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Detection of Variable DNA Repeats in Diverse Eukaryotic Microorganisms by a Single Set of Polymerase Chain Reaction Primers

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We cloned and sequenced a variable DNA repeat from *Trichomonas vaginalis*, a flagellated protozoan parasite. Targeting of this repeat in the polymerase chain reaction resulted in complex and intense product patterns for a wide variety of eukaryotic microorganisms, including the pathogenic protozoan parasites *T. vaginalis*, *Giardia lamblia*, *Leishmania donovani*, three species of *Trypanosoma*, and four species of *Acanthamoeba*; the nonpathogenic protozoans, *Paramecium tetraurelia* and *Tetrahymena thermophilia*; and a yeast, *Saccharomyces cerevisiae*. Each microorganism exhibited a distinctive pattern of repeats. For example, a characteristic pattern was exhibited by six clinical *T. vaginalis* isolates. Eight *G. lamblia* isolates exhibited either one of two characteristic pattern types. There was no reaction with human DNA or DNA from the prokaryotes *Ureaplasma urealyticum* and *Mycoplasma hominis*. This approach may facilitate detection of a wide variety of eukaryotic microorganisms by use of a single primer set and holds promise for the development of typing schemes for both *T. vaginalis* and *G. lamblia*.

Trichomonas vaginalis is a protozoan parasite that afflicts an estimated 180 million women per year worldwide (15, 24). The parasite causes vaginitis and possibility cervicitis in women and urethritis in members of both sexes (21–23). Wet-mount examination, culture, and direct immunofluorescence methods for the diagnosis of *T. vaginalis* infections have been compared (10, 24, 26, 37). Culture was considered the most reliable diagnostic method, but even redundant culture techniques may miss (i) parasites present in low numbers, (ii) defective parasites, or (iii) microorganisms that do not survive the transfer to culture medium. Compared with culture techniques, polymerase chain reaction (PCR) methods offer the potential advantages of enhanced sensitivity and the ability to detect nonviable or defective microorganisms (19, 40).

During development of PCR-based methods for the detection of *T. vaginalis*, several repeated and nonrepetitive genomic *T. vaginalis* DNA clones were sequenced, primers were constructed, and PCRs were carried out. A specific, single-band PCR test for *T. vaginalis* was developed and will be reported separately (unpublished data). One of the candidate primer sets for *T. vaginalis* detection proved to be surprisingly versatile in the detection of variable DNA repeat patterns in a variety of eukaryotic microorganisms. Variable DNA repeats are ubiquitous, variable-length, multiple-copy DNA sequences that have been used in DNA fingerprinting or typing of microorganisms (7, 8, 36, 38, 43, 47) and in human forensic science (9, 11, 17, 18). This study focused on PCR detection of characteristic DNA repeat patterns in *T. vaginalis* and other eukaryotic microorganisms.

MATERIALS AND METHODS

Cells and cell culture. The organisms used in these studies included clinical isolates of *T. vaginalis* from patients in Seattle, Wash. (isolates S13, PHS 2J, S981, S1190, S1202, and S1220); Brooklyn, N.Y. (isolate B34, kindly supplied by William McCormack); and Charlottesville, Va. (isolate C7, kindly supplied by Michael Rein). *T. vaginalis* isolates S13, PHS 2J, B34, and C7 have been described previously (25) and were maintained in liquid nitrogen prior to use in the current studies. Isolates S981, S1190, S1202, and S1220 were fresh clinical isolates from patients attending the Sexually Transmitted Diseases Clinic, Harborview Medical Center, Seattle, and the Prostatitis Clinic, University of Washington Medical Center, Seattle. Prior to use, each trichomonad isolate was cloned in agar and was then grown to the mid-log phase in Diamond's medium at 37°C in 5% CO_2 (23).

Acanthamoeba castellani, *Acanthamoeba rhisodes*, *Acanthamoeba hatchetti*, and *Acanthamoeba culbertsoni* cultures were generous gifts from Thomas Fritche (Department of Microbiology, University of Washington, Seattle). *Acanthamoeba* cells were washed twice in sterile phosphate-buffered saline (PBS) and were subsequently treated identically with *T. vaginalis* cells.

