Essays in Agricultural and Food Microbiology

Edited by

J. R. Norris *and* **G. L. Pettipher**

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Preface

Microbiology is a subject which is not widely taught to school children but it forms an essential part of many agricultural, food technology, food science and microbiology courses taught in Universities and other higher education establishments. Whilst the balance of microbiology in these courses will vary considerably, the broad nature of the topics covered is similar. Thus, an agricultural course will contain a certain amount of food microbiology in addition to the detailed treatment of microbiology in relation to agricultural production, whilst a food microbiology course will inevitably go back beyond the farm gate, since many of the problems associated with the microbiological status of food have their origins on the farm.

This collection of fourteen essays is designed to cover some of the many diverse aspects of microbiology taught on agricultural, food and microbiology courses. Each author has been encouraged to take a simple introductory approach and the primary readership is defined as the early stage undergraduate student, although many topics will be of relevance to the senior school pupil specializing in biology. Essays on the microbiology of soil, water, degradation of organic compounds, waste disposal systems and diseases of crop plants will be of direct interest to the agricultural student. Essays on specific food commodities lead the interest from agricultural practice into the food industry. Whilst the spoilage of food by micro-organisms is covered, the positive use of microbiological processes in the manufacture of fermented foods, fermented beverages and mushroom production is also included in these and other contributions. The essay on microbial food poisoning covers aspects of both agricultural and food microbiology which may lead to public health problems. These essays should provide a sound basis on which to build an advanced knowledge of the subject from more specialized books and journals. The teacher of microbiology needs to have a working knowledge of certain specialist subjects, often as diverse as microbial ecology and microbial genetics. This collection of essays should be of use to both teacher and student alike as it is written by specialists who are able to provide an overview of their area of microbiology, its current status and likely future developments.

In contributing this brief preface, we would like to thank the contributors for writing and amending their scripts and the staff of John Wiley & Sons for their help in preparing this publication.

Contents

Essays in Agricultural and Food Microbiology
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1

The Microbiology of Soil

G. LETHBRIDGE and J. M. LYNCH

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1 Introduction

At a time when current agricultural policy is receiving much criticism, and agricultural research is undergoing a change of emphasis, it is all too easy to forget that soil is our most important natural resource. As a support matrix and reservoir of nutrients and water for plant growth, soil determines agricultural productivity and hence human welfare on this planet.

Soil is more than just an elaborate inert plant growth medium; it contains large populations of microorganisms (bacteria, fungi, algae and protozoa) along with smaller numbers of soil animals such as nematodes, earthworms and insects. Although both groups of organisms are important components of an intact soil, and to ignore either of them results in gross over-simplification of a complex ecosystem, the soil animals are outside the scope of this essay.

The activities of soil microorganisms have an important bearing on soil fertility and plant productivity. Their beneficial effects include: the conversion of nutrients in forms unavailable to plants into plant-available forms, the promotion of nutrient uptake by plants through the activities of root-associated microorganisms and increased soil aggregate stability, resulting in improved soil structure and aeration. Their harmful effects include plant disease (see Essay 14), both temporary and permanent losses of nutrients from the plant-available pools by immobilization and denitrification respectively and the inhibition of seed germination and seedling growth by microbial metabolites.

The aim of this essay is to review our current understanding of microbial life in soil, but before we can do this it is necessary to describe the physicochemical nature of the environment in which soil microorganisms live.

2 Structure and Chemical Composition of the Soil Environment

Soil consists of a complex structural matrix composed of mineral particles (sand, silt and clay) associated with organic matter in various stages of decomposition. In a good agricultural soil the mineral fraction would account for about 50 per cent by volume, compared to 10–15 per cent for the organic fraction. The remaining space is accounted for by water and air in an elaborate system of pores and channels. The contribution of living organisms to the soil volume is negligible.

2.1 The Mineral Fraction

The mineral fraction of soil consists of three types of particles classified on the basis of size into sand, silt and clay (Table 1). They are derived from pre-existing rock by physical, chemical and biological weathering. Soils are assigned to a particular textural class on the basis of their mineral particle size distribution.

