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ROLE OF BACTERIA IN THE GASTRAL CAVITY OF ANTHOZOA

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ABSTRACT - Bacterial density of the coelenteric fluid (CF) of some anthozoan species was determined *in situ* and under laboratory conditions. In all species examined bacterial density of the CF was higher in the gastral cavity than in ambient water ranging from 10^5 up to 5×10^6 cells. ml^{-1} . Incubation experiments with the CF indicate that a bacterial population within the coelenteron is efficiently controlled by the CF, favoring bacterial growth if bacterial densities are low in the CF and showing clearance activity at high bacterial densities. Feeding of pre-starved anthozoans caused a rapid response of coelenteric rod-shaped bacteria. Evidence is presented that coelenteric bacteria are digested periodically although they contribute less than 1% to the respiratory carbon losses of the anthozoans tested. Therefore, it is more likely that the coelenteric bacterial population provide other substances which cannot be synthesized by the host.

Key words : microbes-invertebrate association, bacteria-anthozoa association, coelenteric bacteria, anthozoa, coelenteric fluid.

RESUME - La densité des bactéries du fluide gastrique de différents anthozoaires a été déterminée *in situ* et au laboratoire. Pour toutes les espèces étudiées, la densité des bactéries dans le fluide gastrique était plus élevée que dans l'eau ambiante et comprise entre 10^5 et 10^6 cellules m^{-1} . Des expériences d'incubation du fluide gastrique montrent que la population bactérienne est contrôlée efficacement par ce fluide gastrique, favorisant la croissance de bactéries lorsque la densité est faible mais révélant des activités de digestion lorsque la densité est élevée. En nourrissant des anthozoaires mis à jeun plusieurs jours, on provoque une réaction rapide des bactéries de forme bacillaire. Certaines observations montrent que les bactéries gastriques se développent et sont digérées par cycle dans le coelenteron. Néanmoins, la contribution en carbone de ces dernières, comparée aux pertes d'énergie par la respiration, représenterait moins de 1%. Il est probable que cette population bactérienne fournisse des substances qui ne peuvent être synthétisées par l'hôte.

Mots clés : association entre microbes et invertébrés, association entre bactéries et anthozoaires, bactéries du coelenteron, anthozoaires, fluide gastrique.

INTRODUCTION

The nutrition of anthozoan species and especially corals is the subject of extensive research. The high biomass concentrations in coral reefs surrounded by the nutritive desert of tropical waters put the main effort on the nutrition of corals. The classic works of Odum and Odum (1955) and Johannes *et al.* (1970) indicate that anthozoans utilize a variety of food sources such as zooplankton (Porter, 1974; Sebens, 1977), detritus (Lasker, 1978) and dissolved organic matter (Stephens, 1962; Schlichter 1978) in addition to the photoautotrophic nutrition of anthozoan species harboring zooxanthellae (Trench, 1971; Muscatine and Porter, 1977).

The importance of bacteria as a food source for anthozoan species has been investigated by DiSalvo (1971a, b) and Sorokin (1973a, b), who both fed labeled bacteria to reef

corals. Sorokin (1973a) found that corals consume organic phosphorus bound in the cells of planktonic bacteria more actively than inorganic phosphate at the same concentrations. Reimer (1971), investigating the nutritional mode of the zoanthid *Zoanthus sandwicensis*, found a large bacterial population within the coelenteron and discussing the possible sources of nutrients for *Zoanthus* the author speculated that zoanthids may be able to farm a bacterial flora within the coelenteron and perhaps feed on them. Quite recently, first direct evidence has been presented on the existence of a bacteria - anthozoan association in the giant sea anemone *Stoichactis giganteum* (Herndl *et al.*, 1985). Bacterial density in the coelenteron of *S. giganteum* was found to be controlled by the coelenteric fluid. Below a distinct value of coelenteric bacterial density, bacteria are farmed; above this threshold bacteria are digested by the coelenteric fluid.

