Studies on Giant Amoeboid Organisms

1. The Distribution of *Leptomyxa reticulata* Goodey in Soils of Great Britain and the Effect of Bacterial Food on Growth and Cyst Formation

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SUMMARY: Giant amoeboid organisms may be isolated from soil and other materials by the use of suitable edible bacteria supplied on a base of non-nutrient agar.

Leptomyxa reticulata is widely distributed in the soils of Great Britain. The common occurrence of this organism in soils which have been unmanured or treated with artificial fertilizers for 100 years or more proves that it is a soil inhabitant.

Degrees of pH between 4·1 and 8·7 had no effect on the abundance of growth when a suitable bacterial strain was supplied as food on non-nutrient agar.

Ninety-two very varied strains of bacteria tested as food for *L. reticulata* varied greatly in edibility. Bacteria producing red, violet and blue pigments were mostly inedible. There was no correlation between Gram-staining and edibility.

Certain bacterial strains induced the formation of cysts by *L. reticulata*. This property was not correlated with their edibility.

The use of the recently improved dilution culture method of counting holozoic soil Protozoa (Singh, 1946*a*, *b*), based on the use of edible bacteria as food supply (Singh, 1941, 1942, 1945), revealed the occurrence of giant amoeboid organisms (*Leptomyxa reticulata* Goodey, 1914) in important numbers in arable soils. Goodey (1915), using 0·5–1·0 % agar containing nutrient broth, isolated three new organisms from a few soils receiving large quantities of farmyard manure. Two of these (*L. reticulata* and *L. flabellata*) are multinucleate forms, and the third (*Gephyramoeba delicatula*) is uninucleate. These interesting organisms, though little studied, have been classified by Goodey as proteomyxan rhizopods and placed in two new genera. Until the life cycles of these forms are completely worked out an attempt at classification is premature. An account on the nuclear division and cyst formation in *Leptomyxa reticulata* with certain remarks on its relationships will be published later.

Sandon (1927), in studies of the distribution of Protozoa fauna in soils of various parts of the world, could not find either *L. reticulata* or *L. flabellata*, although *Gephyramoeba delicatula* was recorded on a few occasions. During the past few years the writer has found *Leptomyxa reticulata* commonly present in arable soils. Sandon's failure to find *L. reticulata* and *L. flabellata* was probably due to the use of hay infusion and nutrient agar to isolate protozoa from soil. Goodey (1915) observed that in hanging-drop preparations in 1·0 % hay infusion and egg albumen, toxic substances produced by the bacterial activity check the growth of these organisms and finally kill them. It has been the experience of the writer during the past several years that agar containing nutrients is not satisfactory for the culture of protists which, however, develop

normally on bacteria as the exclusive source of food supply (Singh, 1945, 1946*a*, 1947*a*, *b*). Nutrient media not only encourage the development of inedible micro-organisms but possibly also of those which produce toxic substances. Thus it is essential to use a non-nutrient substrate like plain agar or silica jelly and a suitable bacterial food supply in order to isolate and grow holozoic protists, and for quantitative studies, if reliable and consistent results are to be obtained. The present work deals with *L. reticulata* Goodey.

Method of isolation and culture

The method of isolation was similar to that used in the case of Acrasieae and myxobacteria (Singh, 1947*a*, *b*). One or two loopfuls of a readily edible bacterium growing on nutrient agar slope cultures (2–5 days old) are spread on the surface of non-nutrient agar (1·5 % washed agar in 0·5 % NaCl; pH 6·8–7·0) in the form of a disk or 'bacterial circle' of about an inch in diameter. It is preferable to use Gram-negative bacteria, because they do not encourage the development of lytic actinomycetes which are very commonly present in soils. Several such circles are made in each Petri dish. These circles are then inoculated at the centre either with small crumbs of soil, diluted soil suspensions or with small portions of some other substrate, and the plates are incubated at 21–23° for 2 weeks or more and examined at intervals under the low power of a microscope. In these crude cultures cysts of amoebae in large numbers, together with fruiting bodies of myxobacteria and *Dictyostelium* spp., are usually present in addition to the giant amoeboid organisms.

