Use of Genome Selected Repeated Sequences Increases the Sensitivity of PCR Detection of *Tropheryma whippelii*

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The availability of the *Tropheryma whippelii* genome offers the putative possibility of choosing logical DNA targets. We applied a PCR assay (targeting repeated sequences of *T. whippelii*) to samples from patients with Whipple's disease and to those from members of a control group. When compared to the results seen with regular PCR, the sensitivity of repeat PCR was significantly enhanced ($P = 0.02$) without alteration of its specificity.

An ideal PCR target should be both specific and sensitive. The analysis of bacterial genomes has revealed the occurrence of repeated sequences in various species (13). For *Coxiella burnetii*, a PCR assay targeting a repetitive element existing in 19 copies in its genome has been demonstrated to be much more sensitive than assays targeting single-copy genes (22). The recent sequencing of two *Tropheryma whippelii* genomes (3, 17) has revealed the presence of seven repeated sequences which have the same size (677 bp) in both genomes, with the percentage of nucleotide sequence similarity ranging from 99.4 to 100%. Our aim was to verify whether using primers targeting repeated sequences in the *T. whippelii* genome would enhance the sensitivity of DNA detection in comparison to that of regular-PCR assays without losing specificity.

We selected within the repeated sequences two primers, 53.3F (5' AGAGAGATGGGGTGCAGGAC 3') and 53.3R (5' AGCCTTTGCCAGACAGACAC 3'), targeting a 164-bp fragment repeated seven times in the genomes of two different *T. whippelii* strains. These sequences were found within either open reading frames encoding proteins of unknown function or intergenic spacers. The high level of specificity of these primers was estimated a priori by using BLAST software to compare their sequences with all DNA sequences in GenBank. A total of 98 samples (detailed in Table 1) were obtained at the time of diagnosis or during follow-up of 24 patients with histologically proven Whipple's disease. All samples had previously been tested using our regular PCR, targeting the 16S–23S rRNA gene intergenic spacer and the *rpoB* gene (7). The control group included 48 duodenal biopsies and 36 saliva samples from 84 patients with diagnosis results that excluded the presence of Whipple's disease. DNA was extracted as previously described (5). PCR mixes were prepared using a Fast-Start DNA Master SYBR Green kit (Roche, Mannheim, Germany) and following the manufacturer's instructions. PCR was performed in a LightCycler thermocycler (Roche). All PCR

products were sequenced as previously described (9). We compared the sensitivity levels of regular- and repeat-PCR assays on DNA extracted from 10-fold dilutions of a suspension of 10^6 *T. whippelii* strain Marseille-Twist (ATCC VR-1528) bacteria. We also estimated the in vitro specificity of our primers by applying them to the control group and to DNA extracted from 40 bacterial strains (Table 2). Statistical analyses were performed using chi-square tests.

All PCR results are summarized in Table 1. In comparisons of the detection capacities of the two assays, we were able to detect 1 DNA copy of standard control DNA when our repeat PCR was used and only 10 copies when our regular PCR was used. When clinical samples were tested, our repeat PCR (54 [55%] positive results among the 98 samples from patients with Whipple's disease) was significantly ($P = 0.02$) more sensitive than regular PCR (33 [33.6%] positive results out of 98). Sequences obtained from all PCR products were 100% homologous to *T. whippelii*. When the results were analyzed for each specimen, repeat PCR applied to saliva was significantly ($P =$

TABLE 1. Analysis of 98 samples from 24 patients with Whipple's disease tested with regular PCR and repeat PCR

Sample(s)	No. of samples	No. (%) of samples positive by:	
		Regular PCR	Repeat PCR
Control group			
Duodenal biopsies	27	13 (46.6)	14 (51)
Saliva specimen	34	10 (29.5)	20 (60)
Stool specimen	3	1	2
Presumably sterile specimens	34	9	20
Blood	17	2	6
Cardiac valve	8	4	7
CSF ^a	5	1	2
Aqueous humor	1	1	1
Synovial fluid	1	0	1
Synovial biopsy	1	0	0
Lymph node biopsy	1	1	1
Total	98	33 (33.6)	54 (55)

^a CSF, cerebrospinal fluid.

