ARE ALL THE SOIL BACTERIA AND STREPTOTHRICES THAT DEVELOP ON DEXTROSE AGAR AZOFIERS?

By

PAUL EMERSON *Research Fellow in Soil Bacteriology, Iowa State Agricultural College*

It has been noticed many times that there are a large number of organisms other than *Azotobacter* which develop on nitrogen-free dextrose agar plates, when these plates are made directly from a soil suspension. Apparently, no one has made any attempt to determine the nature of these organisms, and the questions arise: Are they all azofiers or nitrogen fixers, and if so, to what class do they belong? If they are not azofiers, where do they get their supply of nitrogen?

In order to answer these questions, the following experiment was carried out. One hundred gm. of soil² were placed in 200 c.c. of sterile water, thoroughly shaken for 5 minutes, dilutions of 1 to 1000, 1 to 10,000 and 1 to 20,000 made, and 12 dextrose agar plates prepared by the usual method. The following modification of the mannite medium recommended by J. G. Lipman (3) was used:

Distilled water	1000 c.c.
Agar agar	12 gm.
Dextrose	15 gm.
K ₂ HPO ₄	0.2 gm.
MgSO ₄	0.2 gm.
CaCl ₂	0.02 gm.
10% Fe ₂ Cl ₆ solution.....	1 drop.

The solution was made neutral to phenolphthalein by the use of 10 per cent NaOH before the addition of the agar. The plates were incubated for 4 days at room temperature, the colonies counted and the plate that contained approximately the correct average number of colonies was set aside for further study. The count showed that the soil contained per gram of dry soil, 2,400,000 bacteria that were able to develop on nitrogen-free medium.

---

¹ Received for publication January 13, 1917.

² Miami silt loam.

The area of the selected plate was then divided into 4 equal parts, each part containing the same number of colonies. Each of these areas was considered to contain a fair representation of all the bacteria in the original soil that were able to develop on this medium. All the bacterial colonies in one of these areas were picked out and transferred to slants of the same medium. In this manner 30 colonies were separated, replated to insure their purity and their azofying power determined both in soils and in solutions. In the purification of these organisms it was found that 3 colonies developed two distinct organisms. These were isolated and purified and their azofying power determined with the others. The total number of organisms studied was therefore thirty-three.

No attempt was made to classify these organisms further than to divide them roughly into 4 groups, each having distinct cultural characteristics that were easily recognized. The grouping and the number of organisms in each group were as follows:

Group 1-- <i>Nocardia</i> "Actinomyces"	10 colonies.
Group 2--Small jelly-like colonies resembling <i>Bacillus radiculicola</i>	10 colonies.
Group 3--Flat, thin, fluorescent colonies	7 colonies.
Group 4--Thick, dense, white shiny colonies, opalescent, raised in center and growing somewhat in concentric rings	6 colonies.

The soil medium for the azofication tests was prepared as follows. One hundred gm. of air-dried sieved soil of the same type as that from which the organisms had been isolated were placed in tumblers, covered with a thick layer of cotton securely fastened with a cord, and sterilized in the autoclave for 4 hours at 15 pounds pressure. The sterilization was repeated 3 times, 1 day being allowed to elapse between operations. Thorough sterilization of the soil was proven by careful plate tests.

The solution used in the azofication tests was the same as that employed in the isolation work, except that 10 gm. of dextrose were used instead of 15. Fifty c.c. of this solution were placed in flasks and sterilized in the usual manner.

The inoculum was prepared by inoculating the organism into tubes containing 5 c.c. of the above solution. After incubating 3 days, sufficient growth appeared to insure good inoculation which was accomplished by flaming the ends of the tubes and the plugs, and pouring the entire contents of the tube into the medium to be inoculated. This operation took place under an inoculating hood in order to avoid any contamination. In the case of the soil tests no dextrose was added other than that in the 5 c.c. of inoculum, it being assumed that the complete sterilization by heat had rendered soluble sufficient nutrient to make the addition of any carbon supply unnecessary; however, sufficient sterile water to make the content up to the optimum, 22 per cent, was added immediately after the addition of

TABLE I
DETERMINATION OF NITROGEN FIXATION

NOCARDIA

Lab. No.	Solution			Soil				
	Mg. N Found	Blank	Mg. N Fixed	Mg. N Found	Duplicate Mg. N Found	Average Mg. N	Check Mg. N	Mg. N Fixed
1	2.66	0.56	2.10	309.4	315.0	312.2	298.2	14.0
3	5.74	0.56	5.18	316.4	319.2	317.8	298.2	19.6
4	3.22	0.56	2.66	313.6	308.0	310.8	298.2	12.6
8	.....	.....	Lost	298.7	303.8	301.2	298.2	3.0
8X	7.56	0.56	7.00	.....	.....	.....	.....	Lost
9	3.64	0.56	3.08	316.4	310.8	313.6	298.2	15.4
10	0.42	0.56	0.00	322.0	322.7	322.3	298.2	24.1
13	4.62	0.56	4.06	309.4	306.6	308.0	298.2	9.8
26	2.66	0.56	2.10	318.5	315.7	317.1	298.2	18.9
27	2.24	0.56	1.68	312.9	323.4	318.1	298.2	19.9

