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MICROBIOLOGY OF SOIL

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The discovery that certain microorganisms isolated from soil could produce antibiotics when grown in pure cultures in the laboratory has served to focus attention as never before on the subject of soil microbiology. The main emphasis, however, is on problems entirely unrelated to the processes which go on in the soil. The fact that an organism was isolated from soil and then used in medicine, fermentations, or some such way, does not bring such work in the actual field of soil microbiology. Practically all except the more fastidious animal and plant pathogens can be found in the soil at one time or another. Even some of the fastidious organisms are quite regularly found. That is not surprising considering that all sorts of materials find their way back to the soil—the dumping place of much of our refuse. In addition, the soil serves as a good matrix for the preservation of microorganisms, provided competition between the various forms is not too severe.

Waksman (1) reviewed the literature up to 1932, and then supplemented his book with a review (2) covering the years 1932–36. A decade later, Norman (3) very briefly surveyed the field and discussed the status of the science, especially the lack of support allotted to it. It is the purpose of the present paper to review some of the more important work published since 1936. The coverage of the literature cannot be complete owing to the limitation of space; more attention will be paid to some work, less to others. Responsibility for the selection of the references discussed will be the reviewer's, who will be influenced a great deal by his own interests.

Autotrophic Soil Bacteria

Probably the most interesting members of the autotrophic group are those bacteria that oxidize ammonia to nitrite and those that oxidize nitrate to nitrite. They depend upon the carbon dioxide of the soil as the sole source of carbon. Having such a limited physiology naturally makes them rather difficult to handle in experimental work. The presence of some types of organic matter is inhibitive to cultures in the laboratory but does not seem to be

so under natural conditions. Pandalai (4, 5) expressed the opinion that the heterotrophic flora mixed with the autotrophic allowed the latter to function in the presence of inhibitive substances and that this association was symbiotic. The latter view was also shared by Desai & Fazal-Ud-Din (6). Stapp (7) observed that bacteria which were usually chromogenic were frequently associated with the nitrifiers and that they stimulated nitrification. Imsenecki (8) isolated a myxobacterium (*Sorangium symbioticum*, n. sp.) from a clear zone on a silica gel plate which he considered to be identical with Winogradsky's "nitrocystis."

The proper pH value of soil for nitrification has long been a subject for discussion. Using ammonia, ammonium sulfate, and urea in six typical Arizona soils, Caster, Martin & Buehrer (9) found that complete nitrification would not occur above pH 7.7 ± 0.1. There was, however, some nitrite formed even above this threshold. Another interesting point is that ammonia at a concentration as high as three hundred parts per million was not toxic to the nitrifying bacteria and any failure of the ammonia to nitrify to nitrate could be attributed to the high alkalinity of the soil. Analytically they could account for practically all of the added ammonia which indicated that losses by volatilization from the soil or by spontaneous decomposition of ammonium nitrite were negligible. This was not corroborated, however, by Jewitt (10) working with Sudan Gezira soil. Nitrification of various materials in his experiments was normal but appreciable ammonia was lost from alkaline soil when it was added as ammonium sulfate. Fraps & Sterges (11) previously had reported that in the nitrification process nitrogen might be lost by the decomposition of nitrite. Fraps & Sterges (12) also found variability in the nitrification of ammonium sulfate in Texas soils, some nitrifying only 60 per cent of it even though calcium carbonate was added. The addition of phosphate increased the nitrification of most of these, the monocalcium phosphate with calcium carbonate being the best. In this connection, Pikorvska (13) showed that bacteria isolated from different soils varied in their nitrifying ability and Verona (14), that small amounts of phosphomolybdic acid were stimulatory.

The effect of light on nitrification still seems to have its supporters. Dhar & Mukerji (15) again stated that nitrification was due to sunlight alone and that denitrification also occurred with a loss of nitrogen gas. Singh & Nair (16) took a less radical view

and reported that light helped the bacteria to oxidize ammonia to nitrite but did not assist in the further oxidation to nitrate, nor in the ammonification of organic matter. Waksman & Madhok (17) found that biological nitrification was the all important process in the formation of nitrates in soil and that photonitrification did not play an important part in normal soil processes. On the other hand, Puri, Rai & Kapur (18) concluded from their work that nitrites were oxidized to nitrates in soil by a purely physico-chemical process, quite independently of microbiological and photochemical agencies. The oxidation depended upon the base exchange capacities of the soils.

A new and direct method of studying nitrification in soil was proposed by Lees & Quastel (19) which they called the "perfusion technique." By a suitable apparatus, the soil was intermittently perfused by an aerated solution of nitrifiable material. The soil was maintained at near the water-logged state, the excess moisture draining off through the soil. The "perfusate" was then mixed and aerated and again made to drain through the soil. The process was continuous and samples were withdrawn from the reservoir for analysis at various times. One especially interesting fact was brought out by their work, i.e., nitrification in soil takes place wholly at soil surfaces where ammonia is combined or absorbed. An increase or diminution of such receptor sites caused an increase or decrease in the rate of nitrification. The rate is, therefore, a function of the base-exchange capacity of the soil. Little or no nitrification took place in the "perfusate" or soil solution. These and other observations seem to make this a very useful method.

The process of sulfur oxidation by *Thiobacillus thiooxidans* has been elucidated by the work of Vogler & Umbreit (20) and Umbreit, Vogel & Vogler (21). There must be direct contacts between the cells and the sulfur particles in order for the sulfur to be dissolved in the fatlike globules which are usually in the ends of the cells. Umbreit & Anderson (22) observed three types of cells under the electron microscope but the bipolar appearance seen by the light microscope was not brought out. Knaysi (23) found the cell protoplasm gram negative, the vacuolar content, gram positive. One new species isolated from coprolite, *Thiobacillus coprolitícus*, was described by Lipman & McLees (24).

The anaerobic reduction of sulfates to hydrogen sulfide by *Vibrio desulfuricans* will be included here although the organism

is a heterotroph. Aleshina (25), supposedly working with this species, reported that chitin was decomposed and ammonia liberated. Butlin & Adams (26), however, were able to demonstrate that it was a facultative autotroph. More astounding was the report by Starkey (27) that spores were formed in cultures isolated at 55°C., whereas no spores were observed in those isolated at 30°C. When first purified, the high temperature strain could be changed into the low temperature and vice versa. Later each apparently became stabilized to its own temperature range, since neither strain could be induced by this reviewer to grow at the other temperature range. A sporulating strain with an optimum temperature of 30°C. was found by Iya & Sreenivasaya (28) in soil where elementary sulfur was being deposited. It preferred 6 per cent of sodium chloride, but would grow slowly without any.

