Phylogenetic analysis of pbp genes in treponemes

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Background: β-Lactamases are the main cause of bacterial resistance to penicillin, cephalosporins, and related β-lactam compounds. The presence of the novel penicillin-binding protein (pbp) Tp47 in Treponema pallidum has been reported to be a well-known mechanism for turnover of β-lactam antibiotics. Although, T. pallidum remains sensitive to penicillin, clinically significant resistance to macrolides has emerged in many developing countries. The genome sequence of T. pallidum has shown the presence of genes encoding pbp, but there are no current reports of the presence of mobile plasmids.

Methods: The phylogenetic analysis is used to study the diversity of chromosomal pbp genes and its relatedness to Tp47 in Treponema species.

Results: In our study, genes encoding penicillin-binding proteins that showed significant similarity to each other appeared in separate clusters.

Conclusion: Tp47 showed no substantial similarity to other β-lactamases in treponemes. The relatedness of Treponema denticola to other treponemes, including T. pallidum, and the reported presence of natural mobile antibiotic determinants highlight the importance of investigating the diversity of pbp genes in Treponema species. This will lead to a greater understanding of its potential to develop additional antibiotic resistance via horizontal gene transfer that could seriously compromise the treatment and control of syphilis.

Keywords: Treponema pallidum; penicillin-binding proteins (pbp); Tp47

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and benefit each other. The motility of spirochetes allows them to occupy unique ecological niches, such as the guts of certain arthropods and the rumen of cows and sheep (4, 10, 11).

During the past three decades, and especially since 2004, there have been many reports of antibiotic resistance in treponemes, especially T. pallidum. Research studies have confirmed antibiotic resistance associated with macrolides such as erythromycin and azithromycin (12–19). The oral treponemes, T. denticola, primarily associated with periodontitis, have shown resistance to tetracycline. The research has also shown that tetB and ermA genes are now extensively distributed in the T. denticola population (20). The most important observation was that three of the T. denticola isolates were able to transfer their ermA determinants to Enterococcus faecalis recipients (20–22). The presence of a natural mobile antibiotic resistance determinant in the genus Treponema is very alarming as it could move antibiotic resistant genes between different treponema species. In another study, with Treponema hyodysenteriae, intestinal treponemes, 4 of 32 isolates were found to be resistant to penicillin and produced β-lactamases (23). Mobashery et al. reported penicillin-binding protein Tp47 in T. pallidum with an ability of this protein to turn over β-lactam antibiotics. Tp47 is strongly inhibited by products of the β-lactamase reaction, and, therefore, T. pallidum remains sensitive to penicillin (24). As the number of cases increase, there could be a potential for mutations in Tp47 or the presence of a mobile element that can cause multidrug resistance. The present state of knowledge on the diversity of pbp genes among clinical and ecological groups of treponemes has not been studied. Examining the natural patterns of occurrence of pbp genes in treponemes is an important starting point in understanding how these genes are related to each other and will help to uncover the ecological and evolutionary relationships existing between them.

Results and discussion
Treponemes are difficult to culture in vitro, a hindrance to experimental approaches such as mutational analysis to identify antibiotic resistance determinants. The report of azithromycin resistance in penicillin allergic patients has emerged as a clinical and public health challenge worldwide. T. pallidum continues to be one of the most penicillin-susceptible microorganisms, but mutation in Tp47 or presence of mobile plasmids with genes encoding β-lactamases could be one of the contributing factors leading to future penicillin resistance in T. pallidum. The genome of treponemes, Treponema azotonutricium ZAS-9 (25); Treponema brennaborense DD5/3, DSM 12168; T. denticola ATCC 35405 (26); T. pallidum (pallidum Nichols) (27); Treponema pallidum pallidum SS14 (28); Treponema paraluisnuciculari Cuniculi A (29); and T. primitia ZAS-2 (25), are analyzed for the presence of diversity of pbp genes. Phylogenetic calculations using maximum likelihood (ML) methods (30) are shown in Fig. 1. The novel penicillin-binding protein gene Tp47, Treponeme species (Gene ID 11850998, Gene ID 10884263), forms a separate cluster and does not show substantial similarity to any other pbp genes among the Treponema species used in this study. The analysis of genome sequence of T. pallidum suggests that it lacks genetic elements such as plasmids, bacteriophage, and transposons that are commonly associated with horizontal gene transfer mechanisms. The pbp gene for T. denticola ATTC 35405 (Gene ID 2739572) has shown 68% similarity to T. paraluisnuciculari cuniculi A (Gene ID 10884451) and 65% to T. brennaborense DD5/3, DSM 12168 (Gene ID 10580463). T. paraluisnuciculari is the causative agent of rabbit venereal spirochetosis (29). T. denticola, an oral spirochete associated with periodontal disease, has been reported for the presence of natural mobile antibiotic resistance determinant (20). T. brennaborense DD5/3, DSM 12168 (Gene ID 10580463) have shown 68% similarity to T. pallidum (pallidum Nichols) (Gene ID 2611355). T. brennaborense has been isolated from a cow suffering from digital dermatitis. T. denticola ATTC 35405 (Gene ID 2739453) has shown 66% similarity to T. primitia ZAS-2 (Gene ID 10681574) and T. paraluisnuciculari Cuniculi A (Gene ID 10884181). T. primitia has been isolated from termite hindguts. T. azotonutricium ZAS-9 does not show substantial similarity to any pbp genes among treponemes used in this study.

