacteria and actinomycetes. He compared the growth of a considerable number of pure fungi on three different media; each medium was acidified and rose bengal and streptomycin was added to each one. He found that the substitution of rose bengal and streptomycin for sulphuric acid (pH 4) in fungus agar increased the kinds of fungi isolated by approximately 14% and the numbers as much as 100%. It appeared that the greater part of the increased count was due to the development of types which were sensitive to the acid.

Similar results were obtained by Martin and Harding (1950). They stated that rose bengal and streptomycin were essentially as effective as acid in preventing growth

of bacteria and of streptomycetes. The use of the new medium increased the kinds of fungi by approximately 27%.

Hesseltine (1952) investigated the possibility of growing and isolating yeasts in the presence of aureomycin which had been found to be highly effective against Gram-positive, Gram-negative and acid-fast bacteria. Three different concentrations of aureomycin were employed; 50/"g, 250/"g and 500/"g per ml of medium. They were tested on 14 types of yeasts. At a concentration of 50/"g, 6 types grew as well as on the control without aureomycin; 7 types had their growth slightly retarded over the control and 1 had its growth reduced to one half the control. Higher concentrations yielded only poor growth.

Bakerspiegal and Miller (1953) compared oxgall, crystal violet, streptomycin and penicillin as bacterial growth inhibitors in the platings of soil fungi. They found that oxgall at a concentration of 1:100 and 1:40 compared favourably with crystal violet dye, streptomycin and penicillin G in repressing the development of bacteria in soil platings. Crystal violet was less effective than the other three at the concentrations employed.

Miller and Webb (1954) compared acid, rose bengal and oxgall as bacterial inhibitory agents for the isolation of

yeasts from soil. The medium used was potato dextrose agar. The authors stated that on the average, more yeasts were found when rose bengal was used. In one series of samplings, however, more yeasts were isolated on the medium to which oxgall had been added.

Olson and Bonner (1957) reported the results of a comparison between the yeast and mould count in cottage cheese obtained on acidified (pH 3.5) potato dextrose agar, and potato dextrose agar to which 100 ppm of aureomycin had been added. Of 105 samples of commercial cottage cheese 82 showed a higher count on the plates poured with the agar containing the aureomycin. The average number of colonies per plate on the aureomycin agar was 115, whereas the average for the plates with the acidified agar was 89 - an increase of 25% on the aureomycin agar.

Taber, as reported by James (1959), suggested replacing streptomycin with neomycin which is thermo-stable and may therefore be sterilized with the medium.

Application of Statistics to Microbiological Data

Fisher, Thornton and McKenzie (1922) were the first to apply theoretical statistics to platings of bacteria from soil. They showed that platings of bacteria follow a Poisson distribution, and suggested the chi-square test,

using $\chi^2 = \frac{\sum(x - \bar{x})^2}{\bar{x}}$ as an index of dispersion to check whether a set of platings may be considered a random sample, and if any deviation from the Poisson distribution were significant. The authors concluded that it was possible to obtain complete conformity with the theoretical distribution, and that any significant departure would imply a lack of randomness of sampling, or faulty technique resulting in too great a variation in individual data.

Waksman (1922) introduced the concept of standard deviation, standard error and probable error into soil bacteriological work, and the use of the coefficient of variability as a measure of reliability for the estimation of numbers of bacteria, of actinomycetes and of fungi in soil. He noted a considerable increase in the probable error in determining numbers of fungi as compared to numbers of bacteria and numbers of actinomycetes. This increase was due to the fact that the fungi exist (in the soil), both in the form of spores and in the form of aerial mycelia, and because of the great abundance and wide variations in numbers of spores formed in a single head of many fungi. We should, therefore, accept results with a higher coefficient of variability and allow for a lower level of significance for testing the χ^2 values. The

author stated, however, that if a high enough dilution was employed (1/100 of that used for bacteria) the probable error and the coefficient of variability were appreciably reduced.

Wilson and Kullman (1931) showed that the chi-square test may be used to determine whether observed variations between replicate plates on Rhizobia are due to chance or to technique. The calculated values of χ^2 will be distributed in a known manner if the replicate samples are from a Poisson series.

Jensen (1934) found that when applying goodness of fit tests to soil fungi, the results were within the permissible limits. This meant that the findings of Fisher, Thornton and McKenzie (1922) with regard to bacteria could be applied also to soil fungi.

MATERIALS AND METHOD

Preliminary Studies

A measure of the variation which might be expected with mixed yeast and mould cultures obtained from commercial butter samples was determined from a number of preliminary platings. The plating technique used has been described in the "Standard Methods for the Examination of Dairy Products" (1953). Twenty plates were prepared for each of 7 cultures. These plates were incubated at 70°F for 5 days before counts were made of the number of organisms on each plate.

Additional tests were carried out to determine the size of sample necessary in order to obtain reliable results. Two of the series (V and VI) mentioned above, and an additional set of 50 plates (Series VIII) were used. Each of these series was subdivided into groups of 2 according to the manner in which the plates had been poured. Thus, plates 1, 2 and 3 were included in group I; 4, 5 and 6 in group II, etc. Similarly, this same data was subsequently divided into groups of 4, 5 and 6 plates. The means and variances of these groups are compared in Table 3.

It is to be expected that, where quantity (number)

determinations of a particular event are made and where that event occurs with a uniform low probability, the distribution observed will be that of a Poisson. A number of workers (Fisher, Thornton and McKenzie, 1922; Wilson and Kullman, 1931) have indicated that the organisms, particularly bacteria and moulds, in their experiments have followed a Poisson distribution. Where this has been the case the results must often be transformed before the data may be analyzed using normal curve methods (e.g., Analysis of Variance). In order to determine the nature of the material used here, a series of 200 plates was prepared using a butter-water suspension with a count of approximately 35 yeasts per ml. The suspension was prepared by the addition of 55.5 ml of sterile butter to 500 ml of sterile distilled water (1:10 dilution). To this was added an unknown yeast colony from a plate taken at random. The inoculated suspension was shaken for one half hour on a mechanical shaker after which time 2 pilot plates were prepared to check the approximate yeast count in the suspension. One ml of the suspension was delivered onto the plates, and they were poured with 10 ml of acidified (pH 3.5 ± 0.1) malt agar. After solidification, the plates were incubated at 70°F for 5 days. The stock suspension was kept refrigerated during this period.

A count was made of the organisms on the pilot plate after 5 days, and the stock solution was diluted with a sterile water-butter suspension until a yeast count of from 30 to 35 was obtained. The adjusted yeast suspension was plated out onto 200 plates (1 ml per plate) starting with plate No. 1 and continuing systematically to plate No. 200. Each plate was poured with 10 ml of acidified malt agar and incubated at 70°F for 5 days. Counts were made of the number of organisms on each plate. The counts were grouped according to size and then tested against the expected normal and Poisson distributions following the methods outlined by Snedecor (1946).

Main Study

The purpose of this study was to assess the relative efficiency of 5 different treatments used in determining yeast and mould counts in butter. The experiment was divided into 2 sections.

Section I In the first section, 2 basic media, potato dextrose agar and malt agar, were compared when in combination with each of two additives, tartaric acid and a mixture of streptomycin and rose bengal. The 4 treatments possible are listed below.

(a) Potato dextrose agar, plus acid (pH 3.5 ± 0.1)

(b) Malt agar, plus acid (pH 3.5 ± 0.1)

(c) Potato dextrose agar plus rose bengal (1:15,000) and streptomycin (30 γ per ml of medium)

(d) Malt agar plus rose bengal (1:15,000) and streptomycin (30 γ per ml of medium)

The experimental procedure outlined in "Standard Methods for the Examination of Dairy Products" (1953) was followed exclusively in the preparation of the acid plates. In the preparation of the non-acid (rose bengal and streptomycin) plates, the dye was added before sterilizing the medium and the heat-labile antibiotic was added aseptically to the remelted medium just prior to pouring the plates. Butter samples were taken at random from shipments obtained from provincial creameries. For each sample studied, 5 plates (subsamples) were used to measure the effect of each treatment. In all 64 samples were studied. An analysis of variance was carried out for each sample and for all samples combined. Because of the lack of continuity (uniformity) from one sample to another all data were transformed ($\sqrt{\text{Individual plate count}}$) prior to conducting the overall analysis.

Section II Subsequently it was decided to determine the effect of aureomycin on yeast and mould counts and a second experiment was conducted in which aureomycin at a

concentration of 100 γ per ml of medium was compared with a streptomycin and rose bengal check. The basic medium used was potato dextrose agar. The experimental procedure was similar to that used in Section I. The check plates were prepared as before but in the preparation of the other treatment, the dye was omitted and the streptomycin was replaced by aureomycin. For each treatment in each sample, 4 plates (subsamples) were used. The 64 samples studied were the ones used in the first section. Each sample was analysed separately (t-test) and a combined analysis of variance was carried out on all samples using transformed data ($\sqrt{\text{Individual plate count}}$).

