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## SOIL MICROBIOLOGY AS A FIELD OF SCIENCE<sup>1, 2</sup>

#### By Dr. SELMAN A. WAKSMAN

NEW JERSEY AGRICULTURAL EXPERIMENT STATION, RUTGERS UNIVERSITY

THE question is frequently raised concerning the position occupied by soil microbiology in the field of knowledge, especially its relation to other biological sciences, on the one hand, and to agricultural sciences, notably soil science and crop science, on the other. In order to elaborate upon this relatively new branch of science, which is frequently only vaguely understood and often little appreciated, it is sufficient to

<sup>1</sup> Journal Series Paper, New Jersey Agricultural Ex-periment Station, Rutgers University, Department of Microbiology.

<sup>2</sup> The writer has been called upon recently to define 'soil microbiology' and to list a number of outstanding workers in this field of science. He has also been requested to differentiate the work of a microbiologist in relation to related sciences. If an excuse is needed to define a field of science, with which one has been concerned for more than three decades, this excuse is presented here.

define first the student of the subject, namely, the soil microbiologist.

#### WHAT IS A SOIL MICROBIOLOGIST?

A soil microbiologist, as the name implies, is a microbiologist who is concerned with soil microorganisms. His objective is to determine the nature of the microorganisms inhabiting the soil. He is concerned with the activities of these organisms and their characteristics of growth. He analyzes the interrelationships of the various elements of the microbial population of the soil, the reactions with which they are concerned, the influence of soil conditions on the microorganisms comprising the soil population, and the effects of the activities of the population on the. soil and on the plants which develop in it. Finally,

he seeks means of bringing the resources of the soil population to the service of man.

In common with other microbiologists, the soil microbiologist is a biologist whose major interests center about the microscopic forms of life. He is also a biochemist, since he is concerned with the chemical reactions brought about by these microscopic living systems. He is interested in problems involving inorganic and physical chemistry: their importance is evident in the various reactions between inorganic compounds produced by microorganisms and soil materials, and the function of inorganic materials in the development of the microorganisms. He deals with soil structure, which is greatly affected by microbial development, and this as well as other effects of the soil microorganisms involves some consideration of physical chemistry. He deals with a special medium, the soil, in which these organisms live, not in pure culture but in the form of highly complex populations; these comprise many thousands of species, which exert upon one another a variety of associative and antagonistic effects. Because of these complex problems, the cooperative efforts of groups of workers bringing their special training to bear upon such problems is often required.

The soil microbiologist has, therefore, certain functions to perform which distinguish him, on the one hand, from other microbiologists and, on the other hand, from other soil investigators. His principal interest is often only in one of the fields listed below, but he has also a certain perspective recognition of the work of his fellows in other branches of the science, as well as in the related sciences of microbiology, plant physiology and biochemistry. The major fields or interests of a soil microbiologist may be briefly listed under the following categories:

(1) The microbiological population of the soil, including the bacteria, fungi, actinomycetes, protozoa, algae and other lower forms of plant and animal life: The soil microbiologist develops methods for the isolation and for the determination of the abundance and distribution of the various members of this population. These methods are quite distinct from corresponding methods employed by other microbiologists who study animal or plant diseases or those who are concerned with fermentations. Some of the microorganisms found in the soil are characteristic of this particular natural substrate, and occur very seldom, if at all, in other natural media. This is true of certain bacteria, such as the Azotobacter group and the nitrifying bacteria. These organisms may be found in other substrates, such as water basins and on dead plant and vegetable matter; they find in the soil a natural habitat. The relative abundance and the distribution of these organisms in the soil depend upon the nature of the soil, its treatment, the crop grown and a variety of other factors. Various groups of fungi, including some mucors and species of Rhizopus, Trichoderma and Penicillium, may be found in a variety of substrates, such as spoiled food or mildewed cloth, but it is in the soil that these species find their natural habitat and from which they are distributed by wind and water. The specific nature of these and of many other fungi, as well as their relative abundance in a soil depend entirely on the nature of the soil, its treatment and various environmental factors. Certain Basidiomycetes and Myxomycetes, for example, are characteristic of forest soils and of other soils rich in plant residues. The actinomycetes are represented in the soil by certain groups which are characteristic of the soil and are found only to a very limited extent in other substrates. The genus Streptomyces, for example, which comprises those forms that produce a well-developed aerial mycelium, is represented in the soil by hundreds of species, whereas the genus Micromonospora occurs largely in high temperature composts and in lake bottoms. Many other groups of microorganisms are found largely in the soil and only seldom elsewhere. The fact may thus be emphasized that the soil possesses a marked and characteristic microbiological population. Special methods are developed for the study of this population. Media are selected with a view to allowing the development of the greatest number of organisms in a limited space. Extensive use is made of enrichment methods and of selective culture methods, all of which have become essential tools in the hands of the soil microbiologist.

