APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2006, p. 2268–2271 Vol. 72, No. 3
0099-2240/06/$08.00+0 doi:10.1128/AEM.72.3.2268–2271.2006

Thermophilic Lifestyle for an Uncultured Archaeon from Hydrothermal Vents: Evidence from Environmental Genomics†

Hélène Moussard,[1] Ghislaine Henneke,[1] David Moreira,[2] Vincent Jouffe,[1] Purificacion López-García,[2] and Christian Jeanthon[1]*

Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197, Centre National de la Recherche Scientifique, Ifremer & Université de Bretagne Occidentale, Institut Universitaire Européen de la Mer, 29280 Plouzané,[1] and Equipe Diversité et Evolution Microbiennes, Unité d'Ecologie, Systématique & Evolution, UMR 8079, Centre National de la Recherche Scientifique, Université de Paris-Sud, 91405 Orsay,[2] France

Received 25 July 2005/Accepted 28 December 2005

We present a comparative analysis of two genome fragments isolated from a diverse and widely distributed group of uncultured euryarchaea from deep-sea hydrothermal vents. The optimal activity and thermostability of a DNA polymerase predicted in one fragment were close to that of the thermophilic archaeon *Thermoplasma acidophilum*, providing evidence for a thermophilic way of life of this group of uncultured archaea.

Molecular phylogenetic surveys of microbial communities in deep-sea hydrothermal vents have recently revealed the existence of archaeal lineages that were unanticipated from classical microbiological methods. Using primers specific for the archaeal 16S rRNA gene or specific probes, the diversity and abundance of members of the DHVE2 lineage within the deep-sea hydrothermal vent *Euryarchaeota* (DHVE) have been well documented (11–12, 16–17, 19, 24–25). Based on their ecological distribution, members of DHVE2 lineage can be considered likely candidates to represent a group having a significant impact in hydrothermal habitats. Since they escaped cultivation despite valuable efforts (17), their phenotypic and metabolic features are unknown. Instead of cataloging single genes, the capture of large environmental DNA fragments from naturally occurring microbial assemblages has recently been developed to explore the physiological potential of uncultured microorganisms and is now becoming a common method to characterize microbial communities (1–2, 4, 10, 22).

The alvinellid polychaete *Alvinella pompejana* colonizes the walls of black smokers on the East Pacific Rise (6), and its tubes provide sites for attachment for microorganisms discharged by the hydrothermal emissions. In an effort to get a more comprehensive view of uncultured archaea from deep-sea hydrothermal vents, we recently constructed a fosmid library from microbial assemblages associated with the polychaete tubes (15). Based on the Bayesian phylogenetic analysis (13) of the 16S rRNA gene sequences they contained, four fosmid clones containing an archaeal 16S rRNA gene (Alv-FOS1, Alv-FOS2, Alv-FOS3, and Alv-FOS4) clustered within the DHVE2 lineage (Fig. S1 in the supplementary material).

To get more insight into the physiology and genetics of members of the DHVE2 lineage, the ~40-kb inserts of the two fosmids that appeared the most distant in the 16S rRNA tree, Alv-FOS1 and Alv-FOS4, were sequenced completely. The inserts of clones Alv-FOS1 and Alv-FOS4 comprised 40,482 bp and 41,331 bp, respectively. In both genome fragments, neither 23S nor 5S rRNA genes were present in the immediate surroundings of the 16S rRNA gene, but a Met-tRNA gene was. The situation found in the DHVE2 genome fragments is not common but is similar to that observed in the genomes of *Thermoplasmatales* spp. and *Methanopyrus kandleri*, a methanogenic hyperthermophile, and in genomic fragments of uncultured planktonic euryarchaeota whose closest cultured neighbors are the *Thermoplasmatales* (7, 14, 18, 23).

A total of 41 and 44 predicted open reading frames (ORFs) longer than 50 amino acids were identified in Alv-FOS1 and Alv-FOS4, respectively. Most of the predicted ORFs of Alv-FOS1 and Alv-FOS4 (24 of 41 and 25 of 44, respectively) showed sequence homologies to the products of known genes (Table S1 and Table S2 in the supplementary material; Fig. 1). Others were homologous to conserved hypothetical proteins (5 of 41 ORFs and 6 of 44 ORFs for Alv-FOS1 and Alv-FOS4, respectively) or were predicted proteins of unknown function without homologs in databases (12 of 41 ORFs and 13 of 44 ORFs for Alv-FOS1 and Alv-FOS4, respectively).