In this paper, evidence is given that the bacteria - anthozoan association observed in *S. giganteum* is not restricted to this single species but can be detected in other anthozoans as well, in both symbiotic and asymbiotic forms.

MATERIAL AND METHODS

Gut samples of the anthozoan species examined were sucked out of the gastral cavity by means of a syringe following the method described by Porter (1978). Due to the size of *S. giganteum*, a PVC-tube with an inner diameter of 1,6 cm was mounted on a syringe with a capacity of 60 cm³. Gut samples of 3 to 4 specimens and one sample of ambient water were taken per run.

Gastral samples of *Leptopsammia pruvoti* (Madreporaria) and *Parazoanthus axinellae* (Zoantharia) were sucked out using a blunted needle (1 mm inner diameter) mounted on a 20 ml-syringe. 10 polyps of *L. pruvoti* and 20 polyps of *P. axinellae* were sucked out per run. For *Anemonia sulcata* (Actiniaria), a blunted needle with a 4 mm inner diameter mounted on a 20 ml-syringe was used. Three specimens were sucked out per run. The entire sampling procedure was performed using SCUBA. Sampling intervals varied between 2 to 4 h in *L. pruvoti*, *P. axinellae* and *A. sulcata*, while *S. giganteum* specimens were sucked out twice a week.

The syringe needle was inserted into the gastrovascular cavity of the polyp through its mouth and the coelenteric fluid (CF), together with solids, slowly extracted until either the column wall and the oral disk collapsed over the skeleton (*L. pruvoti*) or, in the case of other species tested, until the smooth column collapsed. Within 5 min after collecting the CF, the samples were fixed with formalin to a final concentration of 5% and kept at 4°C in darkness until analysis.

Additional laboratory experiments were performed in order to evaluate the role of the CF in controlling bacterial density within the coelenteron on *P. axinellae*, *A. sulcata*, *S. giganteum* and *Cladocora cespitosa* (Madreporaria).

For sampling the CF in laboratory experiments, a blunted needle (2 mm inner diameter) mounted on a 10 ml- syringe was used. The sample volume was always 4 ml in *S. giganteum* and *A. sulcata* specimens, while in *P. axinellae* and *C. cespitosa* the sample volume varied between 2-5 ml according to the amount of polyps sampled. Between 6-10 polyps of *C. cespitosa* and *P. axinellae* were sampled per run in 2 to 4 h intervals.

One half on the CF sucked out in this manner was fixed immediately by adding formalin, while the remaining CF was incubated in pre-sterilized scintillation vials for 4 h at ambient water temperature and then fixed as mentioned above. The development of coelenteric bacterial density within the CF could therefore be investigated by comparing

the bacterial density of the immediately fixed sample with that of the incubated one.

Bacterial density in the CF was determined using the acridine orange epifluorescence direct counting (AODC) technique (Hobbie *et al.* 1977). The number of bacteria and wet biomass were calculated using the expressions of Linley *et al.* (1981). Conversions to dry biomass and carbon equivalent of the wet biomass were obtained using the coefficients 0.2 (Troitsky and Sorokin, 1967) and 0.1 (Luria, 1960), respectively.

RESULTS

Bacterial density of the CF of anthozoans obtained from in situ investigations

Bacterial densities in the CF of *L. pruvoti* and *P. axinellae* varied considerably during the investigation period. Rod-shaped bacterial density ranges between 0.5 to 7.37×10^5 cells ml⁻¹ CF ($\bar{x} = 3.09 \times 10^5$, S.D. = 2.08×10^5 , n = 22) in *L. pruvoti*, while coelenteric cocci vary between 2.74×10^5 cells ml⁻¹ CF and 40.19×10^5 cells ml⁻¹ ($\bar{x} = 15.35 \times 10^5$, S.D. = 10.3×10^5 , n = 22).