The actual yeast and mould counts obtained on the 4 treatments used in section I, and on the 2 treatments used in section II, are given in Appendix I and II respectively.

RESULTS AND DISCUSSION

Preliminary Studies

The individual counts of the 7 series of 20 plates used in measuring the variation which occurs in yeast and mould cultures are presented in Table 1. The counts of an eighth series consisting of 50 plates are given in Table 2. It was apparent from these data that the variation in this material was rather high. The coefficients of variability were 28.9, 25.6, 33.9, 61.0, 11.1, 25.1, 13.8 and 20.7 respectively for series I to VIII. The lowest variation was associated with those series which had high average counts. (The values were 4.9, 3.7, 3.6, 4.0, 25.4, 5.9, 66.5 and 33.8 respectively for series I to VIII.) This is what might be anticipated where the number of micro-organisms delivered per plate is not strictly controlled and the presence of an unbroken spore head could greatly influence the count on any one plate. This would be of greater significance where the mean number of organisms per plate is low and numerically small deviations would be expressed in a greatly increased variation. This may account for the extremely high coefficient of variability for series IV. In general, the variation of these mixed yeast and mould cultures was high and this may be important

TABLE 1

Counts of Yeasts and Moulds Per Plate for Series I to VII

Plate No.	I	II	III	IV	V	VI	VII
1.	4	2	4	4	26	5	70
2.	3	3	3	6	18	3	49
3.	5	6	3	9	24	1	60
4.	7	3	2	3	26	3	61
5.	5	4	4	3	24	5	48
6.	5	2	6	1	25	6	63
7.	4	6	2	9	25	6	56
8.	4	5	3	1	27	7	72
9.	3	3	5	1	25	8	74
10.	4	2	6	2	27	7	68
11.	7	8	2	5	20	9	67
12.	6	2	2	7	24	7	62
13.	5	4	4	3	22	5	85
14.	5	3	4	1	28	9	78
15.	8	5	3	5	27	5	71
16.	3	3	2	4	30	4	66
17.	5	4	6	7	26	8	77
18.	7	3	3	3	27	7	67
19.	4	4	6	1	28	6	63
20.	4	2	2	4	29	6	64

TABLE 2

Counts of Yeasts and Moulds
for Series VIII

Counts Per Plate			
30	15	17	33
28	21	27	28
24	24	30	34
20	30	32	25
27	27	20	13
29	30	32	22
29	20	25	33
26	26	24	24
27	27	26	23
31	24	24	31
22	22	35	33
22	19	34	
25	28	38	

in detecting small differences between treatments.

The high variation observed in these series raised the question of the number of subsamples which must be used within each treatment in order to obtain a reasonably reliable estimate of performance of that treatment and which will permit selection of those treatments which perform most efficiently the function required of them. It follows that the greater the size of sample, the more confidence we place in our estimate and the more readily (other things being equal) we will be able to detect small significant differences. However, because the numbers of plates which can be physically accomodated is limited and because it would be desirable to use treatments on a number of samples rather than on a single sample, the number of subsamples must be kept to a reasonable level. The mean and standard deviations of the subgroups within series V, VII and VIII are presented in Table 3. A survey of this data indicated that the subgroups provided reasonable estimates of the means of the series, i.e., the mean and standard deviation had not changed appreciably with reduction in numbers from 20 or 50 to 3, 4, 5 and 6. Variation appeared to be quite uniformly distributed with no indication of consistent increases or decreases in number from the first to the last plate. The final criterion in

TABLE 3

Means and Standard Deviations of the Different Groupings in Series V, VII and VIII

Series V

Number of Plates Per Group									
20		6		5		4		3	
$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
25.4	2.8	23.8	3.0	23.6	3.3	23.5	3.6	22.7	4.2
		24.6	2.6	25.8	1.1	25.3	1.3	25.0	1.0
		26.7	2.6	24.2	3.4	24.0	2.3	25.7	1.2
				28.0	1.6	26.8	3.4	23.7	3.5
						27.5	1.3	25.7	3.3
								27.7	2.1

Series VII

Number of Plates Per Group									
20		6		5		4		3	
$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
66.5	9.4	58.5	8.5	57.6	9.2	60.0	8.6	59.7	10.5
		66.5	6.8	66.6	7.2	59.8	10.2	57.3	8.2
		75.5	6.5	72.6	9.0	67.8	4.9	67.3	9.8
				69.2	6.8	75.0	8.3	65.7	3.3
						70.0	7.5	78.0	7.0
								73.0	6.1

TABLE 3 - Continued

Series VIII

Number of Plates Per Group									
50		6		5		4		3	
$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
26.3	5.3	26.3	3.7	25.8	3.9	25.5	4.4	27.3	3.1
		26.2	3.7	28.4	1.9	27.8	1.5	25.3	4.7
		23.7	2.7	21.0	3.7	25.5	4.4	27.3	1.6
		24.8	3.6	26.2	4.3	21.3	4.5	25.0	5.2
		25.5	6.1	23.6	3.2	26.8	4.7	20.3	5.1
		25.2	3.9	26.8	5.8	24.8	2.2	27.0	3.0
		33.7	3.3	25.4	4.3	22.8	5.6	25.3	5.1
		23.3	6.4	32.8	5.3	28.5	5.7	24.3	2.6
				24.4	7.8	24.8	1.0	21.3	5.9
				28.8	4.9	35.0	2.2	29.7	2.6
						25.0	8.8	25.7	6.0
						25.5	5.0	24.7	1.1
								35.7	2.1
								31.7	3.2
								20.0	6.3
								26.7	5.5

determining sample size was the standard error of a mean difference. It may be seen that in our calculation of the difference needed before treatment means were significantly different (least significant difference), one of the values used was subsample number (i.e., number of samples used in the calculation).

$$\text{L.S.D.} = t_{\text{Edf}}\sqrt{\frac{\text{E.M.S.(Variance) x 2}}{\text{Subsample No.}}}$$

This meant that if the error mean squares (or the standard deviations or variances) were equal, then the L.S.D. needed when 6 subsamples were used was $\sqrt{\frac{50}{6}}$ (approximately 2.8) times as large as that required when 50 subsamples were used. The value was 3.5 when only 4 subsamples were used. When 20 subsamples were compared with 6 and 4 subsamples, the values were 1.80 and 2.25 respectively. This also meant that in material such as we had here, where the variance averaged about $5^2 = 25$ or less (see Table 3), there must be a difference of approximately 6 units ($2 \times \sqrt{\frac{25 \times 2}{6}}$) between means before they would be regarded as being significantly different. It is extremely unlikely that a difference of less than 6 units would be of any practical significance to the dairyman who must use this technique, i.e., the treatments would have to have greater differences before the one could be regarded as better or easier than the

other. Thus, it would seem that sample sizes of from 3 - 6 would be adequate for our purposes here.

The counts of the 200 plates used in studying the distribution of yeasts in a sterile water-butter suspension are presented in Table 4. The mean count for all plates was 33.8 with a standard deviation of 7.00 (C.V. = 20.7%). The individual counts were grouped according to magnitude. When this grouped data was tested against the Poisson distribution expected with this mean, the χ^2 value of 32.17 exceeded that permissible (0.05 = 26.30; 0.01 = 32.00) with 18 - 2 = 16 degrees of freedom. If in a Poisson distribution the mean = variance, the poor fit here may be explained on the basis of the higher variance (mean = 33.8; variance = 48.43) of this data. This was further supported by the original data which placed approximately one-quarter of the individuals in the extremes of the distribution. The χ^2 value for the fit of this data to the expected normal distribution is 9.95 (P = 0.10). This meant that the individuals were so distributed that we were justified in using normal curve methods of analysis. A square root transformation of the original data would improve the continuity of the data.

