NOTES

Lack of Deoxyribonucleic Acid-Deoxyribonucleic Acid Homology Between *Thermoplasma acidophilum* and *Sulfolobus acidocaldarius*

CLAUS CHRISTIANSEN, E. A. FREUNDT, AND O. VINTHER

Institute of Medical Microbiology, Bartholin Building, University of Aarhus, DK-8000 Aarhus C Denmark

Deoxyribonucleic acids (DNAs) from the type strains of *Thermoplasma acidophilum* (strain 122-1B2) and *Sulfolobus acidocaldarius* (strain 98-3) are compared. The guanine-plus-cytosine contents of the DNAs are 45.0 and 39.9 mol%, respectively. DNA-DNA hybridization experiments show homologies slightly above the background level of 0.1% in the experiment.

In a previous paper (1) describing the flagellation and swimming motility of *Thermoplasma acidophilum*, we reviewed and discussed current ideas of the taxonomic position of this organism. As an alternative to the alleged association of this organism with the *Mollicutes* (4, 8), the suggestion was made that it might be regarded as a wall-defective variant (L-phase variant) of a thermophilic and acidophilic bacterium as, for example, *Sulfolobus acidocaldarius* (2) or the *Sulfolobus*-like organisms (5). Among the observations that might be adduced in favor of this theory, mention was made of the relatively close values of the guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) of *T. acidophilum* and the *Sulfolobus*-like organisms, viz., 46 mol% (3, 7) and 39 to 45 mol% (5), respectively, although these values are significantly lower than those reported for *S. acidocaldarius*, viz., 60 to 68 mol% (2).

To detect any possible genetic relationship between *T. acidophilum* and the *Sulfolobus* group, we decided to carry out DNA hybridization experiments with representatives of the two groups of organisms. However, since we were unable to obtain any of the two strains (MT3 and MT4) representing the *Sulfolobus*-like organisms described by De Rosa et al. (5), we had to attempt hybridization between *T. acidophilum* and *S. acidocaldarius*. The choice of the latter organism appears to be less logical from its reported high G+C content. The strains used were the type strains of each organism: *T. acidophilum* 122-1B2 (ATCC 25905; AMRC-C165) and *S. acidocaldarius* 98-3 (DSM 639) (kindly provided by S. Searcy, Zoology Department, University of Massachusetts, Amherst).

T. acidophilum 122-1B2 was grown at 56°C in the medium described earlier (1, 3), and *S. acidocaldarius* 98-3 was grown at 75°C in the medium described by Langworthy et al. (6), supplemented with sucrose (15 g per liter).

Harvest of organisms, DNA extraction, buoyant density analysis, and DNA-DNA hybridization were performed as described previously (3). Labeling of DNA was done by adding [³H]thymidine (1 mCi/liter) for the last 16 h of growth. *T. acidophilum* 122-1B2 labeled well under these conditions, whereas incorporation in the DNA of *S. acidocaldarius* 98-3 did not occur. Therefore, DNA from both strains was labeled with ³²P to a specific activity of 4×10^7 cpm by nick translation (9). The in vivo-labeled DNA from *T. acidophilum*, as well as the in vitro-labeled DNAs, were used in the hybridization experiments.

The G+C content of *T. acidophilum* was found to be 45.0 ± 0.3 mol% (mean of three different experiments with standard deviation), thereby confirming the results of earlier studies (3, 7). Determination of the G+C content of the DNA of strain 98-3 of *S. acidocaldarius* unexpectedly yielded a value of 39.9 ± 0.3 mol% (five experiments). This is, in fact, on line with the values found by De Rosa et al. (5) for their *Sulfolobus*-like strains MT3 and MT4 and, hence, is highly different from the value of 61 mol% reported by Brock et al. for strain 98-3 (2). Since the distinction made by De Rosa et al. (5) between *S. acidocaldarius* and *Sulfolobus*-like strains was based mainly on the supposed marked difference in G+C compositions, it may be concluded from our data that this distinction cannot be maintained.

