Microbial Habitat in Soil

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In soil there lives a great variety of microbes undergoing continual changes in their population but nevertheless maintaining comparatively stable levels. Such a paradoxical feature usually referred to as a dynamic equilibrium of soil microbes is found to be closely related to the characteristics of microbial habitat in soil[1,2].

Differentiation of microbial habitat in soil

Excepting burrowing animals most of soil organisms live exclusively in pore space in soil and thus the size of these organisms or pore space is one of the most important factors for their lives. Soil microbes may be divided into two groups by size; microbes less than several μm in size (group I) and those larger than several μm (group II). Bacteria belong to the group I and other microbes such as fungi, algae and protozoa to the group II. Pore space in soil is also classified into two groups; capillary pores which are less than several μm in diameter and non-capillary pores which are larger than the former. Thus it is expected that bacteria may live in both capillary and non-capillary pores and other microbes only non-capillary pores.

The present author showed that most of microbes in non-capillary pores can be dispersed into water by gentle shaking of soil suspension and those in capillary pores by vigorous treatment such as sonic oscillation. Fig. 1 indicates that, in soil at a field condition, bacteria live more abundantly in capillary pores and fungi in non-capillary ones,

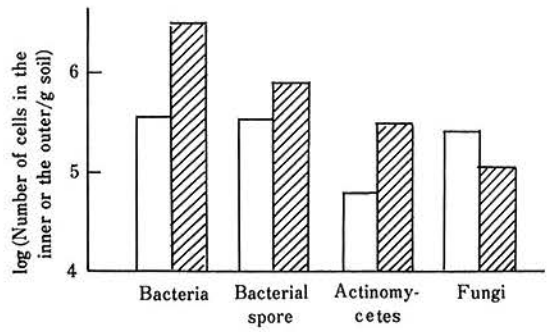

Fig. 1. An example of numbers of various microbes in capillary and non-capillary pores of soil. White column indicates microbes in non-capillary pores and striped column those in capillary pores.[3]

although some fungi such as fusaria prefer capillary pores rather than non-capillary ones[3,4].

Soil moisture or water is an impotrant factor for microbial life in soil. Assuming equilibrium state of soil water system, the forces to retain water in pores can be approximately expressed as

$$pF=\log 0.15-\log r,$$

where r is radius of curvature of the capillary pore and pF is the logarithmic expression of energy of water holding[5]. Based on the equation it is considered that capillary pores retain moisture more strongly than non-capillary ones and as a result bacterial population in the former may vary less widely with changes of moisture content of soil as compared with that in the latter. Fig. 2 shows changes of bacterial population in capillary and non-capillary pores when air dried soil was percolated with glycine solution for 5 days and then dried again. As expected, the wider variation in bacterial population was observed

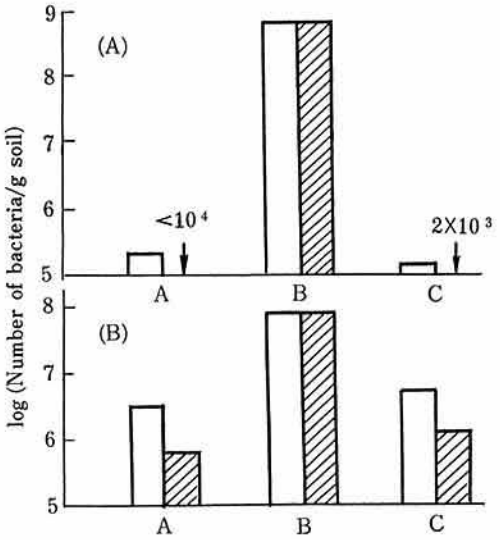

Fig. 2. Variation of bacterial number in the non-capillary (A) and in the capillary pores (B), when soil was percolated with glycine solution. White and striped columns indicate numbers of total bacteria and Gram-negative bacteria, respectively. A is the soil before percolation. B is the soil after 65 hr percolation. C is B dired for 2 days at 50% relative humidity.[3)]

in non capillary pores[3)].

Microbial lives are affected or restricted by the availability of oxygen; aerobic metabolisms go on unless the oxygen concentration falls to a very low critical level and anaerobic metabolisms occur when the oxygen concentration decreases to the low level. Usually the critical value of the oxygen concentration is between 2 and 6 μM. The oxygen supply to microbes in soil may be limited by an inadequate diffusion of oxygen through the gaseous or aqueous phase from the outside of the microstructure of soil towards its inner portion. Consequently anaerobic processes such as denitrification occur more frequently in capillary pores than non-capillary ones depending on moisture content and sizes of soil crumbs.