This transformation was applied to the actual yeast and mould counts of less than 50 organisms per plate. All the

TABLE 4

Counts of Yeasts on the 200 Plates Used to Study the Distribution of Platings of Yeasts

Count per Plate				
40	45	30	32	28
43	45	38	36	30
38	30	24	27	43
38	33	28	36	33
40	40	30	29	41
40	35	34	36	37
42	42	34	30	35
37	43	37	29	41
45	30	41	35	31
45	37	35	27	29
36	42	31	39	27
48	37	26	27	23
38	43	24	36	19
41	39	30	40	31
37	43	36	33	32
45	42	23	31	32
38	42	23	36	30
52	46	35	31	24
40	32	26	26	21
47	34	37	30	24
37	37	27	38	28
46	28	42	30	30
38	29	38	43	23
28	30	34	54	26
45	25	29	28	31
37	30	36	25	28
40	25	42	33	30
39	29	29	27	19
41	32	37	27	32
30	40	31	39	24
39	33	34	40	38
46	43	28	40	29
36	38	28	39	26
37	25	21	28	24
33	29	40	38	30
34	35	37	25	23
32	27	41	35	25
36	30	32	29	21
39	33	32	30	32
39	37	35	31	23

counts were transformed for the overall analyses of variance in Sections I and II.

Main Study

Section I (Comparison of 4 treatments). The means for each of the 4 treatments in each of 64 samples used in this study are presented in Table 6. A survey of these results suggests that no treatment is consistently superior throughout. A summary of the analysis of variance for each sample is presented in Table 7. It may be seen from these analyses that it was not possible to detect significant differences in all samples. This was not surprising in view of the high variation in this material and the small number of micro-organisms in many of the samples. In order to overcome this difficulty the data from all samples was combined and analysed (Table 5). This analysis detected significant differences between samples ($P < 0.01$) and between treatments ($P < 0.01$). The total variance for treatments may be partitioned into variances due to differences between media, differences between additives and interaction. Each component part had one degree of freedom and was significant when tested against the experimental error.

The implied superiority of one medium over another or

TABLE 5

Combined Analysis of Variance for the 64 Samples of Section I

Source of Variation	d.f.	M.S.	F(calc)	F(0.05)	F(0.01)
Samples	63	251.25	39.89	1.39	1.59
Treatments	3	111.06	20.01	2.65	3.89
A	1	27.40	4.94	3.89	6.76
B	1	29.40	5.30	3.89	6.76
AB	1	54.26	9.78	3.89	6.74
Exper. Error	189	5.55	10.26	1.27	1.40
Sampl. Error	1024	.54	-	-	-
Total	1279	-	-	-	-

TABLE 6

Average Number of Organisms
Per Treatment in Each Sample

Sample No.	Treatments (a) Potato Dextrose Agar plus Acid	(b) Malt Agar plus Acid	(c) Potato Dextrose Agar plus Rose Bengal and Streptomycin	(d) Malt Agar plus Rose Bengal and Streptomycin
1.	26.0	24.0	15.7	26.8
2.	4.4	4.6	4.6	7.2
3.	1.7	1.6	2.8	2.4
4.	3.2	3.2	3.0	3.1
5.	4.0	2.6	4.0	4.6
6.	8.2	4.2	8.8	7.8
7.	5.4	3.5	5.0	6.0
8.	3.2	2.9	3.0	3.4
9.	5.2	3.0	6.0	5.8
10.	3.4	4.0	3.4	3.9
11.	10.4	8.8	10.4	11.0
12.	2.2	2.5	1.6	2.6
13.	1.8	1.8	1.4	2.0
14.	4.1	4.2	3.8	4.6
15.	3.4	2.2	4.0	2.8
16.	5.8	2.8	3.6	3.6

TABLE 6 - Continued

Sample No.	Treatments (a)	(b)	(c)	(d)
17.	3.8	3.2	3.8	4.0
18.	6.8	4.2	6.6	5.8
19.	10.4	10.6	10.0	9.8
20.	2.2	2.2	2.1	2.6
21.	2.4	2.8	3.4	3.4
22.	4.2	3.4	3.8	4.4
23.	2.0	1.9	1.8	2.4
24.	3.8	2.4	3.4	3.2
25.	1.8	1.4	2.4	2.0
26.	1.7	2.0	1.8	2.3
27.	4.6	3.2	3.2	4.1
28.	2.8	1.6	2.8	2.8
29.	2.2	1.2	1.8	2.2
30.	6.2	3.6	7.6	6.2
31.	7.2	6.8	7.2	7.2
32.	5.2	4.8	4.8	4.8
33.	5.6	5.6	5.6	6.0
34.	5.4	5.2	5.4	5.8
35.	1.4	1.4	1.0	1.4
36.	6.4	7.0	6.2	7.0

TABLE 6 - Continued

Sample No.	Treatments (a)	(b)	(c)	(d)
37.	3.6	3.4	3.2	3.6
38.	2.0	2.4	2.2	2.1
39.	2.6	3.2	2.6	2.6
40.	4.2	4.0	3.8	3.8
41.	11.0	11.2	10.6	11.2
42.	1.8	2.0	2.0	1.6
43.	5.0	3.0	3.4	2.2
44.	7.6	6.6	7.7	7.2
45.	7.0	6.4	7.6	8.0
46.	2.5	1.6	2.9	3.2
47.	13.6	11.0	14.6	10.0
48.	8.8	9.2	9.8	9.6
49.	3.2	2.6	3.2	2.8
50.	3.8	2.8	2.8	3.8
51.	6.6	6.8	11.6	7.2
52.	11.0	6.8	10.0	6.8
53.	6.4	6.2	7.4	7.4
54.	7.8	7.4	7.4	7.0
55.	7.4	7.0	7.0	8.8
56.	7.4	7.4	7.8	8.4
57.	2.0	1.6	1.8	2.0

TABLE 6 - Continued

Sample No.	Treatments (a)	(b)	(c)	(d)
58.	5.4	4.8	4.8	4.4
59.	6.6	6.6	6.8	7.2
60.	7.2	7.8	8.0	8.8
61.	8.5	7.8	7.4	8.0
62.	7.4	4.0	8.2	7.2
63.	1.8	1.4	1.0	2.2
64.	4.4	4.5	4.5	7.1

TABLE 7

Summary of Analysis of Variance (F values)
for Each Sample Employed in Section I

Sample No.	F Values for Main Effects, (Treatment Levels) (A) Media	(B) Additives	(AB) Interaction	Types of Organisms
1.	11.64	6.28	27.65	Moulds
2.	29.00	19.00	5.70	Moulds
3.	1.30	15.85	0.37	Moulds
4.	<1	<1	<1	Mixed
5.	3.95	23.68	23.26	Mixed
6.	59.58	41.74	21.57	Mixed
7.	<1	2.62	5.11	Yeasts
8.	<1	<1	<1	Mixed
9.	19.23	38.43	11.10	Yeasts
10.	2.89	<1	0	Mixed
11.	1.00	8.45	8.45	Mixed
12.	27.22	7.33	1.00	Mixed
13.	3.10	<1	1.70	Mixed
14.	35.10	9.20	<1	Moulds
15.	1.00	<1	6.7	Mixed

Legend:

$F_{(1,16)}$	5%	1%
	4.49	8.53

TABLE 7 - Continued

Sample No.	F Values (A) Media	(B) Additives	(AB) Interaction	Types of Organisms
16.	13.05	1.49	11.70	Mixed
17.	<1	1.00	2.69	Mixed
18.	52.24	8.62	12.07	Mixed
19.	1.29	3.09	<1	Mixed
20.	<1	<1	<1	Mixed
21.	6.00	67.75	3.75	Moulds
22.	1.25	5.13	32.00	Mixed
23.	<1	<1	2.63	Mixed
24.	5.60	<1	3.40	Mixed
25.	5.88	6.48	0	Moulds
26.	1.72	1.24	<1	Mixed
27.	<1	<1	2.9	Mixed
28.	2.42	2.55	2.55	Mixed
29.	2.40	1.30	7.50	Mixed
30.	54.31	54.20	3.57	Moulds
31.	<1	<1	<1	Mixed
32.	<1	<1	<1	Mixed
33.	<1	<1	<1	Mixed
34.	<1	3.10	1.43	Moulds
35.	1.85	<1	<1	Mixed

TABLE 7 - Continued

Sample No.	F Values (A) Media	(B) Additives	(AB) Interaction	Types of Organisms
36.	4.45	<1	<1	Moulds
37.	<1	<1	1.64	Mixed
38.	<1	<1	1.46	Moulds
39.	2.78	1.61	3.83	Mixed
40.	<1	<1	<1	Mixed
41.	5.91	<1	<1	Mixed
42.	<1	<1	2.86	Mixed
43.	32.00	22.11	2.73	Mixed
44.	8.32	2.21	<1	Mixed
45.	<1	11.57	<1	Mixed
46.	<1	8.96	3.38	Moulds
47.	164.73	<1	10.39	Yeasts
48.	<1	16.75	<1	Moulds
49.	4.70	<1	<1	Yeasts
50.	<1	<1	2.09	Mixed
51.	48.04	79.40	57.26	Mixed
52.	53.98	<1	2.78	Yeasts
53.	<1	7.82	<1	Moulds
54.	1.89	1.27	<1	Yeasts
55.	2.63	3.45	9.25	Mixed

TABLE 7 - Continued

Sample No.	F Values (A) Media	(B) Additives	(AB) Interaction	Types of Organisms
56.	<1	15.37	2.74	Moulds
57.	<1	<1	<1	Mixed
58.	<1	<1	<1	Mixed
59.	<1	<1	<1	Mixed
60.	3.11	5.29	<1	Moulds
61.	0	1.70	3.51	Mixed
62.	118.00	100.00	35.95	Mixed
63.	4.60	<1	19.21	Mixed
64.	3.63	19.52	32.15	Mixed

of one additive over another was not readily apparent from Tables 8 and 9. The sample differences were almost equally divided with respect to media although the differences in favour of potato dextrose agar were often larger, significantly so in 13 out of 17 instances. The pattern was similar when additives were compared, although the trend in favour of the rose bengal and streptomycin additive was more evident. In 21 of the 23 instances where significant differences between additives were noted, the higher counts were obtained from media to which rose bengal and streptomycin had been added.

