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Alvinella pompana
Bacteria
Freeze-fracture

Alvinella pompejana
Bactéries
Cryofracture

Ultrastructural characteristics of *Alvinella pompejana* associated bacteria

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ABSTRACT

A freeze fracture morphological study of *Alvinella pompejana* associated epibiotic bacteria has confirmed the large variety of bacterial species already observed by scanning and thin section transmission electron microscopy. Moreover, freeze-fracture reveals striking differences in the membrane structure: the usual fracture along the hydrophobic part of the membrane for some bacteria, but only an unusual cross-fracture through the membrane for others. This suggests that some bacteria contain non-fracturable lipids typical of certain archaebacteria. This class of microorganism is usually encountered in exceptional ecological niches; it is not surprising, therefore, to find them in the vicinity of deep hydrothermal vents.

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RÉSUMÉ

Caractéristiques ultrastructurales des bactéries associées du "ver de Pompéi"

L'étude morphologique par cryofracture des bactéries épibiontes du "ver de Pompéi" confirme la grande variété des espèces déjà observées par microscopie électronique à balayage et en transmission sur des coupes minces. De plus elle met en évidence des différentes frappantes au niveau de la membrane : certaines bactéries se fracturent le long de la partie hydrophobe de la membrane, mais beaucoup d'autres ne montrent que des fractures transversales. Ceci suggère qu'une partie au moins des bactéries observées comporte des lipides non fracturables caractéristiques de certaines archaebactéries. Cette classe de microorganismes se rencontre le plus souvent dans des niches écologiques particulières, il ne serait donc pas surprenant d'en trouver au voisinage des sources hydrothermales des grands fonds marins.

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INTRODUCTION

A large variety of epibiotic bacteria of *Alvinella pompejana* from deep hydrothermal vents has already been observed by transmission and scanning electron microscopy (Gaill *et al.*, 1984; Desbruyères *et al.*, in press).

Considering the extreme living conditions in the immediate vicinity of the smokers of the *Alvinella* habitat, it appeared relevant to ask whether these associated bacteria belong to the usual, eubacterial, or to the archaebacterial class.

Archaebacteria are microorganisms which have recently been assigned to a particular class. They are commonly encountered in exceptional niches: methanogens in the absence of oxygen and presence of hydrogen and carbon dioxide; halophiles at high salt concentration; thermoacidophiles at high temperature and acidic pH. A