Sand and silt particles are composed primarily of mineral forms unaltered from the parent material (quartz and felspars). Compared to clay these coarse particles have a small surface area and little cation exchange capacity. Consequently they do not retain films of water and organic matter, and as a result are sparsely colonized by microorganisms.

In contrast, the clays are far more influential in terms of microbial activity in soil. Clay particles are multi-layered aluminosilicates. They are so fine (the largest are of an equivalent size to bacterial cells) as to be colloidal in nature. The silicon oxide and aluminium oxide or hydroxide layers are arranged in either a 1 : 1 ratio (Si–Al–Si–Al–Si) or a 2 : 1 ratio (Si–Al–Si Si–Al–Si). The latter are referred to as expanding lattice clays (e.g. montmorillonite), since they have an internal surface area between the adjacent silicon oxide layers

Table 1. Soil particle size in relation to the size of microbial cells and plant roots (after Lynch, 1983)

	Diameter or width (μm)
Mineral particles	
Sand	50–2000
Silt	2–50
Clay	<2
Microorganisms	
Viruses	0.02–0.3
Bacteria (including actinomycetes)	0.2–2.0
Cyanobacteria	2–5
Fungi	0.5–50
Algae	3–50
Protozoa	5–600
Plant roots	
Root hairs	10–14
Roots	>40

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which can absorb water (and nutrients), causing them to expand upon wetting and shrink upon drying. The 1 : 1 clays (e.g. kaolinite) are stable in this respect, because they only possess an external surface area, the alternating silicon and aluminium layers being held close together by hydrogen bonds.

When clay minerals are being formed some of the silicon and aluminium ions are replaced by ions with lower valencies (e.g. Si^{4+} by Al^{3+} and Al^{3+} by Mg^{2+} or Fe^{2+}). This process, known as isomorphous substitution, imparts an abundance of negative charges over the surfaces of clay particles, enabling them to bind cations such as Ca^{2+}, Mg^{2+}, H^+, Al^{3+}, Na^+, K^+ and NH_4^+ from the soil solution. These ions are adsorbed strongly enough to prevent them leaching out of the soil during heavy rainfall, but they are still available to plants and microorganisms. The adsorbed ions are in equilibrium with a pool

Table 2. Influence of soil colloids on substrate decay and microbial growth (from Burns, 1981)

Colloid surface phenomenon	Effect on substrate decay and/or microbial growth (relative to that in absence of clay/humic colloid)
Juxtaposes microbe (or enzyme) and substrate	Stimulation
Orients enzyme beneficially relative to substrate	Stimulation
Functions as buffer during metabolism	Stimulation
Adsorbs inhibitory metabolites	Stimulation
Retains H_2O film = prevents desiccation	Stimulation
Concentrates inorganic nutrients	Stimulation
Supplies inorganic micronutrients (clay)	Stimulation
Protects microbes from predators	Stimulation
Inactivates phages	Stimulation
Produces soluble substrate (humic-enzyme complex)	Stimulation
Adjusts C:N ratios (humic)	Stimulation
Allows co-metabolism of adsorbate (humic)	Stimulation
Performs a biological decay in a biological sequence	Stimulation
Adsorbs microbe (or enzyme) distant from substrate	Inhibition
Intercalates substrate = inaccessible to microbe (clay)	Inhibition
Incorporates substrate into humic polymer = inherited resistance	Inhibition
Inactivates enzymes due to structural changes	Inhibition
Masks active site of enzyme	Inhibition
Increases viscosity = retards O_2 diffusion	Inhibition
Entraps microbe in colloidal aggregate = limited O_2, nutrients, etc.	Inhibition

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of equivalent ions in the soil solution. A measure of this interchangeability is described as the cation exchange capacity of the clay. Anions can also be adsorbed to clay minerals (albeit to a lesser extent than cations), either directly to the much smaller number of positive charges on the clay surface, or via polyvalent metal ion bridges. Adsorption to clay surfaces is not restricted to small ions. Large molecules such as proteins and even microbial cells (which carry a preponderance of negative charges) are adsorbed via charged groups on their surface. Indeed the adsorbed state is the predominant one for soil microorganisms. As a result of their large and highly charged surface areas, clays exert a tremendous influence on microorganisms in soil; some of these effects are summarized in Table 2.