Seventeen ORFs were predicted to represent core components of information-processing systems, e.g., involved in DNA replication, conformation, and repair and in translation and transcription (20, 26). Among them, a putative family B DNA polymerase exhibited the six conserved motifs indicative of the family B DNA polymerases and the tree motifs 3′ to 5′ of exonuclease motifs, suggesting that the protein exhibits both activities (27). The putative family B DNA polymerase exhibited the highest similarities to homologs from *Thermoplasmatales* (51 to 53% identity). This was confirmed by the Bayesian phylogenetic analysis of these sequences, which showed the emergence of the Alv-FOS1 DNA polymerase in a very well supported group (posterior probability [PP] = 1) with homologs from the *Thermoplasmatales* (Fig. S2A in the supplementary material). Within this group, the Alv-FOS1 DNA polymerase branched with those of *Thermoplasma* spp. with a relatively low posterior probability (PP = 0.67). Additional phylogenetic analyses carried out using a set of well conserved

* Corresponding author. Present address: UMR 7144, Equipe Phytoplancton Océanique, Station Biologique, Place Georges Teissier, 29680 Roscoff Cedex, France. Phone: 33-298-292-379. Fax: 33-298-292-324. E-mail: jeanthon@sb-roscoff.fr.

† Supplemental material for this article may be found at http://aem.asm.org/.

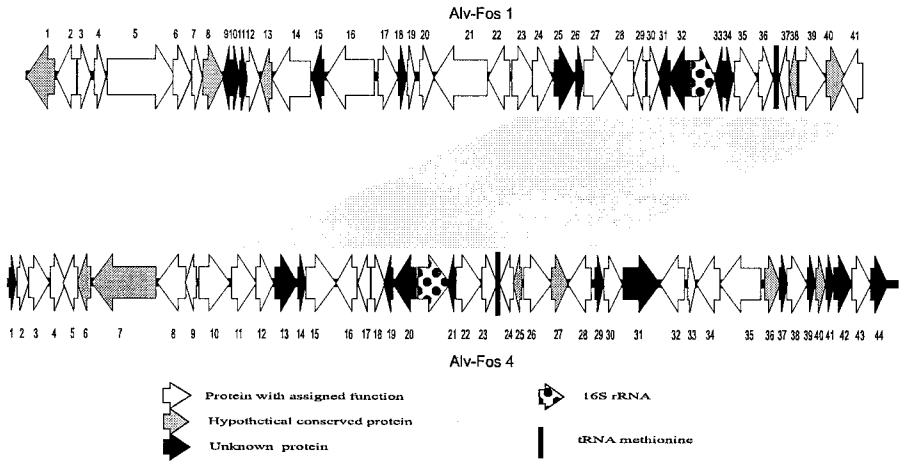

FIG. 1. Schematic representation of the euryarchaeal clones Alv-FOS1 and Alv-FOS4. Open reading frames (ORFs) categorized in proteins with assigned functions, conserved hypothetical proteins, unknown proteins, and rRNAs are differentiated as indicated in the inset. The tRNA genes are indicated by black bars. ORF numbers of the FOS1 and FOS4 fragments refer to Tables S1 and S2 in the supplementary material, respectively. Shading between the clones indicates homologous regions with the percentage of identities of deduced amino acid sequences.

protein-coding genes predicted in both genome fragments and the identification of individual genes and gene clusters specific to the DHVE2 lineage and the *Thermoplasmatales* provides further insight into the relationship between both lineages (Tables S1 and S2 in the supplementary material). In contrast with these findings, the genome fragments analyzed here also encoded proteins that have not been predicted in genomes of *Thermoplasmatales* spp. (e.g., DNA topoisomerases VI subunits). Bayesian phylogenetic analysis showed that the A subunit of Alv-FOS4 topoisomerase emerged at a position occupied by the *Thermoplasmatales* genus in phylogenies reconstructed using 16S rRNA genes (Fig. S2B in the supplementary material). If the DHVE2 lineage shared a common origin with *Thermoplasmatales* (as supported by above phylogenetic analyses), this suggests that the *Thermoplasmatales* spp. have lost the genes encoding DNA topoisomerases VI after their divergence from the DHVE2 lineage.