In *P. axinellae* values of rods range between less than 10^3 and 6.1×10^5 cells ml⁻¹ ($\bar{x} = 2.13 \times 10^5$ cells ml⁻¹, S.D. = 1.36×10^5 , n = 22) and of cocci from less than 10^3 to 54.94×10^5 cells ml⁻¹ ($\bar{x} = 11.7 \times 10^5$ cells ml⁻¹, S.D. = 15.7×10^5 , n = 22). Mean rod-shaped bacterial density in ambient water is 1.41×10^5 cells ml⁻¹ (S.D. = 0.4×10^5 , n = 22) and the mean density of coccoid bacteria is 2.23×10^5 cells ml⁻¹ (S.D. = 0.7×10^5 , n = 21). Therefore, total bacterial density is approximately 6 times higher in *L. pruvoti* and 4 times higher in *P. axinellae* than in ambient waters. Although large deviations of bacterial densities occur in *L. pruvoti* and *P. axinellae*, no distinct diurnal cycles could be observed. Within the coelenteron of the symbiotic sea anemone *S. giganteum*, bacterial densities are generally above those of ambient waters. Mean bacterial density of the CF is 2.49×10^6 cells ml⁻¹ (S.D. = 1.07×10^6), i.e. 2.6 times higher than in ambient waters (Herndl *et al.*, in press). Rods contribute 90% to the number of bacteria and 97% of bacterial biomass. Mean rod-shaped bacterial density is 2.3×10^6 cells ml⁻¹ (S.D. = 1.1×10^6) and therefore 14 times higher than the density of rod-shaped bacteria in the adjacent water. The biovolume of coelenteric bacteria did not differ significantly from that of ambient waters. Mean volume obtained for rods is $0.6 \mu\text{m}^3$ (S.D. = 0.14, n = 100) and for cocci $0.13 \mu\text{m}^3$ (S.D. = 0.03, n = 30).

The mean bacterial density in the coelenteron of the symbiotic sea anemone *A. sulcata* is 2.33×10^6 cells ml⁻¹ (S.D. = 2.2×10^6 , n = 7), consisting of up to 90% of cocci. The volumes of both rod-shaped and coccoid cells of the CF were significantly higher (ANOVA, $P < 0.001$) than those of ambient waters.

Bacterial fluctuations in the CF

Figure 1 demonstrates that in *P. axinellae* the regulative function of the CF against bacteria depends upon the initial bacterial density in the coelenteron. *C. cespitosa* demonstrates very low generation times of coelenteric bacteria at low rod-shaped bacterial densities below 0.1×10^5 cells ml⁻¹ CF, but at 0.2×10^5 cells ml⁻¹ clearance activity of the CF already begins (Fig. 2). Coelenteric coccoid bacterial density shows no such marked fluctuations in turnover rates as do rod-shaped bacteria. During the clearance phase, however, rods and cocci show quite similar slopes, although clearance of rods starts at lower bacterial densities than cocci clearance.

In *A. sulcata*, cocci dominate the bacterial population in the coelenteron during the course of incubation experiments; this corresponds with the values of the *in situ* investi-

