Symbiosis, 4 (1987) 51–62
Balaban Publishers, Philadelphia/Rehovot

Review article

The Possible Nutritional or Detoxification Role of the Epibiotic Bacteria of *Alvinellid polychaetes*: Review of Current Data

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Received February 13, 1987; Accepted April 12, 1987

Abstract

Alvinella pompejana and *Alvinella caudata*, two species of Alvinellidae discovered at hydrothermal vents on the East Pacific rise are always covered by numerous bacteria. To our knowledge such abundant epibioses have never been described in the marine environment. A prominent role of chemoautotrophic bacteria in deep-sea hydrothermal vents ecosystems is now well established. In many cases, bacteria are intracellular (e.g. in *Riftia pachyptila* and *Calyptogena magnifica*) and symbiotic relationships seem evident even if the exact metabolic pathways are not completely described. For *A. pompejana* and *A. caudata* the relationship with their associated bacteria is less clear. In this paper morphological descriptions; ^{13}C:^{12}C ratios; radiolabeled incorporation of bicarbonate and thymidine localizations are reviewed. Possible trophic relations are discussed and the role of bacteria in detoxification of the surrounding environment of the worms is considered.

Keywords: submarine hydrothermal vents, Alvenellidae, *Alvinella, Paralvinella*, epibiotic bacteria, annelid polychaet, East Pacific Rise, bacteria-animal association

Abbreviations: SEM: Scanning Electronic Microscopy, EPR: East Pacific Rise

1. Introduction

Among all the new species of animals discovered in submarine hydrothermal ecosystems of the East Pacific Rise (EPR), there are annelid polychaets *Alvinella* spp. and *Paralvinella* spp. These species belong to a new family of polychaete: the Alvinellidae, (Desbruyères et Laubier, 1986). At present two species of *Alvinella* are described: *Alvinella pompejana* and *Alvinella caudata*, and four species of Paralvinella: *Paralvinella grasslei*, *Paralvinella palmiformis*, *Paralvinella pandorae* and *Paralvinella bactericola*. Both species of *Alvinella* occur in large colonies on black and white smokers and moiré water diffusers and it is these species which live closest to the hot water extruding from the vents and are exposed to the highest concentrations of chemical (e.g. metallic sulfides) and temperatures (from 20°to 40°C). *Paralvinella* species live in different types of microhabitats: on the vestimentiferan tubes (*P. grasslei*, *P. palmiformis* and *Paralvinella pandorae*) within the *Alvinella* colonies on white smokers, on moiré water diffusers (*P. grasslei*) and on sediments covered by bacterial mat (*P. bacteriocola*). The Alvinellidae are strictly hydrothermal and known to exist from 17°S to 50°N with specific biogeographical patterns (Desbruyères et al., 1982; Desbruyères et Laubier, 1986). *Alvinella pompejana*, *A. caudata* and *P. grasslei* live in tubes, the inner part covered with numerous bacteria (rod shaped and filamentous) (Fig. 2F), *P. palmiformis* and *P. pandorae* secrete mucus colonized by bacteria (Tunnicliffe et al., 1985). Beyond differences at the morphological level *Alvinella* species differ from *Paralvinella* species by the presence of numerous bacteria covering their posterior part and forming a felt-like mat visible to the naked eye (Fig. 1). The morphological study of these bacteria revealed four types of epidermic associations according to their localization on the worms (Desbruyères et al., 1983; Gaill et al., 1984; Gaill et al., 1987).

1. Cluster-like bacteria associations (Fig. 2A,B) located in the intersegmentary spaces. These associations are composed of rod shaped, cocooid and filamentous bacteria.
2. Filamentous bacteria associated with epidermic expansions of *A. pompejana* (Fig. 2C).
3. Filamentous bacteria inserted on the posterior part of parapods of *A. caudata* (Fig. 2D).
4. Single bacteria distributed on the animal tegument, without any particular location (Fig. 2E). Four morphological types are represented: rod shaped, prosthecaded, spiral-curved and unsheathed filamentous bacteria. Some subcuticular bacteria are also observed in the two

