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Phylogenetic Analysis of Genetic Diversity of Hemolysins in *Leptospira*

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Abstract

Leptospirosis is a zoonotic disease distributed worldwide. It has now been identified as one of the emerging infectious diseases. The genomic sequences of *Leptospira* species have helped to identify the genetic diversity of hemolysins among different pathogenic and saprophytic *Leptospira* species. The phylogenetic analysis reveals that hemolysin *SphH,* a pore-forming protein on various mammalian cells in pathogenic *Leptospira,* does not show significant similarity to hemolysins in saprophytic *Leptospira biflexa*. The *Leptospira interrogans* hemolysin gene also does not show significant similarity to *L. biflexa* and *Leptospira borgpetersenii*. The genetic diversity among hemolysin genes in *Leptospira* suggests that the gain of more hemolysin genes during the course of their evolution may have contributed to their virulence.

Keywords: Leptospirosis; Hemolysins

Introduction

The genus *Leptospira* contains both pathogenic and saprophytic species belonging to the order spirochaetales. They have diverse ecological habitats that range from soil and water to the tissues of the mammalian host [1]. The availability of the genome sequence has shown evidence of substantial genetic diversity within *Leptospira*. Today, more than sixteen pathogenic and saprophytic species are recognized. The common pathogenic *Leptospira* species include *L. interrogans*, *L. borgpetersenii*, and others. They cause a systemic illness in humans known as *Leptospirosis* [2]. For instance, Weil's syndrome that includes hemorrhage, renal failure and jaundice. The carriers are wild and domesticated animals including rodents, cattle, pigs and dogs [3,4]. This makes *Leptospira* common among farmers, butchers, hunters, and others with direct animal contact. The pathogenic *Leptospira* is shed continually in the urine of the chronically infected animals, and may enter the human body through the skin or mucous membranes when in contact with the contaminated water or soil [4]. Since the animal species serves as a reservoir, leptospirosis is considered as one of the most widespread zoonotic diseases [1,5]. One of the most prominent virulence factors that contribute to the pathogenesis of the leptospirosis is hemolysins [4,6-8]. Hemolysins are the cytolytic toxins that are found in a variety of organisms such as alpha toxin (*Clostridium perfringens*), phospholipases, delta toxin (*Staphylococcus aureus*), and heat stable hemolysin (*Pseudomonas aeruginosa* [6,9]. They are categorized as enzymatic, pore formation, or surfactant based on their mechanism of action on the target cell membranes [6]. The hemolysins in *Leptospira* are considered as phospholipases that act on erythrocytes and other cell membranes containing the substrate phospholipids, leading to disruption of target cell membranes [10,11]. The genome sequence provides more information on the hemolysin in both pathogenic and saprophytic species. Although, the genome of *L. biflexa* contains putative hemolysins, it lacks the pore forming hemolysin capable of degrading tissues, such as *SphH* that are found in pathogen species [8,12-14]. Research studies have shown that *SphH* can cause direct membrane damage of erythrocytes in sheep and mammalian cells, and may play a vital role in the pathogenesis of leptospirosis [6]. Lee et al. [6] have shown that the cytotoxic mechanism of hemolysin *SphH* in *L. interrogans* serovar lai was due to pore formation on several mammalian cells. The absence of pore forming hemolysin in *L. biflexa* strongly supports *SphH* involvement in the virulence within the mammalian host. The diversity of the hemolysins in pathogenic and saprophytic *Leptospira* has yet to be investigated. The analysis uncovers the genetic diversity of hemolysin genes that will explain the ecological and evolutionary connections that exist between them. A significant challenge is to understand how these genetic differences may contribute to the biological differences.

Material and Methods

The NCBI (National Center for Biotechnology Information) database was the source of the list of beta lactamases for treponemes with complete sequenced genomes. Molecular Evolutionary Genetics Analysis version 5.05 (MEGA5) was used for statistical analyses [15]. 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 Maximum likelihood method using a General Time Reversal (GTR) model with a Gamma distribution (+G). The GTR+G model was chosen based on the fact that it had the lowest value of both AICc and BIC among all models currently handled by MEGA5 [15]. The bootstrap confidence levels shown were determined by generating one thousand bootstrap trees. The node values thus presented indicate the degree of confidence in inferring that the nodes do indeed occur at those locations. The tree is condensed so that only values above 50% are shown. The gene sequences used in this study is available for electronic retrieval from the GenBank nucleotide sequence database.

Results and Discussion

The genome sequences of *Leptospira* species: saprophytic *L. biflexa*, and pathogenic *L. borgpetersenii* and *L. interrogans* provide an opportunity to look for the virulence factors that are specific to

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pathogenic and saprophytic species. Both of them contain many divergent genes, which may contribute to differences in disease manifestation [8,12,14]. One of the key virulence factors is the presence of the pore forming hemolysin gene [6,7,10]. The novel approaches to study the diversity of hemolysin genes among saprophytic and pathogenic *Leptospira* species and will provide additional perspectives on leptospiral evolution [8,12,16-18]. The genome of *L. biflexa Patoc 1 (Ames)*, *L. biflexa Patoc 1 (Paris)*, *L. borgpetersenii sv Hardjo-bovis JB197*, *L. borgpetersenii sv Hardjo-bovis L550*, *L. interrogans sv Copenhageni Fiocruz L1-130* and *L. interrogans sv Lai 56601*, are analyzed to explore the diversity of hemolysin genes in *Leptospira* species (Table 1). Phylogenetic tree reconstruction calculations using Maximum Likelihood methods [15,19] are shown in Figure 1. The *SphH* hemolysin gene (Gene ID: 2771882) in *L. interrogans* do not show similarity to hemolysins in other *Leptospira* species, including *L. borgpetersenii*. *L. borgpetersenii* is also known to cause Leptospirosis. *SphH* is one of the key virulence factors and plays a significant role in the pathogenesis of leptospirosis. The hemolysin gene for *L. borgpetersenii* (Gene ID: 4409296, Gene ID: 4407235) have shown 86% similarity to *L. interrogans* (Gene ID: 2771573, Gene ID: 1153279). *L. biflexa serovar Patoc strain 'Patoc 1 (Ames)* (Gene ID 6388383- LBF-3274, Gene ID: 6220944- LEPBI-I2477) have shown no significant similarity to pathogenic *Leptospira species*. There is no substantial similarity of hemolysin genes among *L. biflexa* species.