A large colinear region (~16.6 kb) between Alv-FOS1 and Alv-FOS4 contained 18 ORFs, the 16S rRNA-encoding genes, and the Met-tRNA-encoding genes (Fig. 1). The colinear section included genes encoding putative proteins involved in the metabolism of coenzymes and cofactors, nucleotides, amino acids, and nucleic acids (Tables S1 and Table S2 in the supplementary material). The only difference between both gene clusters corresponded to a small protein of unknown function (ORF34 of Alv-FOS1) absent in Alv-FOS4. Most of the unknown proteins encoded by the syntenic region contained strong predicted transmembrane helices, suggesting that their products were membrane anchored. No synteny identical to that observed between Alv-FOS1 and Alv-FOS4 could be identified in other genomes.

To determine some biochemical properties of the Alv-FOS1 family B DNA polymerase (ORF16), the gene was amplified by PCR, cloned into the prokaryotic expression vector pAHRS (5), and transformed into *Escherichia coli* DH5α (Clontech) according to standard procedures (21). DNA polymerase activity was performed in a final volume of 20 μl containing 20 mM Tris-HCl (pH 9), 25 mM KCl, 1.5 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1 mg/ml bovine serum albumin, 0.1% Tween 20, 0.35 μM [methyl-1′,2′-^{3}H]dTTP, ammonium salt ([^{3}H]dTTP; 119 Ci/mmol; Amersham Biosciences), 200 μM of each four deoxynucleotide triphosphates (dNTP; Q-Biogene), and 4 μg

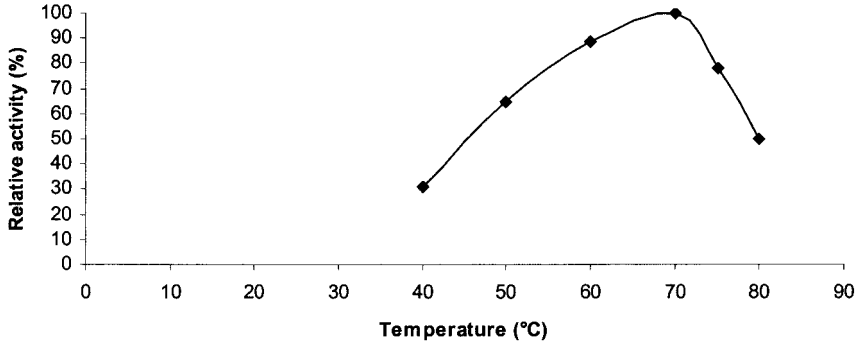

FIG. 2. Effect of the temperature on Alv-FOS1 DNA polymerase activity. FOS1 DNA polymerase (1.4 pmol) was assayed under standard conditions for 20 min at the indicated temperature. DNA polymerase activity was determined by measuring the incorporation of [^{3}H]dTTP into activated calf thymus DNA.

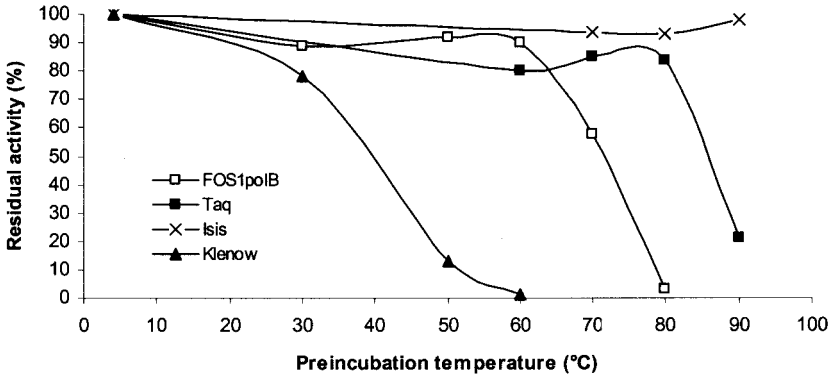

FIG. 3. Thermostability of Alv-FOS1 DNA polymerase compared with that of *T. aquaticus* (*Taq*) and *P. abyssi* (Isis) and the Klenow fragment of *E. coli* DNA polymerase I. The residual DNA polymerase activity was measured for 20 min at 65°C after a 30-min preincubation of the enzymes at increasing temperatures.

of activated calf thymus DNA (Sigma-Aldrich). Under these conditions, the DNA polymerase activity was found to be 0.3 U/ml. The Alv-FOS1 DNA polymerase activity was optimal at 70°C (Fig. 2). However, the optimal temperature of Alv-FOS1 DNA polymerase could not be accurately determined, since activated DNA was not stable above 75°C.