The interpretation that these significant differences reflect qualities of the media or additives per se was suspect because of the significance of the interaction, media x additives, and the relationship of the variance of the interaction to the variances of the media and the additives. This may be illustrated as follows:

Source of Variation	D.F.	Components of Variance
Samples	(r-1)	
Media (A)	(m-1)	$s^2 - rs^2_{AB} - ras^2_A$
Additives (B)	(a-1)	$s^2 - rs^2_{AB} - mrs^2_B$
Media x Additives (AB)	(m-1)(a-1)	$s^2 - rs^2_{AB}$
Experimental error	(r-1)(m-1)(a-1)	s^2

TABLE 8

Average Number of Organisms per Medium,
(Potato Dextrose Agar and Malt Agar)

Sample No.	Potato Dextrose Agar (a-c)/2	Malt Agar (b-d)/2	(P.D. - M)
1.	20.8	25.4	-4.6
2.	4.5	5.9	-1.4
3.	2.3	2.0	+0.3
4.	3.1	3.1	0
5.	4.0	3.6	+0.4
6.	8.5	6.0	+2.5
7.	5.2	4.7	+0.5
8.	3.1	3.1	0
9.	5.6	4.4	+1.2
10.	3.2	3.9	-0.7
11.	10.4	9.9	+0.5
12.	1.9	2.5	-0.6
13.	1.6	1.9	-0.3
14.	4.0	4.4	-0.4
15.	3.7	2.5	+1.2
16.	4.7	3.2	+1.5
17.	3.8	3.6	+0.2
18.	6.7	5.0	+1.7
19.	10.3	10.2	+0.1

TABLE 8 - Continued

Sample No.	(a-c)/2	(b-d)/2	(P.D. - M)
20.	2.1	2.4	-0.3
21.	2.9	3.1	-0.2
22.	4.0	3.9	+0.1
23.	1.9	2.1	-0.2
24.	3.6	2.8	+0.8
25.	2.1	1.7	+0.4
26.	1.7	2.1	-0.4
27.	3.9	3.6	+0.3
28.	2.8	2.2	+0.6
29.	2.0	1.7	+0.3
30.	6.9	4.9	+2.0
31.	7.2	7.0	+0.2
32.	5.0	4.8	+0.2
33.	5.6	5.8	-0.2
34.	5.4	5.5	-0.1
35.	1.2	1.4	-0.2
36.	6.3	7.0	-0.7
37.	3.4	3.5	-0.1
38.	2.2	2.1	-0.1
39.	2.6	2.9	-0.3
40.	4.0	3.9	+0.1

TABLE 8 - Continued

Sample No.	(a-c)/2	(b-d)/2	(P.D. - M)
41.	10.8	11.2	-0.4
42.	1.9	1.8	+0.1
43.	4.2	2.6	+1.6
44.	7.6	6.9	+0.7
45.	7.3	7.2	+0.1
46.	2.7	2.4	+0.3
47.	14.1	10.5	+3.6
48.	9.3	9.4	-0.1
49.	3.2	2.7	+0.5
50.	3.3	3.3	0
51.	9.1	7.0	+2.1
52.	10.5	6.8	+3.7
53.	6.9	6.8	+0.1
54.	7.6	7.2	+0.4
55.	7.2	7.9	-0.7
56.	7.6	7.9	-0.3
57.	1.9	1.8	+0.1
58.	5.1	4.6	+0.5
59.	6.7	6.9	-0.2
60.	7.6	8.3	-0.7
61.	7.9	7.9	-0.0

TABLE 8 - Continued

Sample No.	(a-c)/2	(b-d)/2	(P.D. - M)
62.	7.8	5.6	+2.2
63.	1.4	1.8	-0.4
64.	4.4	5.8	-1.4

TABLE 9

Average Number of Organisms per Additive, (Acid, and Rose Bengal and Streptomycin)

Sample No.	Acid (a-b)/2	Rose Bengal and Streptomycin (c-d)/2	Acid - R.B. & St.
1.	25.0	21.2	+3.8
2.	4.5	5.9	-1.4
3.	1.7	2.6	-0.9
4.	3.2	3.0	+0.2
5.	3.3	4.3	-1.0
6.	6.2	8.3	-2.1
7.	4.4	5.5	-1.1
8.	3.0	3.2	-0.2
9.	4.1	5.9	-1.8
10.	3.7	3.6	+0.1
11.	9.6	10.7	-1.1
12.	2.3	2.1	-0.2
13.	1.8	1.7	+0.1
14.	4.2	4.2	0
15.	2.8	3.4	-0.6
16.	4.3	3.6	+0.7
17.	3.5	3.9	-0.4
18.	5.5	6.2	-0.7

TABLE 9 - Continued

Sample No.	(a-b)/2	(c-d)/2	Acid - R.B. & St.
19.	10.5	10.0	+0.5
20.	2.2	2.3	-0.1
21.	2.6	3.4	-0.8
22.	3.8	4.1	-0.3
23.	1.9	2.1	-0.2
24.	3.1	3.3	-0.2
25.	1.6	2.2	-0.6
26.	1.8	2.0	-0.2
27.	3.9	3.6	+0.3
28.	2.2	2.8	-0.6
29.	1.7	2.0	-0.3
30.	4.9	6.9	-2.0
31.	7.0	7.1	-0.1
32.	5.0	4.8	+0.2
33.	5.6	5.8	-0.2
34.	5.3	5.6	-0.3
35.	1.4	1.2	+0.2
36.	6.7	6.6	+0.1
37.	3.5	3.4	+0.1
38.	2.1	2.3	+0.2
39.	2.9	2.6	+0.3

TABLE 9 - Continued

Sample No.	(a-b)/2	(c-d)/2	Acid - R.B. & St.
40.	4.1	3.8	+0.3
41.	11.1	10.9	+0.2
42.	1.9	1.8	+0.1
43.	4.0	2.8	+1.2
44.	7.1	7.5	-0.4
45.	6.7	7.8	-1.1
46.	2.0	3.1	-0.9
47.	12.3	12.3	0
48.	9.0	9.6	-0.6
49.	2.9	3.0	-0.1
50.	3.3	3.3	0
51.	6.7	9.4	-2.7
52.	8.9	8.4	+0.5
53.	6.3	7.4	-1.1
54.	7.6	7.2	+0.4
55.	7.2	7.9	-0.7
56.	7.4	8.1	-0.7
57.	1.8	1.9	-0.1
58.	5.1	4.6	+0.5
59.	6.6	7.0	-0.4
60.	7.5	8.4	-0.9

TABLE 9 - Continued

Sample No.	(a-b)/2	(c-d)/2	Acid - R.B. & St.
61.	8.2	7.7	+0.5
62.	5.7	7.7	-2.0
63.	1.6	1.6	0
64.	4.4	5.8	-1.4

It might be seen from this that the variance for each of the main effects (media or additives) was made up, in part, of variance due to interaction between these main effects. Where this interaction was significant the amount added might be sufficient to obscure the true relationship between main effects. This difficulty could be overcome by using the interaction as the error term in testing for significance of the main effects. The following results were obtained when the main effects were tested against the interaction.

TABLE 10

Combined Analysis of Variance. Main Effects (A and B) Tested Against Interaction (AB).

Source of Var.	d.f.	M.S.	F (calc)	F (0.05)
Samples	63	251.25	39.89	1.39
Treatments	3	111.06	20.01	2.65
A	1	27.40	1	161.00
B	1	29.40	1	161.00
AB	1	54.06	9.78	3.89
Exper. Error	189	5.55	10.26	1.27
Sampl. Error	1024	0.54	-	-
Total	1279	-	-	-

In this modification of the analysis of variance neither media nor additives contributed significantly to total variance. This meant that the superiority of the potato dextrose agar and of the rose bengal and streptomycin additive was due primarily to the contribution of the interaction to variance.

