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Effects of Bacterial Prey Species and Their Concentration on Growth of the Amoebae *Acanthamoeba castellanii* and *Hartmannella vermiformis*▽

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Two amoebae were presented with six bacterial prey at a range of concentrations, and the growth parameters of the amoebae were deduced. All but one bacterium (*Synechococcus*) resulted in a positive growth response, but the gram-positive bacterium *Staphylococcus aureus* proved to be difficult to digest and the heavily pigmented bacterium *Klebsiella ozaenae* induced unusual amoebic behavior prior to ingestion.

Protozoa are the major predators in microbial food webs (2), but the effects of different groups vary depending on the specific habitat. In biofilms, amoebae are considered more important than flagellates and ciliates due to their obligatory surface-associated lifestyle (20), but quantitative studies of amoeba-bacterium interactions are rare. Although in some studies workers have obtained amoebic growth parameters (3, 7, 17), in very few studies have workers examined the effect of bacterial prey type (28) or concentration (4) on amoebic growth. In this study we addressed this paucity of information by examining the interaction between two amoebae and six bacterial prey at different concentrations.

MATERIALS AND METHODS

Suspensions of two 6-day-old amoebae, *Acanthamoeba castellanii* strain CCAP 1501/1A and *Hartmannella vermiformis* strain CCAP 1534/7A (Culture Collection of Algae and Protozoa [CCAP]), were prepared as described by Pickup et al. (21). *Escherichia coli* K-12 strain N° 10214 (CCAP, United Kingdom), *Pseudomonas aeruginosa* SG 81 (H.-C. Fleming, Gerhard Mercator University, Germany), *Klebsiella aerogenes* NCTC 9528 (National Collection of Type Cultures), *Klebsiella ozaenae* (J. English, University, Lancaster, United Kingdom), and *Staphylococcus aureus* NCTC 6571 were grown on nutrient agar (Lab M; International Diagnostics Group, Bury, United Kingdom) at 25°C for 1 day and then suspended in Amoeba Saline (AS; CCAP) (19a). *Synechococcus* sp. strain No 8 (K. Harper, Lancaster University, Lancaster, United Kingdom) was grown in BG11 (27) for 14 days at 23°C with a daily cycle consisting of 16 h of light and 8 h of darkness and then centrifuged at 2,504 × *g* for 10 min. Prey suspensions were sonicated for 10 min before use, and cell concentrations were determined by 4′,6′-diamidino-2-phenylindole (DAPI) staining and epifluorescence microscopy (22).

AS agarose plates (1.5%, wt/vol) were prepared as described by Pickup et al. (21). Each amoeba was presented with six bacteria on the agar surface at the following concentrations: *S. aureus*, 0, 1×10^3, 1×10^4, 5×10^4, 1×10^5, and 5×10^5 cells cm^{-2}; and other bacteria, 0, 1×10^5, 1×10^6, 5×10^6, 1×10^7, and 5×10^7 cells cm^{-2}. For each combination, the amoeba and the bacterium were coinoculated into 2 ml AS and then poured onto the agar surface. Once the preparations were dry, three 1-cm^2 areas having a confluent bacterial lawn and five amoeba cells were excised and placed onto 10 μl of sterile distilled water in a Sedgwick Rafter counting chamber. Triplicate controls consisting of each bacterium alone at each concentration and each amoeba alone were prepared. The chambers were incubated at 20°C. Agar cubes were examined daily by light microscopy to determine the concentration of amoebae (cells cm^{-2}). At the beginning and end of the experiment, the agar surfaces of test and bacterial control cubes were fixed in glutaraldehyde (final concentration, 0.4% [vol/vol]). Surfaces were scraped to remove adherent cells and place them in suspension. Bacterial concentrations (cells cm^{-2}) were determined by epifluorescence microscopy.

Analyses were performed using SPSS statistics (SPSS Inc., Illinois), SigmaPlot version 5 (SPSS Inc., Illinois), or Excel (Microsoft, Redmond, WA). Amoeba specific growth rates at each prey concentration were determined by linear regression analysis of ln(amoeba cells cm^{-2}) against time (h). The responses of each amoeba to the prey concentrations were fitted to a hyperbolic function (equation 1) (19):

$$\mu = (\mu_{max} \cdot x)/(K_s + x) \quad (1)$$

where μ is the specific growth rate (h^{-1}), x is the initial bacterial concentration (cells cm^{-2}), μ_{max} is the maximum specific growth rate (h^{-1}), and K_s is the half-saturation constant (cells cm^{-2}). In the case of nonzero intercepts the equation was modified to include negative growth rates (equation 2) (14):

$$\mu = [\mu_{max}(x - x')]/[K + (x - x')] \quad (2)$$

where x' is the threshold concentration (cells cm^{-2}) at which the specific growth rate is 0 and $K = K_s + x'$ (5). The parameters μ_{max} and K_s were compared to determine significant differences (t tests with Bonferroni's correction), using means and standard errors provided by Sigmaplot and a pooled standard error (the square root of the sum of the standard errors arising from the nonlinear curve fit) (26). The yield of amoebae (number of amoebae per prey cell) was determined by dividing the concentration of amoebae produced by the concentration of bacteria consumed. The maximum ingestion rate (number of prey cells per amoeba cell per h) was determined by dividing μ_{max} by 0.5Y, where Y is the yield (10^{-3} amoeba per prey cell) as described by Fenchel (9).

