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NOTES

Effects of Grazing by the Free-Living Soil Amoebae *Acanthamoeba castellanii, Acanthamoeba polyphaga*, and *Hartmannella vermiformis* on Various Bacteria

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Cultures of 10 different bacteria were used to serve as food sources for axenically grown *Acanthamoeba castellanii, Acanthamoeba polyphaga*, and *Hartmannella vermiformis*. The nonpigmented enterobacteriaceae *Escherichia coli* K-12 and *Klebsiella aerogenes* appeared to be excellent feed to all three amoebae. Hardly any growth or ammonium production was observed in tests with *Chromatium vinosum* and *Serratia marcescens*, which share the presence of pigmented compounds. Distinct differences in net ammonium production were detected and were correlated to the amoebal growth yield. In general, growth of amoebae and ammonium production increased in the order *A. polyphaga, A. castellanii*, and *H. vermiformis*.

Protozoa are the most important predators of bacteria in soil (2, 6, 10, 25) and aquatic systems (3, 14, 15). Because of their predatory activity, they play a major role in controlling the bacterial population in soil (1, 4, 12). However, not all bacteria seem to be an equally suitable food source for protozoa. Gram-negative bacteria were able to survive the presence of many protozoa (1), while biologically formed toxins in bacteria may prevent the attack by protozoa (17). Protozoan grazing stimulates microbial activity and enhances the turnover of nutrients, particularly nitrogen, which would otherwise become immobilized in bacterial biomass (2, 11, 16, 27). Therefore, they play an active role in the control of soil fertility and soil nutrient cycling (13, 23, 24). Knowledge about the role and importance of protozoa in soil food chains was already available but was mainly focused on ciliates and flagellates. Food preferences of free-living soil amoebae and nitrogen mineralization as a result of amoebal grazing are still barely examined. Free-living amoebae are the dominant bacterial consumers in soil (5, 10) and are responsible for 60% of the total decrease of the bacterial population (5). Differences in the ability for amoebae to grow on various food bacteria were previously reported (20–22, 25). However, quantitative results concerning the predator-prey relationship, edibility of various bacteria, and the growth capacity and ammonium production of free-living soil amoebae feeding on various bacteria are scarce. The aims of this study were to investigate food preferences of actively grazing *Acanthamoeba castellanii, Acanthamoeba polyphaga*, and *Hartmannella vermiformis* in monoxenic cultures and to quantitate the amoebal growth response and ammonium production in relation to their food source.

Organisms and cultivation. *A. castellanii* (CCAP strain 1501/1B) and *A. polyphaga* (CCAP strain 1501/3C) were obtained as axenic cultures from the Culture Collection of Algae and Protozoa (CCAP; Ambleside, United Kingdom) and were cultured as reported previously (19). *H. vermiformis* strain Atlanta (strain CDC-19) was obtained as an axenic culture from B. J. Fields (Respiratory Disease Branch, Center for Infectious Disease, Atlanta, Ga.) and was cultured as reported previously (26). Exponentially growing amoebal cells were harvested by centrifugation (10 min, 750 × *g*) and washed (three times) with and resuspended in 20 mM buffered Neff Amoeba Saline (19). *Arthrobacter simplex, Agrobacterium tumefaciens, Bacillus megaterium, Bacillus subtilis, Chromatium vinosum, Escherichia coli* K-12, *Klebsiella aerogenes, Micrococcus luteus, Pseudomonas fluorescens*, and *Serratia marcescens* were cultured overnight in nutrient broth (Oxoid, Basingstoke, United Kingdom) at 30°C on a shaking incubator. Bacterial cells were harvested by centrifugation (10 to 15 min, 25,000 × *g*) and washed (three times) with and resuspended in 20 mM buffered Neff Amoeba Saline. Immediately after harvest, the amoebae and bacteria were used to start monoxenic incubations.