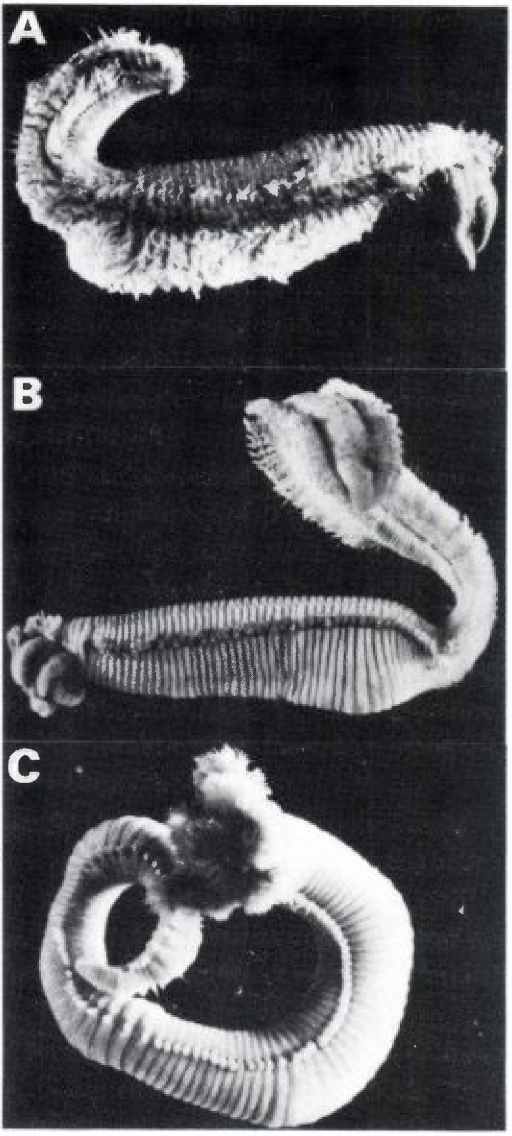

Figure 1. (A) *Alvinella pompejana* — felt-like bacterial mat on the dorsal side of the worm as well as bacterial filaments fixed on the expansions of the posterior part.
(B) *Alvinella caudata* — felt-like bacterial mat only on the posterior part of the worm but numerous bacteria are present in intersegmentary spaces of anterior part.
(C) *Paralvinella grasslei* — no bacterial mat on the surface of this worm.

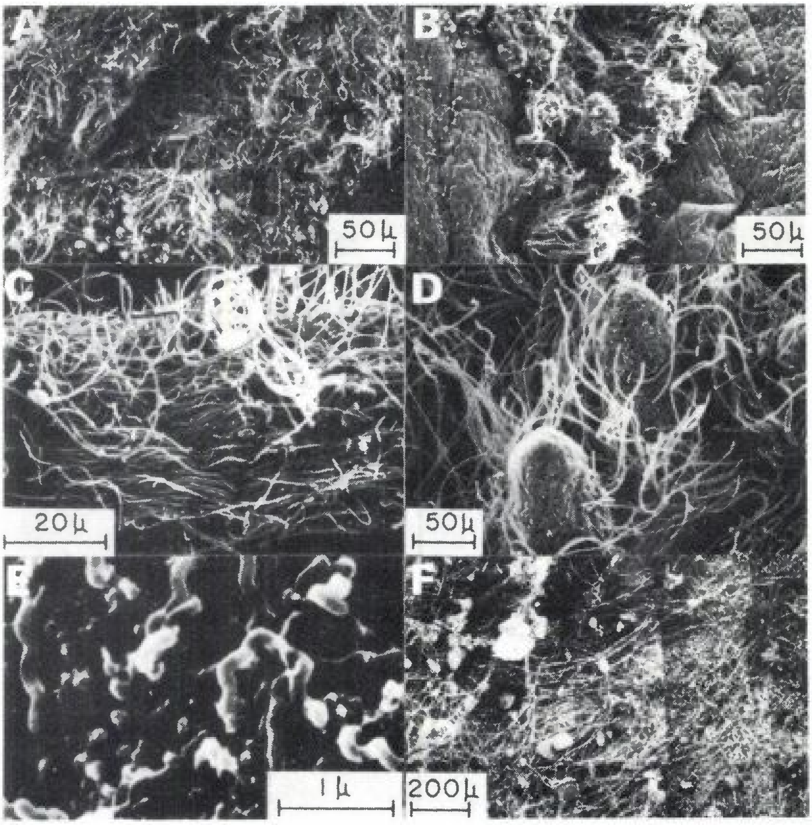

Figure 2. Bacterial associations (SEM observations)

(A) *Alvinella caudata* — filamentous bacteria inserted in an intersegmentary space.

