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Inorganic carbon incorporation and nutritional transfers in the chemosynthetic ectosymbiosis harboured by the Atlantic hydrothermal vent shrimp Rimicaris exoculata

Julie Ponsard (1), Marie-Anne Cambon-Bonavita (2), Magali Zbinden (3), Gilles Lepoint (1), André Joassin (4), Laure Corbari (5), Bruce Shillito (3), and Philippe Compère (1)

(1) Université de Liège, Faculté des Sciences, Département des Sciences et Gestion de l'Environnement, Centre MARE, Bâtiment B6c, Allée du 6 Août n°15, Sart-Tilman, Belgium (julie.ponsard@ulg.ac.be, 0032-(0)4/366.59.75), (2) Ifremer Centre de Brest, Microbiologie des Environnements Extrêmes, LM2E-UMR6197 IFREMER-UBO-CNRS, F-29280 Plouzané, France, (3) Université Pierre et Marie Curie, UMR 7138 « Systématique, Adaptation, Evolution », CNRS, F-72252 Paris cedex 05, France, (4) Université de Liège, Dépt. de Chimie - Chimie de Coordination et Radiochimie, B-4000 Sart-Tilman - Liège, Belgium, (5) Muséum National d'Histoire Naturelle, Département Systématique et Evolution, UMR 7138 « Systématique, Adaptation et Evolution », Equipe Espèces et Spéciation, 57 Rue Cuvier, CP 26, F-75231 Paris Cedex 05, France

The hydrothermal vent shrimp *Rimicaris exoculata* is characterized by its chemoautotrophic bacteria hosted in its gill chambers. It lives in dense swarms of thousands individuals per m^2 on the black smoker chimneys of the Mid-Atlantic Ridge (MAR) vent sites, including the Rainbow site (36° 13'N MAR) where we sampled. First considered as a monoculture of a single phylotype of sulphide-oxidizing epsilon-proteobacteria (Polz & Cavanaugh, 1995), the *R. exoculata* symbionts are recognized as forming real bacterial community that includes various phylotypes and metabolisms. Zbinden *et al.* (2008) and Petersen *et al.* (2010) have identified genes characteristic of methane–oxidizing and sulphide–oxidizing bacteria in the shrimp gill chambers. Furthermore, they observed the occurrence of intracellular sulphur– and iron–enriched granules in filament and rod bacteria, and identified some methanotrophic–like bacteria cells, suggesting that at least three metabolisms (iron, sulphur and methane oxidation) co–occur within the ectosymbiotic community. On the other hand, Schmidt *et al.* (2008) also pointed out that several reducing compounds present in the hydrothermal environment (H_2, CH_4, Fe^{2+} or SH^-) could be used as geochemical energy sources for the shrimp bacterial growth and CO_2-fixation.

The major aims of the work were to determine the environmental reducing compounds really used by ectosymbiotic bacteria of *R. exoculata* as energy supply of their metabolism and to highlight the probable transfer of molecules from bacteria to the shrimp tissues. In the experiments, shrimps were incubated alive in small volumes in pressurized aquarium IPOCAMP^{TM} either for 4, 6 or 10 hours with various reducers (H_2, CH_4, Fe^{2+}, thiosulphate) and in presence of carbon isotope-labelled Na-bicarbonate ($NaH^{13}CO_3$or $NaH^{14}CO_3$). Other experiment was also realized by 1 hour-incubation of shrimps in seawater in presence of ^{14}C-acetate and ^{3}H-lysine. Preferential metabolism and carbon incorporation in the biofilm in the shrimp gill chamber was determined through the quantification of ^{13}C in mass spectrometry and by scintillation counting for ^{14}C. The first data carbon-isotope incorporation allows identifying Fe^{2+} and thiosulphate as suitable energy sources for the bacteria. They also suggest the presence of an internal energy supply in the bacteria owing the carbon fixation also occurs over several hours in pure seawater. The sulphur granules observed in the bacteria reported by Zbinden *et al.* (2008) could be the energy source used. The possible transfer of organic molecules from the bacterial symbionts to the host shrimp was also investigated by examining the ^{13}C, ^{14}C and ^{3}H levels in the tissues. The presence of ^{14}C and ^{3}H incorporated from ^{14}C-bicarbonate, ^{14}C-acetate and ^{3}H-lysine confirm the transfer and the higher levels of radioactivity in the gill chamber integuments support a probable tegumental incorporation pathway of dissolved organic molecules rather than a digestive pathway.

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