Experimental section
The list of pbp genes for treponemes with complete sequenced genomes was obtained from the NCBI (National Center for Biotechnology Information) database as listed in Tables 1 and 2. The Molecular Evolutionary Genetics Analysis version 5.05 (MEGA5) software program was used for the statistical analyses (30). The BLAST (basic local alignment search tool) algorithm was used to calculate the percentage of similarity between known sequences. The phylogenetic tree was constructed via the ML method, using the Kimura 2-parameter model and a discrete gamma distribution with five categories for capturing non-uniformity of evolutionary rates (K2+G). The K2+G model was selected by virtue of the fact that it had the lowest value of BIC (Bayesian information criterion) (30, 31). One thousand bootstrap trees were generated to determine bootstrap confidence levels (32). The resulting (bootstrap) consensus tree was condensed with values >50% as shown in Fig. 1. The gene sequences used in the study are available for electronic retrieval from the Gene Bank nucleotide sequence database (30).
Conclusions

The bifunctional pbp Tp47 had been known for the mechanism of turnover for β-lactam antibiotics in T. pallidum (24, 33, 34). Tp47 has showed no substantial similarity to other pbp genes in treponemes. Analysis of the T. pallidum genome sequence predicted the presence of pbp genes. Cha et al. (24) proposed that if a mutant variant of Tp47 emerges that overcomes the product inhibition of its β-lactamase activity, resistance to penicillin will emerge in T. pallidum. However, this requires a multistep mutational process, which is rarer than the single point mutations observed with macrolide resistance. There are no reports of the presence of mobile plasmids in T. pallidum although the emergence of a natural mobile antibiotic resistant in T. denticola provides no guarantee that it will not move to other Treponema species, including T. pallidum. The diversity among pbp genes across the phylogenetic tree is evident among treponemes that may be representative of their ecological niches. Interestingly, pbp gene for T. denticola (Gene ID 2739572) has shown relatedness to other treponemes, including T. paraluiscuniculi A (Gene ID 10884451); T. brennaborense DDS5/3, DSM 12168 (Gene ID 2739572); and T. pallidum (Gene ID 2611355); and T. primitia (Gene ID 10681574). Thus, the reported presence of a mobile element in T. denticola and the possibility of transfer of antibiotic resistant plasmid may be potentially dangerous, as it could provide a first step toward the acquisition of

Table 1. Summary of pathogenic and saprophytic Treponemes

| Taxa and strain information | NCBI Taxon ID |
|----------------------------|---------------|
| Treponema azotonutricium ZAS-9 | 545695        |
| Treponema brennaborense DDS5/3, DSM 12168 | 906968       |
| Treponema denticola ATCC 35405 | 243275        |
| Treponema pallidum pallidum Nichols | 243276       |
| Treponema pallidum pallidum SS14 | 455434       |
| Treponema paraluiscuniculi Cuniculi A | 545776       |
| Treponema primitia ZAS-2 | 545694        |

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multidrug resistance. The *php* genes for treponemes investigated here could be used for further studies as *Treponema* species are strongly implicated in disease progression. The phylogenetic data presented in this study demonstrate that this organism may possess the potential to acquire antibiotic resistance in the future.

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**Table 2. Strains with accession numbers used in this study**

| GenBank, accession no. | Bacterial strain |
|------------------------|------------------|
| Gene ID: 2611355       | Treponema pallidum pallidum Nichols |
| Gene ID: 6333587       | Treponema pallidum pallidum SS14 |
| Gene ID: 10884451      | Treponema paraluiscuniculi Cuniculi A |
| Gene ID: 2739572       | Treponema denticola ATTC 35405 |
| Gene ID: 10681574      | Treponema primitia ZAS-2 |
| Gene ID: 10580463      | Treponema brennaborense DDS/2, DSM 12168 |
| Gene ID: 10678178      | Treponema azotonutricium ZAS-9 |
| Gene ID: 10581047      | Treponema brennaborense DDS/2, DSM 12168 |
| Gene ID: 10681574      | Treponema primitia ZAS-2 |
| Gene ID: 2739453       | Treponema denticola ATTC 35405 |
| Gene ID: 6333710       | Treponema pallidum pallidum SS14 |
| Gene ID: 10884263      | Treponema paraluiscuniculi Cuniculi A |
| Gene ID: 11850998      | Treponema pallidum pallidum DAL-1 |
| Gene ID: 2739199       | Treponema denticola ATTC 35405 |
| Gene ID: 10580253      | Treponema brennaborense DDS/2, DSM 12168 |
| Gene ID: 10681344      | Treponema primitia ZAS-2 |
| Gene ID: 10884397      | Treponema paraluiscuniculi Cuniculi A |
| Gene ID: 2610950       | Treponema pallidum pallidum Nichols |
| Gene ID: 6333660       | Treponema pallidum pallidum SS14 |
| Gene ID: 2739129       | Treponema denticola ATTC 35405 |
| Gene ID: 10681355      | Treponema primitia ZAS-2 |
| Gene ID: 10675593      | Treponema azotonutricium ZAS-9 |
| Gene ID: 10677805      | Treponema azotonutricium ZAS-9 |
| Gene ID: 10579770      | Treponema brennaborense DDS/2, DSM 12168 |
| Gene ID: 10677463      | Treponema azotonutricium ZAS-9 |
| Gene ID: 2740551       | Treponema denticola ATTC 35405 |

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