Pathogenic Microorganisms in Soil: An Old Problem in a New Perspective

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ABSTRACT

Early investigations on the survival of pathogenic bacteria in soil have been directed mainly to changes in the numbers of viable populations. More reliable techniques to assess the physiological, biochemical and pathogenic capabilities of such organisms are necessary to gain a better understanding of the nature of the diseases they cause and ultimately of the means to control them.

RÉSUMÉ

Des investigations préliminaires sur la survie des bactéries pathogènes dans le sol ont été particulièrement orientées vers les changements survenus dans le nombre de ces bactéries viables. Des techniques plus sûres, pour évaluer les capacités physiologiques, biochimiques et pathogéniques de tels organismes sont nécessaires pour obtenir une meilleure connaissance de la nature des maladies causées par ces bactéries et finalement le moyen de les contrôler.

Since Waksman's classic review (69) on the survival of pathogenic microorganisms in soil almost 25 years ago, the study of the soil as a natural reservoir or as a temporary refuge for pathogenic microbes has lost much of its initial attraction. This may have resulted from earlier reports which suggested that many pathogenic bacteria tend to disappear soon after their exposure to soil conditions (7, 51, 69, 70) and also to the paucity of methods at hand to ascertain the physiological activities of the microorganisms in the complex soil environment. Certain pathogens are known to persist in or inhabit the soil; their occurrence and behaviour while in residence has continued to be a problem poorly understood. With the development of more sophisticated techniques in recent years, experiments designed to study the behaviour of these microorganisms have become feasible. Data obtained from such investigations could provide fresh clues on the etiology, epidemiology and subsequent control of diseases caused by soil-borne organisms as well as contribute to a better knowledge of the nature of soil and water pollution in our environment. This paper aims to focus on recent advances and current research in soil microbiology which may have application to studies on the ecology of soil-borne microorganisms important in human and animal diseases.

Soils are aggregates of minerals, water, humus, and microorganisms in which the latter live, not in pure culture, but as highly diverse and competitive populations (33). The vast array of extracellular compounds in soil provides the microorganisms with a medium of varied physical and chemical properties not normally encountered in the more uniform natural and synthetic laboratory media.

Various soil components may interact to provide a suite of properties quite different than would be predicted from a knowledge of the individual components. For example, the sand fraction and, to a lesser extent, the silt are quite inactive both physically and chemically. Yet the proportion of each in relation to the other soil fractions largely determines the soil texture, pore-space and bulk density which, in turn, influence greatly the soil aeration, temperature and moisture-holding characteristics. Owing to its high surface activity the clay fraction, which occurs as crystalline forms, actively adsorb cations and organic compounds, thus affecting microbial growth. Soil bacteria are known to occur within the pores of soil aggregates and are often embedded in the clay humic complex of soil (12, 23, 76). Three main groups of aluminum silicate clays abound in soils — kaolinite, illite, and montmorillonite. In addition to their chemical variation, these groups differ in plasticity, cohesion and cation adsorption, kaolinite being the lowest in each case and montmorillonite highest (8). For a given soil, therefore, it is important to know not only the clay content but also the predominant type(s) of clay and the properties associated with it. Several workers

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have shown that clays are probably important determinants in the ecology of certain microorganisms (59, 62).

From a quantitative point of view, the availability of organic constituents, however derived, could be the factor most likely to limit bacterial growth (6). Organic materials in nature are accompanied by inorganic nutrients, but the leaching process may soon transfer some of these nutrients (e.g. K^+, Ca^{++} and Mg^{++}) to exchange sites in the soil colloids. Furthermore, well decomposed organic materials ("humus", etc.) form a major part of the soil colloidal system and may provide a large percentage of the exchange sites. Together with a number of other factors, the types and abundance of various ions adsorbed on the colloids determine the composition of the soil solution. So from the moment of entry of organic matter its contribution to the soil and its relationship to soil fertility and chemistry must be considered with respect to the mineral fraction (3, 6, 8).