Monoxenic incubations. Axenically grown amoebal cells and pure bacterial cells were inoculated in sterilized 250-ml Erlenmeyer flasks with cotton plugs containing 25 ml of Neff Amoeba Saline buffer (pH 6.8, 20 mM) with initial cell densities of 10^4 amoebae and 10^9 bacteria per ml. Amoebal and bacterial controls for cell numbers and ammonium concentrations were inoculated in the same way and contained either amoebae or bacteria. Monoxenic cultures of *A. castellanii, A. polyphaga*, and *H. vermiformis* with 10 bacteria (Table 1) and amoebal and bacterial controls were incubated simultaneously on a shaking incubator (25°C, 40 rpm) in the dark for 15 days, and bacterial feed (1 ml) was added every other day at a concentration of 10^9 cells per ml in order to prevent substrate limitation. Homogenous samples of 1 ml were taken every other day under aseptical conditions to quantitate amoebal cell numbers (Bürker-Turk

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TABLE 1. Amoebae cell yield and ammonium production resulting from amoeba growth on various food bacteria[a]

Bacterium	*A. castellanii*		*A. polyphaga*		*H. vermiformis*	
	Amoebae yield[b] (10^5/ml)	Ammonium[c] (mM)	Amoebae yield[b] (10^5/ml)	Ammonium[c] (mM)	Amoebae yield[b] (10^5/ml)	Ammonium[c] (mM)
A. tumefaciens	0.55 ± 0.30	1.66 ± 0.10	0.45 ± 0.12	1.55 ± 0.06	10.84 ± 0.84	2.65 ± 0.24
A. simplex	0.31 ± 0.19	0.59 ± 0.08	0.70 ± 0.17	1.73 ± 0.12	12.21 ± 1.60	3.93 ± 0.26
B. megaterium	1.06 ± 0.14	1.82 ± 0.16	0.76 ± 0.22	1.08 ± 0.18	32.05 ± 3.65	6.44 ± 0.54
B. subtilis	2.94 ± 0.13	1.44 ± 0.21	0.98 ± 0.14	0.51 ± 0.07	32.22 ± 2.43	3.35 ± 0.61
C. vinosum	0.02 ± 0.01	0.43 ± 0.18	0.01 ± 0.01	0.35 ± 0.09	0.62 ± 0.08	0.91 ± 0.02
E. coli K-12	10.46 ± 0.44	11.12 ± 0.41	1.60 ± 0.27	1.73 ± 0.19	167.50 ± 15.6	15.95 ± 0.91
K. aerogenes	3.77 ± 0.45	4.75 ± 0.32	2.08 ± 0.37	3.87 ± 0.22	25.55 ± 1.18	6.18 ± 0.52
M. luteus	0.10 ± 0.02	2.47 ± 0.19	0.51 ± 0.11	3.54 ± 0.12	0.35 ± 0.10	1.00 ± 0.04
P. fluorescens	1.44 ± 0.24	1.27 ± 0.22	1.16 ± 0.31	1.52 ± 0.16	11.80 ± 1.42	2.91 ± 0.26
S. marcescens	0.02 ± 0.01	0.62 ± 0.07	0.01 ± 0.01	0.01 ± 0.01	1.90 ± 0.33	0.60 ± 0.08

[a] Incubations were in 25 ml of medium for 15 days, and data refer to increase in cell numbers and are shown as means ± standard deviations; $n = 3$ for individual experiments.
[b] Initial amoebal density was 0.1×10^5 cells per ml.
[c] Values are corrected for bacterial controls.

counting chamber) and ammonium concentration (18) to reveal amoebal food preferences and ammonium production for amoebae grazing on various bacteria. Examples of amoebal growth responses are shown in Fig. 1 for *H. vermiformis* grazing on *E. coli* K-12 and *A. tumefaciens*. The growth yields and net ammonium production for the predator-prey combinations incubated for 15 days are shown in Table 1 and revealed distinct differences not only between the various bacteria used as feed but also among the amoebae tested. Amoebal growth, to some extent, was detected in all test combinations, but *E. coli* K-12 proved to be a far better feed than indigenous soil bacteria like *A. tumefaciens, A. simplex, B. megaterium, B. subtilis, K. aerogenes*, and *P. fluorescens*. Growth yields varied between 0.01×10^5 and 167.5×10^5 cells per ml for *A. polyphaga* grazing on *C. vinosum* or *S. marcescens* and *H. vermiformis* grazing on *E. coli* K-12, respectively. In accordance with the amoebal grazing activity, distinct differences in the amount of ammonium produced for each monoxenic incubation were detected. The ammonium production in the different monoxenic combinations varied between 0.01 and 15.95 mM for *A. polyphaga* grazing on *S. marcescens* and *H. vermiformis* grazing on *E. coli* K-12, respectively (Table 1).

