Investigation on Winogradsky's Azotobacter Test as to its Applicability to some Rice-field Soils in Japan.

By

Arao Itano and Satiyo Arakawa.

[May 5, 1930.]

In soil microbiology, the attempts have been made to analyse the soil as to its fertility by means of the microbiological methods. It has been known that an intimate relation exists between the soil fertility and the amount of mannite decomposed as well as the amount of nitrogen fixed since LÖHNIS and PILLAI[1], and others demonstrated it experimentally. However there is no experimental report on this subject in this country.

With an aid of the spontaneous culture methods which have been developed by CONN[2] on the direct microscopic count of soil bacteria and WINOGRADSKY[3] on the silica-gel plate culture method for cultivation of Azotobacter, it has been tried to determine the fertility of soil.

This investigation was undertaken to ascertain the applicability of the WINOGRADSKY's azotobacter test to the soils from the rice-field.

Experimental.

I. Soil samples:

Nine different soils from the rice-field of known productivity and which were collected from different locality in Prefecture of Okayama and Hiroshima. The Soils were air dried first and separated by sifting, and the particles less than 0.5 mm. were used in the test. The description of soil samples is given in Table I.

1) LÖHNIS, F. and PILLAI, N. K., 1908, Centbl. f. Bakt. II, 20, S. 781;
BROWN, P. E., 1916, J. Agr. Research, 5, 855;
BURGESS, P. S., 1918, Soil Science, 6, 449;
BEIJERINCK, M. W., 1921, Akad. Wettenschapp. Amsterdam, 30, 431;
CHRITENSEN, H. R., 1923, Soil Science, 15, 329;
WAKSMAN S. A. and KARUNAKER, P. D., 1924, Soil Science, 17, 373.

2) CONN, J. H., 1918, N. Y. (Geneva) Agr. Expt. Station Technical Bull., No. 64.

3) WINOGADSKY, S., 1926, Compt. Rend. Acad. Sci. (Paris) 182, 1061.

Table I.

Description of Soil Samples.

No. of Soils.	% productivity.	p_H	Locality.
1.	81.25	6.23	Ōhara Institute.
2.	62.50	6.09	〃 〃 .
3.	56.25	5.83	Hiroshima-Ken.
4.	62.50	6.61	〃 .
5.	56.25	6.84	〃 .
6.	40.63	5.54	〃 .
7.	100.00	5.19	Okayama-Ken.
8.	50.00	5.48	〃 .
9.	100.00	4.76	〃 .

II. Methods of procedure:

The following six tests were used in the investigation:

Test I. Hundred of the soil particles were planted on the silica-gel medium (WAKSMAN, S. A. and CAREY, C., 1926, J. Bact., V. 12, 87.) in Petrie dish of 9 cm. diameter; incubated at 30°C. for 48 hours. With the fertile soil, fifty or more colonies of Azotobacter should appear at the end of the incubation period while only a few colonies appear with the ordinary soil after four or five days and no colony appears with the poor soil.

Test II. 0.5 g. of mannite is added to 50 g. of soil and made into paste with distilled water; incubated at 30°C. for 48 hours; then microscopically count the number of Azotobacter colonies. The fertility is judged by the quantity of growth.

Test III. 100 parts of starch is mixed with 5 parts of soil and made into a paste form with distilled water and the surface is smoothened up; incubated at 30°C. for 48 hours. Many colonies are expected to grow in case of the fertile soil.

Test IV. One gram of soil is scattered over the silica-gel medium in Petrie dish of 20 cm. in diameter; incubated at 30°C., for 48 hours. 2,500 to 3,000 Azotobacter colonies should appear with the fertile soil; less with an average soil and none with soil.

Test V. The plate culture which was prepared in Test IV and kept for 120 hours, was taken and the total nitrogen was determined by KJELDAHL method and calculated on basis of two grams of mannite. That is, if 20 mg. nitrogen fixed by using 2 g. of mannite, it is taken as the standard; or mannite 100 : nitrogen 1 or Organic carbon 40 : nitrogen 1. With an average soil, a small amount and with a poor soil, only a trace of nitrogen is fixed.

Test VI. The amount of nitrogen fixed in the liquid culture medium, inoculated with the soil sample, as usual, was determined.

Results.

The results of Test I are shown in Table II.

Table II.

Number of Azotobacter Colonies on Plate.

No. of Soils.	Number of soil particles planted.	Hours of incubation.		Total number of colonies.
		48	120	
		No. of colonies.	No. of colonies.	
1.	100.	30*	60	90
2.	〃	22	50	72
3.	〃	—	—	—
4.	〃	89	9	98
5.	〃	100	—	100
6.	〃	—	—	—
7.	〃	—	—	—
8.	〃	—	—	—
9.	〃	—	—	—

Notes : (—) no colony; * average of two to three plates.

Table II indicates that soils No. 1, 2, 4 and 5 formed the colonies, but other six did not form any even after 120 hours of incubation. Soils 4 and 5 are fertile and 1 and 2 are of medium fertility. Further the photograph of plates are shown in Plate XXX.

