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Short communication

Genome size of human oral *Treponema* species by pulsed-field gel electrophoresis

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The genome sizes of seven strains of oral treponemes were determined using pulsed-field gel electrophoresis (PFGE). These strains represent members from six of the currently known cultivable oral treponeme groups. The PFGE fragments were digitally recorded and then quantitated using GIMP v 1.2, an image manipulation program. The results show that the six oral treponeme genomes are comparable in size, ranging from approximately 2.2 to 2.5 Mbp. The genome sizes of these strains are 20–25% smaller than *Treponema denticola* strains, which have genome sizes of approximately 2.8–3.0 Mbp.

Key words: genome sizes; oral treponeme groups; pulsed-field gel electrophoresis

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Spirochetes of the genus *Treponema* are a major component of the bacterial flora of the oral cavity. They are especially prevalent in the anaerobic gingival crevice, having been associated with both oral disease and health (6). The extensive genetic diversity of *Treponema* has been established by using polymerase chain reaction amplification of 16S rRNA cistrons from subjects with a range of periodontal conditions (2). The 16S rRNA sequences are divided into 57 clusters; a cluster being defined as a set of sequences with >99% sequence identity. The clusters were further defined by placement into 10 larger sets called groups. Groups share 90% sequence identity and are given Arabic numerals 1 to 10 (2). Seven of the groups have cultivable members, six of which were examined in this study. The seventh group is represented by the previously studied *Treponema denticola* strain ATCC 33520 (5).

It is already known from whole genome sequencing that the genome sizes of spirochete genera vary widely. For example, *Borrelia burgdorferi* has a genome of 0.9 Mb (not including the genetic contribution of multiple plasmids) (3) and *Leptospira interrogans* has two chromosomes totalling approximately 4.7 Mb (7). Even within genera, genome sizes can be surprisingly varied. *Treponema pallidum*, with a genome size of 1.1 Mb (4), can be contrasted with *T. denticola*, which is approximately 2.8 Mb (TIGR, Rockville, MD; accession number NC002967), 2.5 times the size of the *T. pallidum* genome.

The purpose of this work was to determine the genome sizes of representative strains of the cultivable oral treponeme groups using pulsed-field gel electrophoresis. This approach has been used to accurately estimate the size of the genome of *T. denticola* ATCC 33520 prior to its sequencing (5). Such information allows inferences to be made about the complexity of the genome of each *Treponema* group. The genome size data will be useful to workers who are considering genome-sequencing or typing projects.

Table 1 lists the strains examined in this study and the treponeme group they represent. Strains were obtained from Dr. C. Wyss of the Institut für Orale Mikrobiologie und Immunologie, University of Zürich, the American Type Culture Collection (ATCC, Manassas, VA) or from the culture collections of the Forsyth Institute. All treponeme strains were grown in OMZ-P4 media (1) in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI) at 37°C, containing 80% N_2, 10% H_2 and 10% CO_2.

To prepare intact genomic DNA, *Treponema* cell cultures were centrifuged and resuspended to an OD_{600} of approximately 0.5 in a Tris buffer solution (10 mM Tris-HCl [pH 7.6], 1 M NaCl). The cell suspension was mixed 1:1 (v/v) with 1.6% pulsed-field certified agarose (Bio-Rad, Hercules, CA) and kept at 50°C until 100 μl was poured into each well of a mold to form a set of cell plugs. The treponemes were lysed *in situ* by addition of 1.0 ml lysis buffer – 6 mM Tris-HCl pH 7.6; 1 M NaCl; 100 mM EDTA; 0.5% (w/v) Brij-58 (polyoxyethylene 20 cetyl ether) (Sigma, St. Louis, MO); 0.2% (w/v) Deoxycholate (Sigma); 0.5% (w/v) N-lauroyl-sarcosine (Sigma) – for 18 h at 37°C. The cells were further lysed – 0.5 M EDTA, 1% N-lauroyl-sarcosine (w/v), 50 $\mu g.ml^{-1}$ Proteinase K – for 24 h at 50°C. Plugs were washed twice in TE (10 mM Tris-HCl pH 7.5, 1 mM EDTA) for 10 min at 37°C before being equilibrated for 3 h in the digestion buffer supplied by the manufacturer (New

Table 1. Sizes of restriction fragments, in kb, of the genomes of various *Treponema* species

	Group 1 *T. vincentii* N-9		Group 2 *T. denticola* ATCC 33520	Group 3 *T. lecithinolyticum* OMZ 684^T		Group 4 *T. maltophilum* OMZ 679^T		Group 5 *T. amylovorum* ATCC 700288^T		Group 6 *T. socranskii ss* 04 D11B2		Group 7 *T. parvum* D96NR3		Group 8 *T. pectinovorum* D36DR2^T	
	*Xba*I	*Avr*II	*Asc*I	*Spe*I	*Xba*I	*Spe*I	*Xba*I	*Spe*I	*Nhe*I	*Spe*I	*Xba*I	*Spe*I	*Pac*I	*Not*I	*Pac*I
	454	457	1577	618	495	728	772	421	385	505	466	442	385	475	416
	436	433	1162	330	316	597	702	339	356	394	430	405	330	409	401
	283	295	144	229	200	552	324	277	318	317	315	350	299	374	339
	255	257	86	198	186	316	165	198	299	228	296	320	197	289	315
	203	238	15	198	161	177	129	194	284	215	280	151	160	270	258
	150	195	–	159	142	–	114	142	198	197	195	142	132	241	231
	137	170	–	140	142	–	70	129	179	159	122	95	113	146	198
	120	128	–	116	121	–	52	97	134	158	96	94	112	77	44
	113	105	–	105	121	–	–	93	127	79	73	90	102	–	35
	93	87	–	70	107	–	–	80	87	71	67	78	84	–	25
	77	65	–	70	75	–	–	77	72	63	57	65	81	–	–
	68	37	–	42	70	–	–	65	63	47	51	59	79	–	–
	29	24	–	16	54	–	–	60	50	–	38	44	67	–	–
	23	21	–	–	43	–	–	54	17	–	–	33	56	–	–
	2	–	–	–	34	–	–	44	–	–	–	20	38	–	–
	1	–	–	–	16	–	–	39	–	–	–	15	34	–	–
	–	–	–	–	–	–	–	26	–	–	–	2	30	–	–
	–	–	–	–	–	–	–	23	–	–	–	1	26	–	–
	–	–	–	–	–	–	–	11	–	–	–	–	20	–	–
	–	–	–	–	–	–	–	3	–	–	–	–	16	–	–
	–	–	–	–	–	–	–	2	–	–	–	–	3	–	–
Total	2444	2512	2984	2291	2283	2370	2328	2374	2569	2433	2486	2406	2364	2281	2262
Average	2478		–	2287		2349		2472		2460		2385		2272	

