Growth and Bacteriolytic Activity of a Soil Amoeba, *Hartmannella glebae*

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A soil amoeba, *Hartmannella glebae*, could grow on a variety of gram-positive and gram-negative bacteria, although the rate of growth was faster in the presence of gram-negative bacteria. The amoeba, however, could not use yeasts, molds, or a green alga as a nutritional source. The extract prepared from amoebae grown in the presence of *Aerobacter aerogenes* and *Alcaligenes faeacalis* could lyse intact cells and cell walls of many gram-positive bacteria at different rates. The spectrum of lytic activity was similar to that of egg-white lysozyme, with the exception that several species and strains of *Bacillus*, *Micrococcus*, and *Staphylococcus* were resistant to lysozyme and susceptible to the extract. The gram-negative bacteria tested were resistant.

A wide variety of microorganisms (bacteria, actinomycetes, myxobacteria, bacteriophages, and fungi) has been shown to possess enzymes capable of lysing bacteria and their cell walls (8, 14).

Although it is known that amoebae can be grown in the presence of bacteria, virtually nothing is known about the way bacteria are used for nutrition after being ingested by amoebae. It was of interest, therefore, to determine whether amoebae have a lytic enzyme system to degrade bacterial cell wall macromolecules preparatory to the utilization of the bacteria as nutrients. Castellani (3) did not detect any lytic effect in extracts of a mixed culture containing a soil amoeba, *Acanthamoeba*, and *Cryptococcus pararoseus* Castellani. There have been no other reports concerning the presence of a lytic enzyme in amoebae since Castellani published his findings.

This paper reports that a new lytic enzyme was found in an extract prepared from a soil amoeba, *Hartmannella glebae*, grown in the presence of bacteria. A comparative study of lytic activity in the presence of extract and lytic activity in the presence of lysozyme was conducted against different microorganisms. The ability of amoebae to grow on different microorganisms is also described. A brief report of some of these findings was made earlier (Upadhyay and Foster, Bacteriol. Proc., p. 30, 1967).

**Materials and Methods**

The amoeba was isolated from soil in conjunction with a gram-negative bacterium which was later identified as *Alcaligenes faeacalis*. The organism was identified as *H. glebae* by E. C. Bovee of the University of California at Los Angeles.

To determine the host specificity necessary for amoebae to grow on different microorganisms, a loopful of a uniform suspension, containing cysts of amoebae and *A. faeacalis*, was inoculated at one end of streaks of previously grown cultures of bacteria, yeasts, molds, and a green alga. The streaks were incubated at 30 C, and the distance traveled by the amoebae as they migrated through a streak was measured at different time intervals.

*Aerobacter aerogenes* was used as a nutritional source in the preparation of a crude extract of amoebae. For the inoculum, several petri dishes containing Brain Heart Infusion (BHI) agar were inoculated with a culture of amoebae. The petri dishes were incubated for 4 days at 30 C until mature cysts were formed. *A. aerogenes* was grown similarly and was incubated overnight at 35 C. Both the cysts and the bacteria were collected and suspended in sterile water. A 2-ml amount of the mixture was used to inoculate BHI agar in large pyrex dishes (3-quart size), and the dishes were incubated at 30 C. After incubation for 35 hr, the amoebae were collected before they formed cysts. They were suspended in cold water and separated from bacteria by several differential centrifugations at 1,200 × g for 5 min. The wet weight of the collected amoebae was 15 g. The crude extract was prepared by suspending the cells in 7 ml of water and disrupting them in a sonifier (Bronson Instruments, Inc., Stamford, Conn.). The extract was centrifuged to remove unbroken cells and debris and was stored at −15 C. The details of assay procedures will be described later.

The cell walls were prepared by the method of Salton and Horne (13).

**Results**

**Growth.** Many microorganisms were tested to determine whether they were utilized as nutrients by amoebae. Ability to migrate on a microbial streak was used as a criterion for growth. Typical
growth on plates after 1 day of incubation is shown in Fig. 1. As the amoebae grew, the translucent or opaque growth of bacteria disappeared. The growth of amoebae was almost transparent, and most amoebae were encysted in the growth area, except at the margin (as shown by arrows) where they were in the amoeboid stage. A variety of bacteria could serve as nutrients for the growth of amoebae. The ability of amoebae to grow on representative genera of bacteria is shown in Fig. 2. In general, gram-negative bacteria were a better nutritional source (growth was faster) than were gram-positive bacteria. After 3 days, the amoebae migrated 6.0 cm or more on gram-negative bacteria and between 1.0 to 3.0 cm in the presence of gram-positive bacteria. Amoebae were able to grow on the vegetative cells, but not the spores, of many Bacillus species. The yeasts, Candida, Torulopsis, and Saccharomyces, the molds Aspergillus and Penicillium, and a green alga did not support amoebic growth.

**Lytic activity.** A comparison between crude extract and lysozyme was made to determine the ability of these substances to lyse a variety of bacteria. Trypticase Soy Agar plates, which were dried at 35 °C for 24 hr to remove condensation moisture, were spread uniformly with bacterial suspensions. One drop each of extract and lysozyme, containing 400 µg/ml, was applied to different areas of the plates, and the plates were then incubated overnight at 35 °C. Any inhibition of growth was the result of lytic activity and was not due to the antibiotic effect (Table 1). Micrococcus lysodeikticus and Sarcina lutea were lysed by both the extract and lysozyme. The extract exhibited lytic activity against three strains of Staphylococcus aureus, M. varians, and M. conglomeratus. Lysozyme did not show any activity with these bacteria. All of the gram-negative bacteria tested were resistant to the extract. As shown in Table 2, of the 29 strains and species of *Bacillus* tested, 25 were susceptible to the extract and only 16 were susceptible to lysozyme.

