Characterization of *Thermoplasma acidophilum* Deoxyribonucleic Acid

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The deoxyribonucleic acid (DNA) of the mycoplasma *Thermoplasma acidophilum* has a base composition of 46 mol% guanine plus cytosine (G+C). Depurination can occur to the DNA in the culture conditions, giving rise to a product of lower buoyant density. This effect probably accounts for earlier descriptions of a much lower G+C content in the DNA. The size of the *T. acidophilum* genome, as measured by DNA renaturation, was 30% the size of the *Escherichia coli* genome.

The deoxyribonucleic acid (DNA) base composition of the mycoplasma *Thermoplasma acidophilum* was originally reported to be 25 mol% guanine plus cytosine (G+C), as determined by buoyant density (6). This seemed unusual because thermophilic organisms generally do not have DNA with low G+C contents (23), since DNA with less G+C more easily undergoes thermal denaturation (17). However, Freundt (7, 8) reported *T. acidophilum* DNA to be 45 to 46 mol% G+C. More recently, in surveys of natural populations, Belly et al. (2) reported several buoyant density determinations on *T. acidophilum* DNA, in all the range of 24 to 29 mol% G+C. Our own measurements by thermal denaturation gave variable results. Thus it seemed worthwhile to determine the base composition of the DNA by chromatography of the bases after hydrolysis, since this is the most direct method of determining DNA base composition, and also because this method would detect the occurrence of unusual bases.

MATERIALS AND METHODS

Culture. *T. acidophilum* (isolate 122-1B2; ATCC 25905) was obtained from Paul F. Smith and grown with aeration in 27-liter polypropylene bottles at 59 C, pH 2, in the medium previously described (11), except with 2 g of yeast extract (Difco, Technical Grade) per liter. Effort was made to harvest the cultures during log growth, although, as will be seen later, probably even these cultures were not entirely healthy. Cultures were cooled to about 10 C by passage through a glass coil buried in ice and collected at 10,000 rpm using a Szent-Gyorgyi and Blum Continuous Flow Apparatus in a Sorvall RC-2B centrifuge. The cells were washed once with distilled water and then stored frozen at -70 C.

DNA extraction. DNA was purified from 2 to 3 g of frozen cells by the method of Marmur (16), except that the preparation was deproteinized first with cold unneutralized aqueous phenol and then with chloro-

form-isoamyl alcohol (24:1), and so on as originally described. Calf DNA was either purified from thymus nuclei by the same procedure or was purchased from Sigma Chemicals (St. Louis) and repurified as described above. The glycogen content of the DNA was found (18) to be insignificant (<0.2%).

Hydrolysis and thin-layer chromatography. DNA was first purified free of ribonucleic acid by incubation in 0.3 N KOH for 18 h at 37 C. The DNA was collected by precipitation with cold HClO₄, added to 0.3 N final concentration (19). The resulting precipitate was dissolved in 0.15 M NaCl + 0.015 M sodium citrate, pH 7.0, and the DNA was precipitated with 2 volumes of ethanol. For hydrolysis, about 5 mg of DNA was mixed with 0.1 ml of 70% HClO₄ and hydrolyzed for 1 h at 100 C (3). Replicates no. 1 to 3 of *T. acidophilum* DNA (Table 1) were analyzed directly after acid hydrolysis, since neutralization sometimes results in a loss of purines (9). Replicates no. 4 to 8 were neutralized with 3 N KOH, cooled in ice, and centrifuged, and the clear supernatant was used for chromatographic analysis. Both of these procedures, however, gave essentially similar results.

Chromatography was on MN-Cellulosepulver 300 G (Macherey, Nagel, and Co., supplied by Brinkmann Instruments, Westbury, N.Y.) coated on glass plates (20 by 20 cm). To separate the bases, the two-dimensional solvent system of Randerath (20) was used. Ultraviolet-absorbing spots were extracted from the cellulose by shaking with 1 ml of 0.1 N HCl at 4 C overnight. The bases were identified and measured on a Zeiss PMQ-II spectrophotometer, and calculations were based on a differential extinction method (3).

Other methods of DNA analysis. Thermal denaturation of DNA was measured in 0.15 M NaCl + 0.015 M sodium citrate, pH 7.0, using a Gilford recording spectrophotometer. The base ratios were determined relative to a standard of *E. coli* K-12 DNA (13). The base composition was also calculated from ultraviolet absorbance ratios (26) and from the buoyant density in CsCl solutions (14), using *E. coli* K-12 DNA as a standard.

DNA renaturation. Purified DNA in 0.15 M NaCl + 0.015 M sodium citrate, pH 7.0, was sheared three times in a French pressure cell at 1,360 atm. The DNA was precipitated with ethanol and then redissolved

TABLE 1. DNA base composition determined by thin-layer chromatography^a

Source	Base composition (nmol)				% of purines lost	% G + C from pyrimidines ^b
	A	T	G	C		
Calf						
1	30	30	22	28	10.3	48.3
2	40	42	30	33	6.7	44.0
3	36	39	27	28	6.0	41.8
Mean $\pm$ SE						44.7 $\pm$ 1.9
<i>T. acidophilum</i>						
1	33	40	26	43	28.9	51.8
2	93	114	49	103	34.6	47.5
3	12	13	3	10	34.8	43.5
4	60	50	30	41	1.1	45.1
5	12	16	9	12	25.0	42.9
6	17	33	14	28	49.2	45.9
7	15	24	11	21	42.2	46.7
8	20	27	15	28	36.4	50.9
Mean $\pm$ SE						46.8 $\pm$ 1.1

^a DNA samples were hydrolyzed in 70% HClO₄, and the bases were separated by two-dimensional thin-layer chromatography. The individual bases were extracted from each spot and measured by ultraviolet absorbance. A, Adenine; T, thymine; G, guanine; C, cytosine; SE, standard error.