Giardia lamblia cysts H7 (human, West Virginia), K1 and P1 (human, Portland, Oreg.), PB1 (human, Portland, Oreg.), WB1B (human, Afghanistan), BE1 (beaver, Canada), BE4 (beaver, Canada), and BE5 (beaver, Canada) and *Giardia muris* CuH1 were obtained from Jerry E. Ongerth (Department of Environmental Health, University of Washington, Seattle). Trophozoites were cultivated in TYI-S33 medium (29) with 10% calf serum supplemented with ampicillin (50 μg/ml) and streptomycin (40 μg/ml).

Tetrahymena thermophilia CU427 DNA was obtained from Meng Chao Yao (Fred Hutchinson Cancer Research Center, Seattle, Wash.). *Paramecium tetraurelia* DNA was a gift from Robert Hinrichsen (Fred Hutchinson Cancer Research Center). *Saccharomyces cerevisiae* DNA was a gift from Stephan Zweifel and Walton Fangman (Department of Genetics, University of Washington, Seattle). *Escherichia coli* DH5α and *Ureaplasma urealyticum* UU5 DNAs were from Marilyn Roberts and George Kenny (Department of

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Pathobiology, University of Washington, Seattle). The following *Leishmania donovani* DNAs were gifts from Cindy Tripp and Kenneth Stuart (Seattle Biomedical Research Institute, Seattle, Wash.) *L. donovani* DDB (human, India), *L. donovani* IS clone 2D (human, Sudan), and *L. donovani* D42 (source unknown). Trypanosome DNAs were gifts from Harvey Eisen (Fred Hutchinson Cancer Research Institute). Human DNA was isolated by standard methods from a human lymphocyte culture obtained from Hans Ochs (Department of Pediatrics, University of Washington, Seattle).

DNA isolation. (i) *T. vaginalis* and *Acanthamoeba* spp. DNAs were purified from *T. vaginalis* and *Acanthamoeba* spp. by a recently described method (35). Briefly, mid-log-phase *T. vaginalis* or *Acanthamoeba* cultures (75 to 150 ml) were harvested by centrifugation at 900 × *g* for 5 min at 4°C. The cellular pellet (0.5 ml) was washed twice by centrifugation in 40 volumes of cold (4°C), sterile PBS (pH 7.4), and the pellet was suspended in 1 ml of PBS. Diethylpyrocarbonate (20 μl; Sigma, St. Louis, Mo.) was added to the cell suspension. The cells were subjected to two cycles of centrifugation (900 × *g* for 3 min at 4°C) in 1% (vol/vol) Triton X-100 (Sigma) in reticulocyte standard buffer (10 mM Tris, 10 mM NaCl, 5 mM $MgCl_2$) which had been treated with 100 μl of diethylpyrocarbonate.

The resulting pellet was suspended in 0.5 ml of 10 mM EDTA and 0.1% (wt/vol) sodium dodecyl sulfate and was then incubated at 65°C for 15 min to inactivate the diethylpyrocarbonate. The suspension was then treated with 0.2 mg of proteinase K (Bethesda Research Laboratories, Gaithersburg, Md.) per ml at 37°C for 1 h; this was followed by three cycles of phenol (Bethesda Research Laboratories)–chloroform (Sigma)–isoamyl alcohol (Sigma) (1 vol, 1 vol, 0.02 vol) extraction, two cycles of chloroform–isoamyl alcohol (1 vol, 0.04 vol) extraction, and then 80% (vol/vol) ethanol precipitation. A culture of approximately 10^7 *T. vaginalis* or *Acanthamoeba* cells typically yielded between 100 and 200 μg of DNA (by A_{260} determination).

(ii) *Giardia* trophozoites. Cultures in the late log phase were harvested and placed on ice for 30 min followed by centrifugation at 500 × *g* for 10 min. The cells were washed once in PBS (pH 7.0), suspended in 400 μl of 50 mM Tris (pH 8.0)–50 mM EDTA, and transferred to an Eppendorf tube. Then, 22 μl of 20% sodium dodecyl sulfate was added; this was followed by the addition of 10 μl of 10 mg of proteinase K (Bethesda Research Laboratories) per ml. The tubes were incubated at 37°C for 30 min. This was followed by phenol-chloroform extraction and ethanol precipitation as described above.

