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Comparison of the Genomes of Pathogenic Treponemes of Human and Animal Origin

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The aim of this study was to compare the genomes of two strains of intestinal treponemes, which were isolated from patients suffering from intestinal disorders, with that of *Treponema hyodysenteriae*, the known etiological agent of swine dysentery (bloody scours). The guanine-plus-cytosine contents of the three DNAs were found to be 28.5 to 30.0%. DNA-DNA hybridization in liquid phase indicated a high degree of homology (56 to 95%) among the human strains and with *T. hyodysenteriae*. One of the human strains in particular displayed a very high homology (91 to 95%) with *T. hyodysenteriae*. The overall conclusion is that treponemal strains pathogenic for humans and animals are clustered within the same species (we propose *T. hyodysenteriae*), which suggests the possibility of exchange of pathogenic microorganisms between domestic animals and humans.

Swine dysentery (bloody scours), a mucohemorrhagic enteritis affecting pigs of all countries, was described as a separate disease in 1921 (16). Vibrio-like species, including *Vibrio coli*, were suggested to be responsible, although transient nonhemorrhagic diarrhea was produced by these organisms (11). It was not until 1972, however, that a treponeme, *Treponema hyodysenteriae*, was recognized as the true etiological agent of the disease (8), when it was proven to reproduce hemorrhagic dysentery in experimental animals. Studies by Miao and co-workers showed that *T. hyodysenteriae* is genetically unrelated to *Treponema pallidum*, *T. phagedenis*, and *T. refringens* (12).

There have been numerous observations on the occurrence of spirochetes in the gut of domestic and wild animals (10). More recently, treponemes closely resembling *T. hyodysenteriae* were isolated from the intestines of patients affected by hemorrhagic diarrhea and other intestinal disorders (13, 14). However, the association of the human intestinal treponemes with the disease is not yet formally proven. The relatedness of treponemes isolated from humans and pigs has been tested by several studies. Strains of human intestinal spirochetes were characterized as treponemes by conventional tests: nutritional requirement, fermentation ability, and enzymatic analysis (13). Indirect fluorescent-antibody tests showed that human strains are antigenically closely related to each other and to pathogenic swine strains (7). Electron microscopy studies have shown that human strains are morphologically similar to *T. hyodysenteriae* and different from *T. pallidum* and *T. phagedenis* (6). Preliminary trials of DNA-DNA hybridizations have also suggested some homology between human intestinal treponemes and *T. hyodysenteriae*, but no quantitative determinations were made (1).

The aim of the study presented here was to analyze the genomes of two strains of intestinal treponemes of human origin and one from pigs. The base ratio and DNA homology of treponemes of human origin and of *T. hyodysenteriae* were compared to gather information on the relationship among pathogenic intestinal treponemes.

Two strains of human treponemes which were isolated from patients affected by abdominal pain, diarrhea, vomiting, and acute gastroenteritis (HRM 2) or by constipation and diarrhea (HRM 3) (14) and the reference strain of *T. hyodysenteriae* (P 18) corresponding to ATCC 27087 from the American Type Culture Collection (Rockville, Md.) were used in this study. These treponemes were kindly supplied by A. Sanna of the Institute of Microbiology, Rome, Italy. Lyophylized treponemes suspended in 1 mM EDTA–10 mM Tris hydrochloride buffer (pH 7.6) were lysed with 0.5% sodium dodecyl sulfate for 30 min at 37°C. Lysates were successively incubated for 30 min at 37°C with pancreatic RNase (50 μg/ml) before repeated extractions with water-saturated redistilled phenol. After phenol removal by ether, treponemal DNA was precipitated from the aqueous layer twice with 3 volumes of ethanol (15 min at −70°C). DNA precipitate obtained by centrifugation was dissolved in 0.1 mM EDTA–10 mM Tris hydrochloride (pH 7.6); its concentration and degree of purity were determinated spectrophotometrically.

The G+C content of treponemal DNA was determined from its buoyant density value in CsCl gradient. Starting density of the CsCl solution was $\rho = 1.699$ g/cm^3, corresponding to a refractive index of 1.3992 at 20°C. Buoyant density of DNA (2 μg of DNA per ml of CsCl solution) was established at equilibrium after 48 h of centrifugation at 120,000 × *g* in an analytical-preparative centrifuge (L65; Spinco) equipped with UV optics. Buoyant density of treponemal DNA was calculated relative to that of reference DNA from *Micrococcus lysodeikticus* ($\rho = 1.731$ g/cm^3) by using the relationship $\rho = 1.66 + (0.00098 \times$ G+C content) (15).