From the crude cultures giant amoeboid organisms are purified as follows. A portion of the agar containing these organisms, and very few or no amoebae and other micro-organisms mentioned above, are cut and transferred face downwards to freshly prepared 'bacterial circles'. Within 1 or 2 weeks large numbers of giant amoeboid organisms are seen in these cultures. By repeating this process a few times 'pure mixed' cultures of these organisms living on one species of bacteria are obtained. In order to obtain pure-line culture single cysts are picked with a micropipette from a suspension of cysts in 0·8 % NaCl, and each is inoculated to a 'bacterial circle' in a Petri dish. *Aerobacter* strain 1912 (Singh, 1941) was extensively used in the beginning of the work to isolate giant amoeboid organisms from various soils.

Distribution of Leptomyxa reticulata *in soil*

To study the occurrence of *L. reticulata* in soils, samples were taken from the top 2–6 in. The method of isolation and culture was that described above. Fifty-nine soil samples from Hertfordshire, Berkshire, Bedfordshire, Wiltshire, Kent, Cornwall, Glamorganshire, Breconshire, Pembrokeshire and Aberdeen were examined. *L. reticulata* was found in all the twenty-six arable soils. Of thirty-three grassland soils examined only twelve contained this organism. In addition to the soil samples mentioned above, nine soils of the classical plots of Barnfield and Broadbalk at Rothamsted were also examined. Some of these soils have been treated with farmyard manure, some with artificial fertilizers

only and some have been unmanured, these treatments having been continued for about a hundred years. The presence of *L. reticulata* in all these plots proves that it is a true soil inhabitant. A few actively decomposing composts of straw and sludge that were examined also revealed the presence of *L. reticulata*. No correlation between the pH of the soil and the distribution of *L. reticulata* was found in soils ranging from pH 4·3 to 7·8. A few counts that have been made by the dilution culture method (Singh, 1946*a*) from Barnfield farmyard manured plot revealed the presence of *L. reticulata* up to the soil dilution of 1/1000.

Effect of pH *on the growth of* Leptomyxa reticulata

To test the effects of pH on the growth of *L. reticulata* 'bacterial circles' of *Aerobacter* sp. were made on 1·5 % non-nutrient agar adjusted to pH 4·2, 5·5, 6·0, 6·6, 7·0, 7·5, 8·3 and 8·7. *Leptomyxa reticulata* was inoculated in the centre of some of these bacterial cultures while the others were left uninoculated as controls. The plates were incubated at 20–21°. Within 7–10 days the bacterial cultures were completely consumed and large numbers of *L. reticulata* were present. At the end of 10 days bacteria from the control 'bacterial circles' were gently scraped off and a drop of the indicator was added to the agar in this area and another drop to the agar away from the centre. No change in the pH of the agar was produced by the presence of the bacteria. Similar tests showed that no change in the pH of the agar could be found where *L. reticulata* had grown with the bacterial associate for 10 days. Thus it is clear that pH values between 4·2 and 8·7 have no effect on the growth of *L. reticulata*.

Selection of bacterial food by Leptomyxa reticulata

Ninety-two strains of very varied bacteria were used. They comprise common and rare bacteria isolated from soil, plant pathogens (4752, 5945, 1989, 5944, 5942, 385, 5943, 387, 5241 and 1997; see Singh (1942) for the names of these strains), pigmented and non-pigmented bacteria mostly isolated from soil and a few strains of *Rhizobium*. The following pigmented species obtained from the National Collection of Type Cultures, Lister Institute, were also used (*N.C.T.C. Catalogue*, 4th ed. 1936): *Chromobacterium violaceum* (2537), *Sarcina lutea* (611), *Pseudomonas pyocyanea* (*Ps. aeruginosa*) (1999), *Micrococcus roseus* (2683) and *Sarcina aurantica* (952).

