Number of Colonies for a Satisfactory Soil Plate.

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The uniformity between the number of colonies developing on petri plates carrying equal sized aliquots has been used as the basis for ascertaining the number of colonies satisfactory for one plate. Prucha[1] said in 1916: "Further study is needed to give sufficient basis for drawing definite conclusions, but the results so far point to the conclusion that the average of three plates from the same dilution approaches, reasonably closely, to the average of a hundred plates made from the same dilution, when that average is between one and two hundred colonies per plate."

The following points have served as the basis for determining the number of colonies satisfactory for a soil plate: Soil may be a medium for the growth of all kinds of micro-organisms; the rate at which different bacteria multiply varies considerably, and the antagonisms between organisms are affected by media, etc.

The plan for determining the number of colonies for a satisfactory soil plate was: First, to make many dilutions and platings of a prepared soil and study the numbers of colonies developing in three, seven and ten days incubation. Second, to compare the number of colonies developing from the different dilutions for evidence that plates from the higher bacterial dilution carried one-tenth the number of colonies of the lower dilution when the lower dilution did not give above the maximum number of bacteria that could be developed into colonies on the plates. Third, to give confirmation of the conclusions reached by routine laboratory data.

Unpublished results (obtained in this laboratory) show rather conclusively that practically all micro-organisms can be grown on a simple media. Differences in growth, in addition to being due to the virulence of the organisms and their natural characteristics, result from the media becoming unfavorable for growth, due to the presence of acid or basic reacting substances and specific end products of bacterial metabolisms. The importance of the proper conditions of aeration cannot be overemphasized. It has been noted that duplicate plates from pure cultures often agree well when even more than two hundred colonies are present per plate. Each organism in a pure culture multiplies under similar conditions and unfavorable media and end products stop rate and extent

[1] Prucha, M. J. Journal of Bacteriology, Vol. 1, No. 1, p. 92.

Fig 1. Many colonies but all slow growing.
(Good plate.)

Fig. 2. Many colonies of many characters.
(Doubtful plate.)

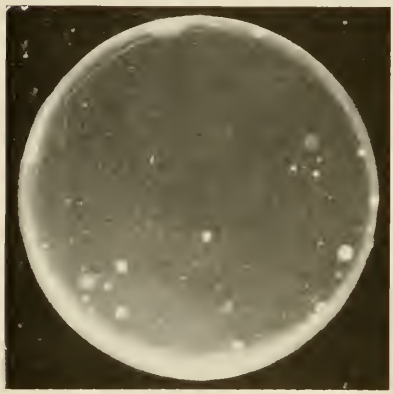


Fig. 3. Poor distribution on plate.
(Unsatisfactory plate.)

Fig. 4 Few colonies rapid growing and poorly distributed.
(Unsatisfactory plate.)

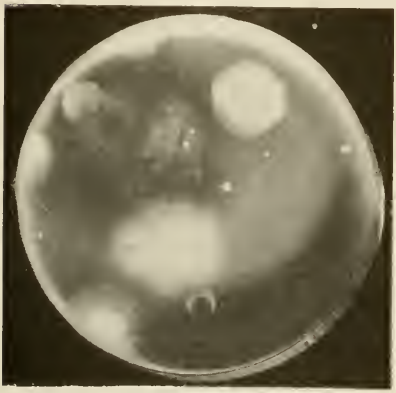


Fig. 5. Few rapidly growing and well distributed colonies.
(Good plate.)

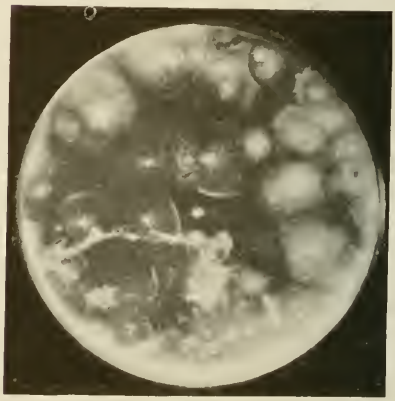


Fig. 6. Rapidly growing colonies.
(Doubtful plate.)

Plate I.

of growth rather than stop the growth of any individual organism. With mixed cultures the media may be suitable for the growth of all the organisms present, but the differences in rate of growth and specific end products cause uneven plates. (See Plate I.)

The literature does not furnish figures on duplicate and triplicate platings where the bacterial dilutions were made from large aliquots (10 cc. or more). In milk it has been noted that platings giving as low as forty colonies are satisfactory.[2] The soil is so much more ununiform than milk that the technic worked out at this station,[3] and depending on large aliquots for diluting and plating, was followed.

Experimental Work.

