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Lewis W. Erdman

Iowa Agricultural Experiment Station

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UNSOLVED PROBLEMS RELATED TO THE INOCULATION OF LEGUMES

LEWIS W. ERDMAN

Almost forty years have passed since Hellriegel and Wilfarth solved the nitrogen problem in soil fertility, by giving a comprehensive explanation of how legumes, with the aid of bacteria living in the nodules on their roots, could grow in a medium devoid of any combined nitrogen. During this time considerable advancement has been made in our knowledge of the subject of soil bacteriology, particularly with reference to inoculation, but all soil bacteriologists will agree that there yet remain many unsolved problems related to the inoculation of legumes. The purpose of this paper is to call attention very briefly to some of these important problems that await solution.

In the final report of the committee of the Society of American Bacteriologists on characterization and classification of bacterial types, the genus *Rhizobium* was proposed to include the nitrogen fixing organisms that produce nodules on the roots of leguminous plants. The type species for this genus was given the name *Rhizobium leguminosarum*. Although this report was adopted at the society meeting December 29, 1919, new literature on the legume organisms is continually being published in our scientific journals, and in the majority of cases the old name *Bacillus radicola* is given for this organism. This name is not recognized by the committee referred to above chiefly because the genus *Bacillus* at the present time includes aerobic rods producing endospores. The legume organism never produces endospores, and hence the name *Bacillus radicola* is incorrect. The unanimous adoption of the correct name for this organism seems to be the first real problem for the soil bacteriologists to solve before much progress will be made with studies on this organism. We should insist upon the use of the name *Rhizobium leguminosarum* by our students and co-workers and all others whose results are to be published.

Under normal conditions of growth all legumes will contain nodules on their roots. Over 7000 species of legumes have been described but only a comparatively few of this number are cultivated. Now are all legumes inoculated by the same organism,

Rhizobium leguminosarum? Can the genus *Rhizobium* be split up into a number of species? There is evidence available to make one believe that it can. Several investigators have already suggested the existence of more than one species. Others have placed the different legume organisms in two or more so-called groups either depending upon morphological, physiological and serological differences, or upon their ability to cross inoculate on members of the same group. At the present time sufficient data are lacking for the description and naming of new species of legume organisms. Fundamental information about this organism will only be obtained after a thorough systematic study of this organism is completed. Such a study will involve a large amount of work; it will necessitate the use of a number of organisms isolated from many different legumes grown under different soil conditions.

If more experimental data were available about the hydrogen and hydroxyl-ion concentration or the critical pH limits for the bacteria which produce nodules on all of our cultivated legumes, it would be possible to answer more of the questions which arise concerning the behavior of legumes on acid and basic soils. Generally speaking very acid soils fail to support legume growth and inoculation likewise is unsuccessful. Some few legumes will grow in medium acid soils, and nearly all legumes will grow in slightly acid soils. But just how acid can soils under different conditions be before different legumes fail to grow and their corresponding bacteria are killed? What is the effect of acidity on the growth of legumes and on *Rhizobium leguminosarum*? Is it due to a toxic hydrogen-ion concentration, the lack of sufficient calcium carbonate in the soil solution, or the soluble aluminum salts? Would it be possible to develop strains of acid-loving bacteria for the inoculation of legumes on acid soils? Why should lime benefit the legume bacteria? Is it because they produce acids? If time permitted many other questions might be asked here that have a direct bearing on this acidity problem which is one of the most important from the standpoint of inoculation.

When knowledge concerning the food requirements of bacteria in general is made more exact, it will be much easier to determine just how this factor affects *Rhizobium leguminosarum*. The statement is generally made that bacteria require the ten essential elements for their growth just the same as is the case with higher plants. But what is the function of each of these elements in the life processes of bacteria? As an example, take iron. Of what value is it to the legume organism? Does it serve as a

catalyst and thereby aid in the reaction involving the fixation of atmospheric nitrogen? Progress has been made in the improvement of different media for the study of this organism, but there is still much to be learned which will help to solve some of these problems.