Nonsymbiotic Nitrogen-Fixing Bacteria

The literature on *Azotobacter* continues to be voluminous and often contradictory. Of special interest were the publications by Russian workers in which it was shown that increased yields of many crops were obtained by the application of "azotogen," a peaty material carrying *Azotobacter* cells. This was applied to seed or to the plant roots. Allison (29) has reviewed the literature to which the reader is referred. Allison *et al.* (30), employing two soils in extensive greenhouse experiments, found no significant effect on yield or growth of plants by the use of pure cultures of *Azotobacter* or "azotogen." Clark (31) also obtained negative results in his study of the possible effect of the same materials on the rhizosphere flora. *Azotobacter* added to cropped and uncropped soil disappeared more rapidly from the former. In no case could the Russian work be substantiated. Katznelson (32) reported that soils showed marked differences in regard to their ability to support *Azotobacter* even in the presence of molybdenum, lime, and sources of energy. This inability to survive seemed possibly to be due to unsuccessful competition with other soil organisms, presence of toxic substances, or absence of certain nutrients such as phosphorus or potassium. His experiments indicated that it was possible by soil amendments to establish *Azotobacter* and to stimulate its development in soils originally inimical to it.

The distribution of *Azotobacter* in soils has received considerable attention. Chang (33) reported *Azotobacter*, mostly *A. chro-*

ococcum, in practically all soils of Manchoukuo; *A. vinelandii*, however, fixed more nitrogen. Three-fifths of the Chinese soils examined by Gaw (34) also contained *Azotobacter*. Cultivated soils in Arizona according to Martin (35) usually contained the organism but range soils generally were lacking in it. Sushkina (36) failed to find *Azotobacter* in virgin arid soils in Russia, but after irrigation it appeared even under the meadow type of soil covering. Using the plaque method, positive results were obtained by Peterson & Goodding (37) in 96 per cent of the soils of Nebraska. Their presence was not correlated with phosphorus, exchangeable bases, or pH of the soil. This bacterium was found at pH 5.3, but Stockli (38) failed to find it in soils of a pH value less than 6.0, which is usually considered the critical pH. A salt content of 0.5 to 1 per cent was found to be optimum for *Azotobacter* by Werner (39), which he thought might explain its absence from some soils. The soils of Portugal examined by Louriero (40) were rich in *Azotobacter*, especially *A. chroococcum*. The observations of Swaby (41) are interesting, not because only 26 per cent of the soils tested were positive but because only eight soils had more than thirty *Azotobacter* cells per gram. Similarly, Roberts & Olson (42) found no *Azotobacter*, or, at most, only a few cells, although there was nonsymbiotic nitrogen fixation in their experiments. They concluded some other organism might be involved. *Azotobacter agile* was isolated by Soriano (43) from 25 per cent of the water supplies around Madison, Wisconsin, and San Francisco, California, and in 75 per cent of those around Buenos Aires, Argentina.

One new species of *Azotobacter* has been described recently. Starkey & De (44) isolated *A. indicum* from tropical acid soil (pH 4.9 to 5.2). It grew slower but fixed as much nitrogen as *A. chroococcum* and produced a great amount of tough slime. Although it grew from pH 3 to 9, it lived longer under acid conditions. Another bacterium, which does not belong to this genus, will be included here for convenience. *Azomonas insolita*, isolated by Stapp (45) also from tropical soil, produced acid and gas from most carbohydrates, grew under vary acid conditions (pH 3.3), and fixed a moderate amount of nitrogen. More observations on the occurrence and abundance of these bacteria seem desirable.

Under some conditions, *Azotobacter* may be efficient in fixing nitrogen if associated with other microorganisms. Richards (46) grew *Aerobacter aerogenes* with *A. chroococcum*, the former serving

to break down the starch used in the medium. Nitrogen fixation was shown by Jensen (47) to take place in cultures of *Azotobacter* associated with certain cellulose-decomposing bacteria belonging to the genus *Corynebacterium*, but not when *Cytophaga*, *Cellvibrio*, fungi, or actinomyces were the associated organisms. This was followed by a more detailed study by Jensen & Swaby (48) on the quantitative relationship between cellulose decomposition and nitrogen fixation and the nature of the organic breakdown products of cellulose that serve as energy material for *Azotobacter*.

Hervey & Greaves (49) noted that the presence of ciliates in liquid cultures stimulated nitrogen fixation. Killed ciliates had the same effect but their filtrates or ash had no effect. A chance contamination (probably by *Bacillus circulans*) was reported by Lind & Wilson (50) as being beneficial to *A. vinelandii*. The contaminant made iron available from an old preparation of humates; it had no effect in the presence of fresh humates. Another factor in the fixation of nitrogen is the effect of molybdenum which Bortels (51) discovered and which was corroborated by Horner *et al.* (52) and others. One part per million of molybdenum gave a tenfold to thirtyfold increase in the nitrogen fixed; vanadium was less effective. By adding an "auxin" to agar, Armandi (53) obtained more color and growth of *A. chroococcum* with nearly a twofold increase in nitrogen fixation. Jones & Greaves (54), however, refute the claim that this organism requires certain accessory food substances. A large number of materials were tested but none of them were needed for normal growth and metabolism of the organism.

Azotobacter can utilize a wide range of substrates but not all strains of a species are identical in this respect. Six strains isolated by Guittoneau & Chevalier (55) fixed 9 to 11 mg. nitrogen per gm. of phenol consumed. They also found (56) that strains varied in their ability to utilize sodium salicylate. By adding sodium benzoate or benzoic acid to soil at rates of 2.5 or 5 per cent, Reuszer (57) isolated a strain of *Azotobacter* producing a green pigment although it had never been found previously in that soil. One wonders if aseptic conditions were maintained. Katznelson (32), however, reported that 1 per cent of calcium benzoate, ethyl alcohol, and butyl alcohol completely suppressed *A. chroococcum*. It would appear, therefore, that there is a great variation between species, or perhaps between strains of the same species in their tolerance of such substances, and that benzoates should be used with caution.