England Biolabs, Beverly, MA). At the end of the equilibration period, fresh digestion buffer was added along with 20–100 units of the desired restriction enzyme. Digestion was for 16 h at 37°C. The digest mix was replaced by 0.5× TBE (45 mM Tris-borate, 1 mM EDTA) and the plugs were equilibrated for 2 h prior to electrophoresis.

Restriction endonucleases that cut rarely in bacterial genomes were selected and tested empirically and six-base cutting endonucleases recognizing the tetranucleotide CTAG were found to work well for several treponemes. Those enzymes giving the clearest patterns were selected for each species examined.

Pulsed-field electrophoresis was performed in a CHEF-DRIII apparatus (Bio-Rad) using agarose gels (1–1.5% [w/v]) in 0.5× TBE buffer. Running conditions varied according to the fragment sizes being separated. A typical set of conditions to separate fragments between 100 and 400 kb was as follows: initial switch time 1.2 s, final switch time 54 s, field strength of 6 volts/cm with a 120° field angle for 25 h at 14°C. Typically, at least two sets of conditions, one for >400 kb fragments, the other for <400 kb fragments, were used per genome. Gels were post-stained in 0.5× TBE containing GelStar® (Cambrex Bio Science, Rockland, MD) according to the manufacturer's instructions. Figure 1 shows an example of a PFGE run.

To quantitate fragment size within digests, the gels were digitally recorded using an AlphaImager™ 2200 documentation system (Alpha Innotech Corporation, San Leandro, CA). Digest lanes were displayed as plots of optical density versus distance using GIMP v 1.2 (GNU Image Manipulation Program, authored by Peter Mattis and Spencer Kimball; http://www.gimp.org/). Fragment mobilities were determined as distances from the top of the gel lane to band intensity maxima. The mobility data of bands of known length, derived from bacteriophage lambda DNA multimers (monomer = 48 kb) and *Saccharomyces cerevisiae* YPH80 chromosomes ([New England Biolabs] 945, 915, 815, 785, 745, 680, 610, 555, 450, 375, 295 and 225 kbp) were fit to curves whose R-squared values were greater than 0.99. The curves were then used to calculate the size of the unknown bands. A genomic size for each *Treponema* strain was calculated with two independent restriction enzymes in this manner.

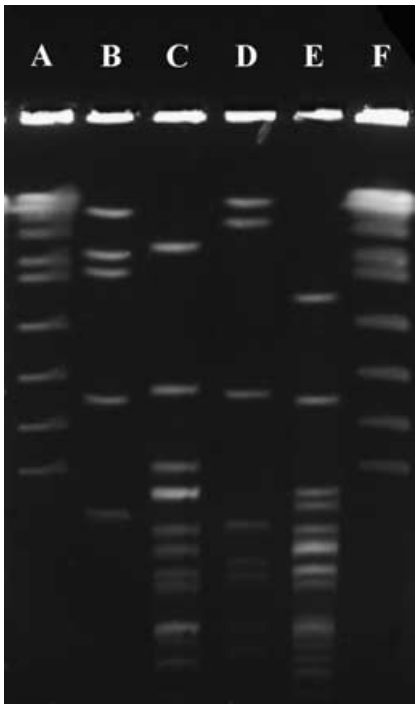

Fig. 1. Pulsed field gel used to size *Treponema lecithinolyticum* OMZ 684 and *Treponema maltophilum* OMZ 679. Lanes A & F, yeast markers (745, 680, 610, 555, 450, 375, 295 and 225 kbp); lane B, OMZ 679 digested with *Spe*I; lane C, OMZ 684 digested with *Spe*I; lane D, OMZ 679 digested with *Xba*I; lane E, OMZ 684 digested with *Xba*I.

As a positive control, the genome size of *T. denticola* ATCC 33520 was determined. The result obtained, 2,984 kb, agrees closely with that previously obtained, 3,030 kb (5). The genome sizes for seven species representing six distinct groups of treponemes are surprisingly consistent at 2.2–2.5 Mbp. The genomes of these species are 20–25% smaller than those of *T. denticola,* but more than twice as large as those of *T. pallidum,* an obligate parasitic species. The smaller genome sizes of the oral treponemes surveyed in this study may indicate their having a reduced metabolic potential compared to *T. denticola* genomes. While all examined cultivable treponemes have genome sizes >2.2 Mbp, it is possible that several of the more than 40 uncultivated human oral treponemes

have significantly reduced genomes, possibly even smaller than *T. pallidum.*

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