A rapid laboratory test to distinguish different strains of legume organisms would be very acceptable to all soil bacteriologists. At the present time the only positive method of testing the purity or efficiency of a culture is to inoculate the legume seed or the culture medium, and grow the plants to see if the organism is capable of producing nodules. This method is not only laborious but time consuming as well. It is necessary to wait from three to six weeks before the plant roots are examined. Another objection is the difficulty of growing plants under absolutely sterile conditions. Sand cultures have proved to be the most satisfactory for this test and it is necessary to grow the plants in the greenhouse, where chances for contamination are great. In the case of those legumes whose bacteria cross inoculate, even in this method it is impossible to determine whether or not the organism came from a specific legume or some other member of the same group which cross inoculates. From the practical standpoint this is not an objection to the method, but from the scientific point of view it is. For example, if the alfalfa and sweet clover organism are not intentional in every way, the investigator needs a test that will enable him to identify absolutely the organism with which he is working.

Many of the problems connected with the inoculation of legumes could undoubtedly be solved if the factors which influence the penetrability of the cells of the plant roots were more clearly understood. To what extent do conditions of isolation and cultivation of the organisms on different media in the laboratory affect their physiological efficiency? What effect does physical condition and chemical composition of different soils have on the invading power of *Rhizobium leguminosarum*? In what way does the stage development of plants, the rate of growth of roots, the number of root hairs, and the character of the root tissue affect the number and size of the nodules on different legumes?

Very closely related to the physiological efficiency of this organism is the problem of its longevity in the soil and on culture media in the laboratory. The working out of the life cycle of *Rhizobium leguminosarum*, starting with its introduction into the soil, then following with its growth and activity and various changes on through the life of the plant and back to the soil

again, would make a most interesting study. How long can it live in the soil without the presence of the host plant? What effect does longevity have on the physiological efficiency of this organism? Recent experiments have shown that the yield and quality of canning peas are increased by inoculation even on soils containing the pea organism. Would the same practice prove beneficial on old alfalfa or clover fields or other legumes? It has been observed that certain commercial cultures kept in the laboratory from six to ten years were able to produce nodules on legume plants. Is it possible that there is a definite resting stage in the life history of this organism which enables it under favorable conditions to live over a period of years? A study of all the factors which influence the longevity of *Rhizobium leguminosarum* in the soil, on the seeds, and in the culture medium would make a valuable contribution to our present knowledge concerning this organism.

A considerable amount of experimental data is to be found in the literature showing the amount of nitrogen that is fixed in the soil under specific conditions by different legumes. These data do not necessarily hold true for all conditions, and hence it is desirable to learn how much nitrogen legumes fix in the soil under different conditions. How much of the combined nitrogen in the soil do plants utilize, and how much is actually taken from the atmosphere? Is there any gain in nitrogen to the soil if the entire legume crop is cut for hay and removed from the land? These questions are difficult to answer because of the fact that the gain in nitrogen is so small that often times the amount which is reported as an increase may well be within the limit of error for the method used in determining the total nitrogen in the crop. If satisfactory answers are to be found for these questions it will necessitate additional studies on the fixation of nitrogen by different legumes under different soil and climatic conditions and over a long period of years.

Finally another problem of primary interest to soil bacteriologists is concerning the mechanism of nitrogen fixation by microorganisms. What is the first product of nitrogen fixation, and how is it accomplished by bacteria? If this question could be answered correctly it would be of far reaching importance not only to the bacteriologist, but to the industrial world as well. Manufacturers of commercial nitrogenous fertilizers would welcome a clear conclusive explanation of how microorganisms can fix nitrogen from the atmosphere with apparently remarkable ease, whereas without them electricity must be employed to bring about

the same results. Probably no other field in the subject of soil bacteriology offers more wonderful possibilities for research than this question of nitrogen fixation by bacteria.

Enough has been said to point out some of the more important problems connected with the inoculation of legumes by *Rhizobium leguminosarum*. The task of solving these problems can not be accomplished by one or several investigators working over a period of one or two years. The efforts of all soil bacteriologists, few in number as they are, must be enlisted with a view of coördinating their experiments and results in the hope that satisfactory abstractions and conclusions may be reached.

LABORATORY OF SOIL BACTERIOLOGY,
IOWA AGRICULTURAL EXPERIMENT STATION.