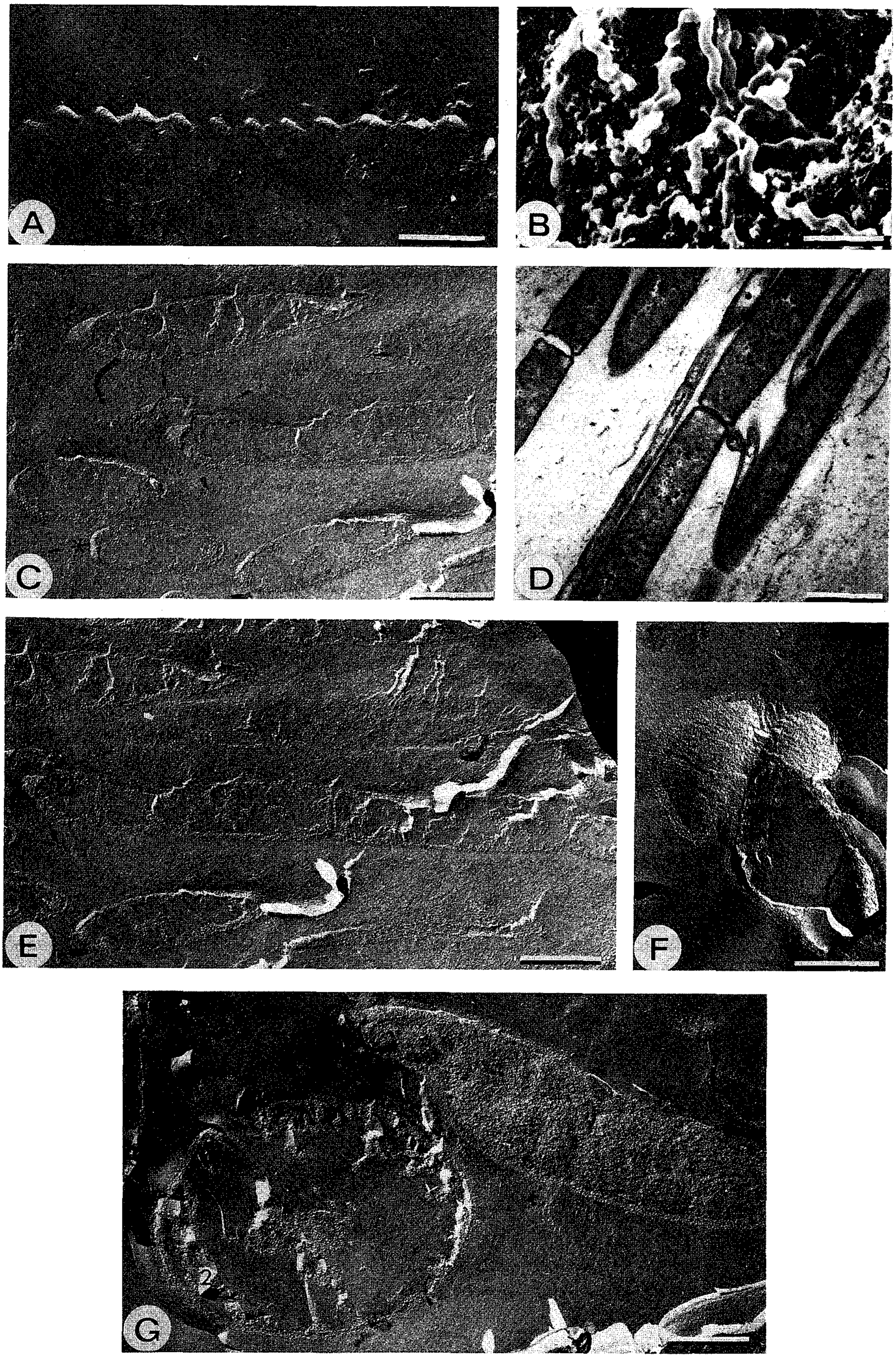

Figure 1
A, C, E, F, G: freeze-fracture images; A, F, G1: usual fracture; C, E, G2: cross-fracture; B: scanning images; D: thin-section images; Bar = 1µm.

A, C, E, F, G : images de cryofracture; A, F, G1 : fracture habituelle ; C, E, G2 : fracture transversale ; B : images de microscopie à balayage ; D : images de coupes minces ; Le trait équivaut à 1 µm.

variety of phylogenetic arguments has led some authors to propose archaebacteria as a third primary kingdom, the two others being prokaryotes and eukaryotes, and to suggest that they may have played a key role in the early history of life (for a review, see Woese, 1981).

In the present study, the use of freeze-fracture confirmed the large variety of epibiotic bacteria, and furthemore revealed some striking morphological features at the membrane level which led us to suggest that certain of these bacteria probably belong to the archaebacterial class.

MATERIAL AND METHODS

Samples associated with *Alvinella pompejana* were collected in the vicinity of hydrothermal vents on the East Pacific Rise during the *Biocyarise* cruise. After arrival at the surface, epibiotic bacteria from the dorsal integument (especially from epidermal expansions) were subsequently fixed with osmium and glutaraldehyde or glutaraldehyde alone, using a classical procedure (Gaill *et al.*, 1984).

For freeze-fracture experiments, freezing was performed on conventionnal gold planchettes after addition of glycerol as a cryoprotectant or between two thin copper plates according to an ultra-rapid freezing procedure (Gulik-Krzywicki, Costello, 1978) in which no glycerol was added.