The strain of *Leptomyxa reticulata* was derived from a single cyst and was growing on *Aerobacter* sp. To test the selection of bacterial food by *Leptomyxa reticulata* the method of 'bacterial circle' described before was used. In each Petri dish two 'bacterial circles' were made on non-nutrient agar from a growth of the bacterium to be tested, derived from a 4–5-day agar slope culture usually on nutrient agar. Small pieces of agar containing *L. reticulata*, cut from actively growing cultures, were inoculated at the centres of these bacterial cultures, which were incubated at 19–20°. The plates were then examined under low power of a microscope after 7 and 15 days' incubation.

The bacteria tested fell into three groups; some of them were completely eaten (readily or slowly), others were partly eaten over a small area for a few

days, after which *L. reticulata* either slowly died or formed multinucleate cysts; the remaining bacteria were either inedible or were eaten very slightly on rare occasions. *L. reticulata* completely consumed 44·5 % of the bacterial strains tested. Table 1 shows the relation between pigment formation by bacteria and

Table 1. *The relation of pigment production by bacteria to their edibility by* Leptomyxa reticulata

Bacterial strains	Total strains tested	Completely eaten (%)	Partly eaten (%)	Not eaten (%)
Colourless and yellow	65	52·3	24·6	23·1
Orange and brown	13	46·1	30·8	23·1
Red, violet, blue and green	10	0	30	70

the feeding reaction of *L. reticulata*. It is interesting to note that red, violet and green bacteria were not suitable food. This resembles the feeding reaction observed in the case of soil amoebae (Singh, 1945), except in the case of two red strains and a green one which were partly eaten by *L. reticulata*.

Table 2. *The edibility of Gram-positive and Gram-negative bacteria by* Leptomyxa reticulata

	No. strains tested	Completely eaten	Partly eaten	Not eaten
Gram-positive	41	19	10	12
Gram-negative	52	23	15	14

In Table 2 is shown the relation between Gram-staining and the edibility of bacteria by *L. reticulata*. No correlation between edibility and Gram-staining exists, as was also the case with soil amoebae (Singh, 1945).

Table 3. *The difference in food specificity of* Leptomyxa reticulata *and a large soil amoeba tested on eighty-four varied strains of bacteria*

The edibility of the bacterial strains by the two organisms was similar for 62 % of the strains.

	% bacterial strains
Eaten by *L. reticulata* and by amoeba	29·7
Inedible to both	32·2
Eaten by *L. reticulata* but not by amoeba	13·1
Inedible to *L. reticulata* but eaten by amoeba	25

When the feeding reactions of certain holozoic organisms (two species of soil amoebae and myxoamoebae of two species of *Dictyostelium* and *Leptomyxa reticulata*) were compared on eighty-four strains of varied bacteria, it was found that *L. reticulata* differed from soil amoebae in 38 % cases and from myxoamoebae of *Dictyostelium* spp. in 47 % cases. The differences in its feeding reaction from a large soil amoeba are shown in Table 3.

Effect of bacterial food on the formation of cysts in Leptomyxa reticulata

Under suitable cultural conditions and with certain strains of bacterial food supplies a single large multinucleate individual will produce multinucleate cysts. Up to twenty or more cysts in clusters may be produced from each individual.

Table 4. *The production of multinucleate cysts by* Leptomyxa reticulata *on varied strains of bacteria*

Edibility of bacterial strains	No. bacterial strains tested	Relative amounts of cyst formation by *L. reticulata*		
		Large number	Few	None
Completely edible	40	15	8	17
Partly edible	25	5	6	14
Non-edible or slightly edible	27	0	9	18

The formation of cysts in the presence of pure cultures of bacteria was tested on non-nutrient agar as in the edibility tests. In Table 4 is shown the relation between edibility of bacteria and the formation of cysts. Among the forty strains of completely edible bacteria only fifteen lead to the production of cysts in large numbers. In the presence of the remaining twenty-five strains few or no cysts were formed. The same is true of the bacteria that were only partly consumed by *L. reticulata*. Inedible or slightly edible strains produced few or no cysts.