Agarose gel electrophoresis. Agarose gels consisted of 1.4% (wt/vol) agarose (Fisher Scientific, Fair Lawn, N.J.) in 90 mM Tris buffer (Fisher Scientific), 90 mM boric acid (Sigma), and 25 mM EDTA (pH 8.3). Each PCR mixture (20 μl) was combined with 5 μl of 0.5% sodium dodecyl sulfate–50 mM EDTA (pH 7.4, adjusted with NaOH)–45% (vol/vol) glycerol (Sigma)–0.1% (wt/vol) bromphenol blue (Sigma). Molten gel solutions and electrophoretic running buffers contained 0.5 μg of ethidium bromide (Sigma) per ml for visualization of nucleic acids under UV illumination. Electrophoresis was conducted at 100 V for 3.5 h.

Genomic *T. vaginalis* libraries. Total genomic *T. vaginalis* 981 DNA was digested to completion with the restriction enzyme *Sau*3A (Bethesda Research Laboratories). Enzyme was removed by phenol-chloroform extraction and ethanol precipitation as described above. The *Sau*3A-digested DNA was used to construct a total genomic DNA library in the plasmid vector pUC18 as well as a repeat-enriched genomic

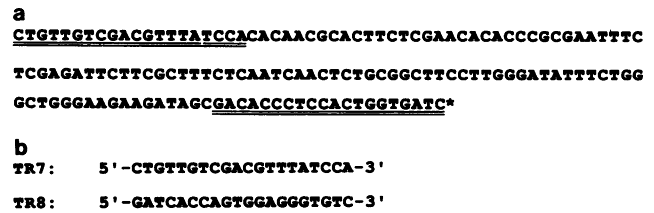

FIG. 1. (a) Nucleotide sequence of *T. vaginalis* genomic clone pT17rp. Computer-assisted homology searches of the GenBank international data base (release 63) revealed no significant matches with known nucleotide sequences. Primer sites are doubly underlined. (b) Oligonucleotide primers synthesized for the detection of genomic T17 sequences.

library. The *T. vaginalis*-pUC18 recombinants were transformed by the method of Hanahan (12) into *E. coli* host JM107 and were plated onto agar medium containing 5-bromo-4-chloro-indolyl-β-D-galactopyranoside isopropyl-β-D-thiogalactopyranoside (48), and 100 μg of ampicillin per ml. The nucleotide sequences of 12 *T. vaginalis* genomic clones were determined. Computer-assisted homology searches were conducted by comparing newly sequenced *T. vaginalis* genomic DNAs with the GenBank data base, release 63 (Riverside Scientific, Bainbridge Island, Wash.). Sequence information was used to construct oligonucleotide primers for the PCRs.

Nucleotide sequencing and PCR. Nucleotide sequencing was performed in conjunction with a rapid miniprep method (33). Oligonucleotide primers (see Fig. 1) were synthesized at the Howard Hughes Research Institute (Seattle, Wash.). PCR buffer consisted of 10 mM Tris HCl (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$. $MgCl_2$ concentrations were varied over the range of 0.5 to 5 mM, with 1.5 mM being optimal. *Taq* polymerase (5 U/100 μl of reaction; Perkin-Elmer Cetus, Norwalk, Conn.) or replitherm (Epicenter Technologies, Madison, Wis.) was used as the thermostable enzyme. The *G. lamblia* product patterns were more intense when replitherm was used, but in general, the two enzymes were used interchangeably. Thermal cycles for PCR consisted of 7 min at 94°C and then 30 to 40 cycles of sequential variation between 94, 50, and 72°C for 1 min each; this was followed by a 7-min extension cycle at 72°C.

