I.—STUDIES ON SOIL ACTINOMYCES. PART I. INTRODUCTION.

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Although, during recent years, a considerable amount of work has been done on the *Actinomyces* occurring in the soil, our knowledge of their exact nomenclature, systematic position among micro-organisms, mode of occurrence, distribution in the soil and general physiological activity, as related to soil conditions and plant growth, still remains comparatively obscure.

The name *Actinomyces* has been used as a generic designation for these organisms by the great majority of authors: but many workers have referred to them as *Discomyces*, *Leptothrix*, *Coniomycetes*, *Nocardia*, *Streptothrix Cohn* (or *Cohnistreptothrix*), *Oospora*, etc. (*vide* Breed, and Conn, *J. Bact.*, 1919, **5**, 585; 1920, **6**, 489). Wright's adoption (*J. Med. Res., N.S.*, 1904–5, **8**, 349) of the terms *Actinomyces* for the anaerobic sporeless forms and *Nocardia* for the aerobic conidia-formers, which has found acceptance by some bacteriologists, suffers from the use of distinctions which are now known to vary to a considerable extent with nutrition and are, therefore, not reliable.

Among the leading systematic biologists, Bergey and co-authors (Determinative Bacteriology, 1923) and Buchanan (General Systematic Bacteriology, 1925) classify these organisms with the bacteria; Dreschler (*Bot. Gaz.*, 1919, **67**, 65 and 147) with true fungi and Lehmann and Neumann (Atlas und Grundriss der Bacteriologie, 1920) as a separate group possessing characteristics intermediate between those of bacteria and fungi. Since all these authors based their classifications on almost identical morphological characteristics as shown in culture media, it may be inferred that such distinctions are not sufficient. Waksman (Principles of Soil Microbiology, 1927) has adduced a certain amount of evidence, on physiological grounds, to show that as a genus, *Actinomyces* are distinct from bacteria and fungi. More data of a similar type are, however, required to place the organisms in a definite, systematic position.

The mode of occurrence of *Actinomyces* in the soil is still not well defined. Our knowledge as to whether these organisms occur exclusively in the form of conidia or as vegetative mycelia interspersed with conidia, though exceedingly important from the point of view

of assessing their real importance in the soil is still inexact. Conn (*Soil Sci.*, 1922, **14**, 149) stated that by his direct staining method the presence of the vegetative mycelia of *Actinomyces*, together with those of fungi, can be demonstrated; but since the staining does not distinguish between the filaments of the two types of organisms, the evidence for their mixed occurrence, thus obtained, is hardly satisfactory. It is necessary to adduce more rigorous proof to demonstrate the exact condition in which *Actinomyces* occur in soil.

Although it has been noted by many workers that *Actinomyces* form quite a large proportion of the colonies appearing on bacterial plates, no attempt has been made to standardise a readily reproducible method that would help in the accurate enumeration of the organisms, vegetative or otherwise. The methods used by the previous workers have not all been identical, so that their results are not really comparable with each other. Other conditions being comparable, the numbers of the organisms being the index of their ability to bring about in the soil the changes characteristic of their species, the necessity for a standard-count medium can hardly be over-emphasised.

A large number of species of *Actinomyces* have been isolated from the soil and their cultural and other characteristics studied by Krainsky (*Zentr. Bakt.*, II, 1914, **41**, 639) and Waksman and Curtis (*Soil Sci.*, 1916, **1**, 99; 1919, **8**, 71), and others : but their exact mode of distribution in the soil, their respective numerical fluctuations with season and general climatic conditions, mechanical composition, physical conditions and reaction of the soil still remain to be determined in proper detail.

Hiltner (*Jahrb. Ver. angew. Bot.*, 1907, **5**, 200) and later Bright and Conn (*N. Y. Agr. Exp. Sta. Tech. Bull.*, 1919, 67) have shown that there is an increase in the number of *Actinomyces* in the soil following the addition of organic manures or treatment with lime and mineral fertilisers. The exact mode of response to such treatments by the different species and their relation to the types of crop raised or to the natural vegetation occurring on the soil has yet to be determined.