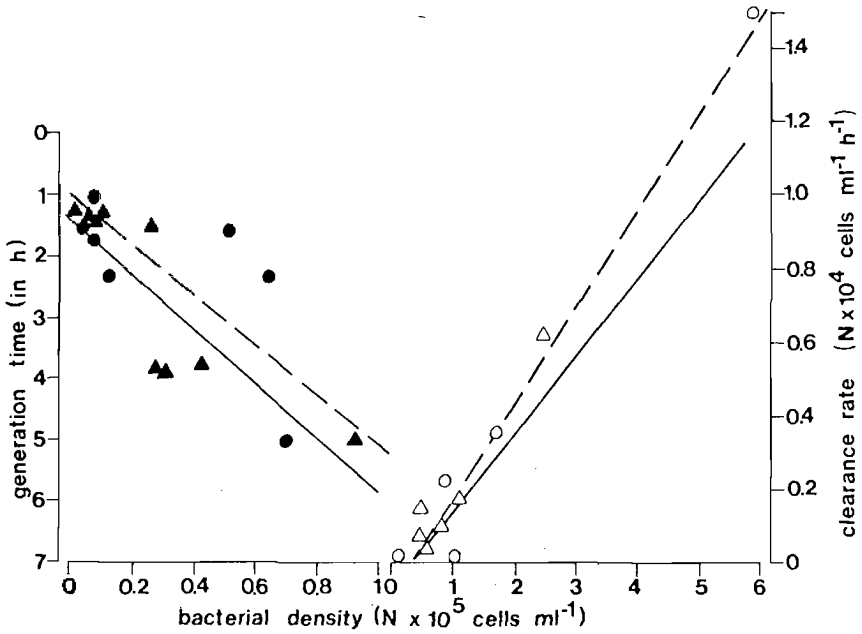


Figure 1 : *P. axinellae*: Dependence between coelenteric bacterial density and the activity of the CF; triangles - rods; circles - cocci; full symbols - values obtained for generation time; open symbols - values obtained for clearance activity; full line - regression line of rods; dotted line - regression line of cocci.

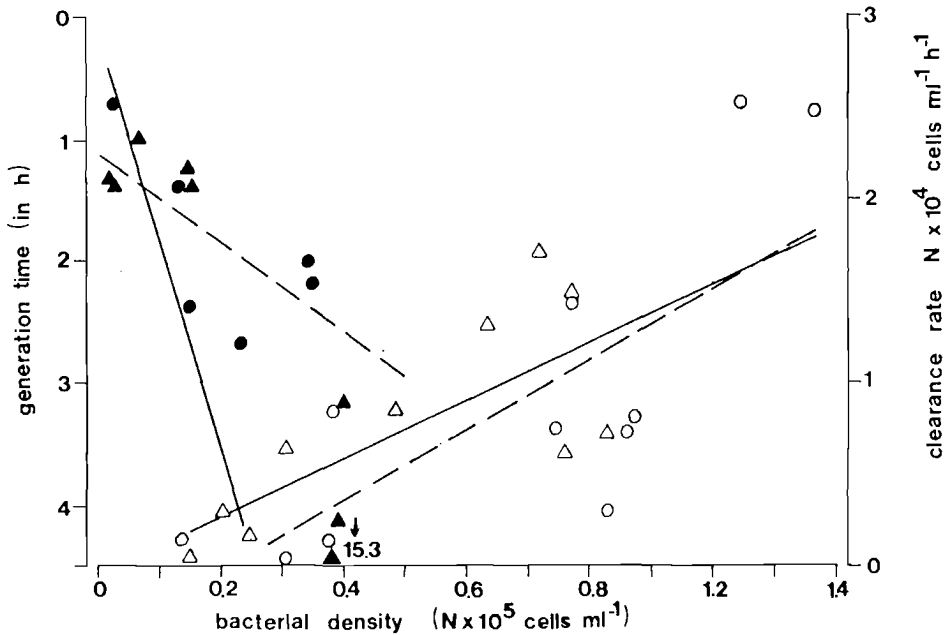


Figure 2 : *C. cespitosa*: Dependence between coelenteric bacterial density and the activity of the CF; for symbols see Figure 1.

gations. Highest clearance rates of up to 1.6×10^6 cells $\text{ml}^{-1} \text{h}^{-1}$ were obtained at an initial coccoid bacterial density in the coelenteron of 6.5×10^6 cells ml^{-1} . While for coccoid bacteria a relation exists between initial bacterial density in the coelenteron vs. both generation time and clearance rate (Fig. 3 a, b), in rod-shaped bacteria a distinct relation is detectable only between density and clearance rate. The build up of rods shows an irregular pattern. *S. giganteum* shows a similar irregularity in the build up of cocci, while decreasing rod-shaped bacterial densities cause higher turnover rates (Fig. 4).

