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## The Behavior of Legume Bacteria in Acid and Alkaline Media

Raymond C. Salter lowa State Teachers College

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# THE BEHAVIOR OF LEGUME BACTERIA IN ACID AND ALKALINE MEDIA.

#### RAYMOND C. SALTER.

Several investigators have noted a very marked difference in the resistance of various legumes to soil acidity. Vast areas of cultivated land in the United States show an acid reaction, and as lime is expensive in some localities it has been suggested that much can be saved by the choice of acid tolerant crops.<sup>1</sup> Red clover, crimson clover, soy bean, cowpea, hairy vetch, lupine and serradella have been reported as acid tolerant, while on the other hand, alfalfa, one of our most useful forage crops, is found to be very sensitive to acid.

This sensitiveness may be due to many factors concerning the nutrition of the plant. Since leguminous plants obtain nitrogen by a symbiotic relation with certain bacteria, it seems probable that the ill effects of the acid may be directly upon the symbiotic bacteria and only indirectly upon the higher plant.

The influence which the acid constituents of the soil may exert on plant growth has been studied chiefly with reference to the growth of higher plants. From the nature of the results of these investigations, it seemed advisable to extend the study to the lower plants. Probably one of the most striking examples of the interdependence of higher plants and bacteria, is the legumes and legume bacteria. Any agent affecting the one will have a corresponding effect on the other.

The growth of legume bacteria can be measured directly by plate counts, and their virulence can be tested by the formation of nodules on the host plant. This property of the organism makes it well suited for a study of the effect of acid and alkali on its development. An increase or decrease which might result will be noted, especially if the result is compared with that from a neutral culture.

Experiments were planned to study and compare the effect of acid and alkali on the bacteria and host plant. Since legumes are found to study with scholar works, resistance, the acid tolerant red clover

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plant and the sensitive alfalfa plant were chosen for comparison. The effect of reaction on the reproduction of legume bacteria was studied in Ashby's mannit solution and in soil.

In Solution. The plan below was followed:

- 1 and 2-1.0 per cent of N/1 sulphuric acid.
- 3 and 4-0.5 per cent of N/1 sulphuric acid.
- 5 and 6—Neutral.
- 7 and 8-0.5 per cent N/1 sodium hydroxide.
- 9 and 10-1.0 per cent N/1 sodium hydroxide.

Ten 500 cc. flasks were innoculated with 1 cc. each of a water suspension of the red clover bacteria. At the end of one and two weeks plate counts were made to show the number of bacteria in each flask. It was found that a neutral or slightly acid reaction in mannit solution is most favorable for the reproduction of the red clover organism. A slight amount of alkali inhibited growth. No growth was found in the presence of one per cent normal alkali. The results of repeated counts supported the preceding statement.

Alfalfa bacteria were studied in the same manner. It was found that *Bacillus radicicola* from alfalfa grew best in a slightly alkaline or neutral solution. The optimum reaction for the growth of the alfalfa organism in mannit solution is somewhere between neutral and 0.5 per cent alkali. Unlike the organism from red clover this strain of legume bacteria was found to be very sensitive to acidity. An acid reaction of 0.5 per cent greatly retards growth. After three weeks no living cells could be found. A repetition of this experiment gave similar results.

In Soil. Twenty-five samples of sterilized Miami silt loam soil were placed in large test tubes and arranged as follows:

- 1 and 2-1.0 gram of CaCO<sub>3</sub> or four tons per acre.
- 3 and 4-0.5 gram of CaCO<sub>a</sub> or two tons per acre.
- 5 and 6—Neutral—untreated.
- 7 and 8-1.0 cc. N/1 sulphuric acid or two tons per acre.
- 9 and 10-2.0 cc. N/1 sulphuric acid or four tons per acre.

After treatment the soil cultures were re-sterilized and when cool, were innoculated with a pure culture of legume bacteria.

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counts show that calcium carbonate in all proportions benefitted the growth of *Bacillus radicicola* from alfalfa. The greatest number occurred in the soil treated with two tons of limestone per acre.