(2) The numerous interrelationships of the soil population, as well as the relations of these organisms to higher plants: These involve a variety of reactions, such as the struggle for development, the competition for nutrients, the favorable and unfavorable effects of one upon the other and the importance of these effects in determining the dominant populations of soil as compared with that of other natural environments. These phenomena also involve the part played by saprophytic soil microorganisms in the elimination of plant parasitic microorganisms. It involves the broad aspects of associative and antagonistic effects of microorganisms, the elucidation of which contributed materially to the development of our knowledge of the production of antibiotic substances. The relationships of microorganisms to higher plants comprise activities which are either adverse or favorable to plant growth; the latter comprise symbiosis of microorganisms with legumes and mycorrhiza formations. The complexity of the reactions taking place in the soil and the difficulty of obtaining a true perspective of the many processes

involved from simplified studies carried out under artificial conditions in the laboratory become selfevident.

(3) The decomposition of plant and animal residues on the surface of the soil as in composts, as well as in the soil itself and the formation of humus: When plants and animals die, or when the residues of their metabolic processes, either in the form of excreta or as dead tissues, find their way into the soil they become subject to attack by large numbers of microorganisms. The nature of these organisms and their relative abundance will depend largely upon the chemical composition of the materials undergoing decomposition, upon the rate of decomposition of the various specific chemical constituents of these materials and upon the environmental conditions, such as reaction of soil, moisture content, aeration and temperature. Among the chemical constituents, the most important are the simple carbohydrates, the starches, pectins and hemicelluloses, the true cellulose and lignin, the proteins and other nitrogenous compounds, the mineral elements, notably the phosphates, sulfates and various bases and their organic and inorganic forms. Some of these constituents, such as the simple carbohydrates and some of the proteins, are attacked very readily and by a great variety of organisms; large numbers of organisms are capable of decomposing cellulose, some of these organisms preferring this carbohydrate to other sources of carbon; some plant constituents, such as lignins and waxes, are attacked only slowly and by a few types of organisms. The rate of decomposition of the carbohydrates may depend to a large degree upon the presence and relative abundance of nitrogenous compounds. The fats, waxes, lignins and certain other compounds may gradually accumulate, because of their relative resistance to decomposition as compared to the other plant and animal constituents. Side by side with the decomposition processes the microorganisms synthesize considerable cell substance, the amount of synthesis being in many cases as much as 30 to 50 per cent. of the material destroyed. The resistant constituents of the plant residues together with the synthesized cell material gradually give rise to the organic matter of the soil commonly known as humus. This material is not of a homogeneous chemical nature, but it possesses certain properties which distinguish it clearly from other forms of organic matter. Its nature depends upon the composition of the material from which it has originated, the extent of its decomposition and the nature of the synthesized microbial cell substances. Although humus is fairly resistant to decomposition, it is subject to slow and gradual attack by a variety of microorganisms. This can be measured by the continuous stream of  $CO_2$  given off

during the decomposition process and by the liberation of the nitrogen as ammonia.

(4) The liberation, through the activities of microorganisms, of the nutrient elements essential for plant life in available forms; namely, the carbon as  $CO_2$ , the nitrogen as ammonia, the phosphorus as phosphate; the sulfur as sulfate, and others: Without these microbial activities, the limited supplies of these elements on the surface of the earth would soon become locked up in the form of plant and animal life, thus bringing to a standstill the development of new life. The varied activities of the soil-inhabiting microorganisms keep these elements in continuous circulation. These processes take place in a series of reactions many of which are brought about by different organisms with different physiological mechanisms. In studying these reactions the soil microbiologist thus contributes to a knowledge not only of soil processes but of microbial physiology as a whole. Let us take the cycle of nitrogen as an illustration. The transformation of the nitrogen in the protein to the amino acid stage, and later to the ammonia stage; the assimilation of the ammonia to build up microbial proteins; the oxidation of the ammonia to nitrate; the reduction of the latter to nitrite and to atmospheric nitrogen-all these involve a series of biochemical mechanisms, the elucidation of which has contributed materially to microbial physiology as a whole.