Table 1 (buoyant density column) indicates values close to 30% G+C for DNA from both treponemal strains of human origin and 28.5% G+C for *T. hyodysenteriae* DNA.

An additional procedure for the determination of the base ratio has been recently developed (3). A DNA sample of about 1 μg was added to 10 μl of a reaction mixture containing 7.5 μM $MgCl_2$; 0.75 mM dithiothreitol; 30 mM Tris hydrochloride buffer (pH 7.4); 5.25 μM each dGTP, dTTP, and dATP (Boehringer GmbH, Mannheim, Federal Republic of Germany); 5.25 μM [5-^{3}H]dCTP (28.5 Ci/mmol; New England Nuclear Corp., Dreieich, Federal Republic of Germany); 0.004 μM [α-^{32}P]dATP (3,200 Ci/mmol; New England Nuclear); and 0.5 U of DNA polymerase I (type 104485 [Boehringer], an endonuclease-containing prepara-

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TABLE 1. Base composition of treponemal DNA

Strain	Origin	G+C content[a] by:	
		Buoyant density	Double labeling
HRM 2	Human	29.7 ± 1.2 (4)	27.3 ± 2.1 (4)
HRM 3	Human	30.0 ± 0.3 (4)	27.7 ± 1.9 (3)
P 18	Pig	28.5 ± 0.4 (4)	30.0 ± 2.0 (4)

[a] Moles percent ± standard deviation; values in parentheses are number of determinations.

tion) (3). After 25 min of incubation at 20°C, nick translation reaction was stopped by 100-fold dilution with 10 mM EDTA–10 mM Tris hydrochloride (pH 7.4). DNA was precipitated with 10% trichloroacetic acid and 1% bovine serum albumin at 0°C for 30 min. Samples were filtered through glass fiber filters (MN85; Macherey-Nagel, Düren, Federal Republic of Germany) which were repeatedly washed with 5% trichloroacetic acid, air dried, and counted for both ^{3}H and ^{32}P in a scintillation spectrometer. After subtraction of background values (samples without DNA) and correction for ^{32}P contamination of ^{3}H channel, base composition was obtained from the relationship 1/percent G+C = [0.01K ($^{32}P/^{3}H$)] + 0.01 by graphic interpolation on a linear plot of 1/percent G+C against the $^{32}P/^{3}H$ ratio, 0.01K being the slope and 0.01 being the ordinate intercept. The K value was determined for each experiment by using two reference DNAs of known composition (*Clostridium perfringens* and *M. lysodeikticus*, 26.5 and 71.9% G+C, respectively).

Table 1 (double labeling column) shows average values of 28% G+C for DNA of human treponemes and 30% G+C for DNA of *T. hyodysenteriae*. These values were close to those obtained by buoyant density determination. It can be concluded, therefore, that the DNAs of intestinal treponemes from humans and pigs have similar G+C contents.

Information on the relatedness of these treponemal strains was obtained by measuring levels of genome homology by DNA-DNA hybridization. The procedure of hybridization in liquid phase was used as previously described (5). The reaction mixture, containing 5.25 μM cold dGTP, dCTP, and dTTP; 0.04 μM [α-^{32}P]dATP (3,200 Ci/mmol); 10 mM $MgCl_2$; 0.2 mM EDTA; 40 mM NaCl; 50 mM Tris hydrochloride buffer (pH 7.4); a sample of purified DNA (1 μg/ml of reaction mixture); and DNA polymerase I (type 104485 [Boehringer]; 0.05 U/μl of reaction mixture) was incubated for 30 min at 15°C. DNA was denatured by 10 min of heating at 100°C, and 0.02 μg of nick-translated DNA was mixed with 1.5 μg of unlabeled DNA and sonicated to yield fragments of an average size of 1,000 base pairs. Hybridization was carried out for 15 h at 60°C. This temperature was chosen because it is 25°C lower than the T_m value of the analyzed DNA. T_m is related to percent G+C by the following equation: T_m = 69.3 + 0.41 (percent G+C) + 18.5 log M, where M is the ratio of the experimental concentration of NaCl (0.3 in this case) to the reference concentration (0.195 M) (4). Mixtures were then subjected to hydrolysis (37°C for 45 min in 33 mM sodium acetate–30 μM $ZnSO_4$) by single-stranded DNA-specific nuclease S1 from *Aspergillus oryzae* (Boehringer; 30 U/μg of DNA), which was stopped by 100-fold dilution with 100 mM EDTA–10 mM Tris hydrochloride (pH 7.8). DNA was precipitated with 10% trichloroacetic acid and 2% bovine serum albumin at 0°C for 30 min. Radioactivity retained by glass filters (see above) was measured in a scintillation spectrometer.