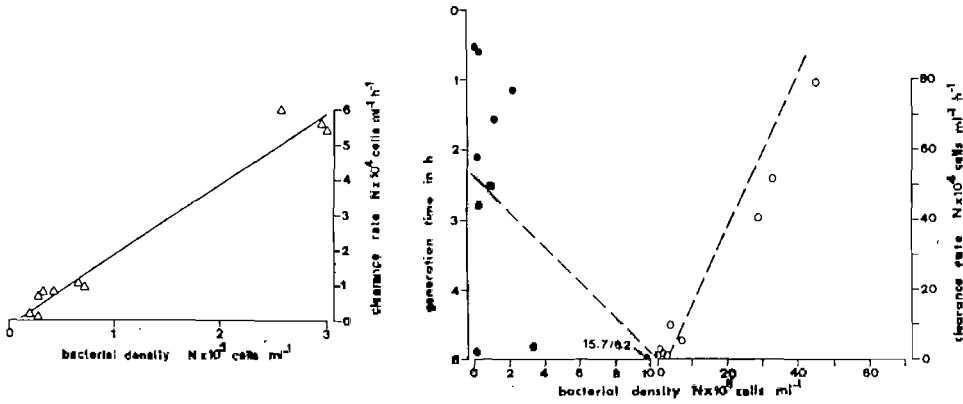


Figure 3 (a et b) : *A. sulcata*: Dependence between coelenteric bacterial density and the activity of the CF; for symbols see Figure 1.

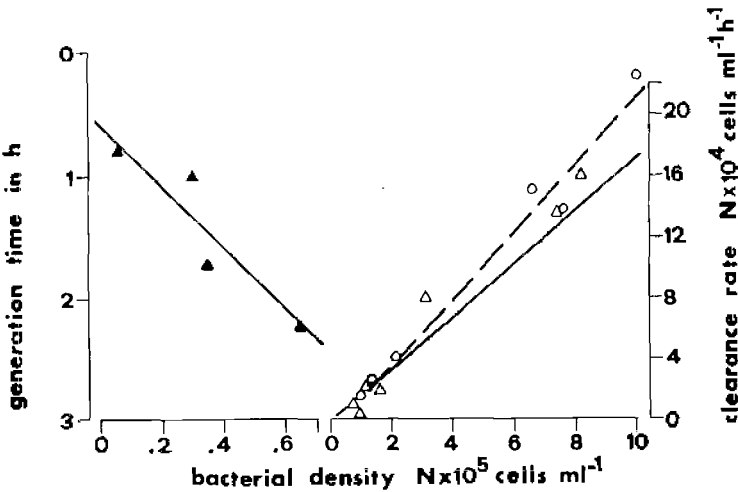


Figure 4 : *S. giganteum*; Dependence between coelenteric bacterial density and the activity of the CF; for symbols see Figure 1; (partly derived from Herndl *et al.*, 1985).

The importance of heterotrophic nutrition on the coelenteric bacterial population examined on *S. giganteum*.

In an attempt to assess the role of heterotrophic nutrition on bacterial density, two *S. giganteum* specimens were pre-starved for 4 days and then fed with 10 g Mytilus-meat. An

increase in bacterial density from 0.77×10^5 cells ml^{-1} to a maximum density of 6.78×10^5 cells ml^{-1} 2 h after feeding was observed; the contribution of rods to the total bacterial density was 78 % (Fig. 5). After this increase, rod-shaped bacteria dropped off continuously during the following hours. 4 h after feeding, rods contribute only 33.7 % and 6 h after feeding they decreased to 29 % of the total bacterial density. While rods respond quickly to changed conditions in the coelenteron, coccoid bacterial density increases more slowly but reaches higher densities than rods after 4 h.