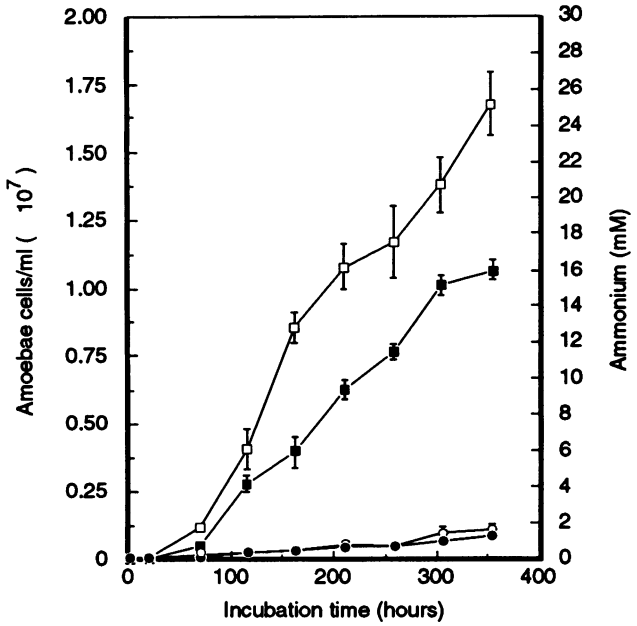

FIG. 1. Typical growth yield and ammonium production curves for monoxenically growing *H. vermiformis* on *E. coli* K-12 (□ and, ■) and *A. tumefaciens* (○ and, ●) as food bacteria. Amoebal cell numbers (open symbols) and ammonium concentrations (closed symbols) are represented as means ($n = 3$ for individual experiments) in which the vertical bars represent the standard deviation.

Protozoa, and especially amoebae, were recognized as the major predators of bacteria in soil, thereby regulating the size of the bacterial population. Amoebal grazing on various bacteria during a 2-week period in monoxenic incubation revealed food preferences as expressed in differences in the amoebal growth yield and concomitant ammonium production depending on the type of bacterial feed. The nonpigmented enterobacteriaceae *E. coli* K-12 and *K. aerogenes* appeared to be a far better food source to all three amoebae than the other bacteria tested. Hardly any growth and ammonium production was observed in tests with *C. vinosum* and *S. marcescens*, which share the presence of pigmented compounds. *A. tumefaciens, A. simplex, B. megaterium, B. subtilis, M. luteus*, and *P. fluorescens*, of which some are indigenous soil bacteria, supported a low to moderate growth of the three amoebae. In general, growth of amoebae and ammonium production increased in the order *A. polyphaga, A. castellanii*, and *H. vermiformis*. Differences in growth yield of amoebae were due to the ability to select their food among different kinds of bacteria (9, 21, 22) and might be explained either by the incapabilities of the amoebae in the uptake or digestion of specific bacteria or by the capability of the bacteria to prevent grazing by means of defense mechanisms, like toxic pigments or special outer membrane structures. Such mechanisms may hamper extensive grazing on bacteria known to be indigenous in soil, like gram-negative *Pseudomonas* and *Agrobacterium* and gram-positive *Arthrobacter* and *Bacillus* (8, 25). Predator-prey relationships can have positive as well as negative effects on the prey organisms. Bacterial numbers may decrease in the presence of amoebae by the consumption of edible bacteria, whereas bacterial numbers of inedible bacteria may increase by a higher rate of mineralization of nutrients in the presence of amoebae (2, 7, 11, 16). Amoebae feeding on bacteria improved the mineralization of nitrogen from soil organic

matter, which previously was immobilized in bacteria, by stimulating the turnover of bacterial biomass (27). Therefore, grazing of rhizosphere microorganizms, especially bacteria, by the free-living soil amoebae has practical importance for agriculture and forestry.