RADICICOLA-LIKE FORMS

2	3.92	0.56	3.36	310.8	315.7	313.2	298.2	15.0
11	1.12	0.56	0.56	311.5	312.7	312.1	298.2	13.9
12	1.26	0.56	0.70	314.3	312.7	313.5	298.2	15.3
17	0.42	0.56	0.00	285.6	294.7	290.1	298.2	0.0
20	2.10	0.56	1.54	309.4	310.8	310.1	298.2	11.9
21	1.82	0.56	1.26	317.8	317.8	317.8	298.2	19.6
21X	0.98	0.56	0.42	320.6	322.0	321.3	298.2	23.1
28X	1.12	0.56	0.56	316.4	315.0	315.7	298.2	17.5
22	0.56	0.56	0.00	312.9	303.8	308.3	298.2	10.1
30	1.26	0.56	0.70	305.2	309.4	307.3	298.2	9.1

FLUORESCENT

15	4.34	0.56	3.78	305.2	306.6	305.9	298.2	7.7
16	0.84	0.56	0.28	303.1	308.0	305.6	298.2	7.4
19	0.98	0.56	0.42	307.3	310.1	308.7	298.2	10.5
23	0.00	0.56	0.00	317.8	319.2	318.5	298.2	20.3
24	0.98	0.56	0.42	311.5	312.9	312.2	298.2	14.0
25	1.26	0.56	0.70	315.0	305.2	310.1	298.2	11.9
28	3.64	0.56	3.08	322.7	324.1	323.4	298.2	25.2

OPALESCENT

5	2.66	0.56	2.10	319.2	317.8	318.5	298.2	20.3
6	0.98	0.56	0.42	317.8	326.2	322.0	298.2	23.8
7	1.12	0.56	0.56	302.4	302.4	302.4	298.2	4.2
14	5.18	0.56	4.62	310.8	318.5	314.7	298.2	16.5
18	1.26	0.56	0.70	310.1	314.3	312.2	298.2	14.0
29	5.88	0.56	5.32	304.3	308.0	306.1	298.2	7.9

the inoculum. This optimum water content was maintained during the incubation period by bringing it up to weight at regular intervals with sterile water. The soils and the solutions were all incubated for 3 weeks at room temperature, and the nitrogen content determined at the end of that period by Kjeldahling. The only variation in the determination from the regular routine of the Kjeldahl determination was that instead of distilling, the digested material was aerated following the Potter and Snyder (4) modification of the method suggested by Kober and Graves (2). Tables I and II show the results of these determinations.

TABLE II
SUMMARY OF TABLE I

	Average Mg. N Fixed	
	Solution	Soils
Nocardia	3.095	15.255
Radicicola-like forms	0.910	13.550
Fluorescent	1.240	13.855
Opalescent	2.286	14.450

It was surprising to find the organisms in the *Nocardia* group developing so abundantly on this medium, but Table II shows that not only are they azofiers, but that they are able to fix on the average a higher amount of nitrogen than the other soil organisms. So little work has been done on this group of organisms by soil investigators that the author was unable to find any definite data on their azofying power. Beijerinck (1) worked with one member of the group, *Streptothrix chromogena*, and came to the conclusion that it was not able to fix atmospheric nitrogen but that it lived on the small amount of nitrogen contained in the medium. A number of investigators have shown the large percentage of these organisms in the soil, and a few have studied their activities. This point is fully discussed in another paper. These results indicate that this group of soil organisms may be quite important in increasing the nitrogen content of soils.

It will be noticed that there is but one organism, No. 17, that did not fix any nitrogen in either soil or solution, and only four, Nos. 10, 17, 22 and 23, that did not fix any in solution. Of these four, three fixed appreciable amounts in the soil. It may be assumed, therefore, that in the light of the above results, 97 per cent of these soil organisms developing on dextrose agar plates were azofying bacteria. What they are, and what their activities are, compared with the known activities of the *Azotobacter* and the *Radicicola* groups, are questions that should be of great importance to the soil bacteriologist. The results become of greater interest when it is realized that no *Azotobacter* were present, showing clearly that there are a large number of organisms in the soil other than

Azotobacter and *Radicalicola* which have the power of fixing atmospheric nitrogen. Apparently, nothing is known at present of the activities of these organisms, but is it not possible that their presence would explain the nitrogen increase usually observed in fallow soils? Numerous instances are on record where authors have reported large bacterial counts on nitrogen-free media, showing that the distribution of these or similar organisms is quite general. It appears, therefore, in the light of the above investigations that if general farming practices favorable to the development of these organisms were followed, the problem of keeping up the nitrogen content of cultivated soils might be much simplified. The importance of further study along this line is apparent.

LITERATURE CITED

- (1) BEIJERINCK, M. W.
1901. Ueber Chinonbildung durch *Streptothrix chromogena* und Lebensweise dieses Mikroben. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 6, p. 2.
- (2) KOBER, P. A., and GRAVES, S. S.
1915. Quantitative ammonia distillation by aeration for Kjeldahl, urea, and other estimations. *In* Jour. Amer. Chem. Soc., v. 35, p. 1594.
- (3) LIPMAN, J. G.
1904. Soil bacteriological studies. *In* N. J. Agr. Exp. Sta. 25th Ann Rpt. p. 263.
- (4) POTTER, R. S., and SNYDER, R. S.
1914. The determination of ammonia in soils. Iowa Agr. Exp. Sta. Research Bul. 17, p. 12.