Results reported in Table 2 indicate very high homology

TABLE 2. Degree of homology between the genomes of intestinal treponemes[a]

Source of unlabeled DNA	% Reassociation[b] with labeled DNA from:		
	HRM 2	HRM 3	P 18
Human treponeme HRM 2	100	56 ± 0.4	ND[c]
Human treponeme HRM 3	60 ± 3.4	100	91 ± 3.9
T. hyodysenteriae P 18	68 ± 4.7	95 ± 5.6	100

[a] DNA from three intestinal treponemes was labeled by nick translation, mixed with a 75-fold excess of unlabeled DNA of treponemal and clostridial (control) origin, sonicated, and annealed; radioactivity resistant to single-strand-specific S1 DNase and measured.

[b] Relative hybridization (± standard deviation from four experiments), or the amount of protected DNA in heterologous annealing compared with homologous annealing value (100% corresponds to 50 to 60% of probe radioactivity), with background value (hybridization with *C. perfringens*) being substracted (all values for *C. perfringens* DNA were 0%, which corresponds to 12% of probe radioactivity).

[c] ND, Not determined.

levels (greater than 90%) between human strain HRM 3 and *T. hyodysenteriae* P 18. The degree of homology between the two subgroups of human strains (HRM 2 and HRM 3) was lower (56 to 60%) but sufficient to infer close relatedness.

The taxonomic value of DNA base composition has been proven by numerous studies: it is widely accepted that organisms having 5% or more difference in G+C content do not belong to the same species. Similarly, an organism might belong to a given genus if its G+C content does not differ by more than 10% from that of a reference DNA (2). The genomes of the three strains of intestinal treponemes analyzed in this study have similar G+C contents (between 27.3 and 30.0%) (Table 1). Note that the G+C content of treponemal DNA was established first by the usual procedure, involving the determination of the buoyant density by CsCl density gradient centrifugation. This technique being unsuitable for analysis of DNAs containing either modified or unusual bases, determinations were independently carried out with a recently developed double-labeling procedure, which proved to be unaffected by the presence of unusual components. Modified bases were indeed shown to occur in treponemal DNA (subjected to isoschizomeric restriction enzymes; P. de Wergifosse and A. M. Agliano, unpublished results); their presence, however, had only insignificant effects on the G+C content of this DNA (compare data in Table 1). The simplest explanation is that methylated bases are present in amounts too small to produce aberrant buoyant density values.

DNA-DNA hybridization techniques yield important information on genetic relatedness among microorganisms. Unfortunately, numerous variants of the basic method are used in different laboratories, making it difficult to compare data from different sources. For the major parameters (molarity of the reaction mixture and hybridization temperature), conditions used in the present work were those most currently used (0.33 M NaCl and t° = T_m − 25°C). It is widely accepted that under these experimental conditions, organisms belong to the same species or to the same genus when their relative hybridization exceeds 60 or 20%, respectively (9). Data in Table 2 indicate more than 68% homology between the DNA of human treponemes and DNA of *T. hyodysenteriae*. The highest homology (91 to 95%) was indeed observed between *T. hyodysenteriae* and human treponeme HRM 3, which could not be distinguished by our test. The overall conclusion is that the treponemal strains isolated from the intestinal tracts of humans and pigs belong

to the same species (*T. hyodysenteriae*) and are genetically closely related. The hybridization data suggest the occurrence within the species of two subgroups, one including the HRM 2 strain and the other including strains HRM 3 and P 18. Domestic animals might be the source of treponemal infection for humans and vice versa, as suggested by the results of this study.

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