Fumes of ethyl alcohol and acetone, however, were readily used by *Azotobacter* according to Kholodny *et al.* (58), whereas vapors of methyl alcohol were unfit. Alcoholic yeast extract (0.5 to 7 per cent) was found by Schroeder (59) to increase growth in proportion to the amount of the extract used. Greaves *et al.* (60) added tyrosine, DL-isoleucine, hydroxyproline, and L-histidine to synthetic mannitol base medium and obtained greatly increased fixation of nitrogen by *A. chroococcum*. Casein and albumin acted similarly but gelatin decreased the fixation. On the other hand, Horner & Allison (61) failed to find that L-histidine was utilized; in fact out of thirty-five nitrogenous compounds tested, including amino acids, purines, pyrimidines, amines, and amides, only urea, aspartic acid, asparagine, adenine, and glutamic acid appeared to be definitely assimilated. As in the case of the long-known effect of nitrate, the addition of nitrogen compounds decreased nitrogen fixation in proportion to the amount of the fixed nitrogen utilized, the unavailable compounds having no effect on fixation. Fedorov (62), nevertheless, found that growth and nitrogen fixation by *A. agile* in solution cultures were stimulated by the addition of 0.01 to 0.5 gm. of *o*-dinitrobenzene per 100 ml. of the medium.

Shtern (63) obtained saltants by irridation which had an increased capacity for nitrogen fixation, the maximum occurring in two days. On the other hand, Dooren de Jong (64) failed to find any permament change induced by treatment with x-ray, and Whelden *et al.* (65) noted a marked decrease in nitrogen fixation proportional to the dosages of the irradiation. In the absence of copper, *A. chroococcum* failed to blacken in Mulder's experiments (66), indicating a beneficial effect of copper. On the other hand, Lewis (67) noted that copper caused a long lag phase in the growth of *A. agile* and that iodine reduced the effect. The ability of the latter to remove the former from solution with the formation of the insoluble copper iodide might easily account for the observation.

Flagellation of *Azotobacter* was restudied by Hofer (68) and all species were found to be peritrichous. Using an old stock culture, Lipman & McLees (69) corroborated previous work of others that a rough black pigmented, a white, or a brown mucoid strain could develop from a parent culture. Emphasis was placed on the appearance of the rough black stage without the use of nitrate, benzoate, or other materials. The dissociation was spontaneous, ap-

parently, and no attempt was made to obtain other forms. The gum produced by *Azotobacter* was analyzed by Cooper *et al.* (70). The polysaccharide was about 90 per cent glucose and 3 to 4 per cent uronic acid residues and belonged to the same class as that of the pneumococcus, Types II and III.

The biochemistry of nitrogen fixation was reviewed and discussed by Burk & Burris (71) and by Burris & Wilson (72). As a result of their own studies using isotopic nitrogen and the work of others, Burris & Wilson (73) concluded that the information at hand favored the view that ammonia was the first stable intermediate formed in nitrogen fixation by *Azotobacter*. Wilson & Burris (74) again reviewed the subject and added to the discussion.

The immediate weather conditions were reported by Bortels (75) to influence nitrogen fixation, i.e., the barometric pressure, moisture, temperature, and light. Seasonal differences in the rate of the process as reported by Roberts & Olson (42) should be expected but one would hardly expect that barometric pressure and light would have any effect. The latter suggests the work of Dhar and his co-workers in India, who have published a series of papers over a period of years on the photochemistry of nitrogen fixation in tropical soils. In a recent paper, Dhar (76) still reports extraordinary gains in nitrogen attributed to the effects of light.

One very curious observation was published by Peklo (77). Aphids, beetles, etc., were found to have *Azotobacter* associated with them which enabled them to gain in nitrogen and fat. He apparently crushed the insects and stained the smears, since no mention was made of any cultures. In view of the extensive work of Steinhaus and others (78) it is very doubtful if Peklo's observations can be substantiated.

Nitrogen may be fixed by other microorganisms in the soil, although they have not received as much attention as is given to *Azotobacter*. De (79) reported fixation by blue-green algae in rice fields, but no benefit of growing *Azotobacter* and algae together. Similarly, Stokes (80) reported that no nitrogen was fixed by mixed cultures of *A. chroococcum* and green algae, owing to the small amount of organic matter secreted by the algae. Bortels (81) included *Nostoc* in his studies of the effect of molybdenum on nitrogen fixation. Very little work seems to have been done on *Clostridium*, at least, in its relation to soil processes. Jensen (82, 83) in

laboratory cultures demonstrated that nitrogen was fixed by *C. butyricum* in symbiosis with certain cellulose decomposing organisms and that molybdenum was essential for the process, as has been noted above for other organisms. Nine strains of *C. butyricum* and one of *C. acetobutylicum* were used in the latter work and in case of five strains of the former, vanadium could replace molybdenum. He stated

> It thus appears that molybdenum, partly replaceable by vanadium, is a specific catalyst of nitrogen fixation in *C. butyricum* as well as in *Azotobacter* and probably in other nitrogen-fixing forms of life.

Symbiotic Nitrogen-Fixing Bacteria

The beneficial effect on soil and the succeeding crop of growing a legume has been known for ages and it is equally well known that there exists a symbiosis between the plant and the bacterium which inhabits the nodules on its roots. Because of the great economic importance of legumes and the scientific interest in nitrogen fixation a tremendous amount of research has been carried on, both on the microorganism and on its relation to the plant. But, as yet, the secrets of the association are still unsolved. The literature on the biochemistry of symbiotic nitrogen fixation was adequately reviewed by Wilson (84).

The symbiosis must depend upon a delicate balance of factors, either as concerns the plant or the microorganism. Chen & Thornton (85) favored the idea that the poor growth of ineffective strains of the bacterium in the nodules indicated that tissues of the host plant provide an environment that was less suited to the ineffective than to the effective strains. Whether this unfavorable factor is normally present in the plant or whether it appears as a consequence of infection by the ineffective strain is not clear. They did find that the ineffective nodules began their growth quite normally and only later showed arrested development. In fact, their data showed no evidence that ineffective strains were really less efficient than the effective strains in fixing nitrogen, if the unit of bacterial mass in the nodules and the length of time before the nodules collapse and disintegrate are considered. One wonders, therefore, if the commonly used term of "parasitic strain" really indicates that condition or whether the blame for the lack of complete symbiosis should not sometimes be placed on the plant.

Nutman (86) approached the problem of "effective and ineffec-

tive strains" from the angle of the genetic constitution of the plant. He found that among many thousands of plants raised from commercial seed, one plant was "resistant" to normally effective bacteria. From this plant a "resistant" line was raised. This factor, however, was only one of several which influenced the symbiosis. He drew the general conclusions that the number and activity of nodules formed on the plant depended upon the interaction of factors, in the bacteria and in the plant, both liable to change by mutation. The relationship, then, would seem to be much more complex than is generally supposed.