(B) *Alvinella pompejana* — filamentous bacteria inserted in an intersegmentary space

(C) *Alvinella pompejana* — filamentous bacteria (100 to 200μm in length) located around the generative part of an expansion.

(D) *Alvinella caudata* — filamentous bacteria inserted in apical portion of parapod digitations.

(E) *Alvinella caudata* — single bacteria without specific location; spiral-curved bacteria showing four spiral revolutions of 0.4μm steps.

(F) *Alvinella pompejana* — bacterial filaments fixed on the inner surface of the worm tube.

species (Gaill, in preparation) but no intratissular or intracellular bacteria have been found.

Paralvinella species are devoid of bacterial epibiosis on the parapods. *P. grasslei* which lives sometimes in the same habitats as *Alvinella* reveals only extremely reduced intersegmentary epibiosis. Epibiotic bacteria have also been observed on some other invertebrates inhabiting hydrothermal ecosystems. The shells of *Calyptogena magnifica*, the periotracum and gills of *Bathymodiolus thermophilis* (Jannasch and Wirsen, 1979; Le Pennec and Prieur, 1984; de Burgh and Singla, 1984), "hairs" of notopodia of *Lepidonotopodium williamsae* (Pettibone, 1984) and of *Hesiolyra bergi* (Blake, 1985) all contain dense assemblages of bacteria. However, Alvinellids differ from the other species in the exceptional abundance and diversity of the bacterial species and their obligate presence (all the individuals observed show these epibiosis).

Whereas a role in nutrition has been quickly alloted to endocellular bacteria contained in other organisms linked to hydrothermal ecosystems such as *Riftia pachyptila* (Jones, 1981; Cavanaugh et al., 1981; Felbeck et al., 1981, 1985) or *Calyptogena magnifica* or *Bathymodiolus thermophilis* (Cavanaugh, 1983; Fiala-Medioni, 1984; Le Pennec et al., 1985; Fiala-Medioni et al., 1986), such a relationship between *Alvinella* species and their epibiotic bacteria is not that clear. Nevertheless, some clues obtained by different approaches led us, for the first time, to think that some direct trophic exchanges could occur between *Alvinella* and some chemoautotrophic epibiotic bacteria. These clues are:

1. Disorganisation of collagenous fibers of the worm's cuticle in some intersegmentary zones (Gaill et al., 1987).
2. Thin filamentous structures link rod shaped bacteria to the cuticle of the worms (Gaill et al., 1987).
3. High vascularization of the underlying epithelium in the dorsal intersegmentary zone (Desbruyères et al., 1983; Gaill et al., in press).
4. Detection by Tuttle (1983) of ribulose biphosphate carboxylase at the surface of *Alvinella.*
5. The high value of $\delta^{13}C$ of *Alvinella* tissues is similar to that of *Riftia pachyptila* tissues.

To test this hypothesis the first *in situ* experiment was realized in 1984 during the French cruise BIOCYARISE on the EPR at the 13°N with the submersible Cyana. Pieces of wall of white smoker including tubes and three

Table 1. ^{14}C bicarbonate experiment: Relative densities of silver grains in the different types of bacteria and tissues after 1 week or 1 month exposure time

	Epibiotic bacteria			Epidermis		Bucal region		Gut		Cardiac Endo-thelium
	Cluster-like associations	filamentous forms on parapods	forms on expansions	Non-glandular	Glan-dular	Gills	Ten-tacles	Walls	Par-ticles	
Alvinella pompejana	+ + +	NP	–	++	±	++	–	–	+	+
Alvinella caudata	+ + +	–	NP	++	±	++	–	–	+	+
Paravinella	NP	NP	NP	++	±	++	–	–	+	+