RESULTS AND DISCUSSION

The sizes of both amoeba populations increased in the presence of five of the six bacterial strains. All concentrations of *Synechococcus* sp. induced encystment of the amoebae even though prey cells were clearly visible within the food vacuoles. Previous studies showed that protozoa ingest *Synechococcus* (8) but digest this bacterium inefficiently (6), possibly because the cell wall is two to five times thicker than the cell walls of other gram-negative bacteria (10), a feature which influences digestibility (11).

For the five remaining prey species, the highest μ_{max} values were recorded with *K. aerogenes* and *E. coli* K-12 (Fig. 1), consistent with the results of Weekers et al. (28). Both amoe-

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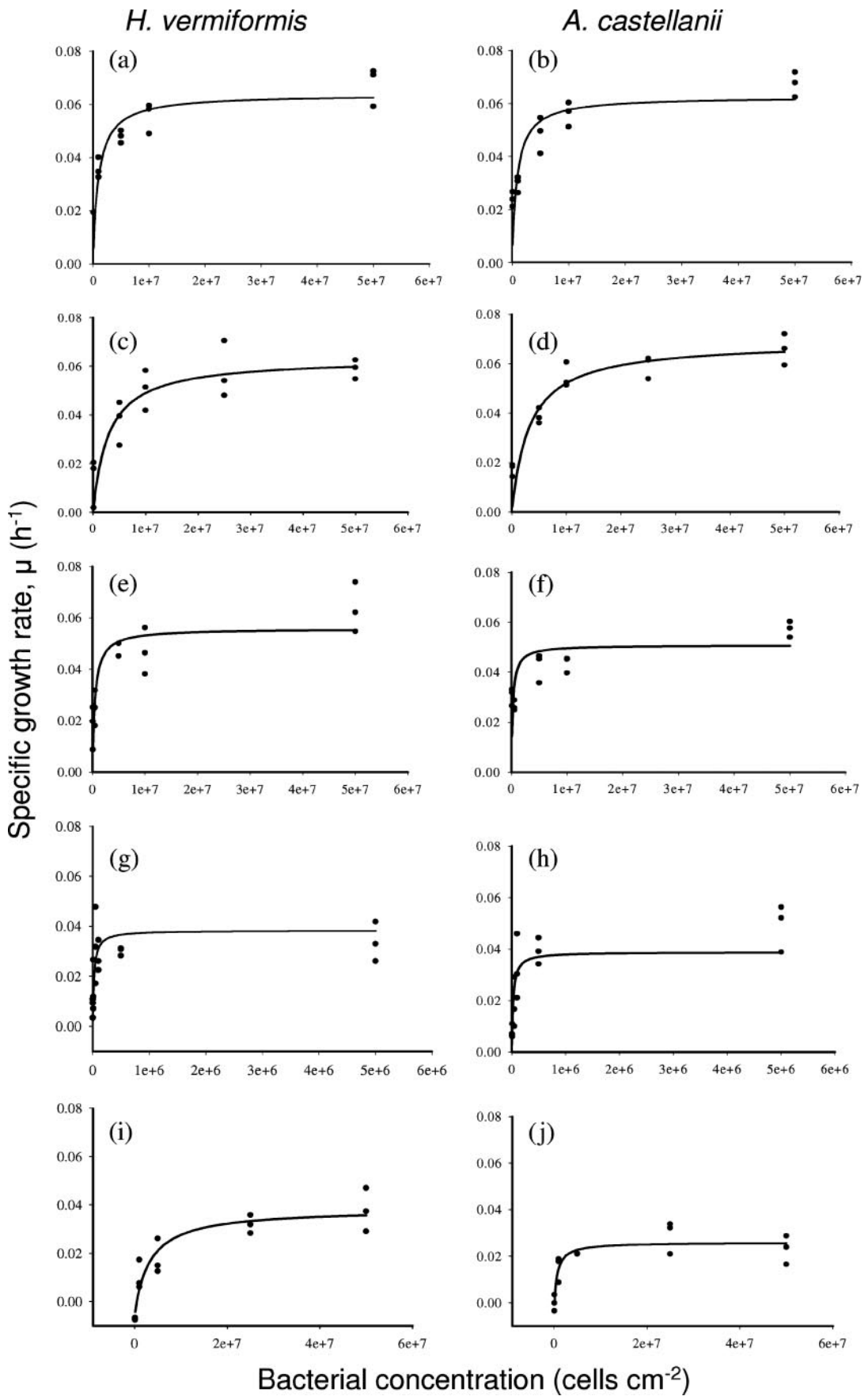

FIG. 1. Responses of the specific growth rates to the initial bacterial concentrations for *H. vermiformis* and *A. castellanii* feeding on *E. coli* (a and b), *K. aerogenes* (c and d), *P. aeruginosa* (e and f), *S. aureus* (g and h), and *K. ozaenae* (i and j) at 20°C on AS agar surfaces.