A black sandy soil was air dried and sieved to unify both the soil and its flora. Triplicate platings were made from 1–40, 1–400, 1–4,000, 1–40,000 and 1–400,000 bacterial dilutions. Counts were made after three, seven and ten days' incubation at 20° Centigrade. Especial care was taken in handling the plates to prevent contaminations. The check plates were in most cases entirely free from bacterial growth and their average has been deducted from the figures given. The results are given in Table I.

[2] Conn, H. W. Public Health Reports. U. S. Public Health Service, Vol. 30, No. 33, August, 1915.

[3] Noyes, H. A., and Voigt, Edwin, in Proceedings of Indiana Academy of Science, 1916, pp. 272-301.

TABLE I.

Decrease in Numbers of Bacterial Colonies on Plates with Increasing Length of Time of Incubation.

No. of Sets of 3 Plates Compared.			Average Counts		
			3 days	7 days	10 days
1.	Colony numbers between	2,900 and 3,000	2,905	2,045	1,689
1.	Colony numbers between	2,000 and 2,100	2,030	1,790	1,610
2.	Colony numbers between	1,700 and 1,800	1,750	1,570	1,003
1.	Colony numbers between	1,600 and 1,700	1,600	1,050	860
1.	Colony numbers between	1,500 and 1,600	1,590	1,400	960
4.	Colony numbers between	1,400 and 1,500	1,456	1,256	1,021
1.	Colony numbers between	1,300 and 1,400	1,380	1,150	840
1.	Colony numbers between	1,200 and 1,300	1,240	960	820
2.	Colony numbers between	1,100 and 1,200	1,135	918	830
1.	Colony numbers between	1,000 and 1,100	1,070	1,010	940
1.	Colony numbers between	900 and 1,000	910	840	825
3.	Colony numbers between	800 and 900	852	778	677
6.	Colony numbers between	600 and 700	659	582	488
4.	Colony numbers between	500 and 600	541	480	440
6.	Colony numbers between	400 and 500	450	409	387
21.	Colony numbers between	300 and 400	340	327	308
11.	Colony numbers between	250 and 300	279	288	275
11.	Colony numbers between	200 and 250	222	220	213
2.	Colony numbers between	175 and 200	195	200	200
3.	Colony numbers between	150 and 175	163	168	172
3.	Colony numbers between	125 and 150	129	136	136
5.	Colony numbers between	100 and 125	115	125	128
5.	Colony numbers between	90 and 100	95	108	109
8.	Colony numbers between	80 and 90	83	91	92
5.	Colony numbers between	70 and 80	72	80	85
4.	Colony numbers between	60 and 70	65	71	79
3.	Colony numbers between	50 and 60	54	57	57
7.	Colony numbers between	40 and 50	43	53	55
7.	Colony numbers between	30 and 40	35	44	46
9.	Colony numbers between	20 and 30	22	30	33
12.	Colony numbers between	10 and 20	11	19	24
6.	Colony numbers between	0 and 10	7	12	15

The table shows the following:

1. Increases in counts resulted from additional incubations when less than 200 colonies were present after three days' incubation.
2. Whether the counts increased or decreased, the counts after seven days' incubation fall between the three- and ten-day counts.
3. Two hundred or more colonies gave unreliable results.
4. The optimum number of colonies is probably much nearer 100 than 200 per plate.

The ratios between the number of colonies developing after ten days' incubation of the 1–40,000 and 1–400,000 bacterial dilutions of soils taken at different times from differently cropped areas are given in Table II. In carrying out the dilutings and platings the lower dilutions were made and plated before the higher dilutions were prepared, since it is believed that multiplications of the organisms have little effect on the higher dilutions under these conditions.

TABLE II.

Colony Counts on Plates of Different Bacterial Dilutions Cover Crop Investigations.