RESULTS

Several genomic clones from *T. vaginalis* isolate S981 were sequenced, including clone pT17rp (Fig. 1). The sequence of pT17rp was nonhomologous with known sequences reported in GenBank and was considered a candidate target sequence for PCR detection of *T. vaginalis*. Primers TR7 and TR8, which were complementary to clone pT17rp, were synthesized and tested in a PCR by using total genomic *T. vaginalis* S1202 DNA as the template. Primers TR7 and TR8 led to multiple product bands, which are collectively referred to as T17 patterns (Fig. 2, lane 1). We were surprised to find that other genomic DNAs, originally intended as negative controls, segregated into two groups: genomic DNAs from the prokaryotes *Mycoplasma hominis* and *U. urealyticum* (Fig. 3a, lane 1) and also the DNAs of higher eukaryotes, including human (Fig. 2, lane 10) and *Salmo salar* (salmon) DNAs, all gave negative or extremely weak T17 patterns (for *M. hominis* and *S. salar*, data not shown). In contrast, all of the protozoa and other unicellular

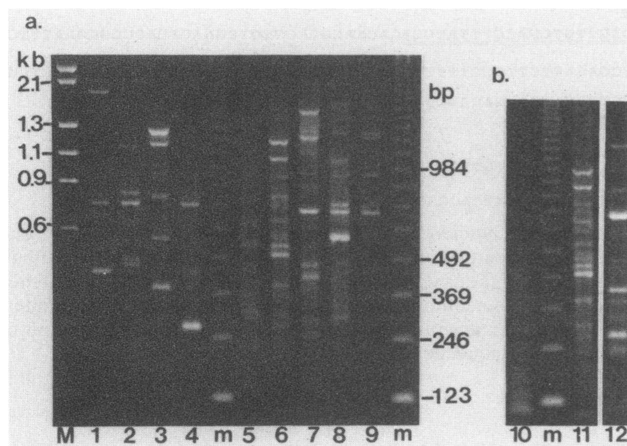

FIG. 2. Ethidium bromide-stained agarose gel electrophoresis of amplified T17 PCR products. Lane M, molecular mass size standard for the upper region of the gel (*Hin*dIII-cut lambda DNA mixed with *Hae*III-cut φX174); lanes m, low-molecular-mass size standards consisting of 123-bp interval ladder fragments (Bethesda Research Laboratories); lane 1, T17 products from *T. vaginalis* S1202; lane 2, *A. culbertsoni*; lane 3, *A. castellani*; lane 4, *A. hatchetti*; lane 5, *A. rhisodes*; lane 6, *Trypanosoma cruzi*; lane 7, *Trypanosoma equiparitum*; lane 8, *Trypanosoma herpetomonas*; lane 9, *S. cerevisiae*; lane 10, human DNA; lane 11, *P. tetraurelia*; lane 12, independent repetition of *T. cruzi*.

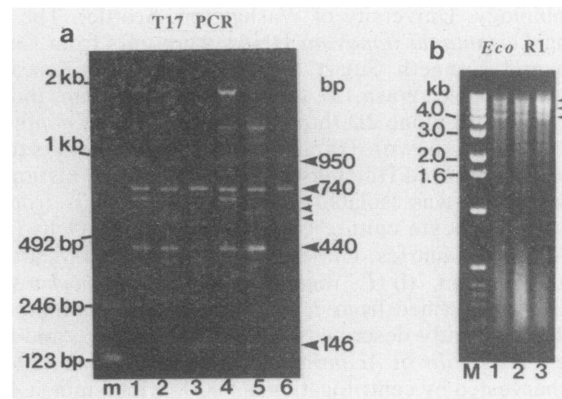

FIG. 4. T17 PCR and restriction digest patterns of clinical *T. vaginalis* isolates. (a) Lane m, molecular size markers, as described in the legend to Fig. 2; lane 1, T17 PCR pattern of *T. vaginalis* S1190; lane 2, *T. vaginalis* S981; lane 3, *T. vaginalis* C7; lane 4, *T. vaginalis* S1202; lane 5, *T. vaginalis* S1220; lane 6, *T. vaginalis* B34. The small arrowheads indicate low-intensity products that were consistently present. (b) Lane M, kilobase-range molecular size markers (Bethesda Research Laboratories); lane 1, *Eco*RI digest of *T. vaginalis* S981; lane 2, *T. vaginalis* S1220; lane 3, *T. vaginalis* C7.

eukaryotes tested gave intense and complex T17 patterns (Fig. 2, lanes 4 to 9).