Critical evaluation of the soil factors affecting the relative abundance and distribution of microorganisms has not always been possible because of the failure of conventional isolation and cultivation techniques to account for all types of microorganisms occurring in soils. For example, the plate count technique, while useful for limited qualitative and quantitative comparisons, accounts for only a small portion of the microbial population in soil. Furthermore, the plating media normally employed for total counts favour the development of fast-growing cells at the expense of the slow-growing and fastidious members of the soil microflora. Thus, the direct microscopic enumeration of microbial cells in soil always yields much larger (ten to a hundred times) estimates than those derived from standard cultural methods (3, 53). Although it might be argued that the higher counts are due to the inability of the microscopic technique to differentiate between living and dead cells, recent improvements using acridine orange stain combined with ultraviolet fluorescent microscopy to distinguish the living from dead cells revealed similarly higher estimates in the microscopic method compared with the plate counts (12). Special procedures employing carbon-free media (38, 39), using capillary tubes to duplicate normal capillary conditions within soil (2, 12, 40), or examining soil suspensions with the electron microscope (57, 60), have led to the discovery of unusual forms of microorganisms. Certain of these unusual forms which could be observed with the electron microscope but not with the light microscope have been estimated to comprise 80% to 85% of the microbial population in podzolic soils (60). These acidic soils are normally developed under temperate, humid climates and are usually low in organic matter (3). Whether the peculiar microorganisms observed in the various methods are morphological and/or physiological variants of known or unknown species remains to be determined, although Orenski, Bystricky and Maramorosch (39) tended to believe that unusual "polyspheroids" observed in American soils belong to a new hitherto undescribed group of microorganisms. However, many bacteria are known to be pleomorphic or to develop bizarre forms under adverse conditions (27). The occurrence of L-forms in both rhizosphere soils (22, 68) and in antibiotic-treated soils (26) has also been reported.

The predominance of anaerobic conditions in waterlogged areas or in certain organic soils has stimulated studies on the contribution of the anaerobic microflora to soil transformation processes (52). The spore-forming clostridia, including several pathogenic species, have been known to inhabit the soil but information on the relationship between soil factors and the preponderance of specific morphological forms remains obscure. For many years the conventional practice of heating soil samples before attempting to isolate clostridia generally encouraged acceptance of the belief that the clostridia occur in soil mainly as spores. However, Smith and Gardner (54) reported that a high proportion of the *Clostridium perfringens* population in various soils was in the form of heat-susceptible cells suggesting the widespread occurrence of *C. perfringens* vegetative cells in soil. Garcia and McKay (18) did not observe immediate mass sporulation of *Clostridium septicum* after inoculation into sterile and nonsterile soils. Moreover, the vegetative cells persisted for up to 30 days in both soils (18). The possible abundance of vegetative clostridia in soil could pose as a serious infection hazard in the light of recent reports (36, 37, 66) that strains low in "sporulating potency" (expressed as the proportion of spores to the total cells) are highly toxigenic. A variety of toxigenic clostridial strains have, in fact, been isolated from soils when the preheating

temperature was lowered (35, 36, 47, 74). This indicates the relative abundance in soil of heat-susceptible vegetative cells of the potentially disease-producing clostridia.

By a special technique, Casida and co-workers (11, 12) isolated a group of slow-growing catalase-negative, coccoid bacteria in apparent abundance from certain soils. Aliquots from soil dilutions one to two logarithmic units beyond the dilution end point for platable soil bacteria were inoculated into a broth medium and incubated up to four weeks. On primary isolation, the cells appeared to be highly pleomorphic, swollen and formed "fried egg" colonies resembling L-forms so that conclusive identification was only achieved after these were continuously subcultured for a long period and displayed less morphological instability (19). The culture was subsequently identified as *Streptococcus sanguis* (19), a species not previously reported as a soil inhabitant but frequently isolated from patients with subacute endocarditis (43, 73). This organism was isolated from fertile soils with pH values ranging from 7.0 to 7.8 but not in arid soils, soils with low organic matter, nor with extremely high or low pH values (19). The isolation of only one species of streptococci from soil does not preclude the probable occurrence of similar pathogenic groups. It may be speculated that further improvements in techniques and media will show the occurrence of previously undetected microorganisms, including pathogens, in soil. In fact, a newly recorded species of *Actinomyces* which may be related to medically important *Actinomyces* spp. may provide interesting clues regarding the epidemiology of human actinomycoses (20).