^b % G+C from pyrimidines = $100 \cdot C/(C+T)$.

and dialyzed extensively against 0.24 M sodium phosphate buffer, pH 6.7. Renaturation was at 65 C in a 1-mm path-length cuvette, and the absorbance was determined relative to a wire screen absorbance standard, as previously described (21). The point of half renaturation ($C_0t_{0.5}$) was used to characterize the complexity of the DNA (4) and was determined using the calculated absorbance at infinite time (21).

RESULTS

Table 1 shows the base composition of *T. acidophilum* DNA as determined by thin-layer chromatography. The pyrimidines were generally more abundant than the purines, which we attributed to depurination of the DNA. Calf thymus DNA was included as a control and showed that the procedures of extraction and analysis did not entirely account for the loss of purines. The most reliable estimate for the base composition is based on only the pyrimidine bases: 47 mol% $\pm$ 1% (standard error) G+C.

No large amounts of unusual bases were observed, although a minor base closely resembling guanine was found. Assuming that it had the same extinction coefficient as guanine, it accounted at most for only 2 to 3% of the total bases in each sample.

T. acidophilum DNA was also analyzed by thermal denaturation. Often these results were variable, which we attributed to depurination of some of the samples. However, an estimate of 45 mol% $\pm$ 1% G+C was made, based on eight samples having the highest denaturation temperatures, which we assumed to be the least

depurinated. The ultraviolet absorbance ratios indicated 44 mol% $\pm$ 1% G+C in determinations on seven different samples.

We believe that the most reliable estimate for *T. acidophilum* DNA is 46% G+C, in agreement with Freundt (7).

To determine if depurination of the DNA could account for low estimates of the G+C content, calf thymus DNA was exposed to *T. acidophilum* culture conditions (59 C, pH 2.0) for 24 h. When this material was analyzed by buoyant density centrifugation, it did indeed band at a lower density than the native DNA, as shown in Fig. 1. The acid-treated DNA appeared to be fully denatured as well as depurinated by this treatment, since there was no hyperchromicity when it was heated from 25 to 95 C. However, after milder treatments, hyperchromicity was observed, and the acid-treated DNA denatured at lower temperatures than untreated DNA.

The size of the *T. acidophilum* genome was estimated by DNA renaturation, with the results shown in Fig. 2. In three determinations on different preparations of *T. acidophilum* DNA, the size of the genome was estimated to be 29.7% $\pm$ 2.2% (standard error) that of *E. coli*.

DISCUSSION

We find the base composition of *T. acidophilum* DNA to be about 46 mol% G+C and suspect that the previously reported value of 25% G+C was a result of depurination. The possibility exists, of course, that we are now

FIG. 1. Equilibrium centrifugation of DNA in CsCl . The ultraviolet-absorbing peaks, left to right, are calf DNA exposed to acidic depurinating conditions, native calf DNA control, and *E. coli* DNA. The identification of these peaks was confirmed in separate experiments. Centrifugation was 2 days at 44,000 rpm.

FIG. 2. DNA renaturation at 65 C in 0.24 M sodium phosphate buffer, pH 6.7. Renaturation of 153 μg of *T. acidophilum* DNA per ml (●●) and 145 μg of *E. coli* DNA per ml (○○) was measured by the relative decrease in absorbance at 260 nm in a 1-mm path length cuvette. The denaturation temperature of the *T. acidophilum* DNA was 87.4 C before renaturation and 85.0 C after renaturation. *E. coli* DNA denatured at 90.2 C before and 87.4 C after renaturation. C_0t is expressed in units of moles of nucleotides times seconds per liter.

culturing an organism different from that originally described, but this seems unlikely since our present cultures do not differ from the descriptions of *T. acidophilum* (2, 5, 6), except for the G+C content of the DNA.

It has been found that 1 day after a culture of *T. acidophilum* has reached maximum density, only 0.1% of the cells remain viable (22). There is only a slight decrease in light scattering and little other indication that the cells are not healthy. By 2 days after maximum density, the fraction of viable cells has decreased to 10^{-5} . Thus, nonviable cells may frequently be used as a source for DNA. If acid from the culture medium has penetrated these cells, then the DNA has been exposed to conditions very similar to standard conditions for preparing completely depurinated DNA (25). The resulting

material, if it is not exposed to alkaline conditions, is of high molecular weight (12, 24) and can still form discrete bands in buoyant density centrifugation. Furthermore, as shown in Fig. 1, the depurinated DNA has a lower density than native DNA. Thus, buoyant density analysis of depurinated DNA will lead to an underestimate of the G+C content of the DNA. This probably accounts for the previous reports of a low G+C content in *T. acidophilum* DNA.

Alternative explanations for the loss of purines are unlikely. For instance, DNA degradation has been reported in other mycoplasmas during the harvesting of the cultures (10). However, this can probably not account for the depurination, since the cells were harvested at low temperatures (0 to 20 C) and quickly frozen. Depurination during subsequent procedures is also unlikely, since calf thymus DNA receiving an identical treatment was not affected. Therefore, the depurination that was seen in the *T. acidophilum* DNA probably occurred before the cultures were harvested.

The molecular weight of the *T. acidophilum* genome, as measured by DNA renaturation, is about 8.4×10^8 , which is slightly less than the value of 9.5×10^8 previously reported by Freundt (8). This size is somewhat smaller than other mycoplasmas in the genus *Acholeplasma* but is larger than that reported for the genus *Mycoplasma* (1, 8, 15). To our knowledge, *T. acidophilum* has the smallest genome yet reported for any nonparasitic organism.

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REPRINT REQUESTS

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