Among the 64 individual samples, 18 had a significant F value for the interaction (AB). When the main effects A and B of these samples were tested against their respective interaction (AB), 8 F values for media (treatment level A) that were previously significant now showed no significance. Of the 8 samples, 6 had a higher average count for potato dextrose agar. This meant that only 7 samples had significantly higher average counts for potato dextrose agar, as compared to the 13 samples indicated previously. (See page 37).

Similarly, 10 F values for additives (treatment level B) lost their significance, and only 13 instead of 21 out of 23 samples now showed significant difference in favour of rose bengal and streptomycin. (See page 37).

A significant interaction implies that the separate effects of the components of that interaction vary from those expressed when they occur in combination. This difference in expression may be one of magnitude or one of direction. For example, in the terms of this study it

could mean that the response of one medium to an additive might be greater than the response of the other medium (magnitude difference), or it could mean that whereas the growth on one medium was stimulated by the use of an additive the growth on the other medium was depressed (direction difference). The mean values for the interactions are presented below:

(a) Potato dextrose agar plus acid: 5.3

(b) Malt agar plus acid: 4.6

(c) Potato dextrose agar plus rose bengal and streptomycin: 5.2

(d) Malt agar plus rose bengal and streptomycin: 5.3

The least significant difference for comparison of these interactions is 0.52 (P = 0.05). Three of the interactions (d, a, c) were not significantly different but all were superior to the fourth (b). This could be interpreted as meaning that there was no difference between media (a - b or b - c not significant), or additives (a - c not significant) per se, but the specific combination of malt agar and of tartaric acid depressed fungal growth.

The performance of malt- and potato dextrose agar in this study was in agreement with that observed in previous investigations by other workers, (Parfitt, 1933; Shadwick, 1938) who were unable to detect differences between these

two media. Some differences had been expected in the performance of the additives.

Bisby, Jamieson and Timonin (1933) have reported that in their material, 26 of 63 species of fungi found in butter were normally found in soil. Martin and Harding (1950) have shown that a medium with a rose bengal and streptomycin additive supported higher numbers of soil fungi than did a similar medium to which (sulphuric) acid had been added. If a similar proportion of the kinds of fungi found in butter also occur in the soil and these make up a sizeable proportion of the total number of fungi in butter, then results similar to those obtained by Martin and Harding might have been expected. If, however, these fungi do not make up a sufficient proportion of the total, and/or if the unknown proportion are yeasts, then the difference between media might be smaller. White and Hood (1931) have reported that milk yeasts grew equally well at a pH of 3.5 or 4.2. If this is the case then the acid medium used here would not inhibit growth and the type of organisms x media interaction might be masked. That this could be a factor may be inferred from the 7 pure cultures of yeasts included in the 64 samples in this study. In general, these cultures showed no differences between media or additives. However, the fact that 3 of these yeast cultures did not have signi-

ficant interactions suggested that the variability of delivery (unbroken spore heads, etc.,) might also have been a factor. By contrast, in 9 of the 15 cultures of moulds, significantly higher counts were obtained with the rose bengal and streptomycin additive.

Section II (Comparison of 2 treatments). The average counts of yeasts and moulds in each treatment and the probability level for the t test for each of the 64 samples are presented in Table 11. Significant differences between media were detected in 21 samples, and in 15 of these the higher counts were associated with the medium containing aureomycin. Higher counts for this medium were obtained in 38 samples. The mean count for the 64 samples grown on medium containing aureomycin additive was 8.7, while the mean count for the same samples grown on the medium with the rose bengal and streptomycin additive was 6.7. The difference between these two means is significant ($P<0.01$) when the mean square for treatments is tested against the sampling error as an estimate of chance variability. (See Table 12). However, when the variance for treatments is tested against the samples x treatment interaction, the F value is not significant. (See Table 13). This meant that while the medium with the aureomycin additive had a

TABLE 11

Probability Levels of the t-Tests for the 64 Samples, and Average Counts of Yeasts and Moulds per Treatment

Sample No.	P Value	Average Count (a) Aureomycin	Average Count (b) Rose Bengal and Streptomycin
1.	<0.01	13.6	19.9
2.	<0.05	3.0	1.6
3.	>0.10	7.4	6.4
4.	<0.05	1.5	0.5
5.	>0.10	3.0	2.6
6.	>0.10	13.2	11.9
7.	>0.10	16.2	17.6
8.	>0.10	16.5	17.2
9.	>0.05	2.2	0.7
10.	<0.05	17.9	20.2
11.	>0.10	13.2	12.0
12.	>0.10	19.8	21.0
13.	<0.01	7.3	5.1
14.	>0.10	19.0	21.3
15.	>0.10	7.2	8.4
16.	>0.10	4.3	4.1
17.	>0.10	13.9	13.0

TABLE 11 - Continued

Sample No.	P Value	Average Count (a)	(b)
18.	>0.10	3.8	2.9
19.	>0.10	9.2	8.9
20.	>0.10	6.0	5.5
21.	>0.10	2.4	2.1
22.	>0.10	5.1	4.9
23.	>0.10	6.8	7.7
24.	>0.10	3.0	3.4
25.	>0.10	3.3	3.1
26.	>0.05	12.3	13.6
27.	<0.05	12.0	10.8
28.	>0.10	4.3	2.7
29.	<0.01	7.1	2.3
30.	>0.10	18.8	19.0
31.	>0.10	4.1	4.7
32.	>0.10	3.8	4.5
33.	>0.10	3.4	2.5
34.	>0.10	2.6	2.2
35.	<0.01	5.7	4.3
36.	<0.05	3.3	2.4
37.	>0.10	8.6	7.4

TABLE 11 - Continued

Sample No.	P Value	Average Count (a)	(b)
38.	<0.01	4.4	1.7
39.	<0.01	12.7	10.1
40.	<0.01	16.9	19.5
41.	>0.10	3.0	2.6
42.	<0.01	9.5	5.0
43.	>0.10	17.1	18.2
44.	<0.01	5.6	7.8
45.	<0.01	7.1	4.0
46.	>0.10	1.7	1.9
47.	>0.10	6.9	6.5
48.	<0.01	22.5	7.9
49.	>0.10	7.1	7.5
50.	<0.01	2.6	1.2
51.	>0.10	2.5	2.1
52.	>0.10	10.1	9.6
53.	>0.10	15.8	16.0
54.	>0.10	9.3	10.6
55.	>0.10	19.0	19.4
56.	>0.10	20.3	19.5
57.	>0.10	3.8	3.3

TABLE 11 - Continued

Sample No.	P Value	Average Count (a)	(b)
58.	>0.10	11.4	11.5
59.	0.05	1.3	1.9
60.	>0.10	14.7	14.8
61.	<0.01	6.4	2.6
62.	<0.01	3.3	1.6
63.	<0.01	9.2	10.8
64.	>0.10	6.6	6.7

TABLE 12

Combined Analysis of Variance for the 64 Samples of Part II. (Treatments Tested Against Sampling Error)

Source of Variation	d.f.	M.S.	F (calc)	F (0.01)
Samples	63	285.34	23.16	1.78
Treatments	1	34.47	37.07	6.73
Exper. Error	63	12.32	13.24	1.51
Sampl. Error	384	0.93	-	-
Total	511	-	-	-

TABLE 13

Combined Analysis of Variance. (Treatments Tested Against Experimental Error, Samples x Treatment Interaction)

Source of Variation	d.f.	M.S.	F (calc)	F (0.01)
Samples	63	285.34	23.16	1.78
Treatments	1	34.47	2.80	7.05
Exper. Error	63	12.32	13.24	1.51
Sampl. Error	384	0.93	-	-
Total	511	-	-	-

higher average count, the pattern was not similar for all the samples, and the trend in the 38 samples with higher average counts for the aureomycin medium was masked by differences in direction and magnitude in the remaining 26 samples.

The lack of consistency in performance may have been a consequence of the small numbers in some samples, and/or it may have been due to a fungi x medium interaction which could be a reflection of differences in additives per se, or a reflection of differences in concentrations of additives. This interaction, however, could not be measured in this survey experiment.

