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Nickel-tolerant mesophiles from deep-sea hydrothermal sources of the Eastern Pacific Rise (12°45' N , 103°59' W)

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Introduction

Deep-sea hydrothermal vents and their specific animal communities shelter a variety of microorganisms that colonize the entire gradient of biologically compatible temperatures from the mouths of the well-known "black smokers" to the surrounding ocean floor (2 °C). Black smokers release many inorganic substances that can promote chemolithotrophic bacterial growth. Heavy metals may be also released by hydrothermal vents: a substantial fraction will be precipitated but one cannot exclude that some will remain bioavailable in some niches of the hydrothermal vent ecosystem and may exert toxic effects (Llanos et al., 2000).

From this perspective, it is of interest to screen the vent ecosystem for bacteria that display plasmid-born resistance to heavy metals as is the case in other natural (or anthropogenic) biotopes that contain high levels of these chemicals (Mergeay, 2000).

The present report focuses on the mesophilic microbial communities of the deep-sea hydrothermal sites Genesis and Grandbonum of the Eastern Pacific Rise (12°45' N, 103°59' W) (depth : 2600 m).

Material and methods

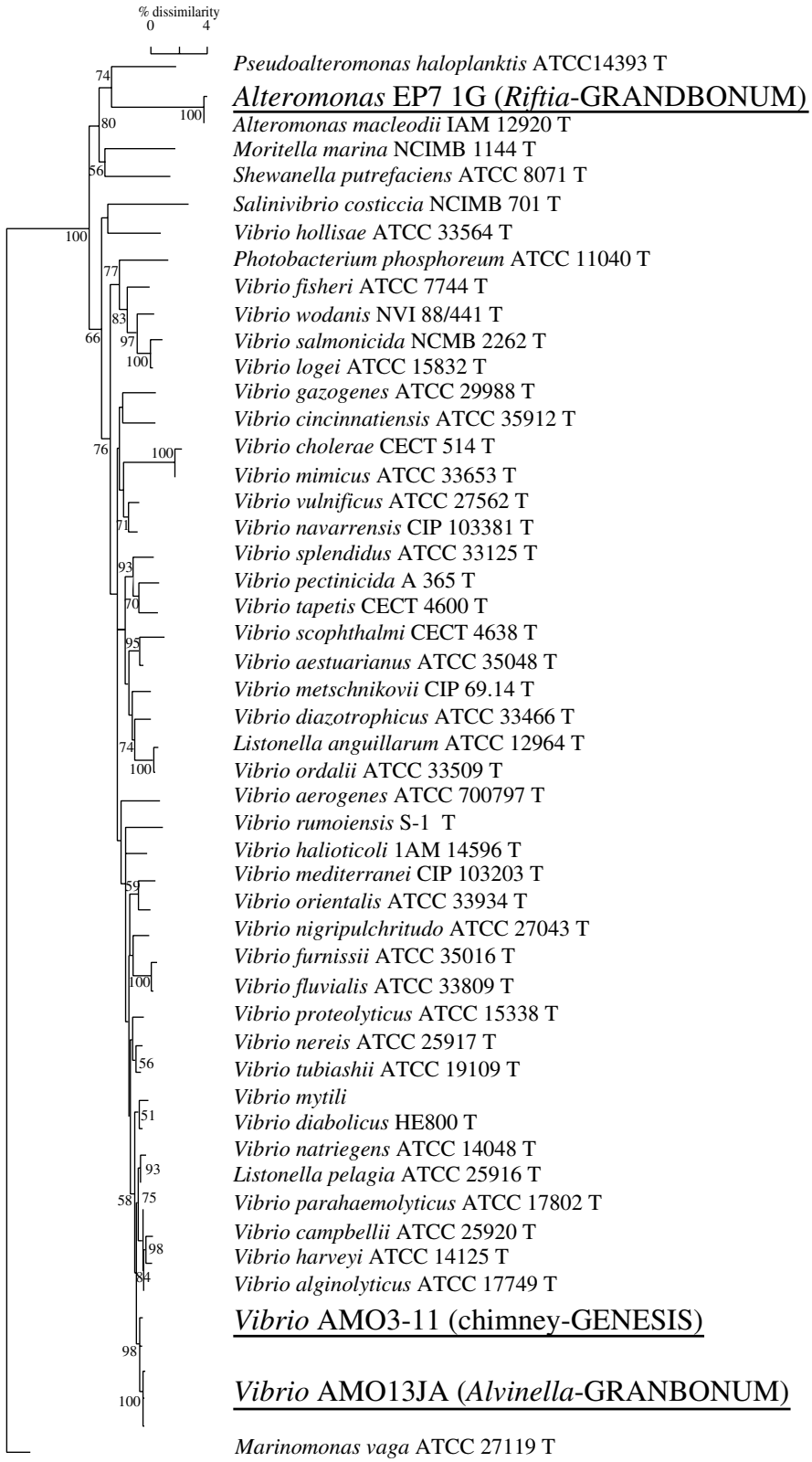
Various samples from the two sites were cultivated under oligotrophic or near autotrophic conditions and thereafter were examined for bacterial tolerance to heavy metals.