This complexity was emphasized by the results obtained by Vincent (87, 88) and later by Erdman (89), both working quite independently. They found great variability in the effectiveness of various strains of *Rhizobium trifolii* on *Trifolium*. This indicated the necessity of selecting proper cultures to obtain maximum nitrogen fixation and growth of the plant. It would seem that the work of these two investigators complicates the manufacture and distribution of cultures which would produce maximum benefit to the plant. This point was brought out further by Nutman (90) wherein he reported that stock cultures of the effective and the ineffective variants maintained on agar showed an occasional tendency to produce new variants in effectivity. If the effective strain was stored in sterilized soil, a considerable proportion of the population proved to be ineffective. Reversion to the effective parent type by plant passage occurred in only two out of more than thirteen thousand nodules. Plant passage had no effect upon the ineffective stock culture. Although conditions of the experiments were different, these results would seem to be at variance with those of Krassilnikov (91). After prolonged culture in filtrates of clover, he found that nodule bacteria from vetch, pea, alfalfa, and bean acquired the ability to form nodules on clover. Fermentative changes were also noted, but not cultural or morphological. Whether inoculation by an effective strain can occur after an infection by an ineffective strain was restudied by Virtanen & Linkola (92). It was established that effective strains could not usually form nodules after a prior infection by an ineffective strain. Differences in this respect were noted, however. They ascribe this to an immunity in the plant set up by the first infection. An attempt was made to divide the roots and inoculate the two halves with effective and ineffective strains, but the results

were inconclusive, owing perhaps to the lack of complete bacteriological control.

The question of nitrogen fixation by excised nodules was reexamined by Machata, Burris & Wilson (93). Isotopic nitrogen and postulated intermediate compounds were used in these experiments with inconclusive results. During five years of study on the subject in their laboratory, one hundred and thirty-three samples of nodules from plants grown under a variety of conditions and subject to numerous diverse treatments were studied. It was concluded that unequivocal evidence of nitrogen fixation was lacking and that the inconsistent results reported in the literature may well have arisen from inadequate bacteriological control, most of the nodules having been grown in unsterile conditions in regard to other bacteria.

The fact that molybdenum was essential for nitrogen fixation by *Azotobacter* suggested to Jensen & Betty (94), among others, that it might also be essential for symbiotic nitrogen fixation. Their results indicated that molybdenum stimulates the process, besides presumably being required for general metabolism of the plant, and that vanadium cannot replace it. Anderson (95) reported the astounding fact that one ounce of molybdenum trioxide per acre gave a high order of increased yield of clover in pastures in Australia. This work was extended by Trumble & Ferres (96), and others, and clearly showed the need of this element, especially on the sandy soils in the mountainous regions of South Australia.

Attempts have been made in the past to separate the various species of *Rhizobium* by serological methods. Vincent (97, 98) studied the alfalfa and clover bacteria and found great heterogeniety among strains of each group, no relationship existing between the host species and the serological reaction of the organism. Kleczkowski & Thornton (99) reported similar results. Strains derived from peas showed a close resemblance in agglutination reactions to others derived from clover; on the other hand, strains that were totally unrelated in antigenic structure were found among those isolated from either one of the host plants. In a way, this bears out the contention of Wilson (100, 101) that the cross-inoculation groups should be abandoned because nodulation occurred quite promiscuously in his experiments. Further evidence for this position was given by Wilson & Chin (102) in work on the

root-nodule bacteria associated with species of *Astragalus*. The criterion for this work was the production of nodules without regard to whether the plant was benefited or not. Naturally, this has led to considerable criticism. It should be remembered, however, that, as noted above, effective and ineffective strains may be produced in the same nodule and that the latter may originate from the former in a test tube. We should not, therefore, for purposes of taxonomy, require that nodules should be beneficial to the host plant. This reviewer has believed for a long while that only one species of *Rhizobium* should be recognized and that effective strains should be considered as varieties and carry the name of the host plant. It seems to him that the confusion now existing would be clarified. No one could object to a variety of a *Rhizobium* species changing into another variety, whereas if these same organisms were designated as species, he would have valid grounds for objecting (i.e., one species changing into another species).

The significance of hemoglobin in the nodules first noted by Keilin & Wang (103) was reviewed by Virtanen (104) and further discussed by Keilin & Smith (105). No unanimity of opinion has been reached except that its presence in the nodule is correlated with nitrogen fixation.

After a study of the vetch nodule bacterium in culture, Gaw (106) concluded that the morphological variations observed in no sense represented stages in a life cycle and that regular stages were not passed through. Lilly & Leonian (107) also working with pure cultures demonstrated that there was a close relationship between the iron content of the medium and the need for accessory growth factors. As to the effect of growth factors on nodule formation, Guyot (108) reported that thiamine increased nodules on alfalfa and peas but the results were erratic on beans, peas, and soybeans.

Various explanations have been suggested for the decline of the legume bacteria in soil. In the past the main emphasis has been placed upon the presence of a bacteriophage. Vandecaveye *et al.* (109) presented more proof of the presence of a phage in soil and nodules. The only logical explanation for the poor growth of alfalfa in their experiments seemed to be that the lysis of the alfalfa nodule bacterium caused a drastic reduction in symbiotic nitrogen fixation. Katznelson & Wilson (110) made a survey of soils in New York State and found the phage present in practically all the alfalfa fields examined. They appeared to be undecided whether

this should be considered a normal condition or whether it might be a factor in limiting symbiosis.

The question of whether antibiotics were active against the nodule bacteria was investigated by Trussell & Sarles (111). In liquid culture, certain strains were definitely adversely affected. Taking this as a lead, Robison (112) isolated from soil by the giant colony technique two cultures each of bacteria, actinomyces, and fungi which were antagonistic to the legume bacteria. When these were added to sterilized soil in a greenhouse experiment, they appeared to interfere with nodulation. This would suggest that antagonists may be responsible for the decline of legume bacteria in soil.

Autochthonous Soil Bacteria

A large percentage of the colonies appearing on plates made from a soil suspension belong to a group commonly called the "autochthonous" or the "indigenous flora." They are usually slow growing and not very active physiologically and consequently some are difficult to classify. They have been studied by various workers but their identity and relationships have not been fully worked out. Topping (113, 114) made an attempt to group some of them according to their morphology and reaction to Gram's stain. Both of these characters, however, are quite unstable in this group. She did demonstrate that higher plate counts could be obtained if yeast extract was included in the medium. Lochhead and his co-workers (115, 116) attacked the problem of characterizing this group by determining the nutritional requirements of the organisms as regards amino acids, specific growth factors, yeast extract, and soil extract. Seven groups were established, varying from those bacteria that would develop in the simple basal medium to those that failed to grow even with the above additions unless soil extract was also added. The latter group comprised 19 per cent of the isolates from soil. The factor, or factors, present in the soil extract was not concerned with the ash constituents but was present in the acetone extract and was adsorbed by charcoal and recovered by elution. It was noted that the extract from a fertile soil was much more effective than that from a poor soil. Topping (114), however, failed to find that soil extract had any special value. No doubt variations in methods of preparation have been factors in studies of soil plating media.