The use of methods similar to that of Casida (11) should provide additional impetus to search for more slow-growing fastidious members of the soil population. Thus, a similar method led to the successful isolation of *Sphaerophorus necrophorus* in pure culture from mixed populations containing faster growing microorganisms (10). This human and animal pathogen has been found to survive for up to ten months under swamp pasture (31) and is suspected to persist longer under continuously waterlogged conditions or "filthy ground" (31, 50). This possibility has long been associated by veterinary pathologists with the prevalence of bovine foot rot (a *Sphaerophorus necrophorus* infection) in such conditions, but there have been few attempts to establish its occurrence, prevalence and distribution in soil because of difficulties in the isolation and cultivation of this bacterium. Recent studies in our laboratory indicate that the use of immunofluorescence may prove useful in detecting this organism outside the host.

The seeming inadequacies of cultural procedures have contributed to limitations on the design of experiments for defining the physiological activities of soil inhabitants. It is not sufficient to isolate and describe the types and relative numbers of microbial communities under various environmental conditions; the influence of specific soil properties on the distribution and physiological behaviour of the microflora must be assessed, if not anticipated. Attempts along this line have not been too successful but the novel approach pursued by Stotzky and co-workers (59) demonstrates the value of extensive studies on soil-pathogen interactions. More than five years ago, Stotzky and Martin (61) observed a high correlation between the rate of spread of *Fusarium* wilt of banana (Panama disease) and the predominant type of clay mineral in soil. A particular type of clay, the three-layered silicate mineral, montmorillonite, occurred consistently in all "long-life soils (areas supporting wilt-resistant bananas)", but was absent from all "short-life soils (areas supporting wilt-susceptible bananas)". Subsequent studies revealed that soils without montmorillonite contained the pathogenic fungus *Histoplasma capsulatum* which also occurs in man, domestic animals and the habitats of certain birds and bats (62). Stotsky concluded that the absence of montmorillonite in most soils from which *H. capsulatum* was isolated suggested that "clays are important determinants of its (*H. capsulatum*) ecology". Mineralogical analyses to follow up the suggestion of Zeidberg (75) that red-yellow podzolic soils provided the best natural habitat for *H. capsulatum* indicated that one of the characteristics of the red-yellow podzolic soils is the absence of montmorillonite-type clay mineral. Further studies on the rates of metabolic growth of several bacteria, actinomycetes and fungi showed that the growth rate of these bacteria and actinomycetes were more rapid in soils containing montmorillonite whereas the growth rate of fungi was almost twice as rapid in soils not containing the clay mineral. Adding montmorillonite to soils normally without such clay also in-

creased the growth rates of bacteria and decreased that of fungi (58, 63, 64).

The influence of other soil properties has yet to be ascertained in a manner analogous to Stotzky's approach. Controlled experiments, however, have shown that the soil type (29, 46, 71), moisture (1, 45), aeration (31), temperature (1, 46), pH (14), and organic matter (18, 28, 29) influence the population dynamics of pathogenic microorganisms. Whether the metabolic rates of growth would reflect such numerical changes in viable populations is conjectural in the absence of sufficient data. Studies on *C. septicum* in glucose-supplemented soils showed that the dehydrogenase activity of this anaerobe seemed to parallel the growth indicated by plate counts of viable cells (18). By contrast, Klein and Casida (24) reported that *Escherichia coli* cells recovered after residence in soil appeared to have an altered metabolism although they reverted to a "laboratory metabolism". Such observations suggest that results from plate counts should be supplemented by other growth parameters to provide a clearer picture of the behaviour of pathogens in soil.

The relative abundance of many natural and synthetic compounds has created in the soil a highly competitive environment where microorganisms co-exist and exert an assortment of synergistic and antagonistic effects (69). Antagonism between microbial populations has been cited frequently as the main biological factor restricting the establishment of pathogenic microbes in soil. A recent and interesting example is the seasonal disappearance of a toxin produced by *C. botulinum* type F in mud samples from a small stream. The absence of the toxin has been attributed to inhibition of *C. botulinum* by an antibiotic producer, *Bacillus licheniformis* (72). No direct evidence, however, was presented to show that an antibiotic was the significant factor involved. The capacity of various indigenous soil organisms to produce antibiotics and related toxins is well documented (32, 69). Nevertheless, how these compounds are produced and to what degree they affect the survival of soil-borne pathogens are questions which have not been answered with certainty. Since these substances are probably mainly proteins, it is quite likely that they are susceptible to adsorption and possible inactivation by clay minerals or are degraded by soil microbes. Some amphoteric and basic antibiotics have been found to be strongly adsorbed by the clay minerals bentonite and illite, while the acidic or neutral antibiotics were adsorbed less strongly by these clay minerals but strongly by montmorillonite (41, 42). Soulides *et al* (55) reported no release of either streptomycin or Terramycin from a montmorillonitic soil, while both were released from a kaolinitic soil.