The results of Test II were not satisfactory to judge the fertility although some Azotobacter colonies were formed.

In Test III, no Azotobacter colony appeared at all in any of the soils tested.

The results of Test IV and V were not distinct owing to the fact that so much water condensation over the surface of medium took place everytime, in course of incubation, that prevented from getting definite results. Consequently no count of colony was obtained, and only the estimate is given in Table III.

Table III.

Growth of Azotobacter in Test IV.

No. of Soils.	Growth of Azotobacter.	
	48 hours.	120 hours.
1.	++	+++
2.	+	+++
3.	±	+
4.	++	+++
5.	++	++++
6.	−	+
7.	−	±
8.	−	±
9.	−	±

Notes: (−) no growth; (±) indefinite; (+) growth; (++) more growth; (+++) much growth; (++++) abundant.

Table III indicates that somewhat similar results to those of Test II were obtained. Further Plate XXXI shows the photograph of the plates.

The results of Tests V and VI are given in Table IV.

Table IV.

Total Nitrogen fixed in Tests V. and VI.

No. of Soils.	Tests.		Turbidity.
	V.	VI.	
	mg. N.	mg. N.	
1.	20.70	8.30	++
2.	17.93	5.91	++
3.	3.19	1.38	+
4.	18.04	12.60	+++
5.	19.95	8.78	+++
6.	2.01	1.34	+
7.	2.02	1.33	+
8.	3.50	3.44	+
9.	1.92	1.35	+

Notes: (+) turbid; (++) marked; (+++) strong.

Among the results obtained in Tests V and VI, there is parallelism as to the amount of nitrogen fixed but in Test V much more nitrogen fixed than

in Test VI. Examining the results of Test VI, in conjunction with the P_H value of soils, it is interesting to note that large quantity of nitrogen fixed in those soils of which P_H values larger than 6.0 and very small where $P_H < 6.0$.

Summary and Conclusions.

The results obtained may be summarized as in Table V:

Table V.

Summary of the Results.

Soils.			Tests.						
No.	% productivity.	P_H	1	2	3	4		5	6
			No. of colony.			48 hrs.	120 hrs.	mg. N.	mg. N.
1.	81.25	6.23	90	×	×	++	++++	20.70	8.30
2.	62.50	6.09	72	×	×	+	+++	17.93	5.91
3.	56.25	5.83	—	×	×	++	+++	3.19	1.38
4.	62.50	6.61	98	×	×	++	++++	18.04	12.60
5.	56.25	6.84	100	×	×	−	±	19.95	8.78
6.	40.63	5.54	—	×	×	−	±	2.01	1.34
7.	100.00	5.19	—	×	×	−	±	2.02	1.33
8.	50.00	5.48	—	×	×	−	±	3.50	3.44
9.	100.00	4.76	—	×	×	−	±	1.92	1.35

Notes: (×) no result; (−) no colony; (±) doubtful; (+) form colony; (++) more; (+++) many; (++++) abundant.

As the above table indicates, Tests 2, 3 and 4 were failure for one reason or another. But the results of Tests I and VI were parallel although it was impossible to get result on some soils. Also the amount of nitrogen fixed in liquid culture medium was far less than that of silica gel plate although they were in parallel. As a whole no agreement between the productivity of the soils and the Azotobacter test was reached.

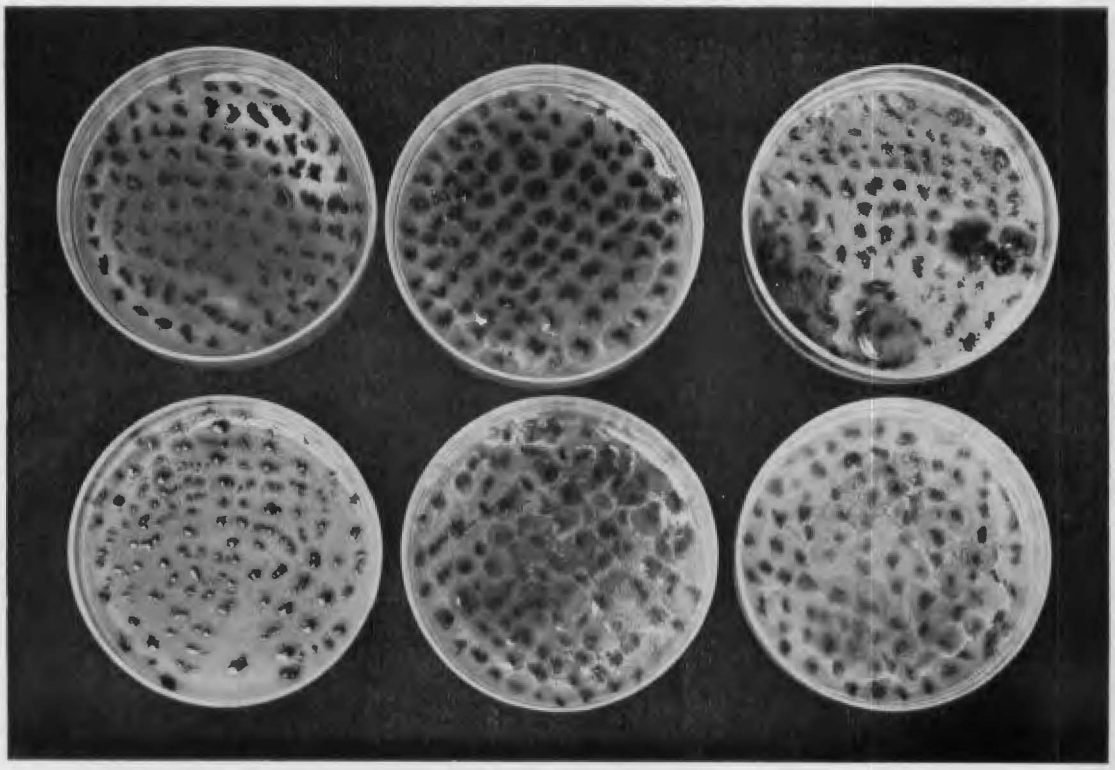
From the foregoing summary, it may be concluded that it is essential to know about the P_H values and the amount of phosphate present in the soils before the Azotobacter test is applied since two factors play a big role. It is more so in case of rice-field soils because the better crop of rice is obtained in the soil of which P_H value 4 than at the larger P_Hs.