More work, especially with divergent soil types, should be done to determine the relative value of soil extracts.

It is doubtful whether the nutritional grouping will be of any value as far as bacterial classification is concerned. It has, apparently, been of value in determining shifts in population due to soil treatment. For instance, Hildebrand & West (117, 118) found that soybeans, carbohydrates, and acetic acid induced an increase in bacteria requiring known amino acids and growth factors and a decrease in those with very simple nutritional needs. Incidentally, this shift in population was associated with a decrease in strawberry root rot in that soil. Katznelson & Chase (119) corroborated the observation of Taylor & Lochhead (120) that in a soil of a given type the relative incidence of the nutritional groups is very similar regardless of the fact that one was a fertile and the other a poor soil. Easily decomposable materials stimulated a temporary change, whereas slowly decomposable substances had a more profound and persistent effect.

The function of the autochthonous flora is probably concerned with the decomposition of the more resistant soil organic matter rather than with easily and quickly decomposable substances which are sometimes added to soil. Certain groups, therefore, should be expected and have been found to be able to attack ring and heterocyclic compounds. Plotho (121) isolated from soil strains of *Proactinomyces* which had this ability, each strain being specific for a particular substance. The mechanism of the oxidative destruction of the benzene ring was studied in more detail by Evans (122), who also included a good review of the literature on the utilization of aromatic compounds by soil microorganisms. A pure culture of a *Vibrio* oxidized completely both phenol and benzoic acid with the formation of intermediate products which were confirmed by isolation. Cholesterol was found by Turfitt (123) to be decomposed by two new species of *Proactinomyces*.

Additional work was done by Taylor & Lochhead (124) and by Taylor (125) on the occurrence and characterization of *Bacterium globiforme*. Of ninety soils selected from various fields in Canada, eighty-nine contained this organism. Large numbers were present if the reaction of the soil was above pH 5.0, but no relationship with soil fertility was found, although higher numbers were sometimes found in fertile soils owing to the greater bacterial population.

From time to time, studies are made on the relationship of the

crown gall organism and its nonpathogenic counterpart found in soil. These species are now included in the new genus *Agrobacterium* in *Bergey's Manual* [(126), p. 227] and, as the classification indicates, they are very close systematically. Coleman & Reid (127) in a serological study of *A. radiobacter* and *A. tumefaciens* found that they were identical in the S phase but dissimilar in the M phase. Their results suggested that the two species represented a single species and that in their M phase they bore to each other the same relationship that had been found between the various types of the pneumococcus. Studies on the nutrition of these two organisms by Starr (128) verified the previously reported fact that these two species could grow in a purified inorganic medium whereas certain other species of the genus required vitamins. The work on the S and M phases had not been published when he did his work and he did not go so far as to suggest that they might be identical except for pathogenicity.

Although not a function alone of the autochthonous flora, the decomposition of hydrocarbons will be mentioned here. ZoBell (129) reviewed the extensive literature on the subject and concluded that hydrocarbon-oxidizing microorganisms were widely distributed in soil, water, and recent marine sediments and especially abundant, of course, in oil-soaked soil. The nature of the organisms varied greatly, nearly a hundred species of bacteria, yeasts, and molds having been shown to have that ability.

Sporeforming Bacteria

An antithesis to the autochthonous flora considered above is the rapid growing versatile group of aerobic sporeformers. They generally constitute only 5 to 10 per cent of the soil flora and probably are not important functionally except in special instances. During the past decade they have been extensively studied as to their characterization, classification, and relationships. The fact that they may appear anywhere because of the formation of heat and drought resistant endospores makes them of general interest to those working in food, dairy, medical, and other laboratories. A special interest has recently been shown in certain species due to their ability to form antibiotic substances. Smith, Gordon & Clark (130) obtained and studied a large number of authentic named species of the genus *Bacillus*, and also included numerous isolations from soil. The variability of each species was determined

so far as possible from laboratory studies, in other cases by the appearance in the collection of variants listed as individual species. Cognizance was taken of the different stages of growth, i.e., rough, smooth, mucoid, rhizoid, and dwarf, and of variation in other characters. Many named species were, therefore, found to be merely stages of growth or variants of a "basic species." For instance, *Bacillus subtilis*, which normally had a rough surface, might appear in bakery products as a slimy organism which had previously been called *B. panis;* if the growth had a folded surface, it was either *B. vulgatus* or *B. mesentericus* (European strain); if a black pigment was formed, it was either *B. niger* or *B. aterrimus;* and if red, it was *B. globigii*. Many of these variants spontaneously changed to the basic species (*B. subtilis*), others were more stable and required considerable manipulation to induce the change. Lysis by a particular bacteriophage isolated from soil was used to good advantage on certain species, especially *B. cereus*, *B. megatherium*, *B. pumilus*, and *B. brevis*. The fermentation of carbohydrates was valuable if ammonia nitrogen instead of peptone was used in the basal medium, and a liberal interpretation was placed on the results. Adaptive enzymes were often found. Many strains unable to utilize a particular carbohydrate could be induced to do so by ageing and serial transfer on the medium containing that substance.

Of special interest was the finding that the rhizoid *Bacillus mycoides* would easily dissociate into a nonmotile *B. cereus* and that these dissociants could not be distinguished from certain cultures of *B. cereus* of soil origin or from nonpathogenic cultures of *B. anthracis*. They postulated, therefore, that *B. anthracis* was a pathogenic variant of the soil *B. cereus*. For convenience, however, *B. anthracis* was retained as a separate species by Smith in the sixth edition of *Bergey's Manual* (126, p. 706).

Gibson (131, 132) and Gibson & Abdel-Malek (133) studied the *Bacillus subtilis* group and came to practically the same conclusions as were published somewhat later by Smith *et al.* (130). The two groups of workers disagreed in one point, however; Gibson maintained that *B. licheniformis* was distinct from *B. subtilis*, whereas Smith and his co-workers considered it as a vigorous strain of *B. subtilis*. Lamanna (134) by the use of precipitogens from spores corroborated Gibson's results but he made different recommendations as to the names to be used for the two species.

Lemoigne and co-workers (135) studied the production of β-hydroxybutyric lipids and acetylmethylcarbinol as a means of separating the genus into four divisions, namely, those species positive in both respects, those negative in both respects, and those positive in one and negative in the other test.