After freeze-fracture in a Balzers Baf 301 apparatus and shadowing with Pt-C, the replica were observed in a Philips 301 electron microscope.

RESULTS

Most of the bacterial morphologies observed by thin-section transmission (Fig. 1*d*) or scanning (Fig. 1*b*) electron microscopy were found when using the freeze-fracture method, as shown in Figure 1.

The fracture appearance is variable and probably depends on the type of bacteria. Some bacteria are fractured (Fig. 1*a, f, g*1) in a very similar manner to usual bacteria such as *Escherishia coli* (Fig. 2*a*). Many other bacteria are almost exclusively cross-fractured (Fig. 1*c, e, g*2); in some cases a small part at one end of the bacteria is partially fractured (* in Fig. 1) along the membrane or the wall. Both types of fracture were observed in bacteria associated with the epidermal expansions.

It should be noted that the interior of the cells which appears on cross fractured images is not uniform. This observation is not a variance with the thin-section images, where the material appears to be more randomly distributed. Possible perturbations of the cytoplasm could be more easily observed in the plane of the replicated surface than in sections with a certain non negligible

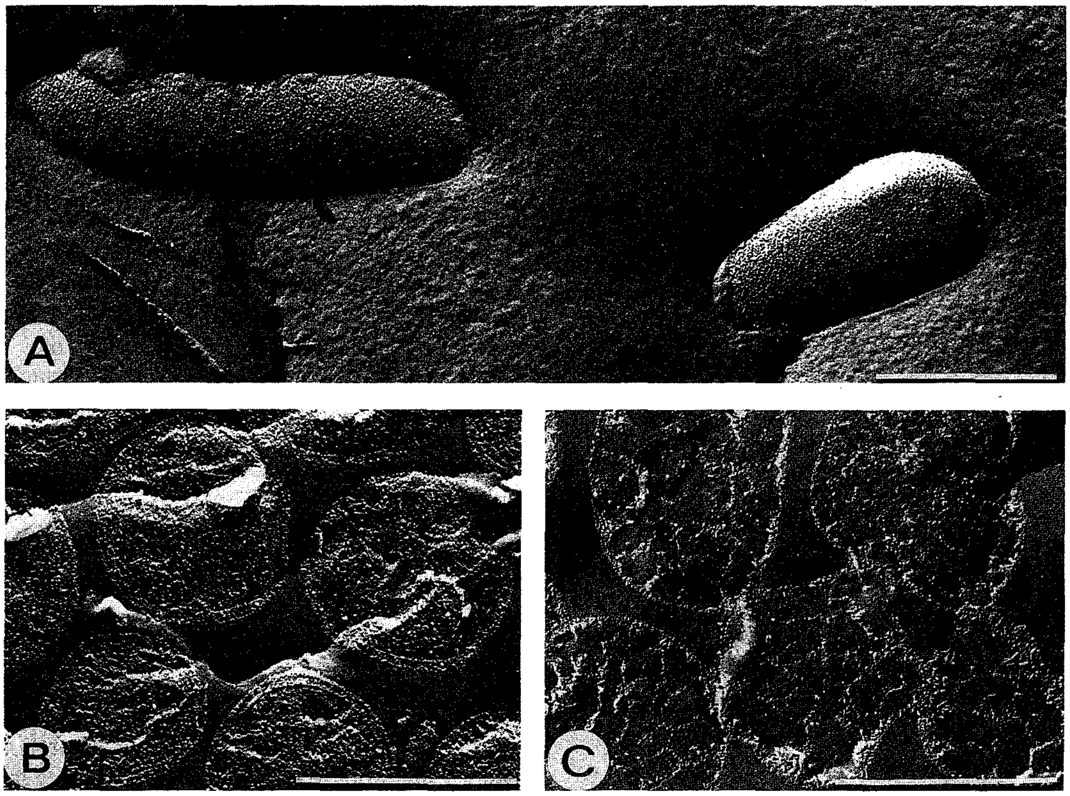

Figure 2

Freeze-fracture images:

A: Escherishia coli: *main type of fracture (cross-fractures may also be observed but with a much lower frequency);* *B,C:* Sulfolobus solfataricus *(cells kindly provided by Pr. De Rosa and Dr. Gambacorta); C: the bacteria were osmotically perturbed; however, the cross-fracture typical of bipolar lipids can still be observed. Bar = 1 μm.*

Cyofracture :

A : *Escherishia coli* : type de fracture le plus souvent observé ; toutefois on peut aussi observer des fractures transversales mais avec une fréquence beaucoup plus faible ; B, C : *Sulfolobus solfataricus* (les bactéries ont été très aimablement fournies par le Professeur De Rosa et le Docteur Gambacorta) ; C : les bactéries ont subi une perturbation osmotique, cependant on peut encore observer la fracture transversale typique des lipides bipolaires. Le trait équivaut à 1 μm.

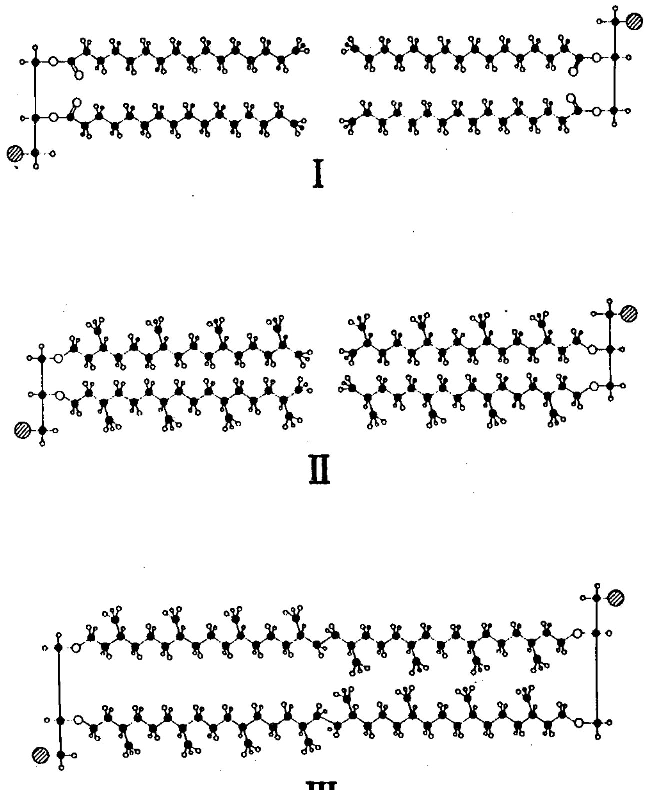

Figure 3

Three examples of lipids, apposed as in lipid bilayers. Filled circles, carbon atoms; open circles, oxygen atoms; small circles, hydrogen atoms; hatched circles, polar residues.
I - lipid of eukaryotes and prokaryotes; the hydrocarbon chains are linear (palmitate in this case) and ester-linked to glycerol.
II and III - lipids of archaebacteria; the hydrocarbon chains are branched (isopranyl) and ether-linked to glycerol.
Each molecule of type II contains two C_{20} chains linked to one glycerol; lipids of this type are commonly found in halophiles and methanogens. III is a dimer of II; it consists of two C_{40} chains ether-linked at both ends to glycerol groups; lipids of this type are mainly found in thermoacidophiles (see *text*).