Selectivity of the T17 PCR. Several lines of evidence indicate that the T17 PCR conditions were selective. First, the primer annealing temperature of 50°C and the extension temperature of 72°C (see Materials and Methods) are temperatures that allow specific annealing of other primers with similar A+T (adenosine plus thymidine) compositions (Fig. 3). Higher annealing temperatures caused the T17 patterns to fade. More important than the A+T composition was the actual sequence of the 3′-terminal nucleotides of the primers, which might cause priming at multiple, short genomic sequences. Such mispriming should be ubiquitous and, most likely, should be enhanced in the case of complex genomes. However, a number of total genomic DNAs (e.g., human [Fig. 2, lane 10] and *U. urealyticum* [Fig. 3a, lane 1] DNAs as well as *S. salar* and *M. hominis* DNAs) representing both more and less complex genomes were negative or weak in the T17 PCR. Also, while many T17 bands exhibited a higher intensity than that of the original 146-bp target sequence (Fig. 4), misprimed sites would be expected to be less intense. Finally, the T17 patterns, including the low-intensity bands, were highly reproducible.

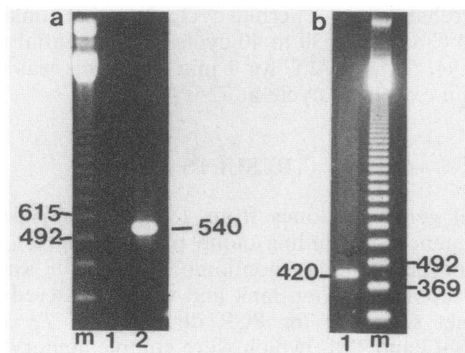

FIG. 3. General PCR reactivity of T17 PCR-negative genomic DNAs. (a) Lane 1, *U. urealyticum* DNA in T17 PCR performed as described in the legend to Fig. 2 and the text; lane 2, same as lane 1, except that primers UU1 (5′-AGAAGACGTTTAGCTAGAGG-3′; 20mer) and UU2 (5′-ACGACGTCCATAAGCAACT-3′; 19mer) were substituted for TR7 and TR8 (see text). The predicted product length was 541 bp, from bases 216 through 757 of the *U. urealyticum* urease gene sequence (5). (b) Identical to the T17 PCR of human DNA (Fig. 2, lane 10), except that primers GK-3 (5′-CTGTGTTA GACTCCTGTTTT-3′) and GK-4 (5′-TTGTCCCTAAAGGTGAAA TA-3′) targeting the human X-linked PGK gene (29) were used. The predicted product length was 420 bp. Numbers next to the gels are in base pairs.

To determine whether DNAs which were negative or weak in the T17 PCR were free of *Taq* polymerase inhibitors, we targeted the phosphoglycerate kinase (PGK1) gene (34) in the same human DNA preparation that was found to be weakly reactive in the T17 PCR. We also targeted the urease gene (5) in the T17 PCR-negative *U. urealyticum* DNA. For these PCRs, the same buffers, thermocycle times, and temperatures as those in the T17 PCRs were used. As shown in Fig. 3, T17 PCR-negative DNAs were reactive in other PCRs, demonstrating both a lack of *Taq* polymerase inhibition and adequate DNA template quality. These findings indicate high stringency of the T17 PCR and strongly suggest selectivity of the reaction for multiple target sequences that are present in lower eukaryotes but that are not present in the prokaryotes or higher eukaryotes tested.