Taxa and Strain Information	NCBI Taxon ID
Leptospira biflexa Patoc 1 (Ames)	355278
Leptospira biflexa Patoc 1 (Paris)	456481
Leptospira borgpetersenii sv Hardjo-bovis JB197	355277
Leptospira borgpetersenii sv Hardjo-bovis L550	355276
Leptospira interrogans sv Copenhageni Fiocruz L1-130	267671
Leptospira interrogans sv Lai 56601	189518

Table 1: Summary of pathogenic and saprophytic *Leptospira*.

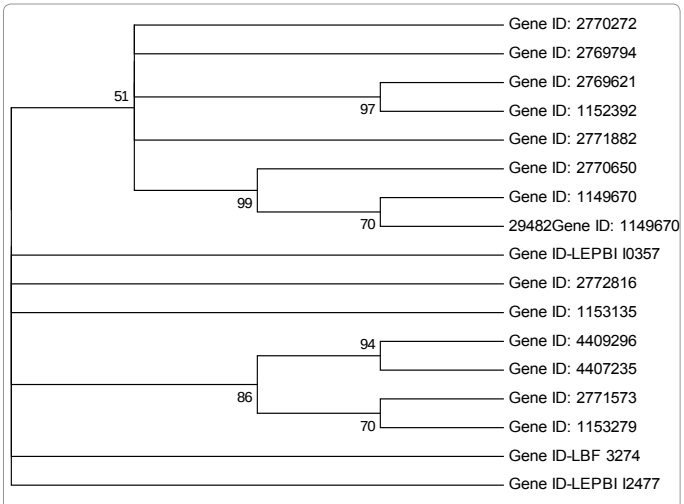

Figure 1: Phylogenetic analysis based on hemolysin gene sequences from the NCBI database. Phylogenetic analysis based on hemolysin gene sequences constructed after multiple alignment data by CLUSTAL W. The clustering was performed with the Maximum likelihood method (GTR+G model) using the software package MEGA version 5.05. Bootstrap confidence levels are shown as percentages on nodes (consensus tree; only branches with confidence values above 50% are shown).

GenBank, accession no.	Bacterial strain
LEPBI_I2477, GeneID:6220944	*Leptospira biflexa serovar Patoc strain 'Patoc 1 (Paris)*
LEPBI_I0357, GeneID:6223575	*Leptospira biflexa serovar Patoc strain 'Patoc 1 (Paris)*
LBF-3274,GeneID:6388383	*Leptospira biflexa serovar Patoc strain 'Patoc 1 (Ames)*
Gene ID: 4409296	*Leptospira borgpetersenii serovar Hardjo-bovis JB197*
Gene ID: 4407235	*Leptospira borgpetersenii serovar Hardjo-bovis L550*
Gene ID: 2772816	*Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130*
Gene ID: 2771882	*Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130*
Gene ID: 2771573	*Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130*
Gene ID: 2770650	*Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130*
Gene ID: 2770272	*Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130*
Gene ID: 2769794	*Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130*
Gene ID: 2769621	*Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130*
Gene ID: 1153279	*Leptospira interrogans serovar Lai str. 56601*
Gene ID: 1153135	*Leptospira interrogans serovar Lai str. 56601*
Gene ID: 1152392	*Leptospira interrogans serovar Lai str. 56601*
Gene ID: 1149670	*Leptospira interrogans serovar Lai str. 56601*
29482Gene ID: 1149670	*Leptospira interrogans serovar Lai str. 56601*

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

The *L. interrogans* hemolysin gene (Gene ID- 2772816, 1153135) does not show substantial similarity to *L. biflexa*. The results suggest that *L. interrogans* have gained hemolysin genes that have no orthologs in either *L. biflexa* or *L. borgpetersenii* (Table 2). The research literature has shown that disease caused by *L. interrogans* is more severe than with *L. borgpetersenii*. The ability to cause severe disease and infect mammals suggests that the horizontal gene transfer helped to expand their ability to survive in diverse habitats.

Conclusion

The genus *Leptospira* exhibits extensive genetic diversity among hemolysin genes. The saprophytic *L. biflexa* contains putative hemolysins with no substantial similarity to pathogenic *L. borgpetersenii* and *L. interrogans*. Picardeau et al. (2008) [8] study has shown that *L. biflexa* has few insertional sequence (IS) elements as compared to *L. interrogans* and *L. borgpetersenii* [8]. The presence of large numbers of IS elements in pathogenic *Leptospira* species suggests that it has undergone horizontal gene transfer [3,4,8]. However, studies are needed to correlate directly strain-specific hemolytic genes with their disease outcomes. The hemolysins are key virulence factors, and there is also the possibility of hemolysin playing a role in nutrient acquisition. The coupling of hemolysis with heme utilization could serve as an effective iron acquisition strategy that would help in progression of infection [6,8]. The future study might attempt to correlate strain-specific hemolytic genes to their ecological niche and different disease outcomes.

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