Plot Supporting	Average of Triplicate Plates.	
	Dilution 1-40,000	Dilution 1-400,000
Nothing		
Nov. 14, 1914	69.7 colonies	8.3* colonies
Feb. 6, 1915	73.0 "	11.6 "
Mar. 2, 1915	107.2 "	10.7* "
Mar. 27, 1915	62.0 "	7.7* "
April 15, 1915	94.0 "	6.0 "
Millett		
November 14, 1914	99.3 "	11.3* "
February 6, 1915	92.0 "	18.6 "
March 2, 1915	172.7 "	22.7 "
March 27, 1915	88.3 "	11.4 "
April 15, 1915	193.3 "	27.0 "
Soy Beans		
November 14, 1914	75.3 "	10.3 "
February 6, 1915	125.3 "	26.3 "
March 2, 1915	165.7 "	17.0* "
March 27, 1915	112.0 "	12.0* "
April 15, 1915	80.0 "	10.7 "
Nothing		
November 14, 1914	62.3 "	8.3 "
February 6, 1915	83.3 "	10.6 "
March 2, 1915	82.0 "	19.3 "
March 27, 1915	113.3 "	11.0* "
April 15, 1915	145.0 "	12.7 "
Hairy Vetch		
November 14, 1914	109.0 "	8.7 "
February 6, 1915	163.0 "	20.6 "
March 2, 1915	225.3 "	36.7 "
March 27, 1915	175.0 "	16.0* "
April 15, 1915	185.7 "	29.3 "
Winter Rye (Sown early)		
November 14, 1914	122.0 "	8.7 "
February 6, 1915	226.0 "	31.6 "
March 2, 1915	245.5 "	13.7 "
March 27, 1915	114.3 "	16.0 "
April 15, 1915	200.0 "	27.6 "
Nothing		
November 14, 1914	65.3 "	9.7 "
February 6, 1915	129.7 "	22.0 "
March 2, 1915	173.7 "	13.3 "
March 27, 1915	72.3 "	6.7* "
April 15, 1915	110.3 "	15.0 "
Winter Rye (Sown late)		
November 14, 1914	70.0 "	7.0* "
February 6, 1915	68.7 "	14.0 "
March 2, 1915	145.0 "	16.3 "
March 27, 1915	127.0 "	15.7 "
April 15, 1915	167.0 "	27.3 "
Crimson Clover		
November 14, 1914	44.7 "	3.3* "
February 6, 1915	91.0 "	15.6 "
March 2, 1915	254.3 "	23.3 "
March 27, 1915	46.0 "	6.7 "
April 15, 1915	122.2 "	16.7 "

TABLE II—Continued.

Colony Counts on Plates of Different Bacterial Dilutions Cover Crop Investigations.

Plot Supporting	Average of Triplicate Plates.	
	Dilution 1-40,000	Dilution 1-400,000
Nothing		
November 14, 1914	62.0 "	6.3* "
February 6, 1915	93.0 "	17.3 "
March 2, 1915	181.0 "	13.3 "
March 27, 1915	43.0 "	3.0* "
April 15, 1915	96.0 "	9.7 "
Buckwheat		
November 14, 1914	50.0 "	10.3 "
February 6, 1915	121.3 "	21.3 "
March 2, 1915	163.0 "	15.0* "
March 27, 1915	86.0 "	6.4 "
April 15, 1915	119.7 "	14.0 "
Natural Growth of Weeds		
November 14, 1914	54.7 "	5.7* "
February 6, 1915	100.3 "	23.3 "
March 2, 1915	152.5 "	33.6 "
March 27, 1915	82.3 "	11.0 "
April 15, 1915	108.0 "	13.7 "
Average of all 60 comparisons	117.15 "	14.8 "
Average of 15 comparisons*	94.4 "	9.4 "

These results show:

1. The ratio between the number of colonies on plates from the 1–40,000 and 1–400,000 bacterial dilutions is dependent on the number of organisms present rather than on the cropping system or the time of the year the soil samples were taken.
2. The averages show:
   (*a*) That the average of all comparisons for the 1–40,000 bacterial dilutions was too great for satisfactory plates.
   (*b*) That the results from the two dilutions tend to check when the number of colonies on the plates from the 1–40,000 bacterial dilutions is under 100.

Table III has been compiled to show the ratios between the counts of the two dilutions when the number of colonies developing on the 1–40,000 bacterial dilution is under 100. These results are the cases where the counts from twenty-four soil samples averaged under 100 on the 1–40,000 bacterial dilution.

TABLE III.

Colonies on 1-40,000 and 1-400,000 Bacterial Dilutions.
(Counts on 1-40,000 Bacterial Dilutions between 47 and 100.)

