Note

Active Bacterial Flora Surrounding Foraminifera (Xenophyophorea) Living on the Deep-Sea Floor

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Bacteria form unique ecosystems by coexisting with large organisms. Here we present the first evidence of active flora surrounding xenophyophorea revealed through clone analyses of environmental ribosomal RNA gene sequences. The flora included eight phyla in the xenophyophorean cells with agglutinated test. The major operational taxonomic units were unique from that in the near-surface sediment. This flora appears to be formed by coexistence with xenophyophores.

Key words: microbial flora; deep sea; xenophyophore; foraminifera

Bacteria form unique ecosystems on benthic structures, substrates and coexist with large organisms on the deep-sea floor. For example, giant foraminifera (xenophyophorea) can have a considerable influence on nearby microflora. In areas deeper than the carbonate compensation depth, foraminifera construct fragile agglutinated tests (technical term for shells) composed of benthic sediment and organic cement secretion.1,2) Xenophyophores are the largest foraminifera (about 25 cm in size) making tests, and are widely distributed at a depth of 7,111 m on the Izu-Ogasawara Trench slope (32°47.65’N, 141°52.59’E). Surface sediment was collected at approximately 50 cm from the collected xenophyophore (Fig. 1A). Both samples were immediately stored at −80°C. Since the xenophyophore was fragmented in the sampler, we isolated the top 3.5 cm from the push-core sample as a “xenophyophore sample,” a mixture of xenophyophorean cells and the loosely agglutinated test. We used the upper 3.5 cm of the reference push-core sediment as a “reference sample.” Total RNAs were extracted from the frozen samples individually using an RNA PowerSoil Total RNA Isolation Kit (Mo Bio Laboratories Inc., Solana Beach, Calif.). The amounts of total recovered RNA were 69.4 ng of total RNA/g (xenophyophore sample), and 73.5 ng of total RNA/g (reference sample). The total RNAs were treated with DNase I, Amp Grade (Invitrogen, Carlsbad, CA) and with RNase inhibitor SUPERaseIn (Ambion, Austin, TX). cDNAs were synthesized using SuperScript III (Invitrogen, Carlsbad, CA) and with RNase inhibitor SUPERaseIn (Ambion, Austin, TX) from the cDNAs. Reactions without reverse transcriptase were performed to exclude DNA contamination. PCR products were ligated into pGEM-T vectors and cloned into Escherichia coli DH5α-competent cells. We sequenced 309 xenophyophore sample and 210 reference sample clones using 27F and 1492R primers. The former average sequence lengths were 1,413 bp and the latter were 1,411 bp. Both sequences were grouped into operational taxonomic units (OTUs) defined at 97% sequence similarity.7) The coverage values were 88.5% (xenophyophore sample) and 83.8% (reference sample).8) The OTUs were aligned using Greengenes alignment8) and were phylogenetically analyzed at the phylum level using ARB software.9) The closest related species were selected by BLAST.

We collected a xenophyophore that was rippled approximately 7 cm in diameter (Fig. 1A). This is the first report of a xenophyophore from the Izu-Ogasawara Trench. A molecular phylogenetic tree based on the partial small subunit (SSU) rDNA sequence using S8F and S8 primers10) by neighbor-joining (NJ) analysis and the maximum likelihood (ML) method confirmed the

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Abbreviations: BLAST, Basic Local Alignment Search Tool; NJ analysis, neighbor-joining analysis; ML, maximum likelihood; NCBI, National Center for Biotechnology Information; OTU, operational taxonomic unit
monophyly of xenophyophores (Fig. 1B). Nucleotide BLAST analysis showed the highest similarity to Shinkaiya lindsayi (NCBI accession no. EU649778) in the conserved regions, but the sequence similarities were less than 94%, suggesting that the two xenophyophores were different species. These results suggest that the bacterial clones obtained from the total RNA associated with the xenophyophore described as a genetically new phylotype.

Bacterial 16S rRNA clones obtained from the total RNA of the xenophyophore sample showed that the bacterial OTUs belonged to eight phyla, Proteobacteria, Gemmatimonadetes, Acidobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Planctomycetes, Chlorobi, and candidate divisions (Figs. 2 and 3), suggesting that the active flora were comprised of these bacteria. The same phyla and candidate divisions were detected in the bacterial 16S rRNA clones obtained from total RNA of the reference sample, suggesting that no difference existed between active flora in the xenophyophore and the reference sediments at the phylum level.

At the OTU level, high genetic diversity was observed. The OTUs within the Proteobacteria subclasses showed the highest diversity and the largest proportions (Fig. 2A and B). Although OTU0001 in Alphaproteobacteria was the most abundant, its population comprised less than 6%, suggesting that no dominant OTU was present in this flora. Most of the OTUs were unique to one of the two samples relative to the other and were detected in all the phyla name above (Figs. 2 and 3), with the exception of Chlorobi, a minor phylum including only one OTU (Fig. 3D). Alphaproteobacteria OTU0001 was detected in both samples, but showed a significantly lower population ($p < 0.05$, Fisher’s exact test) in the xenophyophore sample compared to the reference sample (Fig. 2A). This

Fig. 1. Phylogenic Analysis of the Collected Xenophyophore. A, Sampling at the sea floor. Collected new xenophyophore (arrowhead) and reference sediment (arrow) are shown. Hollow arrowheads show other xenophyophores. Scale bar, 10 cm. B, Molecular phylogenetic tree based on partial SSU rDNA sequences and representative foraminifera inferred by NJ analysis and the ML method. An alignment scoring 975 nucleotide positions (gaps excluded) was created using Clustal X. Bootstraps support values higher than 50% (1,000 replicates for NJ analysis, 100 replicates for the ML method), and are represented as NJ/ML. Scale bar indicates estimated number of base changes per nucleotide sequence position.
suggests that population of OTU008 decreased after incorporation into xenophyophorean cells and/or agglutinated test. Unfortunately, we could not identify the localization patterns of the OTUs unique to xenophyophore sample by microscopy, because xenophyophore cells are fragile, and were totally destroyed during sampling. However, we can at least say that OTU008 is not an obligatory symbiotic bacterium, because OTU008 is active in the sediment without the xenophyophore.

OTU008 forms a clade with uncultured bacterium clone S26-96 (EU287396) collected from the Arctic and with clone Ulrdd36 (AM997500) collected from the South Atlantic Ocean with 99% sequence homology (Fig. 3F), suggesting that three clones are same species. They are found in deep-sea sediments, but their metabolisms are unknown. Close clades containing OTU005 and OTU073 did not show significant differences between the two samples (Fig. 2A).

Our results suggest that active phylotypes in surface sediment change greatly after coexisting with xenophyophores. A previous study suggested that xenophyophores contain higher amounts of bacteria than the reference sample, and that the bacterial ratio patterns between the two samples are different. Further analysis of the amounts, localization, ecology of OTU008 and other unique OTUs, and individual differences between xenophyophore sp. Boso2008 should clarify the interaction mechanism between the bacteria and the xenophyophores.

In conclusion, this study provides an initial view of the microflora surrounding xenophyophores in the deep sea, and is the first report of a molecular ecological study of this flora. Description of the bacterial ecology in a micro
habitat, such as co-localization with large organisms, is still rare, especially based on RNA analysis correlating biological activity. Active microbial flora in the xenophyophore test can provide clear evidence of ecology of minor and dormant microorganisms in the environment.

The collected xenophyophore and bacterial rRNA sequences obtained in this study were deposited in the GenBank nucleotide sequence databank under accession nos. AB694014 to AB694522.

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