Quantitative data for lysis were obtained by use of selected genera and species of bacteria which were collected during the logarithmic phase of growth and were suspended in 0.05 M phosphate buffer, pH 7.0. The cell density was adjusted to 150 Klett units (red filter), and 0.2 ml of extract was added to 2.8 ml of the suspension. The mixture was incubated at 35 °C, and the turbidity was monitored at different time inter-

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### Table 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
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<td><em>Aerobacter aerogenes</em></td>
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<td><em>Pseudomonas fluorescens</em></td>
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<td><em>Salmonella typhosa</em></td>
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<td><em>Vibrio cholerae (R)</em></td>
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<td><em>Bacillus megaterium</em></td>
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<td><em>Bacillus subtilis</em></td>
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<td><em>Staphylococcus aureus</em></td>
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<td><em>Staphylococcus epidermidis</em></td>
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<td><em>Sarcina lutea</em></td>
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<tr>
<td><em>Micrococcus lysodeikticus</em></td>
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<td><em>Streptococcus fecalis</em></td>
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<tr>
<td><em>Corynebacterium xerosis</em></td>
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**FIG. 2 Growth of Hartmannella glebae on various bacteria.**

*Fig. 1. Migration of Hartmannella glebae on bacteria.* (A) Bacillus circulans; (B) Escherichia coli; (C) Staphylococcus albus; (D) Alcaligenes faecalis. The arrows indicate the migration of amoebae from the point of inoculation on the left. The growth on B. circulans did not start after 1 day of incubation.
vals. The times (in minutes) required for a 50% reduction in turbidity were: *M. lysodeikticus*, 2; *M. varians* (UT 212a), 20; *S. aureus* (UT 35 and UT 128), 40; *S. lutea*, 5; and *Bacillus megaterium* Penn, 20. *Micrococcus candidans* (UT 213), *S. aureus* (UT 13), Gaffkya tetragea, Streptococcus pyogenes, *S. faecalis*, and all gram-negative bacteria tested, *Escherichia coli*, *A. aerogenes*, *Proteus vulgaris*, *Pseudomonas fluorescens*, and *Salmonella typhi*, were resistant to the extract. Lysozyme (40 μg/ml) did not exhibit lytic activity against the above organisms; these organisms, with the exception of *M. lysodeikticus, S. lutea*, and *B. megaterium*, were susceptible to the extract. Purified cell wall preparations from *M. lysodeikticus* and *Bacillus cereus* ATCC 7064 showed 50% lysis after 8 and 30 min, respectively. Lysozyme did not affect *B. cereus* cell walls. Even though the cells of *A. aerogenes* and *E. coli* were not lysed, their cell walls were partially susceptible.

Extracts prepared from *A. aerogenes* and *A. faecalis*, grown under similar conditions, did not show any lysis against *M. lysodeikticus* and *B. megaterium*. Therefore, the lytic activity was produced by the amoebic extract.

**DISCUSSION**

Various bacteria can serve as nutrients for amoebae. The soil amoebae, *Acanthamoeba castellani* and *Acanthamoeba* sp., have been shown to grow on both bacteria and yeasts (9, 10, 11). Chang (4) demonstrated that gram-negative bacilli were utilized by a strain of *H. glebae* and gram-positive bacilli were not utilized by this organism. This strain of *H. glebae* can grow on gram-negative rods as well as on a variety of gram-positive cocci and rods, including *Bacillus* species. The strain, however, was not able to use yeasts, molds, or a green alga as nutritional sources.

It is well known that amoebae grow at the expense of bacteria they have engulfed. Nero et al. (11) conducted frequent microscopic studies and found no evidence of external lysis of yeast cells by *Acanthamoeba* enzymes. The clearing of growth observed by these investigators was due to devouring of the yeast cells by amoebae. Castellani (3) did not detect any lytic effect of yeast cells from extracts...
of a mixed culture of Acanthamoeba and Cryptococcus pararoseus. Tracey (15) demonstrated chitinase and cellulase activity in some soil amoebae. This is the first report of lytic activity in extracts of H. globae (these extracts can lyse intact cells and cell walls of some bacteria). The lytic effect seems to be caused by an endoenzyme, since no external lysis was noticed on solid media, and engulfed bacteria were observed inside amoeba cells with a phase-contrast microscope.

Bacteriolytic enzymes have been found in several different microorganisms. Myxobacter, now referred to as Myxococcus (6), M. xanthus (1, 7), Aeromonas (5), Pseudomonas aeruginosa (2), the fungus Chalaropsis sp. (8), and Streptomyces (12) attack varying genera of gram-positive bacteria. None of these microorganisms has been shown to lyse gram-negative bacteria. The amoeba extract similarly lysed gram-positive bacteria, although some bacteria were more susceptible than others and some bacteria were completely resistant. The spectrum of lytic activity was found to be similar, in some respects, to egg-white lysozyme. Several species of Bacillus, Micrococcus, and Staphylococcus were resistant to lysozyme but were susceptible to the extract. The cell walls of B. cereus were lysed by the extract but were not lysed by lysozyme.

Further attempts to purify and characterize the lytic enzyme are in progress.

ACKNOWLEDGMENTS
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LITERATURE CITED