Lieske (Morphologie und Biologie der Strahlenpilze, 1921) made some study of the effect of temperature, desiccation, different types of light rays, certain chemical poisons, dyes, etc., on the *Actinomyces* of the soil: Munter (*Zentr. Bakt.*, II, 1916, **44**, 673) observed the effects of salts of different concentrations in culture media. Their investigations, however, require to be followed up, in intensive detail, on the soil itself and with reference to the species which are noted to be active in the soil.

In the course of their cultural studies Krainsky (*loc. cit.*) and Waksman (*Soil. Sci.*, 1919, **8**, 71) have shown that many soil *Actinomyces* secrete cellulases, amylases, proteases and other enzymes. More detailed and quantitative data in regard to the enzymic activities of the organisms are however, required before their physiological activity in the soil can be properly defined.

The influence of air supply on most forms of *Actinomyces* has not yet been definitely established. Since the time when Beijerinck (*Zentr. Bakt.*, II, 1900, **6**, 2) described the whole genus as being facultative anaerobes, many opinions have been expressed by different authors, some describing them as being aerobic, some as being strictly anaerobic, and so forth. Since aerobic and anaerobic conditions prevail side by side in the soil, the subject requires careful revision with quantitative studies of the different metabolic products of the organisms.

Krainsky (*loc. cit.*), Waksman (*J. Bact.*, 1919, **4**, 307) and others have studied extensively the utilisation of different carbohydrate and allied materials by *Actinomyces* for their growth and development, but information is not yet available concerning the quantities of the different compounds thus assimilated, the manner in which various substances are taken up, and the different metabolic products that are formed. Nor is anything known regarding the extent to which the normal activity of the *Actinomyces* may be modified by the other organisms present in the soil.

The investigations of Mace (*Compt. rend.*, 1905, **141**, 147), Munter (*Zentr. Bakt.*, II, 1914, **39**, 561), Waksman (*J. Bact.*, 1920, **5**, 1) and others have shown that *Actinomyces* can decompose proteins into amino-acids and ammonia; but it is not known whether they bring about similar changes in the soil where vast numbers of different types of organisms possessing similar properties are present. The extent to which *Actinomyces* can assimilate and transform mineral matter under different conditions in the soil, likewise remains unknown.

The part played by soil *Actinomyces* in the decomposition of cellulose materials in the manure heap has not, so far, been determined, although the musty odour characteristic of their presence is frequently quite pronounced, particularly when such material is stored in the dark. Perhaps the most characteristic thing about the soil *Actinomyces* is this earthy odour; but little is known about the nature of the odoriferous principle, its relation to the physiological activity of the organism or any effect it may have on the growth and development of the other organisms occurring in the soil. While it is generally recognised that carbohydrates are utilised in its production, it is a common experience that the intensity of odour may vary considerably when the organisms

are grown on different types of carbohydrate material, e.g., from the very faint odour obtained on certain sugars, to the oppressive intensity obtained with starches, suggesting that the chemical structure of the carbohydrate supplied is a determining factor in its production.

Another important physiological aspect of *Actinomyces* which merits detailed investigation is the mode in which the numerous pigments characterising the different species are produced. Conn (*N. Y. Agr. Exp. Sta. Tech. Bull.*, 1921, 83), Waksman and Curtis (*Soil Sci.*, 1916, **1**, 99), Lieske (*loc. cit.*) and others have made interesting observations on pigment formation in different culture media, but no definite knowledge of the subject is yet available. Beijerinck (*Zentr. Bakt.*, II, 1900, **6**, 2) and others have suggested various types of reactions such as quinone-formation, oxidase-action, etc., as representing the mechanism of the pigment-production, but much further work is required to arrive at any definite conclusion on the subject. The relation of the chromogenic principles to the physiological activities of the *Actinomyces* also deserves careful study.

Actinomyces are known to occur in the air, on butter (Jensen, *Zentr. Bakt.*, II, 1902, **8**, 171) and various other materials of plant and animal origin. Though it is probable that many of these forms have been derived from the soil, it is not definitely known whether such varieties exhibit the same physiological characteristics, nor is it known whether a normal soil saprophyte can, under other conditions, become an animal parasite.

The foregoing and allied problems are under investigation in these laboratories and will form the subjects of future communications.

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