NP: this epibiosis not present in this species, – no grain, + light density, ++ medium density, + + + high density

Table 2. ^{3}H thymidine experiment: Relative densities of silver grains in the different types of bacteria and tissues after 1 week or 1 month exposure time

		Epibiotic bacteria		Epidermis		Bucal region		Gut
	Cluster-like associations	filamentous forms on parapods	forms on expansions	Non-glandular	Glandular	Gills	Tentacles	Walls walls
Alvinella pompejana	+	NP	+ + +	++	+	+ + +	+	+
Alvinella caudata	+	+	NP	+ + +	+	+ + +	+	+
Paravinella	NP	NP	NP	+ + +	+	+ + +	+	+

NP: this epibiosis not present in this species, – no grain, + light density, ++ medium density, + + + high density species of Alvinellidae *A. pompejana*, *A. caudata* and *P. grasslei* were incubated with ^{14}C bicarbonate or ^{3}H thymidine in plastic boxes. It appeared that under experimental conditions (described in a previous paper, Alayse-Danet et al., 1986) some bacteria of the cluster-like associations and of the inner surface of the tubes incorporated bicarbonate (Table 1) but not the filamentous bacteria fixed on *A. pompejana* expansions and on *A. caudata* parapods. The latter incorporated ^{3}H thymidine (Table 2) suggesting that these bacteria were actively dividing during the incubation. These results show that autotrophic bacteria as well as certainly heterotrophic bacteria are present among epibiotic associations on both *Alvinella* species and corroborate the isolation of mixotrophic and heterotrophic strains from *A. pompejana* epidermis (Prieur, in press).

Lack of fixation of bicarbonate by filamentous bacteria during the experiment does not demonstrate that these bacteria are strictly heterotrophic because in some facultative chemoautotrophic bacteria the potential to fix CO_2 depends on sulfide and oxygen concentrations (Jorgensen, 1982; Nelson and Jannasch, 1983). The *in situ* incubation chambers were filled with water containing less hydrothermal fluid than the water normally surrounding *Alvinella* in their habitat and perhaps sulfide-oxygen concentrations or the proportions of each were not adequate for CO_2 fixation by filamentous associates. In *A. pompejana* and *A. caudata*, ^{14}C was fixed at the level of the non-glandular epidermis but principally in the gills. This is the same in *P. grasslei* which is practically devoid of epibiotic bacteria. This observation leads us to think that direct passage of metabolites between autotrophic epibiotic-bacteria and underlying epidermis is certainly not a predominant process if it exists.

The labelling of the animal tissues could be the result of carboxylase reactions but it can also correspond to absorption of metabolites released by different bacteria in the microenvironment of the worms (free bacteria and bacteria fixed on the tubes and on the worms). From this experiment and the other observations, the reality of a trophic relationship between *Alvinella* and their epibiotic bacteria and other bacteria of their microenvironment is not demonstrated nor eliminated.

Another effect of epithelial bacteria might be detoxification, Alvinellidae, particularly *Alvinella* live in water rich in different compounds generally toxic for invertebrates, such as metals and hydrogen sulfide. Bacteria of the microenvironment could participate in detoxification of fluid by transforming toxic sulfide to sulfate or other non toxic oxides and by precipitating metals.

At present, it is difficult to involve the more abundant epibiosis of *Alvinella*, the filamentous bacteria fixed on parapods or on expansion in this detoxification role because *P. grasslei*, which is devoid of these bacteria, lives in the same environment and is exposed to the same toxic compounds. Non-specific association must also be considered, but these epibiotic associations appear to be highly specific in species composition and location on the host-surface, suggesting a functional interaction between the bacteria and the polychaet.

2. Conclusions

Results obtained from this first set of experiments led us to design new experiments and analyses:

1. to test temperature and sulfide effects on the epibiotic bacterial metabolism,
2. to look for carboxylases in the Alvinellid gills,
3. to test the hypothesis or the role of the epibiotic bacteria in detoxification.

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