Species-specific T17 PCR patterns. The T17 PCR patterns were similar in appearance to the hypervariable minisatellite repeat patterns that have often been used to type or fingerprint organisms (9, 11, 17–19, 36, 38, 40, 43, 45–47). We reasoned that if the T17 patterns obtained by PCR amplification were useful for microorganism typing, in a manner analogous to forensic DNA typing, then various isolates of a given species should show similar T17 patterns. Figure 4 shows the results of experiments in which the primers TR7 and TR8 were used on a variety of *T. vaginalis* isolates. The

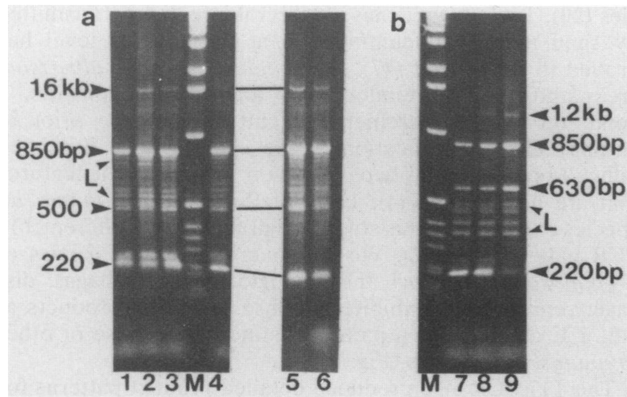

FIG. 5. T17 PCR patterns of *G. lamblia* type 1 (a) and type 2 (b) isolates. Lanes M, kilobase-range molecular size standards; lane 1, *G. lamblia* H7; lane 2, *G. lamblia* K1; lane 3, *G. lamblia* P1; lane 4, *G. lamblia* PB1; lane 5, *G. lamblia* KL; lane 6, *G. lamblia* WB1B; lane 7, *G. lamblia* BE1; lane 8, *G. lamblia* BE4; lane 9, *G. lamblia* BE5. The L and small arrowheads indicate ladder pattern T17 PCR products consistent for each pattern type.

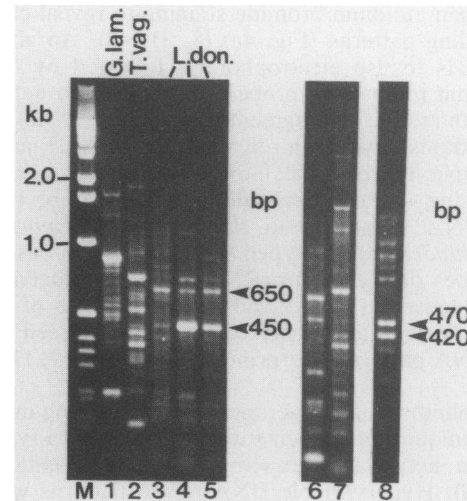

FIG. 6. *G. lamblia* and *T. vaginalis* T17 PCR patterns compared with the PCR patterns of two additional microorganisms. Lane 1, *G. lamblia* WB1B; lane 2, *T. vaginalis* S981; lane 3, *L. donovani* K42; lane 4, *L. donovani* LSB5201; lane 5, *L. donovani* DD8; lane 6, *T. thermophilus* CU47. *T. thermophilus* was left out of the gels shown in Fig. 2. Not surprisingly, *T. thermophilus* exhibits a pattern distinct from those of all of the other microorganisms, including *P. tetraurelia*, its nearest relative in this study.

patterns of the six *T. vaginalis* isolates examined were closely related to one another. Although individual isolates exhibited variations, a consistent *T. vaginalis* T17 pattern was observed. Specifically, PCR-amplified products at 740 and 440 bp and a faint band at 146 bp were present in all of the *T. vaginalis* isolates (Fig. 4, arrowheads). Although slight variations in intensity were noted among isolates, the general pattern of consistently intense PCR products and, in addition, several of the less intense bands clearly distinguished the *T. vaginalis* isolates from the other microorganisms examined (see Fig. 2, 4, and 6). Although similar, the T17 pattern of each *T. vaginalis* isolate also appeared to have features unique to the isolate, since repeated determinations for a given isolate yielded the same pattern (e.g., compare S981, Fig. 4, lane 2, and Fig. 6, lane 2).