Aerobacter sp. (strain 1912) has been used for more than 3 years to keep the cultures of *Leptomyxa reticulata*. This strain is readily and completely consumed by *L. reticulata*, but no cysts are produced after a few subcultures. When the organisms had been grown for over a year on *Aerobacter* sp., subcultures being made every 2–3 weeks, they were supplied with twelve strains of bacteria, some completely and some partly edible, that normally induced the formation of numerous cysts. No cysts were produced on any one of these twelve strains supplied as food. During the past 2 years the original strain of *Leptomyxa reticulata* maintained on *Aerobacter* sp. has been tested twice to see if it could produce cysts on some of those bacteria on which it easily produced cysts when freshly isolated from soil, but without any success. Thus it seems that this strain of *L. reticulata* has lost its property of producing cysts after being subcultured on *Aerobacter* sp. for over a year. In the first few months of its isolation and culture on *Aerobacter* sp., it could produce cysts when grown in association with certain bacteria. A new strain recently isolated from soil on *Aerobacter* sp. easily produces cysts when fed with suitable bacteria. It may be of interest to mention in this connexion that the importance of certain bacteria for the production of cysts in the cultures of *Entamoeba histolytica* has been realized by several workers who have grown these amoebae on a diet of uncontrolled mixed bacterial cultures growing in a very rich medium. It

has also been noticed frequently that under these conditions of growth *E. histolytica* loses its power to produce cysts. It is quite possible that these amoebae after growing for some time with bacteria unsuitable for cysts formation lose their property to produce cysts, as has been observed in *L. reticulata.*

DISCUSSION

The earlier work of Cutler, Crump & Sandon (1922) at Rothamsted showed that an inverse correlation existed between the numbers of bacteria and active amoebae in 85 % of the daily counts taken over a period of one year. Recently it has been shown (Singh, 1941, 1942, 1945) that soil amoebae do not feed indiscriminately on any bacteria. Besides true soil amoebae, other groups of holozoic organisms, such as species of *Dictyostelium* (Singh, 1947*b*, *c*) and giant amoeboid organisms, have been shown to be commonly present in arable soils of Great Britain. Since these groups of soil organisms feed selectively on bacteria like the true soil amoebae, it seems likely that they are of some importance, affecting not only the quantity but also the quality of the bacterial population of the soil. Although *Leptomyxa reticulata* has been found to be present only up to a soil dilution of 1/1000, its volume of more than 1000 times that of a soil amoeba suggests that it is important in soil economy.

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REFERENCES

Cutler, D. W., Crump, L. M. & Sandon, H. (1922). A quantitative investigation of the bacterial and protozoan population of the soil, with an account of the protozoan fauna. *Philos. Trans.* B, **211**, 317.

Goodey, T. (1915). A preliminary communication of three new Proteomyxan Rhizopods from soil. *Arch. Protistenk.* **35**, 80.

Sandon, H. (1927). *The composition and distribution of the protozoan fauna of the soil.* Edinburgh: Oliver and Boyd.

Singh, B. N. (1941). Selectivity in bacterial food by soil amoebae in pure mixed culture and in sterilized soil. *Ann. appl. Biol.* **28**, 52.

Singh, B. N. (1942). Selectivity of bacterial food by soil flagellates and amoebae. *Ann. appl. Biol.* **29**, 18.

Singh, B. N. (1945). The selection of bacterial food by soil amoebae, and the toxic effects of bacterial pigments and other products on soil protozoa. *Brit. J. exp. Path.* **26**, 316.

Singh, B. N. (1946*a*). A method of estimating the numbers of soil protozoa, especially amoebae, based on their differential feeding on bacteria. *Ann. appl. Biol.* **33**, 112.

Singh, B. N. (1946*b*). Silica jelly as a substrate for counting holozoic protozoa. *Nature, Lond.*, **157**, 302.

SINGH, B. N. (1947*a*). Myxobacteria in soils and composts; their distribution, number and lytic action on bacteria. *J. gen. Microbiol.* **1**, 1.

SINGH, B. N. (1947*b*). Studies on soil Acrasieae. 1. Distribution of species of *Dictyostelium* in soils of Great Britain and the effect of bacteria on their development. *J. gen. Microbiol.* **1**, 11.

SINGH, B. N. (1947*c*). Studies on soil Acrasieae. 2. The active life of species of *Dictyostelium* in soil and the influence thereon of soil moisture and bacterial food. *J. gen. Microbiol.* **1**, 361.

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