Here again, the red clover bacteria failed to show any gain in numbers in the presence of a basic substance. Large amounts of calcium carbonate retarded the growth of legume organisms from clover. Apparently red clover does best in neutral Miami silt loam soil.

Effect of Reaction on Growth and Nodule Formation of Higher Plants.—In order to test the effect of reaction on the plant and the formation of nodules it was necessary to grow the plants with a pure culture of Bacillus radicicola. Large test tubes were used. These contained soft filter paper pulp plus mannit solution of known reaction. The same range of reaction was used as in the experiments with solution cultures.

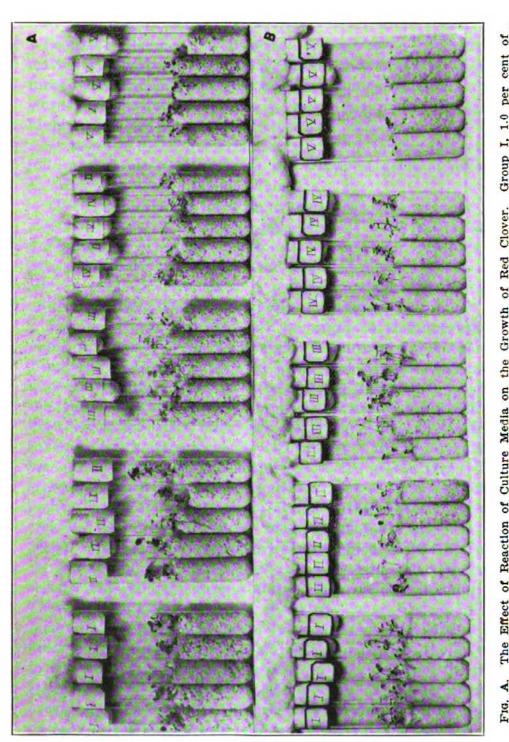
Figure A of Plate VIII shows the results with red clover. The plants developed best in the presence of 0.5 per cent of normal acid and even 1.0 per cent of the acid does not seriously injure growth. The nodules on the roots were carefully counted. The 0.5 per cent of acid also seemed most favorable for nodule formation. But, judging from nodule formation in the presence of alkali, it would seem that the latter was less injurious to the bacteria than to the higher plant.

Figure B of Plate VIII shows the same experiment carried out with alfalfa. An alkaline reaction seems to be favored by this plant. Not only the best growth, but also the greatest number of nodules were found in an alkaline medium. The alfalfa plant and bacteria seem to favor alkali in about the same concentration that the red clover favors acid.

These experiments were repeated in soft mannit agar and in sterilized soil. In all cases the results confirmed those cited above. The filter paper pulp furnished the best medium for the formation of nodules.

Further experimentation is needed to test the effects of different acids or to represent more closely the conditions which actually exist in an acid soil. It is very evident from the experiments performed that alfalfa bacteria are benefitted by an alkaline reaction while the clover bacteria do best in a neutral or slightly acid medium. The difference in behavior of alfalfa and red clover plants in acid soils is characterized by a corresponding difference in the behavior of their symbiotic bacteria.

DEPARTMENT OF NATURAL SCIENCE, IOWA STATE TEACHERS COLLEGE.



of normal Group IV, ver. Group I, 1.0 per Group III, Neutral. ction of Culture Media on the Growth of Red Clover. Group I, 1.0 p Group II, 0.5 per cent of normal sulphuric acid. Group III, Neutral. sodium hydroxide. Group V, 1.0 per cent of normal sodium hydroxide. Group I, 1.0 per cent Group III, Neutral. sulphuric acid. V, 1.0 per cent of normal Culture Media on the Growth of Alfalfa. 0.5 per cent of normal sodium hydroxide. 0.5 per cent of normal sulphuric acid. The Effect of Reaction of of Reaction normal sulphuric acid. hydroxide. The Effect B. The sodium 0.5 per FIG.