Available online at www.sciencedirect.com
ScienceDirect
C. R. Chimie 11 (2008) 268–280
COMPTES RENDUS CHIMIE
http://france.elsevier.com/direct/CRAS2C/

Full paper / Mémoire

Tomography of bacteria–mineral associations within the deep-sea hydrothermal vent shrimp *Rimicaris exoculata*

Louise Anderson [a,*], Sébastien Halary [a,b], Jean-Pierre Lechaire [a,c], Thomas Boudier [b,d], Ghislaine Frébourg [a], Sergio Marco [b,d], Magali Zbinden [a], Françoise Gaill [a]

[a] *UMR 7138, Systématique, adaptation et évolution, Université Pierre-et-Marie-Curie, 7, quai Saint-Bernard, 75252 Paris cedex 05, France*
[b] *Institut Curie, section Recherche, Laboratoire d'imagerie intégrative, Centre universitaire d'Orsay, 91405 Orsay cedex, France*
[c] *Service de microscopie électronique, Institut de biologie intégrative (IFR 83 CNRS), Université Pierre-et-Marie-Curie, 7, quai Saint-Bernard, 75252 Paris cedex 05, France*
[d] *INSERM U759, Centre universitaire d'Orsay, bâtiment 112, 91405 Orsay cedex, France*

Received 11 December 2006; accepted after revision 23 October 2007
Available online 14 January 2008

Abstract

Rimicaris exoculata flourishes around deep-sea hydrothermal vent environments along the Mid-Atlantic Ridge (MAR). Epibiotic bacteria and minerals found within the branchial chamber of the shrimp are of interest in the search for the metabolic energy pathways sustaining shrimp swarms at the Rainbow vent site (MAR). Here we examine the three-dimensional (3D) relationships between epibionts and their associated minerals. The morphology and chemical composition of the minerals were analysed by Energy Filtering Transmission Electron Microscopy (EFTEM) and the 3D organisation was determined by Transmission Electron Tomography (TET). The minerals are preferentially associated with small, rod-shaped bacteria. The iron oxide deposits exhibit three main associations with the bacterial exterior: (1) close, (2) zoned/layered and (3) distant. Iron is the most prevalent element, with a close association with the bacteria, and co-occurs with oxygen. 3D reconstructions reveal a discontinuous network of deposits around the bacteria, showing the intricate nature of these iron oxides. ***To cite this article: L. Anderson et al., C. R. Chimie 11 (2008).***

Résumé

Rimicaris exoculata prospère autour des sources hydrothermales profonds de la ride Médio-Atlantique (MAR). Les bactéries épibiontes et les minéraux se trouvant dans la chambre branchiale de ces crevettes présentent un intérêt dans la recherche des voies métaboliques de transfert d'énergie vers les agrégations des crevettes à Rainbow (MAR). Nous avons examiné les relations tridimensionnelles entre les épibiontes et leurs minéraux associés. La morphologie et la composition chimique des minéraux ont été analysées par microscopie électronique à transmission en perte d'énergie (EFTEM), et l'organisation 3D a été déterminée par tomographie électronique à transmission (TET). Les minéraux sont préférentiellement associés à de petites bactéries en bâtonnet. Les dépôts d'oxyde de fer présentent trois types d'association avec la partie externe des bactéries : (1) proche, (2) en zones/en couches et (3) distante. Le fer est l'élément prédominant. Il présente une association proche avec les bactéries et est co-localisé avec

* Corresponding author. Present address: Department of Geology, University of Leicester, Leicester, LE1 7RH, UK.
E-mail address: lma9@le.ac.uk (L. Anderson).