Cellulose Fermentation

The fermentation of cellulose by soil microorganisms still seems to be a fruitful field for research. Fuller & Norman (136) isolated and described five new species capable of fermenting cellulose to a greater or lesser degree; three species of *Pseudomonas*, one *Achromobacter*, and one *Bacillus*. They found that the presence of xylan in corn stalks allowed a greater destruction of the cellulose and that, in the case of vigorous bacteria, the decomposition of cellulose increased as the lignin decreased. With weak organisms, no difference was noted. They thought that the inhibition of lignin was mainly physical. Alarie & Gray (137) isolated from Quebec soils thirteen cultures of aerobic bacteria that decomposed cellulose, eight of which they assigned to new species; five to the genus *Bacillus*, two to *Vibrio*, and one to *Bacterium*. These were briefly characterized and apparently no effort made to compare them with known species, nor was there any quantitative determination made of the cellulose decomposed. This was apparently slight in most cases, filter paper breaking in a peptone solution usually in one to three weeks. With a majority of the cultures, growth on cellulose agar failed to give a clear zone. Two of the new species of the genus *Bacillus* were separated merely on the ability to ferment dulcitol. These and other considerations suggest that a more thorough study of these newly named species is very essential.

Perlin, Michaelis & McFarlane (138) used an impure culture of one of the above new species (*Vibrio perimastix*) and determined the products of decomposition. It was brought out that alkali treated cellulose was more easily attacked than untreated cellulose, 30 per cent of the former and 15 per cent of the latter being decomposed in two weeks.

The fermentation of cellulose by the *Myxobacteriae* has been studied vigorously by many investigators. Stanier (139) summarized the work up to 1942 and included a proposed classification and a brief description of the species. Fuller & Norman (140) extended the information on the *Cytophaga* group and named three new

species, two of which had a weak action on cellulose which was soon lost in the laboratory. The physiology of these isolates was more versatile than noted in other species, which necessitated a revision of Stanier's key to permit the inclusion of those forms. The question of whether *Cytophaga* could use glucose was settled in the positive by Fåhraeus (141) and Stanier (142); the former later reported (143) that cellulose was split to glucose which was consumed at once. He thought that the mucilage found in cultures was synthesized from simple compounds.

The *Myxobacteriae* decomposing chitin were studied by Stanier (144) and found to belong to the genus *Cytophaga*. Their nutrition was unspecialized and good growth occurred on a variety of media. Singh (145) observed that various species produced an extracellular enzyme capable of passing a cellophane membrane, which could lyse gram negative bacteria to a greater extent than the gram positive.

In soils more acid than pH 5.0, Skinner & Mellem (146) found fungi active, whereas if the pH was above 5.0, both fungi and bacteria were responsible for cellulose decomposition. Various other factors were examined by Reese (147) and two methods for studying cellulose decomposition quantitatively were worked out which involved nutrition and aeration. *Sporocytophaga myxococcoides* and *Cellulomonas* spp. were used. These bacteria were found by Jacobs & Marsden (148) to be inhibited by a substance in sawdust from a variety of coniferous trees. The toxic material could be extracted from the sawdust with water or better with a mildly alkaline solution of inorganic salts. The residue, however, was still very toxic and completely inhibited *Sporocytophaga*, whereas the action of *Cellulomonas* was delayed but not prevented. This antibiotic is probably not that isolated by Frykholm (149) from *Pinus silvestris* and named "pinosylvine" by him. But it is apparently identical with the water extract from Western Red Cedar as reported by Southam (150). A wide variety of bacteria and fungi was found to be inhibited but not killed.

The decomposition products of cellulose under anaerobic conditions were acetic and butyric acids as determined by Pochon (151). The anaerobes were unstable in culture and frequently lost their cellulolytic properties. Rotmistrov (152) considered the anaerobic cellulose bacteria as butyric acid organisms and together with Lokhvitskaya (153) isolated from soil several strains

of *Clostridium butyricum* which after five to seven weeks fermented filter paper like true anaerobic cellulose bacteria.

Under thermophilic and anaerobic conditions, Rotmistrov (154) found that *Clostridium illipsosporogenes* n. sp. produced 5 to 10 per cent of alcohol and 45 to 70 per cent volatile acids. Pochon (155) also isolated a new species (*Terminosporus thermocellulotyticus*) which produced acetic and butyric acids and some alcohol. But in studying this organism, Pochon & Sarciron (156) found practically as good decomposition of cellulose under aeration. In this connection, Murray (157) showed that the bacteria usually considered as anaerobic were really aerobic or facultative, and that humidity was the critical factor in aerobic cultures, saturation of the air with moisture being necessary.

The saprophytic chytrids were shown by Whiffen (158) to have some power to dissolve cellulose, varying from a weak to a fair fermentation (35 to 65 per cent decomposed). Stanier (159) demonstrated that the chytrid *Rhizophlyctis rosea* was able to attack cellulose. His results showed also that cellulose or its hydrolyic products, cellobiose and glucose, were its chief, if not its only, carbon source.

Changes in the Soil Population

Effect of herbicides on the soil population.—In recent years the use of herbicides has increased tremendously, especially since the organic forms have been made. Most popular of the latter are various derivatives of 2,4-dichlorophenoxyacetic acid (2,4-D). Smith *et al.* (160) found no significant effect of 2,4-D on the total plate counts, actinomyces, fungi, and protozoa at concentrations up to 500 p.p.m. The nitrifying bacteria, however, were definitely injured with 100 p.p.m. but they recovered in from ten to forty days. The nitrite-forming group was more sensitive than the nitrate-formers. The applications of this herbicide used in these tests were considerably greater than recommended in practice. Payne & Fults (161), however, found that as little as 0.009 lb. per acre drastically reduced the nodulation of beans grown in treated soil and that 0.075 lb. entirely prevented nodulation. In this case, the injury may have been on the plant rather than on the legume bacteria.

The effects of chlorate are more severe. Lees & Quastel (162) noted a bacteriostatic action on the nitrate-forming bacteria which

caused an accumulation of nitrite in the soil. No bad effect on the nitrite-formers was apparent. Smith and his co-workers (160), however, found a great reduction in the numbers of nitrite-formers when 500 lb. per acre of sodium chlorate were added; no determination was made of the nitrate-formers. Although these investigations did not agree exactly, they showed a definite toxicity of chlorate to the nitrifiers. Nelson (163) increased the bacterial activity by adding organic matter to a chlorate treated soil in a laboratory apparatus. Under practically anaerobic conditions, the toxicity of the chlorate was reduced whereas when nitrate was added, the toxicity still remained. Obviously more work on this herbicide is indicated under better controlled conditions.