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TABLE 2. Bacterial strains tested using the repeat-PCR assay^a

Bacterial strains
<i>Staphylococcus aureus</i>
<i>Micrococcus luteus</i>
<i>Streptococcus A</i>
<i>Streptococcus B</i>
<i>Streptococcus C</i>
<i>Streptococcus pneumoniae</i>
<i>Streptococcus sanguineus</i>
<i>Streptococcus bovis</i>
<i>Propionibacterium acnes</i>
<i>Actinomyces neuii</i>
<i>Actinomyces meyeri</i>
<i>Actinomyces viscosus</i>
<i>Nocardia asteroides</i>
<i>Rhodococcus equi</i>
<i>Bacillus cereus</i>
<i>Bacillus thuringiensis</i>
<i>Corynebacterium</i> sp.
<i>Listeria monocytogenes</i>
<i>Peptostreptococcus asaccharolyticus</i>
<i>Bacteroides fragilis</i>
<i>Fusobacterium nucleatum</i>
<i>Escherichia coli</i>
<i>Enterobacter aerogenes</i>
<i>Salmonella</i> sp.
<i>Shigella</i> sp.
<i>Citrobacter freundii</i>
<i>Yersinia</i> sp.
<i>Campylobacter fetus</i>
<i>Campylobacter jejuni</i>
<i>Pseudomonas aeruginosa</i>
<i>Sphingomonas paucimobilis</i>
<i>Ralstonia pickettii</i>
<i>Pantoea agglomerans</i>
<i>Haemophilus influenzae</i>
<i>Neisseria gonorrhoeae</i>
<i>Neisseria meningitidis</i>
<i>Mycobacterium tuberculosis</i>
<i>Mycobacterium lentiflavum</i>
<i>Mycoplasma hominis</i>
<i>Ureaplasma urealyticum</i>

^a No PCR product was obtained with any of the listed strains.

0.015) more sensitive than regular PCR. The 10 patients whose results were positive when repeat PCR was used but negative when regular PCR was used had not been treated previously. For presumably sterile specimens, repeat PCR was also significantly ($P = 0.045$) more sensitive than regular PCR. Among the 11 patients whose results were positive only when repeat PCR was used, samples had been obtained at the time of diagnosis from 2 untreated patients and from 9 patients treated for less than 3 weeks. For duodenal biopsy specimens, the difference in the results of the two PCR assays was not significant ($P = 0.78$). The only duodenal specimen amplified by repeat PCR and not by regular PCR was obtained from a patient who had not been among those treated at the time of sampling. The repeat PCR failed to amplify any product from the control group or the collection of bacteria, thus confirming the BLAST search results.

PCR tests have been introduced to complement pathological analyses for the diagnosis of Whipple's disease (8), but the initial choice of primers was empirical (1, 4, 7, 10, 12, 14, 15, 18, 20, 21). The recent development of bacterial genome sequencing has provided an important source of potential targets for

PCR (16). We speculated that targeting repeated sequences would increase the detection sensitivity of PCR. In addition, as more than 100 complete bacterial genomes are now available in databases, the specificity of targeted sequences may be evaluated before laboratory tests.

For the first time, a logical choice of DNA targets for PCR assays may be possible using genomic data. In this study, our repeat PCR was more sensitive than regular PCR in testing samples of patients with Whipple's disease. The level of sensitivity is still low at 51%, but it is important that most PCR-negative duodenal biopsies were obtained from patients treated for more than 1 year. Repeat PCR was more sensitive for saliva and presumably sterile specimens, especially in patients treated for less than 3 weeks. We hypothesize that in these cases, the number of *T. whipplei* bacteria present is very low; thus, DNA is only detected by a more sensitive tool. The PCR specificity for the diagnosis of Whipple's disease has been questioned due to the presence of false-positive results, but study results are discrepant (2, 6, 7, 11, 19). Here, *T. whipplei* DNA was never amplified in our control group despite the fact that we had enhanced the sensitivity of our assay. Our new PCR approach for the diagnosis of Whipple's disease is promising. On the basis of our experience, we propose a rationalized strategy to choose DNA targets to perform PCR assays. First, the presence of repeated sequences must be investigated within the bacterial genome of interest. Second, the specificity of DNA targets must be evaluated by comparing the chosen sequences with all sequences available in GenBank. Third, the in vitro sensitivity must be verified with repeat PCR and regular PCR using DNA extracted from 10-fold dilutions of a suspension of the targeted bacterium. Fourth, the specificity must be confirmed using DNA extracted from a bank of bacteria. Then, the in vivo sensitivity and specificity must be verified on human samples. The next step will be to evaluate prospectively this new assay to confirm these good preliminary results.

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