To better evaluate its ability to resist elevated temperatures, we compared its thermal stability with that of DNA polymerases from microorganisms of different thermal classes. The Klenow fragment of *E. coli* DNA polymerase I, the Alv-FOS1, *Taq*, and Isis family B DNA polymerases (from *Thermus aquaticus* and *Pyrococcus abyssi*, respectively) were preincubated for 30 min at temperatures ranging from 4°C to 90°C, and their ability to incorporate dNTPs into the DNA substrate was tested at 65°C for 20 min. Contrary to that of *E. coli*, Alv-FOS1 DNA polymerase was found to be resistant up to 60°C (Fig. 3). However, Alv-FOS1 DNA polymerase was less thermostable than DNA polymerases purified from *P. abyssi* and *T. aquaticus*. Its thermostability was comparable to that of the *T. acidophilum* DNA polymerase (9). Our expression study provides evidence that Alv-FOS1 (and most likely its relatives of the DHVE2 lineage) harbors a thermophilic lifestyle. This physiological trait had previously only been speculated on the basis of the high-G+C content of their 16S rRNA, a typical feature of thermophilic microorganisms (3, 8).

By providing new insight into the evolution, mode of life, and diversity of members of the DHVE2 lineage, our study illustrates the potential of the metagenomic approach. Our analysis identified several genes indicative of metabolic pathways. Unfortunately, this information was not sufficient to predict precise metabolic traits that could suggest useful strategies to elaborate culture media. Better knowledge of the environmental distribution and abundance of DHVE2 members could provide strategies for capturing new genomic sequences of this particular phylogenetic group and/or direct selective isolation attempts.

We are grateful to Nadine Le Bris and Françoise Gaill, chief scientist and project leader of the Phare cruise, for inviting us on board. Didier Flament and Gaël Erauso are gratefully acknowledged for valuable discussions and advice. We thank the captain and crews of *L'Atalante* and the pilots and support crew of the remote-operated vehicle *Victor*. We greatly acknowledge Emmanuelle Morin and Patrick Durand (Irisa-Inria, Rennes) for their help in the use of the Genostar platform.

This work was financed by the CNRS program "Geomex" and by the CNRS-Ministère de la Recherche program "Séquençage à Grande Echelle." H.M. was supported by a grant from the Ministère de la Recherche.

REFERENCES

1. **Allen, E. E., and J. F. Banfield.** 2005. Community genomics in microbial ecology and evolution. Nat. Rev. Microbiol. **3:**489–498.
2. **Campbell, B. J., J. L. Stein, and S. C. Cary.** 2003. Evidence of chemolithoautotrophy in the bacterial community associated with *Alvinella pompejana*, a hydrothermal vent polychaete. Appl. Environ. Microbiol. **69:**5070–5078.
3. **Dalgaard, J., and R. Garrett.** 1993. Archaeal hyperthermophilic genes, p. 535–563. *In* M. Kates, D. J. Kushner, and A. T. Matheson (ed.), The biochemistry of Archaea (Archaebacteria). Elsevier, vol. 26, Amsterdam, The Netherlands.
4. **DeLong, E. F.** 2005. Microbial community genomics in the ocean. Nat. Rev. **3:**459–469.
5. **De Moerlooze, L., I. Struman, A. Renard, and J. A. Martial.** 1992. Stabilization of T7-promoter-based pARHS expression vectors using the parB locus. Gene **119:**91–93.
6. **Desbruyères, D., P. Chevaldonné, A. M. Alayse, D. Jollivet, F. H. Lallier, J.-T. C. F. Zal, P. M. Sarradin, R. Cosson, J. C. Caprais, C. Arndt, J. O'Brien, J. Guezennec, S. Hourdez, R. Riso, F. Gaill, L. Laubier, and A. Toulmond.** 1998. Biology and ecology of the "Pompei worm" (*Alvinella pompejana* Desbruyères and Laubier), a normal dweller of an extreme deep-sea environment: a synthesis of current knowledge and recent developments. Deep-Sea Res. II **45:**383–422.
7. **Futterer, O., A. Angelov, H. Liesegang, G. Gottschalk, C. Schleper, B. Schepers, C. Dock, G. Antranikian, and W. Liebl.** 2004. Genome sequence of *Picrophilus torridus* and its implications for life around pH 0. Proc. Natl. Acad. Sci. USA **101:**9091–9096.
8. **Galtier, N., N. Tourasse, and M. Gouy.** 1999. A nonhyperthermophilic common ancestor to extant life forms. Science **283:**220–221.
9. **Hamal, A., P. Forterre, and C. Elie.** 1990. Purification and characterization of a DNA polymerase from the archaebacterium *Thermoplasma acidophilum*. Eur. J. Biochem. **190:**517–521.
10. **Handelsman, J.** 2004. Metagenomics: application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev. **68:**669–685.
11. **Hoek, J., A. Banta, F. Hubler, and A.-L. Reysenbach.** 2003. Microbial diversity of a sulphide spire located in the Edmond deep-sea hydrothermal vent field on the Central Indian Ridge. Geobiology **1:**119–127.
12. **Huber, J. A., D. A. Butterfield, and J. A. Baross.** 2002. Temporal changes in archaeal diversity and chemistry in a mid-ocean ridge subseafloor habitat. Appl. Environ. Microbiol. **68:**1585–1594.
13. **Huelsenbeck, J. P., and F. Ronquist.** 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics **17:**754–755.
14. **Moreira, D., F. Rodriguez-Valera, and P. López-García.** 2004. Analysis of a genome fragment of a deep-sea uncultivated group II euryarchaeote containing 16S rDNA, a spectinomycin-like operon and several energy metabolism genes. Environ. Microbiol. **6:**959–969.
15. **Moussard, H., D. Moreira, M. A. Cambon-Bonavita, P. López-García, and C. Jeanthon.** Uncultured *Archaea* in a hydrothermal microbial assemblage: phylogenetic diversity and characterization of a genome fragment from an euryarchaeote. FEMS Microbiol. Ecol., in press.