Ammonium sulfamate, sodium arsenite, and sodium borate were not harmful to any of the soil microorganisms according to Smith *et al.* (160). Ammonium thiocyanate, on the other hand, was inhibitive and bactericidal, but the fungi were stimulated. This probably was due to decomposition products, perhaps hydrocyanic acid.

Effect of insecticides on the soil population.—Highly chlorinated hydrocarbons have recently been developed as insecticides. They are not only used on plants but also in soil, as in the control of the Japanese beetle, wireworms, etc. The effects of dichlorodiphenyltrichloroethane (DDT) were studied by Wilson & Choudri (164) and no injury was noted on ammonification, nitrification, the soil population as determined by the plate counts, and the nodulation of alfalfa, red clover, soybeans, and vetch. Pure cultures of various bacteria, actinomyces, and molds were also not affected. Appleman & Sears (165) likewise did not find any interference with nodulation of legumes when less than 100 lb. of DDT were applied per acre. Heavier applications adversely affected nodulation. Payne & Fults (161) found more injury than this, the number of nodules on bean roots being reduced more than a half by 103 lb. per acre.

Benzene hexachloride (BHC) and chlordane proved to be quite toxic to the nitrifiers, especially to the nitrate-formers, in experiments conducted by Smith & Wenzel (166). A fungicidal action was also noted when a heavy application of 500 lb. per acre was added. In the same tests, a chlorinated camphene had no harmful effects on any of the groups of soil microorganisms. In none of these experiments did the protozoa of the soil appear to be affected.

On the other hand, Lloyd (167) reported toxicity to *Paramecium candatum* when more than 1 p.p.m. of BHC was present.

Effect of organic matter on the soil population.—A great amount of work was done in the early years of the science on the decomposition of organic matter in soil. Much of the research was of a biochemical nature and only casual attention was paid to the organisms involved. In later years analyses made during the decomposition process included a determination of the groups of soil organisms. In a paper by Lockett (168) young and mature rye and clover was said to increase the bacteria, actinomyces, and fungi; the extent of the increase depending upon the nature of the organic material. Stevens (169) added the factor of irrigation to cropped and fallow field soils. In his experiments, the microbiological results correlated well with crop production. Bodily (170) used dried and finely ground green manures in soil and found results similar to those that had previously been reported when the fresh green material was added. The increase in numbers of bacteria reached a peak in three days and then dropped rapidly to the sixth day, after which there was a slow decline.

King (171) found that large amounts of stable manure caused great increases in soil microorganisms and postulated that perhaps this caused the reduction noted in the activity of the cotton root rot fungus, *Phymatotrichum omnivorum*. As a result of this work, Mitchell *et al.* (172) undertook to determine the course of the soil population over a period of a year in the black soils of Texas. Soils cropped to continuous cotton and those receiving sorghum and cowpea residues were analyzed for groups of microorganisms. Plate counts far in excess of those generally reported were obtained, the peak coming in April. In the extension of this work Mitchell, Hooton & Clark (173) and Clark (174) reported that the sclerotia of the fungus could be destroyed in soil devoid of susceptible roots by adding organic matter. Especially important was the observation that cutting below the crown encouraged saprophytic fungi and hastened the disappearance of *Phymatotrichum omnivorum* from diseased cotton root systems. It was concluded from these observations that by exploiting microbial antagonisms a practical line of attack against root-rooting parasites could be undertaken.

Fixation of minor elements by soil microorganisms.—The liming of acid soils has been said to reduce the availability of boron. At first, it was thought to be a chemical fixation, but later it appeared

to be biological. Hanna & Purvis (175) measured the carbon dioxide evolved from an acid and limed soil which showed an increased microbial activity especially in the limed soil. The influence of added boron also was greater in that soil. From plate counts, the fungi seemed to be more affected than the bacteria and the possible use of *Trichoderma* species was suggested as a test for boron deficiency. Previous to this work, Ark (176) attributed the little-leaf or rosette disease of fruit trees to a zinc deficiency. He found that the healthy soil contained mostly fungi whereas the diseased soil contained mostly bacteria. Soil sterilization cured the trouble, as did applications of zinc. He isolated three bacteria, two of which produced the disease when inoculated into healthy soil.

A deficiency of manganese has been said to cause the "grey speck" disease of oats, especially on alkaline soils. Various explanations have been offered for the appearance of the disease but none of the chemical or physical factors seemed to fully explain its cause. MacLachlan (177) isolated manganese-oxidizing bacteria and fungi and attributed the deficiency of available manganese in the soil to microbial activity. Although not connected with any study of this disease, Marsh & Bollen (178) obtained an increase in the mold count on certain Oregon soils, a decrease on one, and an increase in the bacterial count in a peat by adding manganese. Carbon dioxide production indicating microbial activity responded roughly inversely to the available manganese present in the soil. Timonin (179) found that a susceptible variety of oats harbored around its roots a denser population of manganese-oxidizing, casein-hydrolyzing, and denitrifying bacteria than a resistant variety when grown in the same soil under identical conditions. Sterilization of the soil by fumigants reduced or completely eradicated the bacteria capable of oxidizing manganese. A positive correlation was obtained between severity of the disease and manganese-oxidizing and cellulose-decomposing microorganisms.

It would appear, therefore, from these and other publications that soil microorganisms may be instrumental in immobilizing some of the minor elements and thus upsetting the nutrition of the plant.

Microorganisms on the Roots of the Higher Plants

The mycorrhiza.—The literature on the ectotrophic and endotrophic mycorrhizal flora affecting trees has been quite adequately

reviewed by Rayner & Neilson-Jones (180) and by Schmidt (181) and need not be repeated here. The status of the problems was aptly analyzed by the former:

A traditional atmosphere of controversy envelops attempts to unravel the tangled skein of mycorrhizal relationships. The habit is so wide spread among vascular plants, and its expression in different groups so varied that contributions to the elucidation of the problem as a whole are necessarily fragmentary.

The mycorrhiza of crop plants has received less attention than that given to trees. Bain (182) reported that the myorrhizal flora of cranberries consisted of four unidentified fungi. There was no indication that these were necessary nor that they produced any injury. Systemic infection was lacking. Magrou (183) noted that potato roots had more mycorrhiza in good soil than in poor soil, and healthy roots more than unhealthy roots. Previously reported fixation of nitrogen by mycorrhiza was refuted by Bose (184).