1631-0748/$ - see front matter © 2007 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.crci.2007.10.007

l'oxygène. Les reconstructions en 3D révèlent un réseau discontinu de dépôts autour des bactéries, qui montre la complexité de ces oxydes de fer. ***Pour citer cet article : L. Anderson et al., C. R. Chimie 11 (2008).***

Keywords: Hydrothermal; Crustacea; Bacteria; Biomineralisation; Iron oxide; Tomography; Symbiosis

Mots-clés : Hydrothermale ; Crustacés ; Bactéries ; Biominéralisation ; Oxyde de fer ; Tomographie ; Symbioses

1. Introduction

The hydrothermal vent shrimp *Rimicaris exoculata* is one of the most dominant creatures in the fauna found around hydrothermal vents on the Mid-Atlantic Ridge (MAR). Dense populations of bacterial epibionts can be found on both the shrimp's mouthparts and the inner surfaces of the branchial chamber [1,2]. It has been suggested, from genetic analysis of samples from Snake Pit (MAR), that the epibiont community consists of one single bacterial phylotype belonging to ε-Proteobacteria [3]. A further suggestion that these bacteria obtain their metabolic energy from sulphide oxidation has not yet been confirmed through cultivation [4,5]. The definitive role of these epibionts remains unknown, although the majority of questions posed regarding this have been in relation to the shrimp diet. The epibionts have been suggested as a primary food source, grazed on by the shrimps [4,6–8]. However, the shrimps have been observed grazing on surface bacterial mats of sulphide chimneys [5].

In this study, we have focussed on examining, in greater detail, the mineral deposits closely associated with those epibionts found on the inner surface of the shrimp's branchiostegite. Gloter et al. [9] identifed these minerals, and Zbinden et al. [2] studied their distribution in the gill chamber. However, no one has yet described the morphogenesis of these mineral assemblages or their three-dimensional (3D) distribution around the bacteria; in the search for further proof of microbially mediated mineral deposition. Zbinden et al. [2] observed three distinct areas on the gill chamber wall, differing from each other not only in the abundance of bacteria and mineral deposits, but also in the nature of the minerals. Taking into consideration the fluid pathway through the gill chamber and the geochemical characteristics of the shrimp environment, those authors went on to theoretically divide the branchiostegite into three chambers of potentially differing micro-chemical-environment/functionality. Of these three chambers, the richest in mineral deposit and most orange-brown in colour was the third antero-dorsal area (upper pre-branchial chamber). The minerals from this location have been classified as 2-line-ferrihydrite (2l-Fh), a type of iron oxide, and may represent an analogue of the solid ferrihydrite (Fh) precursor involved in magnetite biomineralization [9]. The deposits were found to be very homogeneous and the specific mineralogical composition of the iron oxide deposits suggests that bacteria play a direct role in mineral formation [9]. However, this suggestion has not been definitively proven. Numerous authors report the occurrence of iron oxide deposits, commonly accepted to be of microbial origin at hydrothermal vents (e.g. Refs. [2,5,10,11]). More recent studies have highlighted the direct role of bacteria in the deposition of iron and suggested a major contribution of chemoautotrophic iron oxidisers in this process [12–14]. To date, however, the occurrence of such iron oxidisers in association with this shrimp species has not been described.

The samples examined in this study originate from the Rainbow hydrothermal vent site. The vent field is situated on an ultramafic substrate that has been exposed by large-scale faulting, and it is recognised as the richest site on the MAR for metals [15], being enriched particularly in iron but depleted in sulphide [16]. Therefore, the Rainbow site is potentially an ideal location to harbour iron-oxidising bacteria.

Tomographic reconstruction techniques, in conjunction with Transmission Electron Microscopy (TEM) and Cryo-Electron Microscopy methods, are becoming increasingly used in the search for better understanding of mineral–cell interactions and associations. Of note are the recent studies of magnetotactic bacteria by Scheffel et al. [17] and Komeili et al. [18], which used cryo-electron tomography to image sub-cellular structures putatively governing the localization of MamJ (an acidic protein potentially influencing magnetosome formation), and the work of Boudier et al. [19] on integrating Energy Filtering Transmission Electron Microscopy (EFTEM) with tomography to analyse internal granular mineral deposits in bacteria of the vent tubeworm *Riftia pachyptila*.

In this paper, we examine: (1) the different types of bacteria–mineral associations (TEM), (2) the distribution of the principal elements iron and oxygen in relation to the bacterial membrane (EFTEM), and (3) the