Discreteness of microbial habitat in soil

Lives of a great variety of microbes in soil are based on or supported by discrete microhabitats. The concept is derived primarily from the fact that soil is a heterogeneous, discontinuous, and structured environment, dominated by soil particles varying in size from less than 0.2 μm to greater than 2 mm. Such discrete microhabitats are typically recognized as capillary pores. Assuming the pores as spheres with the mean diameter of 4 μm and their total volume per gram soil to be 0.1 ml one may expect that there are 10^{10} capillary pores at least. Since the number of bacteria per g of soil is usually less than 10^8, capillary pores lodging bacteria may be less than 1% of them. Consequently contact interactions between bacteria may not be so frequent in capillary pores of soil.

Microbial interactions are also induced by soluble substances biologically produced. However, since water in capillary pores is often discontinuous by being replaced with air, transfer of such active substances from one organism to another is very difficult. Transfer

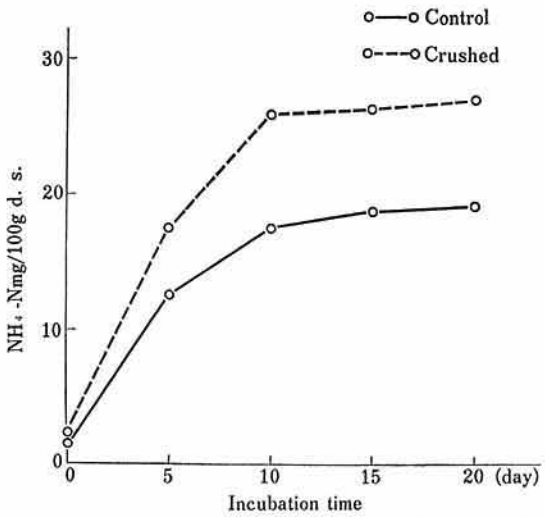

Fig. 3. Effect of mechanical disruption of soil structure on the mineralization of organic nitrogen in soil. K. Hiura, Dr. Thesis of Tohoku University, 1974.

of substances in the pore system may be also obstructed by close or semi-closed pores.

It is to be noted here that organic substances in closed pores are not attacked by microbes for their inaccessibility and becomes decomposable when soil structure is disrupted mechanically. An example is shown in Fig. 3.

Free or adsorbed states of microbial cells in soil

It is a very important problem whether microbial cells are freely suspended in soil solution or adsorbed on soil particles since free and adsorbed cells behave very differently.

Sorption of cells onto soil particles may be determined by a variety of factors. Sorption experiments of *E. coli* onto resin particles as a model show that the growth age of the cells largely affects their adsorbability. Hydrogen ion and salt concentrations are also among important factors.[6] The present author showed that adherence between *E. coli* and clay is determined by size of clay particle and species of ion retained by clay as well as hydrogen concentration of the medium. Fig. 4 shows the stabilities of two types of cell-clay complex as a function of pH; *E. coli*-pyrophyllite homoionic to sodium ion and *E. coli*-pyrophyllite retaining both sodium and ferric ions. The different behaviors were explained as follows; the association of the former is resutled from the interaction between the negatively charged surface of *E. coli* and the positively charged edges of the clay, and that of the latter from the chelating bond between ferric ions on the flat surface of the clay and organic radicals on the cell surface.[7]

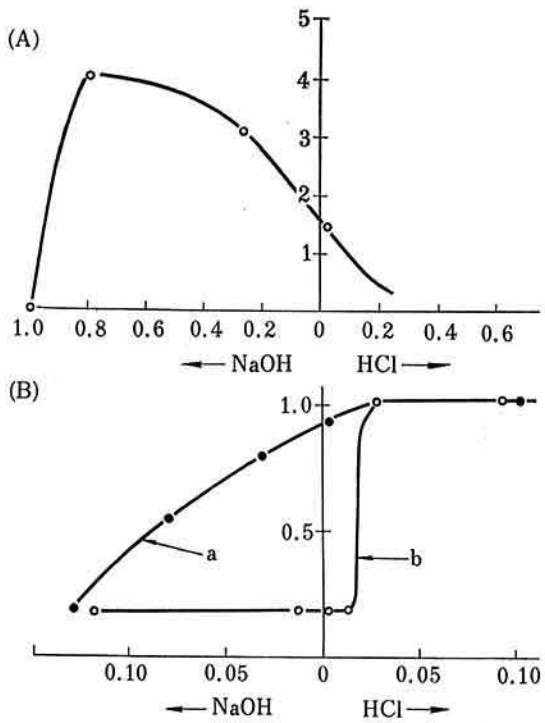

Fig. 4. Effect of HCl and NaOH addition on the stability of the bacterium-clay complex. Ordinate indicates numbers of cells adhered to one clay particle. Abscissa indicates amount of HCl or NaOH added. (A) shows the complex with clay adsorbing Na^+ and Fe^{3+} and (B) that with clay homoionic to Na^+ [7].