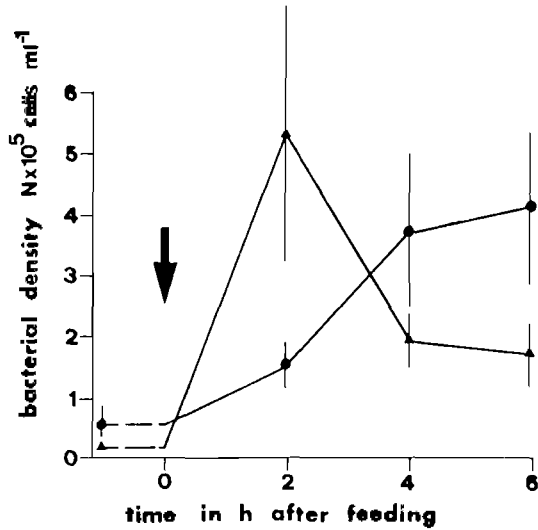


Figure 5 : Development of coelenteric bacterial density after feeding in *S. giganteum*; arrow indicate feeding; triangles indicate rods; circles - cocci; mean and S.D. of 3 experiments.

DISCUSSION

The results indicate that a bacterial population within the gastral cavity is efficiently controlled by the CF in all species examined. The rapid increase of rod-shaped bacteria after feeding in *S. giganteum* (Fig. 5) and the predominance of rods in *S. giganteum* specimens *in situ* reveals the high nutrient availability in the coelenteron, because rods are generally characteristic of waters with a high nutrient amount (Ferguson and Rublee, 1976; Fuhrman and Azam, 1982). On the other hand, cocci dominated the bacterial population in starved *S. giganteum* specimens. One can assume that the coccoid forms represent metabolically inactive (dormant) or starved forms (Stevenson, 1978) during periods of low nutrient availability in the coelenteron, while rod-shaped bacteria are the exploitative cells, capable of taking up high nutrient amounts in short periods of time due to their higher surface to volume ratio (Fuhrman *et al.*, 1980).

Although nutrient availability influences coelenteric bacterial density considerably, the CF is the most important factor in controlling coelenteric bacterial densities - favoring bacterial growth if bacterial densities are low and showing clearance activity at high bacterial densities.

If coelenteric bacteria are digested as soon as they reach a threshold density, anthozoans may derive part of their energy requirements from incorporating bacterial carbon into host tissue. This assumption was tested and it has been found that bacteria contribute less

than 1% to the carbon losses of the anthozoans tested (Herndl and Velimirov, in prep.). Therefore, it is more likely that the coelenteric bacterial population provides trace elements, vitamins (Sorokin, 1973 a) or antibiotic substances (Burkholder, 1973) which cannot be synthesized by the host rather than providing carbon and nitrogen for metabolic activities. A similar phenomenon was described by Wilkinson (1978 a, b, c) between sponges and associated bacteria. He concludes that the digestion of microbial symbionts does not yield a significant amount of energy to the host sponge.

The rapid response of coelenteric bacteria to particle feeding in *S. giganteum* provides evidence that the digestive activity of the CF on bacteria during phago- and pinocytosis of food items might well be an effective defense mechanism against microbial invasion (Disalvo, 1971 a) and a strategy reducing energy losses by exploiting the bacterial biomass itself.

On the other hand, the support of bacterial growth—even during starvation periods—by the CF when the bacterial density is low indicates that these bacteria are either essential for anthozoans or that it is energetically meaningless to clear the CF of bacteria at low bacterial concentrations.

For bacteria the coelenteron of anthozoa is an endobiotic habitat (Sieburth, 1979) and of high nutritive value since the CF is the major pathway of heterotrophic nutrition and excretion of waste products in anthozoans. This specific situation of a protected microhabitat and the high nutritive value of the CF may also account for the low generation times of bacteria at low bacterial densities.

Further investigations, however, are required to understand the role of bacteria in the CF of anthozoa. The use of culture techniques for bacteria will give new insights into the bacteria-anthozoan association.

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