(5) Nitrogen-fixing bacteria and the mechanism of the fixation process: No other group of microorganisms and no other microbiological process are so characteristic of the soil as the reactions involving the fixation of gaseous nitrogen and its transformation into complex organic compounds. Although some of the bacteria capable of fixing nitrogen are found in other natural substrates, such as water basins, and some nitrogen may be fixed under these conditions, it is the soil which may be looked upon as the natural habitat of these organisms. The process of nitrogen-fixation contributes largely to the fertility of the soil and toward making it a medium favorable for extensive plant growth. Whether the leguminous plants harboring the root-nodule bacteria are growing in a wild state, whether they are cultivated in the form of special crops, or whether they are used in a given crop rotation for soil improvement purposes, the continuous, even if slow, increase in the supply of soil nitrogen resulting from the growth of such crops has long been recognized and advantage taken of it in developing systems of husbandry. The knowledge gained from the study of the mechanisms of the fixation process, although not fully elucidated as yet, has greatly contributed to the understanding of one of the most significant biological reactions and of the physiology of a unique group of microorganisms.

(6) The process of nitrification: The soil microbiologist has largely initiated and has been chiefly responsible for the development of the knowledge gained on the autotrophic bacteria as a whole and the nitrifying forms in particular. Volumes have been written on the subject of nitrification, one of the most important processes in the soil and one of the most fascinating chapters in microbiology. The knowledge of the very peculiar physiologic characteristics of a group of bacteria, capable of utilizing the energy obtained by the oxidation of the ammonia to nitrite and of the latter to nitrate, has greatly enriched microbial physiology. These organisms may be present in other substrates, such as water basins and activated sewage disposal systems, but it is the soil that forms their natural habitat. Whether they have found their way from the soil into lakes and seas, and there is ample evidence for this, or whether they were first living in water basins before becoming established in the soil is a purely philosophical question. At present, they are universally present in soil, whereas in the sea they are largely found in areas close to land. There are but few cases on record of soils lacking in nitrifying bacteria. This may be due to excessive acidity or to anaerobic conditions, which either prevent the growth of these bacteria or inhibit the process of nitrate formation. The rate of accumulation of nitrate in the soil was found to correspond to the extent of microbiological activities in the soil as a whole and incidentally to soil fertility.

(7) Various other processes of oxidation and reduction brought about by soil microorganisms. Such processes may become, under certain special conditions, of great theoretical significance and practical importance. These include such reactions as the oxidation of sulfur to sulfuric acid and the oxidation of a variety of other inorganic and organic compounds; among the reduction processes, it is sufficient to mention the reduction of sulfate to hydrogen sulfide, of nitrate to nitrite, to ammonia or even to atmospheric nitrogen, and of various complex organic compounds. The organisms responsible for these processes may develop only when a combination of certain factors are favorable. Sulfate-reducing bacteria develop, for example, when conditions are anaerobic, when sulfate is present and when organic matter is provided. These reactions may either be important in themselves, as in the loss of nitrogen, or they may be responsible for secondary reactions, such as the corrosion of metals accompanying sulfate reduction.

(8) The role of microorganisms in the conservation of the soil: This important function has become recognized only recently. Through the cell material that is synthesized, especially the mycelial structures of fungi, and through the various products of cell metabolism, such as the slimy products of bacteria, microorganisms exert various physical and chemical effects upon the finer soil particles. This results in the aggregation of the particles and consequently in their rapid removal from the soil by flowing waters or strong winds. It is becoming more and more evident that one of the major problems in soil conservation is to supply a sufficient amount of organic matter and other nutrients to the microorganisms of the soil. These agents, through their processes of decomposition and synthesis, take a highly active part in binding the soil, thus preventing the breakdown of the soil structure through atmospheric agencies.

The soil microbiologist is thus found to deal with a highly complicated group of problems, which are primarily biological in nature but which have many chemical and physical phases and applications. Soil microbiology, like the older science of botany, has its ecological aspects, its physiological and biochemical problems and numerous practical applications. The applications bear, first of all, upon soil fertility and crop production. By reason of the diversity of the reactions which take place in the soil, it is necessary for the soil microbiologist to study and understand the physiology of various microorganisms. This is largely the reason why he has particular interest in and has made so many contributions to the field of microbial physiology.

There are also other phases and branches of microbiology to which the soil microbiologist has made important contributions. His field is thus not limited to the study of a few processes by simple methods, and the importance of soil microbiology is not restricted to a knowledge of practical crop production. With this in view, it becomes essential to recognize the relation of this science not only to other major sciences, but especially to those newer or special sciences with which it has had the greatest contact.