Trois exemples de la couche lipidique dans les membranes.
Les cercles pleins sont des atomes de carbone, les cercles ouverts des atomes d'oxygène, les petits cercles des atomes d'hydrogène, les cercles hachurés des résidus polaires.
I : lipide d'eucaryote ou de procaryote ; les chaînes hydrocarbonées sont linéaires (palmitate dans ce cas) et sont liées au glycérol par une liaison ester.
II et III : lipides d'archaébactéries : les hydrocarbures sont ramifiés (isopranyl) et liés au glycerol par une liaison ether.
Chaque molécule de type II contient deux chaînes en C_{20} liées à un glycérol. Les lipides de ce type sont trouvés habituellement dans les halophiles et les méthanogènes. III est un dimère de II, il est constitué de deux chaînes en C_{40} liées par un ether aux deux extrémités à des groupes glycérol ; les lipides de ce type sont principalement trouvés dans les thermoacidophiles.

thickness. However, some perturbations may occur during sample collection (drastic changes in temperature and pressure) and/or during fixation, as well as during the freezing process.

What ever the origin of these perturbations, it seems very unlikely that the strinking differences in fracture behaviour are artefactual; it is more likely tha they reveal differences at the membrane level. These differences can even be observed on the same replica between neighbouring bacteria (Fig. 1*g*).

DISCUSSION

Freeze fracture has been extensively used in the study of membranes or lipid systems. It has been demonstrated that the fracture follows the plane of the membrane between the two layers of lipids (Branton, 1966). Thus a bacterium can be fractured along the middle of the cytoplasmic membrane and also along the outer membrane or the wall (Fig. 2*a*). Even in cells having a high membranar protein content, the fracture can follow the plane of the membrane within the bilayer. In particular cases, exceptions to this rule may occur, resulting in an unusual fracture along the hydrophilic surface, rather than along the hydrophobic membrane interior: this has been shown for an artificial reconstitution of vesicles with a very high cytochrome oxydase content packed in a crystalline arrangement (Costello, Frey, 1982). These authours suggested that such a type of fracture might occur in the case of crystalline membranes. There is no *a priori* reason to assume such a situation in the present study.

Indeed, some natural membranes cannot be fractured in the usual manner because they do not contain a double layer of lipids: one monolayer of lipids with a hydrophilic group at each end of the lipid molecule spans the thickness of the membrane. This is the case for some thermophilic bacteria (Weiss, 1974; pers. observ., *see* Fig. 2*b*, *c*).

The different structures of the lipids encountered in living systems and the possible fractures are shown in Figure 3. Using freeze-fracture, it is thus possible to distinguish between lipids of type I or II and type III. Type I lipids are eukaryotic and prokaryotic, type II and III lipids are archaebacterial. It is very likely that when cross-fracture occurs, as in Figure 1 (*c*, *e*, *g*2), the main lipid constituents are of type III, only found in some archaebacteria. These lipids are highly predominant in some thermophilic bacteria, present to some extent in methanogenes and in unknown amounts in thermophile anaerobes (Langworthy, 1985). As freeze-fracture cannot distinguish between lipids of type I and II, the possibility cannot be ruled out that most if not all bacteria observed belong to be archaebacterial class.

Archaebacteria are usually found in exceptional ecosystems, and it is not surprising to find them in the immediate vicinity of deep sea hydrothermal vents. One might expect to encounter a great variety of methanogenes and thermophiles. An extreme thermophilic archaebacterium methanogene, *Methanococcus Janashi*, has been isolated from black smoker sediment sampled at the East Pacific Rise hydrothermal vent. Furthermore, its culture enabled the authors to establish the structure of particular lipids (Comita *et al.*, 1984).

Several sulphur-dependent archaebacteria from submarine hot volcanic area have already been described (Stetter, Zillig, 1985). Large amounts of sulphur have also been detected in the deep hydrothermal sites. Furthermore many sulphur-dependent bacteria belong to the archaebacterial class (Stetter, Zillig, 1985), and it is remarkable that all these bacteria contain tetraether lipids *i.e.* lipids of type III (Langworthy, 1985).

The present results lead us to pose the following question: do the bacteria from hydrothermal vents, associated or not with benthic species, belong to known types of archaebacteria or to new types still unknown? The establishment of microorganism strains should help us to answer this question.

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