The hybridization experiments with standard nitrocellulose filter techniques with hybridization in $2 \times \text{SSC}$ (0.30 M NaCl, 0.03 M trisodium citrate [pH 7.0] at 65°C) are shown in Table 1.

The experiment with ^3H -labeled *T. acidophilum* 122-1B2 DNA gave results identical to those in Table 1. The maximal homology percentage detected in these experiments between DNA from the two strains is 0.25%, barely above the background value for the experiments. Thus, no genetic relationship can be demonstrated between *T. acidophilum* and *S. acidocaldarius* by means of DNA-DNA hybridization. This, of course, is not necessarily at variance with the proposed classification of *Thermoplasma* and *Sulfolobus*, together with the methanogens and extreme halophiles, in a common phylogenetic group, the Archaeobacteria, as based on analyses of their 16S ribosomal RNA sequence homologies (10, 11).

TABLE 1. Hybridization experiments with DNA from *T. acidophilum* 122-1B2 and *S. acidocaldarius* 98-3

Source of ^{32}P -labeled DNA	Mol% G+C of DNA (buoyant density)	<i>T. acidophilum</i> 122-1B2 ^a	<i>S. acidocaldarius</i> 98-3 ^a	Blank
<i>T. acidophilum</i> 122-1B2	45.0	36,595	365	330
<i>S. acidocaldarius</i> 98-3	39.9	345	42,667	244

^a Each value is the average of four determinations, and the results are given as counts per minute bound per filter.

This research was supported by a grant from the Fogh-Nielsen Foundation.

REPRINT REQUESTS

Address reprint requests to: Dr. E. A. Freundt, Institute of Medical Microbiology, Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark.

LITERATURE CITED

1. Black, F. T., E. A. Freundt, O. Vinther, and C. Christiansen. 1979. Flagellation and swimming motility of *Thermoplasma acidophilum*. *J. Bacteriol.* **137**:456-460.
2. Brock, T. D., K. M. Brock, R. T. Belly, and R. L. Weiss. 1972. *Sulfolobus*. A new genus of sulfur-oxidizing bacteria living at low pH and high temperature. *Arch. Mikrobiol.* **84**:54-68.
3. Christiansen, C., E. A. Freundt, and F. T. Black. 1975. Genome size and deoxyribonucleic acid base composition of *Thermoplasma acidophilum*. *Int. J. Syst. Bacteriol.* **25**:99-101.
4. Darland, G., F. D. Brock, W. Samsonoff, and S. F. Conti. 1970. A thermophilic, acidophilic mycoplasma isolated from a coal refuse pile. *Science* **170**:1416-1418.
5. De Rosa, M., A. Gambacorta, and J. D. Bu'Lock. 1975. Extremely thermophilic acidophilic bacteria convergent with *Sulfolobus acidocaldarius*. *J. Gen. Microbiol.* **86**:156-164.
6. Langworthy, T. A., W. R. Mayberry, and P. F. Smith. 1974. Long-chain glycerol diether and polyol dialkyl glycoside triether lipids of *Sulfolobus acidocaldarius*. *J. Bacteriol.* **119**:106-116.
7. Searcy, D. G., and E. K. Doyle. 1975. Characterization of *Thermoplasma acidophilum* deoxyribonucleic acid. *Int. J. Syst. Bacteriol.* **25**:286-289.
8. Subcommittee on the Taxonomy of *Mycoplasma*-*tales*. 1974. Minutes of meeting, 5 and 6 September 1973. *Int. J. Syst. Bacteriol.* **24**:390-392.
9. Rigby, P. W. J., M. Dickman, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**:237-251.
10. Woese, C. R., L. J. Magrum, and G. E. Fox. 1978. Archaeobacteria. *J. Mol. Evol.* **11**:245-252.
11. Woese, C. R., J. Maniloff, and L. B. Zablen. 1980. Phylogenetic analysis of the mycoplasmas. *Proc. Natl. Acad. Sci. U.S.A.* **77**:494-498.