The following enrichments in artificial sea water (ASW) were carried out :
- a culture in autotrophic conditions (GasPak jar with air + H_2 + CO_2) in presence of a fragment of an active chimney (Genesis);
- a culture with diluted broth inoculated with scrapings from a tube of *Riftia pachyptila* Jones, 1981 (site Grandbonum that lies at a distance of ~5 nautical miles from the Genesis black smoker);
- direct platings from a crushed deep-frozen *Alvinella pompejana* Desbruyères & Laubier, 1980 worm sampled on a black smoker from the Grandbonum site, and an oligo-autotrophic enrichment from the same sample.

Results and Discussion

All these cultures and enrichments gave rise to rather homogenous platings where the CFUs (CFU: colony forming unit) were overwhelmingly resistant to nickel chloride at a concentration of 1 to 5 mM. CFUs resistant to Cu (1 mM) were also found but to a much lesser extent. CFUs clearly resistant to Cd (1 mM) or Zn (1-2 mM) were not found. The obvious general response to nickel, in contrast with other heavy metals, was intriguing especially since no nickel selective pressure was exerted during the cultures or the enrichments. Subsequently, characteristic isolates from these four enrichments and platings were purified and conserved, their 16s rDNAs were amplified and fully sequenced (on around 1500 bp). Three isolates (that from Genesis and the isolates from the *Alvinella* extract) proved to be almost identical (more than 99.5 % similarity) and clustered near *Vibrio natriegens* (Payne et al., 1961) (98.9 % similarity) (Fig. 1).

Deep-sea *Vibrio* have already been described and include some psychrophilic strains (Raguenes et al., 1997; Xu et al., 1998). The DNA sequences of the isolates from *Alvinella* (direct plating of the crushed worm or oligo-autotrophic enrichment for 10 days) were absolutely identical: they are referred to Figure 1 as the isolate AMO13JA. In fact, these isolates are able to grow in presence of 1 mM Ni^{++} only when they are grown with glutamate as a carbon source. With gluconate as a carbon source, they are much more



Type strains are followed by T.
Bootstrap values were calculated and indicated if higher than 50%.

Figure 1. Phylogenetic tree of new nickel-resistant strains.

sensitive to nickel (MIC : lower than 0.5 mM Ni^{++}) (MIC: Minimum Inhibitory Concentration).

In this respect, they are genotypically different from the closely related strain AMO3-11 which is, on all tested media, clearly resistant to nickel with a MIC higher than 5 mM. It would be of interest to see if the genetic determinant for resistance to nickel is transferable from the *Vibrio* sp.AMO3 to the *Vibrio* AMO13JA or is associated with a mobile genetic element (integron, plasmid or transposon). The nickel-resistant *Vibrio* thrive on minimal ASW media with glutamate, gluconate, succinate or lactate as carbon sources but they still reasonably grow on ASW media with no added carbon source. Growth is slightly enhanced in the presence of carbonate (but not in the presence of glucose); they do not appear to be hydrogenotrophs and it is not clear how they were enriched in autotrophic conditions. On all plates (especially in oligotrophic conditions: plates with no added organic carbon source), they produce long swarms that started from round, flat and translucid colonies and finally resolved into fractal forms.

The closest relative of the nickel-resistant isolate found in the culture inoculated with the rusty scrapings of *Riftia pachyptila* Jones, 1981 is *Alteromonas macleodii* Baumann et al., 1972, IAM 12920 (Fig. 1). A deep-sea representative of this genus has already been described (Raguenes et al., 1996). The genus *Alteromonas* is now restricted to a single species, *Alteromonas macleodii*, as 11 species, formerly located in the genus *Alteromonas*, were now accommodated into the new genus *Pseudoalteromonas*, with *P. haloplanktis* ZoBell & Upham, 1944 (type strain, ATCC 14393) as the type species. *Alteromonas* EP7 1G is resistant to nickel in presence of glutamate or gluconate (MIC : 4 mM); it does not grow using glucose or succinate.

The level of nickel-tolerance observed in *Vibrio* AMO3-11 and in *Alteromonas* AMO13JA is similar to that observed in *Ralstonia metallidurans* Goris et al., 2001, CH34 or in *Klebsiella oxytoca* (Flügge, 1816) (see review by Mergeay, 2000). Further studies will examine if these *Vibrio* and *Alteromonas* (both genera belong to the γ-Proteobacteria) share some characteristics of the microbial communities that are specific of the deep-sea hydrothermal sources as piezotolerance. They will also look at the genetic determinants involved in the tolerance to nickel. This may first be carried out by using probes or primers taken from genes that confer resistance to nickel in other bacteria. The main nickel-resistance determinants that were described are *nreB*, found in *Klebsiella oxytoca* and in *Ralstonia metallidurans* 31A, the *cnr* YXHCBA operon of *R. metallidurans* CH34 and the *nccYXHCBAN* in *R. metallidurans* 31A. The *cnr* and *ncc* genes command three-component cation proton antiporter efflux of nickel and cobalt (for a review, see Mergeay, 2000).

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