The question of why one of a number of treatments (agars plus bacterial inhibitor) supported higher counts of yeasts and moulds cannot be answered from the results obtained here, and must be determined from other, more specific studies. It is sufficient to suggest that all the treatments used did not support equal numbers of yeasts and moulds, and that all cultures did not react to the treatments in the same manner. It should also be stressed that although there are indications that certain media support greater numbers of yeasts and moulds this need not mean that these are preferred media. What is required is a medium which accurately measures relative differences

between samples and is, at the same time, physically easy and economical to produce. Antibiotics and dyes may be preferred over acids as bacterial inhibitors even if there is no difference in fungal count because of the ease with which the antibiotics and dyes can be added to the medium, (no effect on pH, consequently there is no need for a pH meter), and because they have no effect on the subsequent jelling property of the medium.

Combinations of dyes and antibiotics may be preferred over antibiotics alone because of the tendency of the dye to reduce the amount of spreading in moulds. This facilitates identification and enumeration of the moulds on the plate. In this connection it should be pointed out that yeasts especially are more easily distinguishable on the coloured medium. At the same time, the presence of two substances with bacteriostatic action decreases the likelihood of contamination due to the development of bacterial resistance to one or both of them. By contrast, the concentration of either of these additives will not be sufficiently high to affect adversely fungal growth.

The choice of antibiotic to be used will be influenced by a number of factors. For example, aureomycin, although possessing a wider antibacterial spectrum and being effective in smaller amounts than streptomycin, is considerably

less stable than the latter. A streptomycin solution stored in a refrigerator will retain its activity for at least three months, whereas a refrigerated solution of aureomycin should be discarded after two weeks (1952). Also, mixtures of antibiotics, unless readily available and stable, possess no advantage over a dye-antibiotic combination as far as preparation is concerned; whether they are more effective bacterial inhibitors is a matter of speculation.

SUMMARY AND CONCLUSIONS

Preliminary Studies

A measure of the variation which might be expected within samples with mixed yeast and mould cultures was determined from a number of preliminary platings. It was found that the within-sample variation was relatively high but was rather uniformly distributed among 7 series of 20 plates each, 1 series of 50 and 1 series of 200 plates respectively. The technique followed could therefore be considered to be adequate.

Additional tests were carried out to determine the number of subsamples per sample necessary to obtain reliable results. There was no appreciable change in variation when the number of subsamples was reduced from 50 or 20 to 6, 5, 4 or 3 respectively.

In a third test, the counts on 200 plates of yeasts were tested against the expected Poisson and normal distributions. The deviation from the Poisson distribution was significant at $P = 0.01$, but the fit to the normal distribution was good. This meant that normal curve methods of analysis could be used.

A square root transformation of the original counts of less than 50 organisms per plate was used in the main

study to improve the continuity of the data.

Main Study

Section I Four different treatments (media plus bacterial inhibitors) for determining the yeast and mould counts in butter were compared. Sixty-four butter samples obtained at random from commercial creameries were used. Five plates were prepared per treatment. The treatments were:

(a) Potato dextrose agar, plus acid (pH 3.5 ± 0.1)

(b) Malt agar, plus acid (pH 3.5 ± 0.1)

(c) Potato dextrose agar plus rose bengal (1:15,000) and streptomycin (30 γ per ml of medium)

(d) Malt agar plus rose bengal (1:15,000) and streptomycin (30 γ per ml of medium)

The analysis of variance of the counts for all samples combined indicated significant differences between media (main effects = treatment level A) at P = 0.05, and between bacterial inhibitors = additives (main effect = treatment level B) at P = 0.05.

However, the interaction media x additives (AB) was also significant at P = 0.01, and the interpretation that the significant differences between media and additives reflected qualities of the media or of the additives per se was suspect. For this reason the main effects A and B were tested against their respective interaction AB and

subsequently showed no significant differences.

A comparison between the 4 overall treatment means (L.S.D.) suggested there was no significant difference between treatments a, c and d, but all 3 were superior to treatment b.

The inferiority of treatment b may have been due to an unfavourable response by a greater portion of the species of yeasts and moulds to the malt agar and acid combination.

Section II Two treatments were compared using the same butter samples as in Section I. Four plates were prepared per treatment. The treatments were:

(a) Potato Dextrose Agar plus aureomycin (100 γ per ml of medium)

(b) Potato Dextrose Agar plus rose bengal (1:15000) and streptomycin (30 γ per ml of medium)

The aureomycin medium had a significantly higher overall mean count but the pattern was not similar for all the individual samples. Of the 64 samples tested, 38 showed higher mean counts on the medium to which aureomycin had been added, while for 26 the mean was higher on the agar which contained the rose bengal and streptomycin.

The lack of consistency in performance may have been a consequence of the small numbers in some samples, and/or it may also have been due to a fungi x medium interaction

which could be a reflection of differences in additives per se, or a reflection of differences in concentrations of additives. This interaction, however, could not be measured in this experiment.

The question of why one of a number of treatments (agars plus a bacterial inhibitor) supported higher counts of yeasts and moulds cannot be answered from the results obtained here, and must be determined from other, more specific studies. It is sufficient to suggest that all the treatments used did not support equal numbers of yeasts and moulds, and that all cultures did not react to the treatments in the same manner.

BIBLIOGRAPHY

AMERICAN DAIRY SCIENCE ASSOCIATION (1930). The Microbiological Analysis of Butter. (Committee Reports). J. Dairy Sci., 13, 394.

AMERICAN DAIRY SCIENCE ASSOCIATION (1933). The Microbiological Analysis of Butter. (Committee Reports). J. Dairy Sci., 16, 289.

AMERICAN PUBLIC HEALTH ASSOCIATION INC. (1953). Standard Methods for the Examination of Dairy Products. 10th ed., 790 Broadway, New York 19, N.Y.

ANTIBIOTICS: A Survey of their Properties and Uses (1952). The Pharmaceutical Press, 17 Bloomsbury Square, London, W.C.1.

BAKERSPIEGEL, A. and MILLER, A. J. (1953). Comparison of Oxgall, Crystal Violet, Streptomycin, and Penicillin as Bacterial Growth Inhibitors in Platings of Soil Fungi. Soil Sci., 76, 123.

BISBY, G. R., JAMIESON, M. C., and TIMONIN, M. (1933). The Fungi Found in Butter. Can. J. Research, 9, 92.

FISHER, R. A., THORNTON, H. G., and MACKENZIE, W. A. (1922). The Accuracy of the Plating Method of Estimating the Density of Bacterial Populations. Ann. Appl. Biol., 9, 325.

HESSELTINE, C. W. (1952). Isolation and Growth of Yeasts in the Presence of Aureomycin. J. Bact., 64, 55.

HOOD, E. G., and WHITE, A. H. (1928). Studies on Moulds and Yeasts in Creamery Butter. Dom. of Canada Pamphlet 92.

JAMES, N. (1959). Plate Counts of Bacteria and Fungi in a Saline Soil. Can. J. Microbiol., 5, 431.

JENSEN, H. S. (1934). Contribution to the Microbiology of Australian Soils. (Numbers of Micro-organisms in Soil and their Relationship to Certain External Factors). Proc. Linnean Soc., N. S. Wales, 59, 101.

LITTMAN, M. A. (1947). A Culture Medium for the Primary Isolation of Fungi. J. Science, 106, 109.

MARTIN, J. P. (1950). Use of Acid, Rose Bengal and Streptomycin in the Plate Method for Estimating Soil Fungi. Soil Sci., 69, 215.

MARTIN, J. P., and HARDING, R. B. (1950). Comparative Effects of Two Bacterial Growth Preventives, Acid (pH4) and Rose Bengal Plus Streptomycin on the Nature of Soil Fungi Developing on Dilution Plates. Soil Sci. Soc. Am. Proc., 15, 159.

MILLER, J. J., and WEBB, N. S. (1954). Isolation of Yeasts from Soil with the Aid of Acid, Rose Bengal and Oxgall. Soil Sci., 77, 197.

OLSON, H. C., and BONNER, M. D. (1957). Methods for Analysis of Cottage Cheese. Tech. Bul. T-71; Oklahoma State University.

PARFITT, E. H. (1933). The Influence of Media upon the Yeast and Mould Count in Butter. J. Dairy Sci., 16, 141.

SHADWICK, Jr., G. W. (1938). A Study of Comparative Methods and Media Used in Microbiological Examination of Creamery Butter. I. Yeast and Mould Counts. Food Research, 3, 287.

SMITH, N. R., and DAWSON, Virginia T. (1944). The Bacteriostatic Action of Rose Bengal in Media Used for Plate Counts of Soil Fungi. Soil Sci., 58, 467.

SNEDECOR, G. W. (1946). Statistical Methods Applied to Experiments in Agriculture and Biology. 4th ed., The Iowa State College Press, Ames, Iowa, U.S.A.

WAKSMAN, S. A. (1922). Microbiological Analysis of Soil as an Index of Soil Fertility: I. The Mathematical Interpretation of Numbers of Micro-organisms in the Soil. Soil Sci., 14, 81.