#### Soil Microbiology and Other Sciences

The soil microbiologist is thus shown to be in a position to make important contributions not only to the knowledge of the soil, plant nutrition, crop production or agronomy, and forestry, but also to several apparently distinct sciences. It is sufficient to mention, in this connection, the sciences of aerobiology, the biology and chemistry of fresh- and salt-water basins, the broad field of ecology and plant pathology, the problems of food preservation, of sanitation and of sewage purification, as well as the domain of public health and medicine. Brief outlines of some of these relations will suffice.

Most of the fungi and bacteria that one finds in the

air, no matter how far away from the land surface, have originated from the soil that has been carried by the wind in the form of dust particles. These organisms, having had their origin in the soil, behave in all respects like typical members of the soil population. This phenomenon not only has a bearing upon the problem of allergy but also upon a certain type of deep-seated infection that is often traced to certain soil-inhabiting microorganisms.

Any survey of the microbiological population of water basins, whether small land-locked or large oceans, will reveal certain facts that have an important bearing upon the close relationships between the nature of such populations and that commonly found in or characteristic of the soil. Bacteria, for example, are found in much greater abundance in those areas of the sea that are close to shore than in regions distant from land. The occasional presence in sea water close to shore of such typical soil organisms as specific fungi, actinomycetes and various bacteria, which are absent from or occur only rarely in waters far away from land, point to their soil origin. Certain special groups of bacteria, such as the nitrifying and aerobic nitrogen-fixing forms, that are found to occur with great regularity in waters close to land and are absent elsewhere in the sea, led to the question whether these organisms occur there only because they have been washed in from land or whether they are true seainhabiting organisms.

The science of ecology owes a great deal to our knowledge of soil-inhabiting microorganisms. One need only mention the effect of root-nodule bacteria on the growth of legumes, which influences, in turn, the growth of grasses and other plants; the effects of mycorrhiza fungi and certain other soil-inhabiting organisms upon forest vegetation and forest succession is another illustration.

Various investigators and technologists concerned with problems that are apparently totally unrelated to soil microbiological processes have come to the soil for an answer to their specific needs. Out of a number of illustrations that may be cited, it is sufficient to draw attention to the following three that recently have aroused great interest: (a) the subject of spoilage and deterioration of textiles, of leather goods, of wooden structures and of many other types of service materials, under conditions of high humidity and high temperature; (b) the search for microorganisms producing antibiotic substances; (c) the search for organisms concerned in various industrial fermentations.

The problem of "mildewing" of cotton cloth, of woolen material and of leather goods has recently received renewed emphasis because of the huge quantities of such materials that are being handled under

moist and humid conditions. The various procedures used for preventing rapid deterioration of these materials brought out the fact that the fungi and bacteria that are responsible for the attack are largely of soil origin. The test organisms used in evaluating the resistance of such materials to "mildew" and to "rot" are typical soil forms. When a very severe type of test procedure is to be used, a soil suspension containing a number of different organisms is employed for inoculation purposes, or the material is actually buried in the soil for a given time under favorable conditions of moisture and temperature. These are not direct problems for the soil microbiologist, but much can be gained from a knowledge of the microbiological population of the soil and of the physiology of its constituent forms. This is true especially of the resistance of these organisms to poisons, their nutrient requirements, especially their ability to attack cellulose, the effects of relative humidity, etc.

The student of antibiotics and of their application to chemotherapy has long recognized the fact that some of the most important organisms that produce antibacterial substances find their permanent or temporary habitats in the soil. Thus, Bacillus brevis that produces tyrothricin is a typical soil bacterium; Penicillium notatum and P. chrysogenum that form penicillin are soil fungi; Streptomyces lavendulae and S. griseus, that give rise, under special conditions of culture, to streptothricin and streptomycin are soil actinomycetes. Investigators in numerous laboratories throughout the country are at this very moment enriching the soil with all sorts of pathogenic bacteria and other microorganisms, in order to stimulate the development of antagonistic forms that could be utilized for the production of desirable antibiotic substances. Literally thousands of fungi, bacteria and actinomycetes are now being isolated from soils or from composts, and tested for the production of such antibiotic agents. Here as well, a knowledge of the composition of the complex microbiological population of the soil and its variation under the influence of treatment would have saved a great waste of time and energy. Stories are told of aeroplanes collecting soil samples from all over the world, and of automobile trips throughout North America gathering bits of soil from which to isolate bacteria or fungi capable of producing specific antibiotic agents.