TABLE 1. μ_{max}, K_s, yield, and calculated maximum uptake rate for *H. vermiformis* and *A. castellanii* feeding on five species of bacteria on AS agar at 20°C

Bacterial prey	*H. vermiformis*				*A. castellanii*			
	μ_{max} (h^{-1})	K_s (10^6 bacteria cm^{-2})	Yield (10^{-3} amoeba cell prey $cell^{-1}$)	Maximum uptake rate (prey cells amoeba $cell^{-1}$ h^{-1})	μ_{max} (h^{-1})	K_s (10^6 bacteria cm^{-2})	Yield (10^{-3} amoeba cell prey $cell^{-1}$)	Maximum uptake rate (prey cells amoeba $cell^{-1}$ h^{-1})
E. coli K-12	0.064 (0.004)[a]	0.989 (0.297)	0.230 (0.216)	554	0.0626 (0.005)	0.8631 (0.356)	0.413 (0.391)	30
K. aerogenes	0.069 (0.003)	3.238 (0.489)	0.520 (0.030)	265	0.0633 (0.004)	2.921 (0.547)	0.022 (0.017)	575
P. aeruginosa	0.056 (0.004)	0.494 (0.194)	0.035 (0.015)	318	0.0509 (0.003)	0.253 (0.761)	0.033 (0.011)	308
S. aureus	0.038 (0.003)	0.023 (0.009)	0.006 (0.002)	1,532	0.0389 (0.003)	0.026 (0.009)	0.007 (0.004)	1,060
K. ozaenae	0.037 (0.003)	3.290 (0.259)	0.008 (0.004)	925	0.0259 (0.002)	5.999 (1.920)	0.005 (0.003)	1,036

[a] The values in parentheses are standard errors of the means.

bae exhibited significantly lower affinity for *K. aerogenes* ($P < 0.025$) (Table 1), which may have been due to the extracellular polysaccharide capsule of *Klebsiella* species in general, which enhances resistance to phagocytosis and/or digestion in mammalian white blood cells (1). Our data do not suggest that *K. aerogenes* resisted ingestion but do suggest that the efficiencies of digestion of this prey by the two amoebae were different; the yield of *H. vermiformis* was highest with this bacterium (Table 1).

The μ_{max} values with *P. aeruginosa* were significantly lower than the μ_{max} values with *E. coli* or *K. aerogenes* ($P < 0.05$) (Table 1), but both amoebae exhibited a higher affinity for *P. aeruginosa* (Table 1). In some studies, amoebae have fed effectively on *P. aeruginosa* (24, 29), but in other studies this species has proved to be toxic (12, 23, 25). In the present study, *P. aeruginosa*, although not toxic, did restrict the movement of amoebae after 52 h, when the bacterial population formed microcolonies, an antigrazing response (13, 16, 29) which slows the ingestion process.

The μ_{max} values with *S. aureus* were significantly lower than those with *E. coli*, *K. aerogenes*, and *P. aeruginosa* ($P < 0.01$), and the K_s values were nearly 2 orders of magnitude lower (Table 1). The high affinity for *S. aureus* (Table 1) could have been due to its smaller size, which facilitates easier ingestion, but the lower yield may have been due to the fact that gram-positive bacteria are generally more difficult to digest (11) or the fact that carotenoids protect this bacterium from oxidation (15). Fecal pellets containing intact *S. aureus* cells were evident in amoeba trails, suggesting that some cells avoided digestion.

K. ozaenae yielded the lowest μ_{max} values and highest K_s values of the five palatable bacteria. Both amoebae required a threshold density of ca. 1×10^6 *K. ozaenae* cells cm^{-2}, below which the specific growth rates were negative. Also, instead of forming characteristic cysts, trophozoites lysed or exhibited unusual behavior. *H. vermiformis* remained stationary until 26 h into the experiment, after which trophozoites began to move and feed normally. The response of *A. castellanii* was more dramatic. At a low prey concentration the trophozoites moved off the lawn, while at higher concentrations they either moved in erratic circles or attempted to burrow into the agar. However, after an initial lag period of 30 h, this amoeba consumed the *K. ozaenae* cells and had positive specific growth rates. The reason why *K. ozaenae* induced such amoebic behavior is currently unclear. There have been no reports that *K. ozaenae* is toxic to amoebae, but in another study the workers found that the pigmented bacterium *Serratia marcescens* was "toxic" to *H. vermiformis* for 3 days, after which the bacterium was ingested (12).

In conclusion, the two species of amoebae exhibited similar growth trends with the six bacterial prey, even though individual parameter values differed. Both species exhibited μ_{max} values with the palatable prey at concentrations greater than 1×10^7 prey cells cm^{-2}, which are concentrations common in natural biofilms (18), suggesting that amoebae are significant grazers of bacteria in these communities.

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