Considering the optimum P_H of the rice-plant in the light of that of Azotobacter, it is evident that the correct indication of productivity by the Azotobacter test alone can not be obtained in case of rice-field.

PLATE XXX.

WINOGRADSKY's Azotobacter Test II.

I. II. III.



Top, left to right. (30°C., 48 hours incubation.)

I. Azotobacter colonies 10%.
II. Ditto 100%.
III. Ditto 25%.

Lower, left to right.

I. Azotobacter colonies 0%.
II. Ditto 98%.
III. Ditto 72%.

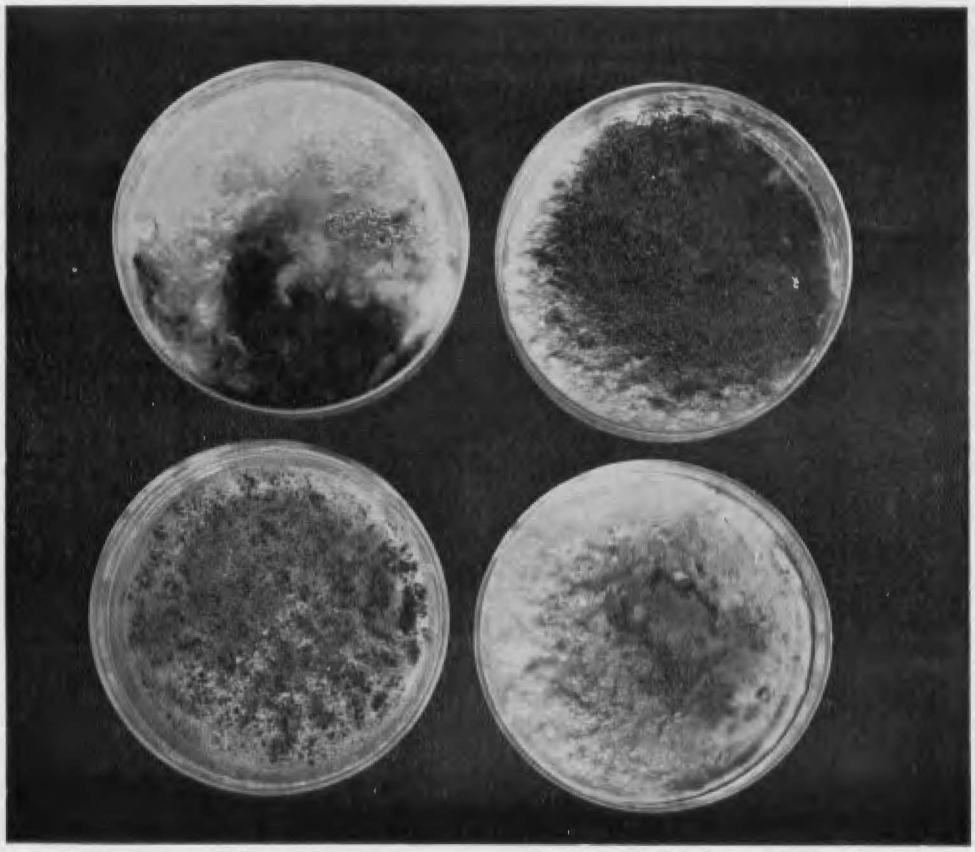
(1/6 of original size.)

Photo. by ARAKAWA.

PLATE XXXI.

WINGRADSKY's Azotobacter Test IV.

I. II.



Top, left to right. (30°C., 120 hours incubation.)

I. Soils not very active.

II. Ditto.

Low, left to right.

I. Soils permanently inactive.

II. Soils very active.

(1/12 of original size.)

Photo. by ARAKAWA.