By their very nature clay particles do not occur as discrete entities, but tend to flocculate and become coated with a thin film of organic matter to form the so-called organo-mineral complex.

2.2 Organic Matter

Soil contains an enormous variety of organic molecules exhibiting a wide range of degradability, from the simple sugars used as microbial growth substrates to the recalcitrant phenolics which may inhibit microbial growth. The soil organic matter is a heterogeneous mixture of animal, plant and microbial remains in the early stages of decomposition, breakdown products of this particulate material (carbohydrates, fats, proteins, amino acids) and a brown, colloidal, polymeric residue called humus, which is synthesized from various components of the previous fraction.

Humus itself can be split into three fractions (humic acid, fulvic acid and humin) in terms of their solubilities in acid and alkali. This also forms the basis of a technique for extracting humic polymers from soil. Humic acid (MW 5000–1 000 000) is soluble in alkali but not acid and fulvic acid (MW $< 10\,000$) is soluble in both. Humin being soluble in neither remains behind in the extracted soil. Residue analysis indicates that these fractions are probably different size classes of the same polymer. Since many of the residues are similar to those found in lignin (phenylpropanes), it is likely that humic polymers are partly derived from the breakdown products of lignin. The remainder may be derived from other aromatic compounds of plant origin (e.g. flavonoids) or from *de novo* microbial synthesis. Humus formation therefore results from the cooperative action of microorganisms capable of modifying and degrading lignins and those that can synthesize phenolic polymers. The final product is remarkably persistent; its turnover rate being of the order of 2–5 per cent per year. Radiocarbon dating has shown that the most recalcitrant fractions have half-lives of hundreds of years. The association of humic matter with clay surfaces to form the organo-mineral complex prolongs the lifetime of the organic matter considerably.

The humic colloids have an extensive internal (as well as external) surface area resulting from their expansion upon hydration. Like the clays they carry a large number of predominantly negative charges arising from the pH-dependent dissociation of carboxyl and hydroxyl groups of the polymer, and as a result exert considerable effects on the soil microorganisms, some of which are summarized in Table 2.

2.3 Soil Aggregation

In soil the components described in the two previous sections do not exist as discrete particles. Instead, they come together in an intermingled state to form soil aggregates or crumbs which are responsible for giving soil its structure (Figure 1). The process of soil aggregate formation involves the flocculation of clay particles, the formation of clay–organic matter complexes, the adsorption of clay and humic colloids onto the surfaces of microbial cells, the meshing together of mineral particles by actinomycete and fungal hyphae, adhesion of the particulates to each other by extracellular polysaccharides predominantly of microbial origin (e.g. slimes and capsules) and the humification of these polysaccharides by interaction with polyphenolics to prevent their degradation. Thus microorganisms play a major role in the formation of good soil structure, which is dependent not only on the formation of soil aggregates but also their stability.

Figure 1. Hypothetical arrangement of a soil aggregate. (From Lynch, 1983.) Reproduced by permission of Blackwell Scientific Publications Ltd.

The effect of aggregation is to produce a soil containing an extensive series of pores and channels through which water (and dissolved nutrients) and gases can pass freely. The attributes of a good soil structure include water retention in dry periods, coupled with good drainage to avoid water-logging in wet periods. Good drainage ensures that there are sufficient gas-filled pore spaces in the soil to allow efficient gas exchange with the atmosphere above the soil to prevent the development of anaerobic microsites which would retard the growth of plant roots. The water-retaining capacity of a soil is a function of its clay and organic matter content; the coarser sand and silt particles being responsible for regulating drainage and aeration.