The rhizosphere.—The soil immediately surrounding the root has been considered by some investigators as representing the rhizosphere, whereas others have included the roots, or their surfaces, with the adjacent soil. This naturally has led to some confusion since the most abundant flora is on the root surface. If a bacterial analysis of the soil adhering to the roots is made, then the moisture content of the soil from which the roots are taken is an important factor. According to Clark (185), roots from comparatively dry soil gave much higher numbers of microorganisms than roots from a moist soil. He attributed this to the adherence of more soil of a lower microbial content to the moist root. In fact, this was proven by analyzing roots from dry soil and from the same soil to which water had just been added. It is obvious, therefore, that some way of reporting results obtained by the plate count method should be worked out to make the data accumulated by different workers comparable.

The use of the buried slide (Cholodny technique) was suggested by Starkey (186) for studying the flora of the rhizosphere. Results were analogous to those obtained by the plate count method. Linford (187) grew seedlings in a glass chamber made of rings and cover slips and made direct observations on the roots magnified up to nine hundred diameters. He confirmed Starkey's observation that microbial activity was not confined to the older roots but also occurred on root hairs.

Modification of the flora of the roots of wheat by adding organic matter was not successful in Clark's experiments (188), al-

though a great increase in microbial numbers occurred in the soil. On the other hand, Morrow *et al.* (189) observed that organisms inoculated on the seed or seedling could be recovered later from the rhizosphere. Several Soviet writers have reported that the flora of nonleguminous plant roots could be modified by inoculation with a resulting increase in crop yield. This literature was reviewed by Allison (29) in connection with the work on "azotogen." In view of the negative results obtained by him and his co-workers (30) and by Clark (31), these claims of the Russian workers should be substantiated before they are accepted.

The qualitative nature of the rhizosphere flora was studied by Lochhead (190) and Timonin (191) and earlier observations corroborated that a great difference existed between the flora of the roots and that of the soil. That the rhizosphere flora is affected by the secretions from the roots was established by West (192) and West & Lochhead (193). Thiamine, biotin, and amino acids were secreted and favored the development of those types of microorganisms that had complex nutritive requirements. This was called the "rhizosphere effect" and was noted to be different between resistant and susceptible varieties of flax and tobacco. Timonin (194) ingeniously grew aseptic flax plants in solution and noted that the incidence of pathogenic fungi was lowered and that of the saprophytic increased by the "rhizosphere effect" of the resistant variety. Katznelson & Richardson (195) sterilized soil by steam, chloropicrin, and formaldehyde and then made analyses of tomato roots. The same "rhizosphere effect" was found under those conditions. The root flora of mangels was studied in more detail by Katznelson (196) in manured and unfertilized soil. A striking selective action on the numbers of bacteria, actinomyces, fungi, ammonifying and denitrifying bacteria and protozoa was exerted by the mangel roots. The "rhizosphere effect" was also noted on algae, aerobic cellulose-decomposing bacteria, and anaerobic bacteria.

Factors Limiting the Soil Flora

A few years ago the main factor limiting the soil flora aside from the physical factors was thought to be the protozoa. A great amount of work was done over a period of years which has now dwindled to practically nothing. Very recently, Anscombe & Singh (197) tested the effect of eight micropredators on eighty-seven

strains of common and rare soil bacteria. Three of the predators were amoebae, two were slime molds (*Myxomycetes*), and three were species of myxobacteria. Thirteen of the bacteria were attacked by only one predator and seven were inedible to all predators. There was, therefore, great variation in the ability of the micropredator to digest the bacteria, or in resistance of the latter to digestion. Previously Singh (198) found myxobacteria common in soil. Gram negative bacteria were more often attacked by them than were the gram positive. He also studied the myxomycetes (199) and came to the conclusion that they are soil rather than dung organisms. Raper (200 to 203) had made some time before an exhaustive study of the nature, growth, and development of the slime mold *Dictyostelium discoideum.* This species lent itself readily to pure culture study since the spores could easily be picked free of any contamination. Raper & Thom (204) mixed the myxamoebae of two species of *Dictyostelium* and found that they subsequently segregated and gave rise to sorocarps typical of the two species. It was, however, possible to graft portions of a pseudoplasmodium which had fed on a colorless bacterium to a portion of another pseudoplasmodium which had consumed a chromogenic bacterium (*Serratia marcescens*). A portion of the resulting sorocarp was colored red, depending upon the position of the graft containing the coloring matter residue of the bacterium. As a result of his observations, Raper (200) considered this slime mold capable of appreciably altering the bacteriological flora of decaying vegetation in soils.

The isolation of antibiotic substances produced by pure cultures of soil microorganisms in appropriate media has aroused great interest. The work has been adequately reviewed by Benedict & Langlykke (205) and others. Although directed towards the control of pathogens, one can prophesy that increasing interest will be taken in the function of these and other organisms in their natural habitat. Newman & Norman (206) reported that antibiotic or inhibitive substances were present in subsurface soil which prevented rapid development of introduced organisms. Aqueous extracts were without effect, but alcoholic extracts of the soil were inhibitive. Another case of antibiotic activity was given by Nickell & Burkholder (207). *Azotobacter vinelandii* was greatly reduced in numbers or killed completely by actinomycete cultures during incubation together in mixtures of soil and crop residues.

Soil Microorganisms and Erosion Control

The influence of soil organisms in reducing soil erosion has been studied quite extensively during the past few years. A binding action by *Azotobacter indicum* and fungi was noted by Waksman & Martin (208) and Martin & Waksman (209, 210), the latter finding a difference in aggregation due to the materials decomposed. Bacteria seemed to be responsible for aggregation only as they produced by-products that function as cementing materials, according to Peele (211) and Myers & McCalla (212). This is in line with the results of Pohlman & Nottingham (213) that merely numbers of bacteria and fungi did not correlate with aggregation. McCalla (214) found that the quality of the organic matter added was more important than the quantity. Going farther in the analysis, Martin (215) attributed 50 per cent of the effect of *Cladosporium* to the substances formed whereas in the case of *Bacillus subtilis* 80 per cent of its effect was due to the by-products. Martin (216) also found that the microbial by-products, such as polysaccharides, were attacked by at least one microbe and usually by several. Hubbell & Chapman (217) reported that by-products by themselves did not form aggregates and that when such were formed living organisms were always observed in the structure, and that bacteria, actinomyces, and fungi each formed a distinct type of aggregate.

Actinomyces and Fungi in Soil

For a review of the literature on soil fungi, the reader is referred to Waksman (218). Although not complete and dealing mainly with the work in his laboratory, it may serve as a basis of references. During the past decade, perhaps the most interesting work was that of Waksman, Umbreit & Cardon (219) on the thermophilic actinomyces and fungi in soil and composts. Mention should also be made of the classification of the actinomyces by Waksman & Henrici (220).

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