16. **Nercessian, O., M. Prokof'eva, A. Lebedinski, S. L'Haridon, C. Cary, D. Prieur, and C. Jeanthon.** 2004. Design of 16S rRNA-targeted oligonucleotide probes for detecting cultured and uncultured archaeal lineages in high-temperature environments. Environ. Microbiol. **6:**170–182.
17. **Nercessian, O., A. L. Reysenbach, D. Prieur, and C. Jeanthon.** 2003. Archaeal diversity associated with in situ samplers deployed on hydrothermal vents on the East Pacific Rise (13 degrees N). Environ. Microbiol. **5:**492–502.
18. **Ree, H. K., and R. A. Zimmermann.** 1990. Organization and expression of the 16S, 23S and 5S ribosomal RNA genes from the archaebacterium *Thermoplasma acidophilum*. Nucleic Acids Res. **18:**4471–4478.
19. **Reysenbach, A. L., K. Longnecker, and J. Kirshtein.** 2000. Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic Ridge hydrothermal vent. Appl. Environ. Microbiol. **66:**3798–3806.
20. **Rivera, M. C., R. Javi, J. E. Moore, and J. A. Lake.** 1998. Genomic evidence for two functionally distinct gene classes. Proc. Natl. Acad. Sci. USA **95:** 6239–6244.
21. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harber, N.Y.
22. **Schleper, C., G. Jurgens, and M. Jonuscheit.** 2005. Genomic studies of uncultivated archaea. Nat. Rev. Microbiol. **3:**479–488.
23. **Slesarev, A. I., K. V. Mezhevaya, K. S. Makarova, N. N. Polushin, O. V. Shscherbinina, V. V. Shakhova, G. I. Belova, L. Aravind, D. A. Natale, I. B. Rogozin, R. L. Tatusov, Y. I. Wolf, K. O. Stetter, A. G. Malykh, E. V. Koonin, and S. A. Kozyavkin.** 2002. The complete genome of hyperthermophile *Methanopyrus kandleri* AV19 and monophyly of archaeal methanogens. Proc. Natl. Acad. Sci. USA **99:**4644–4649.
24. **Takai, K., and K. Horikoshi.** 1999. Genetic diversity of Archaea in deep-sea hydrothermal vent environments. Genetics **152:**1285–1297.
25. **Takai, K., T. Komatsu, F. Inagaki, and K. Horikoshi.** 2001. Distribution of archaea in a black smoker chimney structure. Appl. Environ. Microbiol. **67:**3618–3629.
26. **Tatusov, R. L., D. A. Natale, I. V. Garkavtsev, T. A. Tatusova, U. T. Shankavaram, B. S. Rao, B. Kiryutin, M. Y. Galperin, N. D. Fedorova, and E. V. Koonin.** 2001. The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res. **29:**22–28.
27. **Zhao, Y., D. Jeruzalmi, I. Moarefi, L. Leighton, R. Lasken, and J. Kuriyan.** 1999. Crystal structure of an archaeal DNA polymerase. Structure **7:**1189–1199.