2.4 Water and the Soil Atmosphere

Outside periods of heavy rainfall, well-drained soils contain no free water. Instead, the water is held either as a surface film on the soil colloids or inside the soil pores. When a dry soil is rewetted the pores fill up in order of size, starting with the finest. Although the soil pores are not true capillaries their water retention is primarily a function of capillary effects. Hence, unless a soil is so wet that water drains freely from it by gravity, microorganisms and plants can only absorb water from soil by expending energy in the form of suction forces to overcome the surface tension forces retaining the water in the soil pores. Furthermore, since pure water does not occur in soil, additional energy will have to be expended to take up water bound to solute molecules.

It is obvious from this discussion that expression of the soil water content on a weight or volume basis does not tell us how much of the soil water is available to plants and microorganisms. It is much more important to know the actual availability of water in soil. This is referred to as the water potential, and is a measure of how much work (in terms of suction) an organism must do to absorb water from the soil.

Water potential can be split into two components: the matric and osmotic potentials. The matric potential refers to the retention of water in soil pores by surface tension forces. It is inversely proportional to the radius of the menisci of the water held within the soil pores.

$$\text{Matric potential (dynes cm}^{-2}) = \frac{2 \times \text{surface tension of water (73 dynes cm}^{-1})}{\text{radius of menisci (cm)}}$$

The drier the soil the smaller the radius of the menisci of the capillary water, and hence the larger as a negative value the matric potential. The larger the matric potential the less available the water to microorganisms. In the literature matric potential is often expressed in the non-SI units 'bars' (1 bar = 10^6 dynes cm^{-2}). Water is available to plants over matric potentials ranging from -0.05 bars (field capacity) down to -15 bars (permanent wilting point). Field capacity is the water content of a soil at which free drainage following saturation ceases.

The osmotic potential refers to the reduction of water availability through binding to solutes, but is only important in desert and saline soils.

Those pores not filled with water contain the soil atmosphere which is usually saturated with water vapour. It differs from the atmosphere above the soil in having a reduced partial pressure of oxygen, but an increased partial pressure of CO_2 (generally within the range 0.002–0.02 atm) as a result of plant root and microbial respiration. Gas exchange between the soil and the air above is by diffusion through the pores. This can be restricted when the pores are few in number, very small, tortuous, discontinuous or filled with water. Although oxygen and carbon dioxide are soluble in water it is important to note that gas diffusion is considerably faster through air than water (10 000 times so for oxygen).

As is the case with other nutrients, the distribution of oxygen throughout a soil is heterogeneous, so that even well-aerated soils may have anaerobic pockets where the access of oxygen is impeded, such as in the centre of soil crumbs. Under these conditions the active microorganisms are those capable of using alternative electron acceptors to oxygen, such as nitrate and sulphate. In addition, some volatile microbial metabolites, including methane, ethylene and hydrogen sulphide, may accumulate in the soil atmosphere. All these microbial activities can have deleterious effects on plant root development and will be discussed in greater detail in sections 6 and 7.

2.5 Temperature and pH

Soil temperature is regulated by solar radiation, water content and plant cover. Soils are heated by insolation. The wetter the soil the longer it takes to warm up, because heat is used to evaporate water from the surface. Temperature and fluctuations in temperature are lower under vegetation than in bare soil.

Sub-surface layers are insulated to some extent from the air above the soil, and do not exhibit such wide diurnal and seasonal fluctuations in temperature. In temperate regions the sub-surface soil is cooler than the surface in summer, whereas in winter when the surface is frozen the bulk soil temperature might be comparatively mild.

The pH of most soils lies within the range 4 to 8.5. Acidic soils are found on moorland or under coniferous vegetation where there is high rainfall which causes extensive leaching of bases, whereas alkaline soils are found in chalk and dry grasslands.

The buffering capacity of a soil depends on the cation exchange capacity of the clay and humic colloids, in conjunction with the amount of insoluble carbonates (e.g. calcium) present. Adsorption of hydrogen ions to the soil