Proceedings of the Iowa Academy of Science

Volume 38 | Annual Issue Article 107

1931

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THE EFFECT OF PHOSPHORUS ON NITROGEN FIXATION BY AZOTOBACTER

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Since the isolation of species of Azotobacter by Beijerinck in 1901, many investigations have been conducted to determine the mineral requirements of the organisms of this genus. In most of this work it has been found that increasing concentrations of phosphates up to a certain limit, greatly stimulate the growth of Azotobacter. The question may be asked — are the rather large amounts of phosphorus which seem to be necessary for maximum growth assimilated by the organisms or do they serve another purpose, such as a stimulant or a buffer in the culture medium? In a study of this problem, several investigators have attempted to determine the amount of phosphorus assimilated by the Azotobacter by making an analysis of the cells. The results secured have been quite variable, however, especially when compared with the amounts of nitrogen fixed. Other investigators have studied the effect of various concentrations of phosphorus on the amounts of nitrogen fixed, and in some cases, good fixation has been secured with rather low concentrations of phosphorus in the culture medium. This seems to indicate that phosphorus is not necessary for nitrogen fixation, but only serves as a stimulant to the growth of the organism. In other tests, sterile soil has been inoculated with Azotobacter and the amount of water soluble phosphorus has been determined after a certain period of incubation. In some cases, increases in soluble phosphorus have been secured but in most instances, decreases have been noted.

There is also the possibility that Azotobacter assimilate the phosphorus merely because it is present, while they are able to live on smaller amounts when necessary. If this be the case, they may even be competitors with higher plants for the available phosphorus in the soil. Certain species of Azotobacter might assimilate more phosphorus than others and still be lower in nitrogen fixing power.

The purpose of the work reported here was to study the rate of phosphorus assimilation compared with the rate of nitrogen fixation using some solutions containing considerable available phosphorus and others poor in available phosphorus.

[1] The authors wish to express their appreciation to Dr. P. E. Brown, for reading of the manuscript.

The culture medium used for the growth of the organisms was as follows:

K_2HPO_4	0.5 gram
$MgSO_4$	0.2 gram
NaCl	0.2 gram
$FeCl_3$	trace
$MnSO_4$	trace
Distilled water	1000. cc.
Dextrose	10. grams

The reaction of this medium was adjusted to a pH of 7.5 by adding sodium hydroxide. One hundred cc. portions were placed in 500 cc. Erlenmeyer flasks, sterilized and duplicate flasks inoculated with pure cultures of Azotobacter. The organisms used were *Azotobacter chroococcum, Azotobacter beijerinckii, Azotobacter vinelandii,* and Azotobacter number 2. The last organism is a pure culture obtained from a niter spot in Colorado. The cultures were incubated for 16, 24, 26 and 28 days at room temperature. At the end of each incubation period the total nitrogen was determined by the Kjeldahl method and the water soluble phosphorus by the colorimetric method of Deniges as modified by Truog (1), in both the check solutions (uninoculated) and in the solutions inoculated with Azotobacter. One cc. of the culture solution was diluted to 50 cc., then the molybdate solution and the stannous chloride were added to develop the color. This solution was compared in a colorimeter with a standard solution containing a known amount of phosphorus. The difference between the quantity of phosphorus in the check solutions and that in the inoculated solutions represented the amount of phosphorus assimilated by the bacteria.

Table I shows the amounts of nitrogen fixed and the quantities of phosphorus assimilated by the different organisms studied. The organisms varied considerably in their rate of growth and in the amount of nitrogen fixed. *Azotobacter chroococcum* fixed the most nitrogen at 26 days, *Azotobacter beijerinckii* at 16 days, *Azotobacter vinelandii* at 26 days, andAzotobacter number 2 at 24 days. Although Azotobacter number 2 is a very good nitrogen fixer, it is not very active in phosphate assimilation. This organism and *Azotobacter vinelandii* fixed twice as much nitrogen per gram of phosphorus assimilated as did *Azotobacter beijerinckii* or *Azotobacter chroococcum.*

These results seemed to indicate that Az. number 2 and *Az. vinelandii* might fix more nitrogen than the other two organisms when they are grown in a solution containing a very small quantity of phosphorus. This was tested and it was found

Table I — The Amount of Nitrogen Fixed and the Quantity of Phosphorus Assimilated by Azotobacter After Different Periods of Incubation

Culture	16 Days			24 Days			26 Days			28 Days		
	N fixed mgm.	P assimilated mgm.	N fixed per mg. P assimilated mgm.	N fixed mgm.	P assimilated mgm.	N fixed per mg. P assimilated mgm.	N fixed mgm.	P assimilated mgm.	N fixed per mg. P assimilated mgm.	N fixed mgm.	P assimilated mgm.	N fixed per mg. P assimilated mgm.
Az. chroococcum	1.3	0.18	7.0	4.1	0.74	5.5	3.4	0.64	5.3	2.1	0.41	5.1
Az. beijerinckii	6.3	0.98	6.4	2.3	0.44	5.2	2.6	0.42	6.2	1.9	0.40	4.8
Az. vinelandii	3.0	0.31	9.7	4.0	0.37	10.9	4.0	0.42	9.5	3.3	0.32	10.3
Az. No. 2 *	11.3	1.25	9.0	21.1	1.90	11.1	18.7	2.67	8.5	17.4	1.66	10.5

* A pure culture of Azotobacter which produces a brown to black pigment.

that all the organisms used all the phosphorus present, but Az. number 2 and *Az. vinelandii* fixed from a third to a half more nitrogen than did the other organisms.

When the organisms were grown in a medium containing one gram of dipotassium hydrogen phosphate per liter they did not assimilate any more phosphorus nor did they fix very much more nitrogen than when grown in a solution containing a lower concentration of phosphorus. In other words, practically as much nitrogen was fixed when 0.05 gram of the phosphate was added per liter as when 1 gram was added. This was not true, however, for Az. no. 2, which required about 0.1 to 0.2 gram of phosphate per liter for vigorous growth.

These results as a whole seem to indicate that large amounts of phosphorus are not assimilated by Azotobacter and hence are not necessary for their growth. The beneficial effects of large amounts of phosphates, therefore, are probably due to their buffer action which aids in maintaining a more favorable reaction condition for the growth of the organisms.

REFERENCE

1. TRUOG, E., 1930. The Determination of the Readily Available Phosphorus of Soils. Jour. Amer. Soc. Agron. 22: 874-882.

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