The T17 PCR products of *G. lamblia* exhibited two distinct patterns (Fig. 5). The more common pattern (type 1) consisted of an intense 850-bp PCR product, a medium-intensity product at 500 bp, and a prominent band at 220 bp. Type 1 *G. lamblia* isolates also exhibited a characteristic evenly spaced ladder of about 11 product bands ranging from 500 to 850 bp (Fig. 5). The type 2 pattern observed for *G. lamblia* isolates consisted of prominent bands at 850 and 650 bp, a consistent light band at 1,200 bp, a variable-intensity band at 220 bp, and a distinctive ladder of medium-intensity bands from 340 up to 450 bp. After detecting two *G. lamblia* types by T17 PCR, we learned that restriction fragment length polymorphism (RFLP) data also distinguished two distinct types among *G. lamblia* isolates (31).

The three isolates of *L. donovani* examined had two T17 product bands in common at 450 and 650 bp (Fig. 6). Preliminary data indicate that *Leishmania brazeliensis* and *Leishmania tarentolae* have T17 patterns distinct from those of *L. donovani*.

Genus-level comparisons of T17 PCR patterns. In contrast to species-specific T17 patterns, genus-level comparisons have thus far revealed no interrelationships among the T17 patterns. For example, the three species examined within the genus *Trypanosoma* showed no similarity in their T17 patterns (Fig. 2, lanes 6 to 8). Four species of *Acanthamoeba* had few, if any, common products (Fig. 2, lanes 2 to 5). *G. muris* exhibited a T17 pattern distinct from the two characteristic *G. lamblia* pattern types described here.

DISCUSSION

The T17 PCR-amplified multiple, variable-length target sequences present in the genomic DNAs of lower eukaryotes. Consistent DNA fingerprint patterns were obtained for six *T. vaginalis* isolates; for several isolates of *G. lamblia*, in which two distinct pattern types were seen; and for three isolates of *L. donovani*. The species that are potentially identifiable by the T17 PCR range from the relatively simple budding yeast *S. cerevisiae* through *Acanthamoeba* spp. and the ciliated protozoa *Paramecium tetraurelia* and *Tetrahymena thermophilia* and to the flagellated protozoan parasites *T. vaginalis*, *Trypanosoma* spp., *Leishmania* spp., and *G. lamblia*. Given the broad spectrum of microorganisms that produce T17 patterns by use of a single primer set, this approach holds potential as a generic or universal typing procedure or as a convenient complement to other typing procedures.

There exists a wide variety of variable and hypervariable DNA repeats which have proven to be extremely useful for the typing or DNA fingerprinting of organism and microorganism species, strains, and even individual humans in forensic investigations and population studies (9, 11, 17–19, 36, 38, 40, 43, 45–47). There is a spectrum of variability among repeat DNAs ranging from hypervariable repeats, which are specific for individuals (9, 11, 17, 18, 45, 46), to species-specific repeats (13, 27, 28), and there are conserved repeats (9). With the exception of mitochondrial and ribosomal gene typing, the actual function of the DNA repeats that are used in typing has rarely been determined. There is evidence that some variable repeats function as hot spots for recombination (45).

One approach to DNA typing involves restriction enzyme digestion of the DNA followed by agarose gel electrophore-

sis and then ethidium bromide staining to reveal characteristic banding patterns (Fig. 4a) (8, 31, 43). An alternative approach is to use electrophoresis followed by Southern blotting and radioactive probe hybridization to detect variable-length restriction fragments (16, 18, 31, 32, 42). Both of these methods have been referred to as "RFLP analysis." For example, Hughes et al. (16) accomplished parasite strain typing using an oligonucleotide probe mixture based on repeat DNA sequences in *Plasmodium falciparum* (16). *Giardia duodenalis* was typed by using an M13-based probe (42). Probes derived from rRNA genes have been used to type trypanosomal stocks and trace the origin of different strains (13). A large variety of probes have been used for repeat DNA pattern fingerprinting in humans (9, 11, 17, 18, 45, 46).