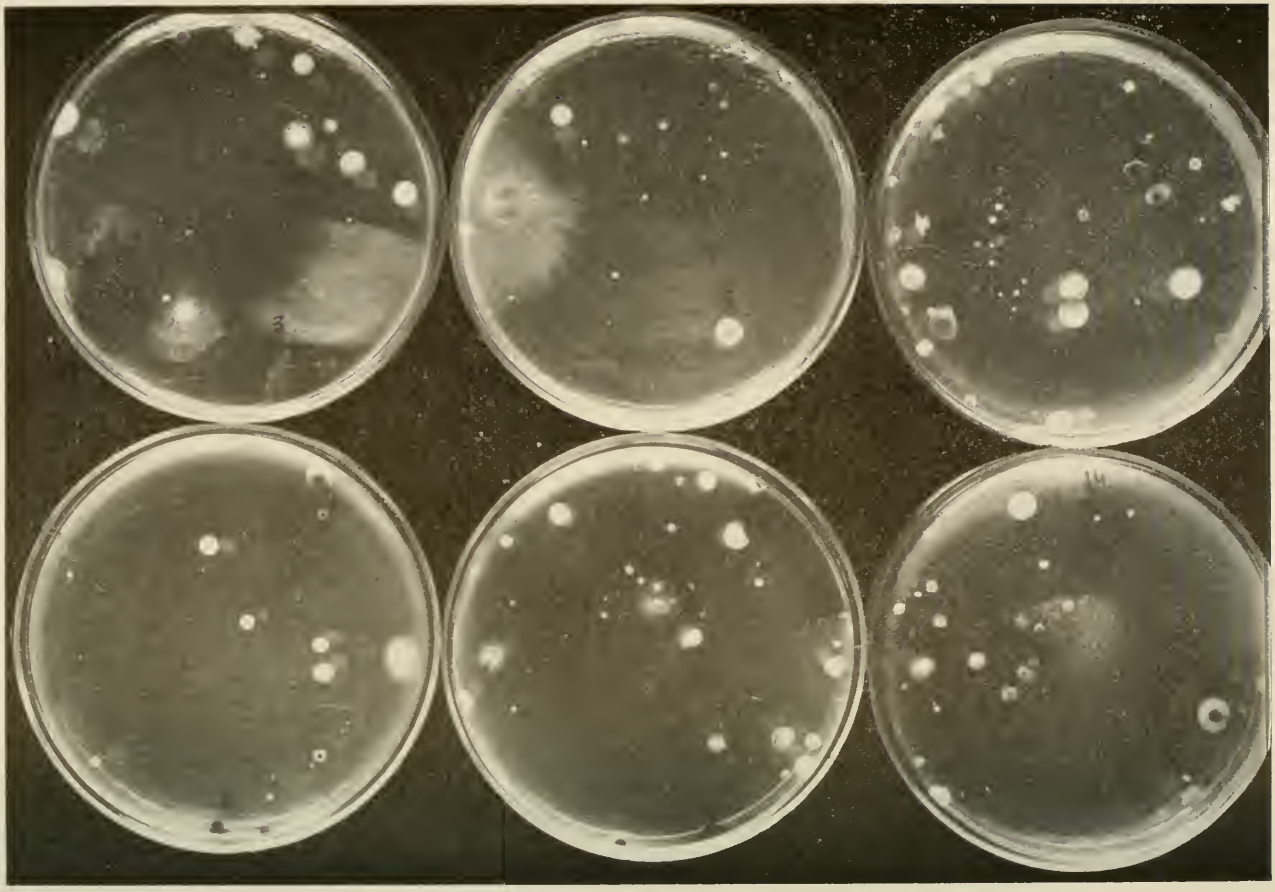
DILUTIONS	
1–40,000**	1–400,000
94.5	12.0
91.7	12.3
88.0	13.5
88.0	11.0
86.3	9.0*
80.3	7.8*
74.0	8.0*
65.3	8.0*
65.0	5.3*
56.0	7.0*
51.0	4.0*
48.0	5.0*
47.0	5.3*
Average all 71.9	8.3
Average * 63.6	6.6

**Counts are averages of triplicate plates.

*These numbers multiplied by 10 are within 15 of the numbers obtained in the lower bacterial dilution.

Table III brings out that, while 100 colonies per plate are quite satisfactory, the 10 to 1 ratio is more nearly approximated when much less than 100 colonies were present per plate.

To further substantiate the evidence that results are reliable when relatively small numbers of colonies are present per plate, the ten-day counts from the 1–40,000 and 1–400,000 bacterial dilutions of a sandy soil, low in organic matter, are given in Table IV.



Pla'e II. Good Petri Plates.

TABLE IV.

Colonies on 1-40,000 and 1-400,000 Bacterial Dilutions.
(Colonies on 1-40,000 Dilutions number under 30.)

DILUTIONS	
1-40,000	1-400,000
29.0	3.3* **
26.7	2.3* **
26.0	3.0* **
25.3	3.0* **
24.7	2.0* **
23.0	3.0* **
22.3	2.1* **
20.7	3.3* **
20.7	2.0* **
20.3	3.0*
20.0	3.0*
19.0	3.0*
17.7	2.0* **
17.3	1.7* **
16.0	3.0*
16.0	2.3* **
13.0	2.0* **
11.7	2.0*
11.3	3.3*
11.0	1.0* **
10.0	2.0*
Average of all 19 1	2.5
Average of * 19.5	2.5
Average of * ** 21.0	2.2

All figures are averages of triplicate plates.

*Counts for 1-400,000 dilution are within 1.5 colonies of 0.1 of number on 1-40,000 dilution.

* **Counts for 1-400,000 dilution are within 0.7 colonies of 0.1 number on 1 40,000 dilution.

Summary.

1. These and other tests (of which these are representative) have shown that thirty is near the optimum number of colonies for a petri plate 100 mm. in diameter. Plate II.

2. The averages of a sufficient number of plates carrying between 10 and 100 colonies are satisfactory for computing bacterial numbers.