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REFERENCES

1. **Alexander, M.** 1981. Why microbial predators and parasites do not eliminate their prey and host. Annu. Rev. Microbiol. **35:**113–133.
2. **Bamforth, S. S.** 1985. The role of protozoa in litters and soils. J. Protozool. **32:**404–409.
3. **Barcina, I., B. Ayo, A. Muela, L. Egea, and J. Iriberri.** 1991. Predation rates of flagellate and cilliated protozoa on bacterioplankton in a river. FEMS Microb. Ecol. **85:**141–150.
4. **Casida, L. E.** 1989. Protozoan response to the addition of bacterial predators and other bacteria to soil. Appl. Environ. Microbiol. **55:**1857–1859.
5. **Clarholm, M.** 1981. Protozoan grazing of bacteria in soil—impact and importance. Microb. Ecol. **7:**343–350.
6. **Clarholm, M.** 1984. Heterotrophic, free-living protozoa: neglected microorganisms with an important task in regulating bacterial populations, p. 321–326. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
7. **Clarholm, M.** 1985. Possible roles of roots, bacteria, protozoa and fungi in supplying nitrogen to plants, p. 355–365. *In* A. H. Fitter, D. Atkinson, D. J. Read, and M. B. Usher (ed.), Ecological interactions in soil. Special Publication 4, British Ecological Society. Blackwell Scientific Publications Ltd., Oxford.
8. **Coleman, D. C., C. V. Cole, H. W. Hunt, and D. A. Klein.** 1978. Trophic interactions in soils as they affect energy and nutrient dynamics. I. Introduction. Microb. Ecol. **4:**345–349.
9. **Danso, S. K. A., and M. Alexander.** 1975. Regulation of predation by prey density: the protozoan-*rhizobium* relationship. Appl. Microb. **29:**515–521.
10. **Elliot, E. T., and D. C. Coleman.** 1977. Soil protozoan dynamics in a shortgrass prairie. Soil Biol. Biochem. **9:**113–118.
11. **Elliot, E. T., D. C. Coleman, and C. V. Cole.** 1979. The influence of amoebae on the uptake of nitrogen by plants in gnotobiotic soil, p. 221–229, *In* J. L. Harley and R. S. Russell (ed.), The soil-root interface. Academic Press, London.
12. **Fenchel, T.** 1987. Ecology of protozoa: the biology of free-living phagotropic protists. Science Tech Publishers, Madison, Wis.
13. **Foster, R. C., and J. F. Dormaar.** 1991. Bacteria-grazing amoebae in situ in the rhizosphere. Biol. Fertil. Soils **11:**83–87.
14. **Gonzáles, J. M., J. Iriberri, L. Egea, and I. Barcina.** 1990. Differential rates of digestion of bacteria by freshwater and marine phagotropic protozoa. Appl. Environ. Microbiol. **56:** 1851–1857.
15. **Gonzáles, J. M., J. Iriberri, L. Egea, and I. Barcina.** 1992. Characterization of culturability, protistan grazing, and death of enteric bacteria in aquatic ecosystems. Appl. Environ. Microbiol. **58:**998–1004.
16. **Griffiths, B. S.** 1990. A comparison of microbial-feeding, nematodes and protozoa in the rhizospere of different plants. Biol. Fertil. Soils **9:**83–88.
17. **Habte, M., and M. Alexander.** 1978. Mechanisms of persistence of low numbers of bacteria preyed upon by protozoa. Soil Biol. Biochem. **10:**1–6.
18. **Kempers, A. J., and A. Zweers.** 1986. Ammonium determination in soil extracts by the salicylate method. Soil Sci. Plant Anal. **17:**715–723.
19. **Page, F. C.** 1988. A new key to freshwater and soil gymnamoebae. Culture Collection of Algae and Protozoa, Freshwater Biological Association, Ambleside, Cumbria, England.
20. **Singh, B. N.** 1941. Selectivity in bacterial food by soil amoebae in pure and mixed culture in sterilized soil. Ann. Appl. Biol. **28:**52–65.
21. **Singh, B. N.** 1942. Selection of bacterial food by soil flagellates and amoebae. Ann. Appl. Biol. **29:**18–22.
22. **Singh, B. N.** 1946. A method of estimating the numbers of soil protozoa, especially amoebae, based on their differential feeding on bacteria. Ann. Appl. Biol. **33:**112–119.
23. **Stout, J. D.** 1973. The relationship between protozoan populations and biological activity in soils. Am. Zool. **13:**191–201.
24. **Stout, J. D.** 1980. The role of protozoa in nutrient cycling and energy flow. Adv. Microb. Ecol. **4:**1–50.
25. **Stout, J. D., and O. W. Heal.** 1967. Protozoa, p. 149–195. *In* A. Burges and F. Raw (ed.), Soil biology. Academic Press, Inc., New York.
26. **Weekers, P. H. H., and C. van der Drift.** 1993. Nitrogen metabolizing enzyme activities in the free-living soil amoebae *Acanthamoeba castellanii, Acanthamoeba polyphaga* and *Hartmannella vermiformis*. J. Euk. Microbiol. **40:**251–254.
27. **Woods, L. E., C. V. Cole, E. T. Elliott, R. V. Anderson, and D. C. Coleman.** 1982. Nitrogen transformation in soil as affected by bacterial-microfaunal interactions. Soil Biol. Biochem. **14:** 93–98.