WHITE, A. H., and HOOD, E. G. (1931). Studies of Methods for Determining Numbers of Moulds and Yeasts in Butter: I. Relation of pH. J. Dairy Sci., 14, 463.

WILSON, P. W., and KULLMAN, E. D. (1931). A Statistical Enquiry into Methods for Estimating Numbers of Rhizobia. J. Bact., 22, 71.

APPENDIX I

Actual Counts of Yeasts and Moulds

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 1	(a)	875	550	660	800	515
	(b)	610	475	570	625	610
	(c)	200	365	195	250	250
	(d)	450	900	750	800	750
Sample No. 2	(a)	18	18	17	20	21
	(b)	11	20	24	26	27
	(c)	8	21	24	23	31
	(d)	45	46	50	50	64
Sample No. 3	(a)	5	2	2	5	2
	(b)	1	6	1	5	2
	(c)	13	6	8	10	4
	(d)	5	6	6	7	5
Sample No. 4	(a)	12	6	8	15	9
	(b)	7	11	12	8	12
	(c)	11	6	8	14	9
	(d)	13	11	7	7	11
Sample No. 5	(a)	21	13	13	17	16
	(b)	5	5	7	8	11
	(c)	14	13	16	16	21
	(d)	26	15	19	24	20

Legend: (a) Potato Dextrose Agar plus acid (pH 3.5 ± 0.1)
(b) Malt Agar plus acid (pH 3.5 ± 0.1)
(c) Potato Dextrose Agar plus rose bengal (1:15000) and streptomycin (30 γ per ml of medium)
(d) Malt Agar plus rose bengal (1:15000) and streptomycin (30 γ per ml of medium)

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 6	(a)	58	77	85	57	61
	(b)	18	15	11	23	21
	(c)	57	93	68	74	97
	(d)	60	72	65	53	54
Sample No. 7	(a)	26	31	29	29	30
	(b)	12	11	13	14	19
	(c)	25	18	35	33	36
	(d)	34	29	38	36	39
Sample No. 8	(a)	7	11	10	11	16
	(b)	7	5	17	6	11
	(c)	5	6	10	11	14
	(d)	12	11	8	8	18
Sample No. 9	(a)	20	20	40	37	26
	(b)	11	11	14	6	7
	(c)	36	34	33	49	34
	(d)	41	33	37	33	25
Sample No. 10	(a)	13	13	12	17	9
	(b)	17	17	16	16	17
	(c)	11	11	9	11	15
	(d)	12	15	12	13	17
Sample No. 11	(a)	110	131	104	90	109
	(b)	78	65	78	97	80
	(c)	97	118	95	125	109
	(d)	128	100	152	112	123
Sample No. 12	(a)	5	4	4	5	6
	(b)	3	8	6	7	9
	(c)	2	2	3	4	3
	(d)	7	6	8	6	7

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 13	(a)	2	2	3	6	3
	(b)	3	5	3	3	3
	(c)	1	3	2	1	3
	(d)	5	1	6	6	3
Sample No. 14	(a)	19	20	28	8	14
	(b)	18	22	20	13	15
	(c)	14	18	16	11	17
	(d)	18	26	22	20	21
Sample No. 15	(a)	12	12	10	13	13
	(b)	5	3	9	4	5
	(c)	12	15	19	25	12
	(d)	9	6	7	11	9
Sample No. 16	(a)	25	33	40	31	38
	(b)	7	10	4	13	7
	(c)	5	14	17	20	18
	(d)	13	18	8	17	11
Sample No. 17	(a)	13	9	15	25	14
	(b)	7	12	13	12	10
	(c)	12	6	22	20	15
	(d)	28	15	14	10	15
Sample No. 18	(a)	41	39	54	39	60
	(b)	26	14	18	19	14
	(c)	43	53	42	34	53
	(d)	33	42	36	27	30
Sample No. 19	(a)	120	97	124	109	98
	(b)	96	119	110	101	108
	(c)	100	100	113	101	105
	(d)	106	72	106	92	102

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 20	(a)	6	4	3	5	8
	(b)	8	4	5	5	4
	(c)	5	3	6	7	3
	(d)	6	6	7	4	9
Sample No. 21	(a)	7	7	6	7	7
	(b)	6	6	12	12	6
	(c)	13	10	12	10	14
	(d)	10	11	8	12	11
Sample No. 22	(a)	14	19	20	17	18
	(b)	10	11	11	14	11
	(c)	20	14	13	16	10
	(d)	17	15	24	26	15
Sample No. 23	(a)	6	4	3	3	6
	(b)	3	5	2	5	4
	(c)	2	3	2	4	7
	(d)	8	8	4	5	3
Sample No. 24	(a)	11	15	14	13	16
	(b)	5	11	5	7	9
	(c)	13	10	12	11	9
	(d)	12	9	10	10	9
Sample No. 25	(a)	1	4	6	5	3
	(b)	2	3	2	2	1
	(c)	3	10	8	6	5
	(d)	4	2	3	3	7
Sample No. 26	(a)	3	4	5	3	2
	(b)	4	1	6	5	4
	(c)	4	4	6	4	1
	(d)	7	6	6	4	4

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 27	(a)	25	20	24	17	22
	(b)	15	11	6	14	9
	(c)	11	17	10	9	7
	(d)	19	16	16	27	15
Sample No. 28	(a)	8	10	8	5	9
	(b)	4	3	3	1	3
	(c)	8	6	8	12	7
	(d)	9	7	8	9	7
Sample No. 29	(a)	6	2	6	6	6
	(b)	2	1	2	1	2
	(c)	4	3	4	2	5
	(d)	4	3	6	2	11
Sample No. 30	(a)	37	51	42	31	31
	(b)	91	17	22	11	12
	(c)	51	66	59	51	64
	(d)	34	33	41	36	47
Sample No. 31	(a)	59	49	57	65	54
	(b)	36	34	52	55	56
	(c)	56	52	44	51	61
	(d)	47	43	55	55	58
Sample No. 32	(a)	29	26	29	27	25
	(b)	20	22	28	25	17
	(c)	26	30	25	23	23
	(d)	24	23	23	24	31
Sample No. 33	(a)	33	29	37	31	28
	(b)	34	36	24	28	33
	(c)	27	31	30	41	29
	(d)	31	34	35	46	29

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 34	(a)	29	31	18	38	28
	(b)	29	24	27	27	27
	(c)	32	34	28	30	25
	(d)	36	35	36	24	38
Sample No. 35	(a)	1	1	5	2	1
	(b)	2	3	1	2	2
	(c)	2	1	1	1	1
	(d)	2	3	2	1	3
Sample No. 36	(a)	33	32	46	50	47
	(b)	46	43	43	57	53
	(c)	33	37	50	38	41
	(d)	39	44	57	59	49
Sample No. 37	(a)	11	17	9	15	13
	(b)	11	14	12	8	8
	(c)	14	10	8	21	6
	(d)	12	16	9	11	17
Sample No. 38	(a)	6	3	3	5	2
	(b)	7	5	4	5	4
	(c)	5	3	6	12	7
	(d)	3	3	9	3	5
Sample No. 39	(a)	5	7	7	6	8
	(b)	10	6	11	8	14
	(c)	2	7	7	8	10
	(d)	6	8	6	8	7
Sample No. 40	(a)	17	22	20	13	14
	(b)	16	18	17	10	18
	(c)	35	13	8	15	13
	(d)	12	21	17	12	11

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 41	(a)	117	118	119	113	130
	(b)	118	131	117	137	130
	(c)	122	98	116	117	117
	(d)	113	120	140	139	123
Sample No. 42	(a)	5	1	3	5	3
	(b)	4	4	6	2	4
	(c)	4	5	4	2	5
	(d)	2	2	3	4	2
Sample No. 43	(a)	24	27	23	22	31
	(b)	8	12	4	13	11
	(c)	3	13	14	11	17
	(d)	7	3	6	4	5
Sample No. 44	(a)	58	48	53	62	63
	(b)	48	31	59	40	47
	(c)	66	55	67	56	54
	(d)	59	57	46	44	54
Sample No. 45	(a)	34	47	56	61	48
	(b)	33	36	40	39	61
	(c)	49	55	58	62	60
	(d)	52	56	56	75	75
Sample No. 46	(a)	8	7	11	11	1
	(b)	1	4	3	3	2
	(c)	7	11	10	10	5
	(d)	5	11	12	12	12
Sample No. 47	(a)	193	163	190	196	196
	(b)	125	123	107	104	139
	(c)	202	195	232	219	215
	(d)	68	103	101	100	123