Chemical activities of cells adsorbed on a resin, Dowex 1 are generally observed to be lower than those of cells freely suspended. The activity-pH curve was shifted to alkaline side by adsorption in many cases, which was interpreted by a hypothetical cationic layer around adsorbed cells. Lag time observed in induced oxidation of organic substance usually became shorter by cell adsorption, which was attributed to the release of repressing substances (some nucleotides) from the cells by adsorption. The apparent activation energy of substrate oxidation was also different between free and adsorbed cells[8,9,10].

As to the growth of bacterial cells adsorbed on a surface it is essentially important to take into consideration that a surface is two dimensional, contrasting three-dimensional liquid medium. Model experiments with a resin, Dowex 1 showed that growth rates are larger with adsorbed cells than with free cells as shown in Table 1. It was also presented that bacterial cells adsorbed on the resin may multiply on the surface and newly divided daughter cells may remain in the adsorped state until bacterial density on the surface

Table 1. Rates in liquid medium and on the surface of an anion-exchange resin Dowex 1, in the chloride form[2)]

Organism	Specific growth rate (hr^{-1})	
	In the liquid	On the surface
E. coil	0.54	2.4
B. subtilis	0.41	0.67

reaches a maximum value and, after having reached to the value, daughter cells may be released into the culture medium. In the case of *E. coli* the maximum value may be between 10^7 and 10^8 cells/g-resin. It is to be noted that released cells in a continuous flow system show a synchronous growth as shown in Fig. 5, indicating that released cells consist of newly divided ones on the surface[11,12)].

Although it is very difficult to distinguish adsorbed cells from free cells in capillary pores of soil, one may approach this by applying the dependency of settling or centrifuging velocity on particle radius carrying cells. Nioh and Furusaka showed by means of centrifugation in two layered sucrose solution system that, with air-dried soil, most of bacterial cells in non-capillary pores are in adsorbed states but ca 30% of cells in capillary pores are in free states (Fig. 6). Fig. 6 also indicates that, when soil is supplied with a nutrient solution to induce bacterial growth, the percentage of cells in free states markedly increases in both capillary and non-capillary pores[13)]. This result may be interpreted as follows; bacterial cells in adsorbed states may multiply on the surface of soil particles and daughter cells may be released into soil solution after the effective surface area being occupied by predecessor cells as in the case of bacterial growth on a resin.

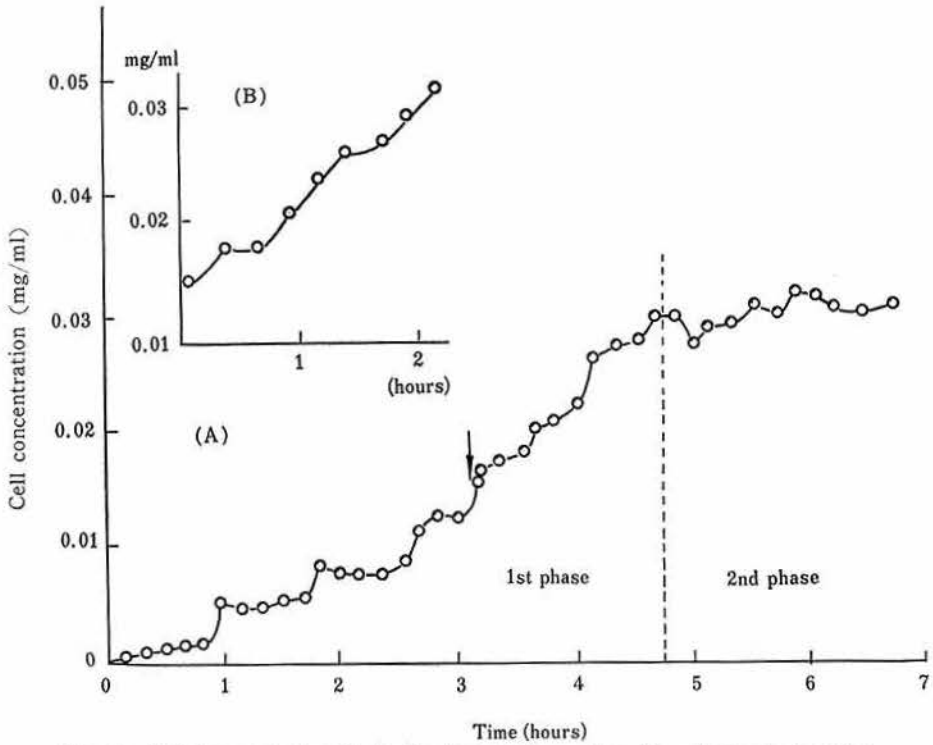

Fig. 5. (A) Increase of cells in flowing medium when *E. coli* adsorbed on the resin was incubated in the flowing medium. An arrow shows that a part of effluent was reincubated in batch system. (B) Growth of the effluent in a batch culture.[12)]