A so-called second generation of DNA typing uses PCR. PCR techniques have been successfully used to type *Theileria parva*, herpes simplex virus, and human papillomavirus (4, 19, 40). Hypervariable DNA repeat patterns which are distinct from the DNA repeat patterns reported here have been targeted by PCR (6, 30). Previous investigators have also described cross-species PCR methods. For example, universal PCR primers are available for amplification of both prokaryotic and eukaryotic ribosomal genes (27, 28).

We would categorize the T17 repeats as conserved, variable repeats, since the repeats were recognized in diverse microorganisms, and yet, repeat pattern alterations were extensive within the genera examined. The term "variable repeat" distinguishes these repeats from the "hypervariable repeats" that often exhibit dramatic differences at the individual organism level (9, 11, 17, 18, 45, 46).

The TR17 patterns obtained for six *T. vaginalis* clinical isolates indicated some genetic variation, but the patterns were consistent with the assignment of these isolates to a single species. Previously, *T. vaginalis* isolates were characterized as expressing both common antigens and isolate-specific antigens (1–3, 22, 39, 41). It was unclear whether the frequently observed isolate-specific antigens were due to genetic variation among isolates or variation at the level of expression. However, genetic variation among *T. vaginalis* isolates was obvious in T17 PCR patterns (e.g., compare isolate C7 with isolate S1220 in Fig. 4a), which were reproducible for each isolate. Genetic variation was less obvious in ethidium bromide-stained RFLP patterns (Fig. 4b). The background smear seen in the restriction digests (Fig. 4b) obscured minor differences in the RFLP patterns. This background is caused by heterogeneous nonrepeated DNA and is typical of the ethidium bromide-RFLP method (e.g., see references 8 and 31).

G. lamblia is a flagellated enteric protozoan that, like *T. vaginalis*, is adapted to colonization of mucosal surfaces. This parasite is an important cause of chronic debilitating diarrhea and diarrhea in travelers (29). *G. lamblia* exhibited two T17 PCR patterns. Pattern type 1 was found in isolates from human hosts from Oregon and Afghanistan. To date, the type 2 pattern has been seen only in isolates from beavers (Fig. 5). Previous probe-RFLP studies (31) of *G. lamblia* also concluded that there were two main *G. lamblia* subtypes, with isolates P1 and BE1 characterized as distinct types, in accord with the results of the present study. *G. lamblia* has a wide mammalian host range (31), so that the pattern types from human and beaver sources observed here, while reflecting parasite subtypes, are not necessarily unique to those respective hosts.

Rapid detection and typing methods are also needed for *Leishmania* (47) *Acanthamoeba* (32), and *Trypanosoma* species (20). *L. donovani* causes visceral infections transmitted by sand flies, and identification at the species level has proved to be difficult (47). *A. castellani* and *A. culbertsoni* are pathogens in granulomatous amoebic encephalitis, a condition that is extremely difficult to diagnose prior to autopsy (32). Identification at the species level has been difficult because it has been based on "morphologic features that are not clear" (44). Each of the four *Acanthamoeba* species examined in this study had dramatically different T17 PCR patterns (Fig. 2). We also examined three species of *Trypanosoma*. *T. cruzi*, the etiologic agent of Chagas' disease, reproducibly exhibited intense T17 PCR products at 1.2, 1.1, and 0.5 kb, a pattern distinct from those of other *Trypanosoma* species (Fig. 2).

The T17 PCR also produced detailed product patterns for microorganisms that are not considered to be pathogenic, such as *P. tetraurelia* (Fig. 2), *T. thermophilus* (Fig. 6), and *S. cerevisiae*. Thus far, the only unifying features apparent in microorganisms detected by the T17 PCR is that they are all unicellular and eukaryotic. The eubacterium *E. coli* did yield a T17 PCR pattern, although it was weak. We have not tested primitive multicellular organisms, which would be interesting from an evolutionary standpoint.

In summary, the T17 PCR identified variable DNA repeats in a spectrum of eukaryotic microorganisms, including a wide variety of pathogenic protozoa. Using a single set of primers, we distinguished characteristic banding patterns of particular species and, in some cases, of subgroups of organisms within species. This approach holds promise as a tool for identification of organisms at the species level and for the development of improved methods for clinical diagnosis.

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