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 48	(a)	79	75	74	87	81
	(b)	72	80	78	94	93
	(c)	85	99	96	97	104
	(d)	97	90	104	87	91
Sample No. 49	(a)	10	10	8	12	11
	(b)	7	7	6	12	5
	(c)	12	8	17	7	10
	(d)	6	13	7	7	9
Sample No. 50	(a)	37	16	10	15	8
	(b)	10	6	20	11	9
	(c)	10	5	9	9	8
	(d)	15	21	7	13	17
Sample No. 51	(a)	28	48	61	45	44
	(b)	43	54	42	44	53
	(c)	120	137	133	151	122
	(d)	56	45	69	44	51
Sample No. 52	(a)	92	96	129	137	148
	(b)	43	52	41	50	51
	(c)	50	98	105	130	137
	(d)	49	61	47	45	33
Sample No. 53	(a)	60	53	33	28	34
	(b)	32	37	45	28	51
	(c)	65	47	62	47	47
	(d)	62	50	59	37	62
Sample No. 54	(a)	63	78	68	50	53
	(b)	62	47	55	52	51
	(c)	78	50	61	57	31
	(d)	38	41	54	51	62

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 55	(a)	43	49	57	71	62
	(b)	40	54	39	63	46
	(c)	49	50	41	52	55
	(d)	60	50	92	88	108
Sample No. 56	(a)	50	60	53	60	54
	(b)	50	46	61	49	60
	(c)	56	59	75	72	51
	(d)	66	85	71	56	75
Sample No. 57	(a)	6	4	3	3	3
	(b)	4	4	2	1	2
	(c)	4	2	1	7	7
	(d)	6	1	4	6	4
Sample No. 58	(a)	26	32	26	28	29
	(b)	24	24	21	33	25
	(c)	23	17	23	30	26
	(d)	24	18	22	22	23
Sample No. 59	(a)	33	55	49	44	40
	(b)	32	57	43	57	38
	(c)	46	39	45	50	46
	(d)	49	54	64	52	46
Sample No. 60	(a)	51	57	55	48	50
	(b)	57	56	74	69	60
	(c)	55	72	69	68	59
	(d)	79	66	81	86	71
Sample No. 61	(a)	89	64	68	53	93
	(b)	62	69	72	56	45
	(c)	74	66	40	49	43
	(d)	55	60	79	71	55

APPENDIX I - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Sample No. 62	(a)	55	50	69	46	56
	(b)	15	15	15	20	17
	(c)	58	69	75	73	63
	(d)	51	54	49	64	45
Sample No. 63	(a)	2	3	3	5	2
	(b)	2	2	2	1	2
	(c)	1	1	1	1	2
	(d)	5	2	7	8	3
Sample No. 64	(a)	18	18	17	20	21
	(b)	11	20	24	26	27
	(c)	8	21	24	23	31
	(d)	45	46	50	50	64

APPENDIX II

Actual Counts of Yeasts and Moulds

	Media	Plate 1	Plate 2	Plate 3	Plate 4
Sample No. 1	(a)	176	183	220	165
	(b)	300	400	385	550
Sample No. 2	(a)	8	7	18	5
	(b)	1	3	3	3
Sample No. 3	(a)	85	46	35	57
	(b)	64	38	36	28
Sample No. 4	(a)	4	2	1	3
	(b)	1	1	0	0
Sample No. 5	(a)	8	9	13	7
	(b)	5	9	8	5
Sample No. 6	(a)	400	130	105	110
	(b)	260	105	110	115
Sample No. 7	(a)	250	330	230	240
	(b)	350	330	225	340
Sample No. 8	(a)	300	310	240	245
	(b)	335	315	280	250

Legend: (a) Potato Dextrose Agar plus aureomycin (100 γ per ml of medium)
(b) Potato Dextrose Agar plus rose bengal (1:15000) and streptomycin (30 γ per ml of medium)

APPENDIX II - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4
Sample No. 9	(a)	11	4	3	2
	(b)	0	0	2	2
Sample No. 10	(a)	335	350	345	365
	(b)	425	455	365	385
Sample No. 11	(a)	253	150	154	150
	(b)	150	130	174	125
Sample No. 12	(a)	405	460	355	350
	(b)	420	515	440	400
Sample No. 13	(a)	54	50	52	56
	(b)	22	22	30	29
Sample No. 14	(a)	320	425	335	365
	(b)	390	480	465	375
Sample No. 15	(a)	38	73	45	56
	(b)	64	83	53	81
Sample No. 16	(a)	24	11	23	16
	(b)	22	14	17	16
Sample No. 17	(a)	214	159	190	210
	(b)	221	158	162	142
Sample No. 18	(a)	8	13	22	16
	(b)	6	13	11	5

APPENDIX II - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4
Sample No. 19	(a)	110	103	60	71
	(b)	76	66	97	78
Sample No. 20	(a)	64	40	26	21
	(b)	46	29	24	23
Sample No. 21	(a)	10	8	2	5
	(b)	7	9	1	3
Sample No. 22	(a)	15	21	33	39
	(b)	25	35	17	20
Sample No. 23	(a)	43	50	41	52
	(b)	68	50	59	60
Sample No. 24	(a)	19	8	5	7
	(b)	14	11	10	10
Sample No. 25	(a)	16	3	11	16
	(b)	8	6	20	6
Sample No. 26	(a)	183	180	132	123
	(b)	243	164	171	269
Sample No. 27	(a)	170	130	133	140
	(b)	129	114	115	109
Sample No. 28	(a)	27	43	10	6
	(b)	5	9	5	10

APPENDIX II - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4
Sample No. 29	(a)	62	51	46	41
	(b)	3	4	8	8
Sample No. 30	(a)	360	300	305	400
	(b)	395	340	375	340
Sample No. 31	(a)	10	17	23	20
	(b)	10	22	32	30
Sample No. 32	(a)	14	19	10	16
	(b)	17	26	27	12
Sample No. 33	(a)	15	9	15	9
	(b)	16	7	3	2
Sample No. 34	(a)	22	5	2	4
	(b)	10	4	4	3
Sample No. 35	(a)	27	40	33	32
	(b)	23	20	17	13
Sample No. 36	(a)	18	6	10	10
	(b)	4	7	6	7
Sample No. 37	(a)	123	53	46	86
	(b)	45	66	55	55
Sample No. 38	(a)	13	24	17	25
	(b)	2	2	9	1

APPENDIX II - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4
Sample No. 39	(a)	184	152	132	179
	(b)	86	104	112	106
Sample No. 40	(a)	275	370	235	275
	(b)	380	395	380	360
Sample No. 41	(a)	17	4	10	8
	(b)	9	7	12	2
Sample No. 42	(a)	98	86	93	87
	(b)	20	32	24	25
Sample No. 43	(a)	281	394	304	211
	(b)	299	376	254	402
Sample No. 44	(a)	30	31	28	37
	(b)	54	59	68	60
Sample No. 45	(a)	65	48	42	46
	(b)	16	5	27	21
Sample No. 46	(a)	3	3	3	3
	(b)	5	2	4	3
Sample No. 47	(a)	53	45	42	49
	(b)	46	40	42	39
Sample No. 48	(a)	489	517	423	690
	(b)	48	76	47	81

APPENDIX II - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4
Sample No. 49	(a)	49	58	48	49
	(b)	60	53	55	56
Sample No. 50	(a)	9	5	7	6
	(b)	1	3	1	1
Sample No. 51	(a)	5	7	13	2
	(b)	6	3	3	6
Sample No. 52	(a)	105	107	92	103
	(b)	122	100	83	75
Sample No. 53	(a)	230	310	255	210
	(b)	235	290	200	310
Sample No. 54	(a)	90	85	110	70
	(b)	90	120	105	135
Sample No. 55	(a)	350	425	320	350
	(b)	410	400	350	345
Sample No. 56	(a)	465	460	355	375
	(b)	320	490	355	365
Sample No. 57	(a)	14	13	13	18
	(b)	11	15	9	8
Sample No. 58	(a)	127	105	139	146
	(b)	135	129	126	140

APPENDIX II - Continued

	Media	Plate 1	Plate 2	Plate 3	Plate 4
Sample No. 59	(a)	2	1	3	1
	(b)	4	4	4	2
Sample No. 60	(a)	201	224	213	226
	(b)	161	241	215	262
Sample No. 61	(a)	43	39	51	33
	(b)	5	7	7	8
Sample No. 62	(a)	12	5	15	13
	(b)	2	2	5	2
Sample No. 63	(a)	93	91	88	72
	(b)	106	121	109	135
Sample No. 64	(a)	36	49	42	48
	(b)	32	48	54	47