The contributions of the soil microbiologist to public health and to medicine has thus recently gained added significance. Knowledge already established by the soil microbiologist includes the disappearance in the soil of most of the pathogenic bacteria causing infections and epidemics in man and in animals, as contrasted to the survival of a few important bacterial pathogens, such as those containing tetanus and gas gangrene and of a number of bacteria and fungi causing plant diseases. The relationship of the soil to a variety of other diseases, such as hookworm and deep-seated actinomycetic infections, is likewise a matter of great importance. Some of these problems require further elucidation.

One could cite other illustrations of contributions of soil microbiology to human welfare, perhaps not so spectacular as the above, but important nevertheless. The dependence of various industrial fermentations upon soil-inhabiting microorganisms is well known. Others include the anaeobic bacteria producing butyl alcohol and acetone, the butylene glycol organisms of both the aerobic and anaerobic types, and many other organisms that produce various organic acids and alcohols. The citric-, fumaric-, gluconic- and itaconicacid-producing fungi are also largely soil-inhabiting organisms; the very name of the fungus producing the last acid has the flavor of the soil (Aspergillus terreus). The industrialist interested in fungi and bacteria producing pectolytic enzymes, as well as proteolytic and amylolytic enzymes, usually goes to the soil in search for appropriate organisms. Some of the vitamin-producing microorganisms are of soil origin.

Although in all the above cases, the soil and its extensive microbiological population were given but little consideration, much benefit could frequently have been derived from a knowledge of the environment in which these organisms live and of their mode of life in the soil.

#### WHAT SHOULD A TRAINING IN SOIL MICROBIOLOGY BE?

On the basis of the above facts one may feel justified in postulating the type of training a prospective worker in soil microbiology should acquire. Soil microbiology as well as its mother science, microbiology, is dependent on general bacteriology and on mycology, as well as on physiology and biochemistry. Soil microbiology does not comprise merely the study of legumes and their utilization, of Azotobacter and its physiology, or of the cycle of nitrogen in the soil, as glorified by the time-honored terms of "ammonification," "nitrification" and "denitrification"; it does not comprise merely the enumeration of bacteria and fungi in the soil, or the isolation and identification of so many hundreds of species of these organisms-it is all that and much more. Soil microbiology, like microbiology or bacteriology, has a much broader concept and has acquired many ramifications. These comprise specific aspects of ecology, taxonomy, physiology, biochemistry and practical utilization. The soil microbiologist carries a certain taxonomic responsibility, since it is highly essential to know the exact nature of the organisms involved in a given reaction or group of reactions, and not merely to list these organisms as bacteria, molds or protozoa. The student who devotes himself to soil microbiology is, therefore, entitled to learn the various phases of this relatively new science.

#### Summary

It can now be definitely recognized that the soil microbiologist is in a position to make important contributions not only to our knowledge of soil processes and plant growth, but also to microbiology, especially microbial physiology, and to the utilization of microorganisms for various industrial, public health and other processes. The soil microbiologist is able to contribute in many ways to man's capacity to survive, by learning to control the activities of injurious microorganisms and by favoring the processes brought about by the beneficial organisms.

The reason why the broader concept "microbiology" rather than the narrower term "bacteriology" has been used throughout this discussion is that the soil microbiologist has often to pay as much attention to the fungi as to the bacteria, and occasionally also to the protozoa, the algae and even the nematodes and other worms, inhabiting the soil in large numbers. Only a recognition of all these lower forms of life and their many interrelationships can help to elucidate this complex and important science.

### OBITUARY

#### FRANCIS BERTODY SUMNER<sup>1</sup> August 1, 1874–September 6, 1945

PROFESSOR SUMNER'S interests were primarily those of a naturalist; he is best known scientifically for his experimental studies on the nature and inheritance of adaptive variations as the key to an understanding of the problem of organic evolution. These studies

<sup>1</sup>Contributions from the Scripps Institution of Oceanography of the University of California, New Series No. 264. have been widely recognized as being of exceptional significance. To him biology is also indebted for the first experimental proof of the origin by mutation of new varieties in any wild species of mammal and the Mendelian inheritance of their characteristics. At the time of his death he had been connected with the Scripps Institution for Biological Research (later the Scripps Institution of Oceanography) of the University of California for thirty-two years, having reached the retiring age of seventy the preceding