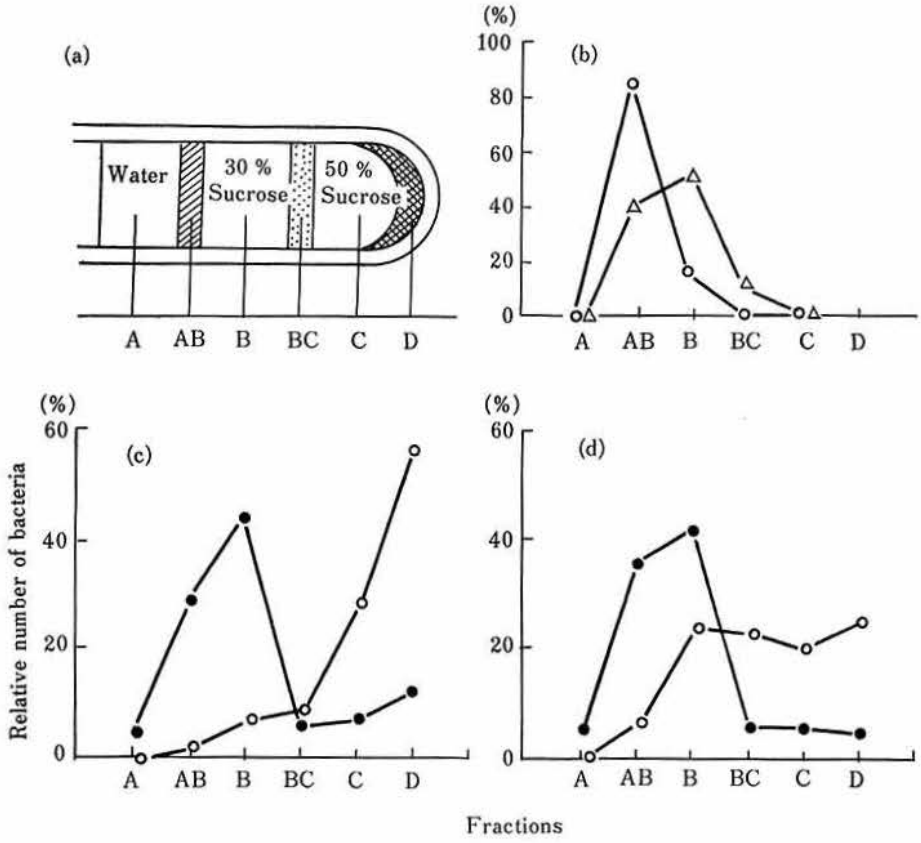

Fig. 6. Fractionation of bacteria in soil in two-layered sucrose solution system by centrifugation. (a) Position of each fraction. (b) Distributions of *E. coil* (opened circles) and *B. subtilis* (triangles) in pure culture. (c) and (d) distributions of bacteria in non-capillary and capillary pores of soil percolated with glycine for 1 hrs (opened circles) and 3 days (closed circles), respectively.[13]

Growth of nitrifying bacteria in soil microhabitat

As to the growth of nitrifying bacteria in soil percolated with ammonium or nitrite solution, a particular situation relating to their microhabitat was proposed[2]. One may make two assumptions; (1) nitrifying bacteria may grow exclusively on some specific solid surface (specific site) exuding sticky substances and thus sorbing so firmly on the surface. Their growth may be ceased when the site is covered by the bacteria or other bacteria, or when the microenvironment directly circumscribing the site is changed unfavorablly. (2) There may be a chance that some cells detach from the site and settle on a vacant site to multiply further. The probability may be proportional not directly to number of bacterial cells, but to the number of sites themselves covered with the cells. The assumptions may interpret relatively low counts of nitrifying bacteria as compared with their activity; the count of nitrifying bacteria by the dilution method may reflect not the number of the organism but that of the site with the organism since it is very difficult to disperse the cells on the site. From the result of percolation experiment by Nishio and Furusaka one may estimate that the mean cell number of nitrifying bacteria per one

specific site is between about 10 and 330[2].

Conclusion

Microbial populations in soil structure are not in a static state but a dynamic state, which may involve two types of equilibria; that is, the equilibrium between various microbes and that between formation and decomposition of soil structure[2] The former may be closely related to differentiation of the microhabitat into capillary and non-capillary pores and the discreteness of capillary water. As to the latter, it should be mentioned that soil particles to be incorporated into soil structure may retain bacterial cells at a probability proportional to their surface area. Among those cells some may be trapped in capillary pores and others in non-capillary pores. Cells in capillary pores may proliferate in response to various nutrient more frequently as compared those in non-capillary pores. As the result of such proliferation one may expect the existence of cells and presumably micro-colonies in free states even in capillary pores of air-dried soil as indicated in Fig. 6.

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