Other natural modes of antagonism have recently attracted considerable attention. Thus bacteriophages active on certain pathogenic bacteria have been repeatedly isolated from a variety of soils (13, 17, 65). Some of these phages isolated have the ability to cause lysis of the host cells or to induce lysogenic conversion of certain bacteria. Compared with the parent strains these lysogenic derivatives show modified morphological and physiological characters and probable alterations in the degree of pathogenicity (16, 30). Among others, lysogenic strains of mycobacteria have been isolated from soil (48).

In addition to parasitism with phages a recently discovered soil bacterium, *Bdellovibrio bacteriovorous* has been found capable of parasitizing on several gram-negative bacteria including, *E. coli, Aerobacter aerogenes, Serratia marcescens, Pseudomonas aeruginosa,* and *Spirillum serpens* (21, 25, 56) as well as possessing the ability to live independently of its hosts (15, 49). Thus, the presence of high numbers of *Bd. bacteriovorous* may be of ecological significance in respect to changes of the bacterial equilibria in certain soils.

OUTLOOK

In recent years, knowledge of soil microbiology has become a prerequisite to the solution of an increasing number of problems associated with the pollution of our environment. These include such problems as: health hazards associated with soil, plant and water contamination (34, 44, 67), dangers from improper disposal of human, farm and industrial wastes (46), the continued alteration of the microbial balance in the environment by indiscriminate use of pesticides and other chemical synthetics (4), and the possible contamination of earth by extra-terrestrial organisms (5, 60). Certain of these problems have been recognized for a long time but research efforts have failed to keep pace. For instance, early investigations on the survival of soil-borne pathogens have been di-

rected mainly to numerical variations of the viable populations. Although such an approach is important, its value is very limited. Evaluation of the physiological, biochemical and pathogenic capabilities of such microorganisms while residing in soil is necessary to gain a better understanding of the epidemiology of the diseases they cause, in order to anticipate their probable behaviour under various soil conditions and to propose possible measures controlling their distribution.

It has been observed that in nature many pathogens seem to survive poorly away from their hosts. However, many do undergo a phase in soil, even if only one of a rapidly declining number (9) and still others continue to persist or adapt to a saprophytic existence in the absence of the host. Microorganisms adapted to a parasitic mode of existence probably do not remain as large, stable populations outside their hosts, but there is no *a priori* reason for saprophytic ability and specialized parasitism to be mutually exclusive (9). It is obviously important to investigate the possible morphological, physiological, genetic and other mechanisms by which such parasites survive under inimical conditions, outside the influence of the host or where the host phase is discontinuous. Investigations of these problems might be facilitated by the use of microorganisms which have a biphasic type of existence, sometimes parasitizing hosts, at other times living freely in soil.

The recent discoveries of soil microorganisms with unusual morphology, L-forms, and the isolation of previously undetected pathogenic species have confirmed the current lack of information regarding the composition of the soil microflora and the need to develop more techniques for recovering a wider spectrum of microbial types in soil. It is hoped that subsequent studies on the behaviour and activities of these organisms would lead to a deeper insight into the mechanisms of survival and persistence of pathogens in soil. Thus we are led to formulate these questions: Are some of the unusual forms recently observed in soils potential pathogens or are they possible unrecognized L-variants of known pathogenic bacteria? Are pathogenic bacteria introduced into apparently harmful soil conditions capable of transforming into L-forms and hence, not readily recovered by conventional techniques? Do L-forms of pathogens survive, stabilize and multiply in soils? If so, what conditions favour their development? Will these forms retain the capability of reinfecting the host and producing the disease characteristic of their parents? Recently, Stotzky (60) asked the fundamental question, "What do microbes look like in soil (are they protoplasts)?" More imaginative research has yet to be done before some of these queries will be answered.

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