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3D modelling of the pathogenic *Leptospira* protein LipL32: A bioinformatics approach

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ABSTRACT

Leptospirosis is a widespread zoonotic disease caused by pathogenic *Leptospira* species (Leptospiraceae). LipL32 is an abundant lipoprotein from the outer membrane proteins (OMPs) group, highly conserved among pathogenic and intermediate *Leptospira* species. Several studies used LipL32 as a specific gene to identify the presence of leptospires. This research was aimed to study the characteristics of LipL32 protein gene code, to fill the knowledge gap concerning the most appropriate gene that can be used as antigen to detect the *Leptospira*. Here, we investigated the features of LipL32 in fourteen *Leptospira* pathogenic strains based on comparative analyses of their primary, secondary structures and 3D modeling using a bioinformatics approach. Furthermore, the physicochemical properties of LipL32 in different strains were studied, shedding light on the identity of signal peptides, as well as on the secondary and tertiary structure of the LipL32 protein, supported by 3D modelling assays. The results showed that the LipL32 gene was present in all the fourteen pathogenic *Leptospira* strains used in this study, with limited diversity in terms of sequence conservation, hydrophobic group, hydrophilic group and number of turns (random coil). Overall, these results add basic knowledge to the characteristics of LipL32 protein, contributing to the identification of potential antigen candidates in future research, in order to ensure prompt and reliable detection of pathogenic *Leptospira* species.

1. Introduction

Leptospirosis is an important and widespread zoonotic disease that can be found all around the world, except for Antarctica. It is caused by pathogenic *Leptospira* species (Leptospiraceae) (Adler et al., 2011; Evangelista and Coburn, 2010; Bulach et al., 2006a,b). The spirochetes are classified into 21 species, with more than 300 serovars of pathogenic *Leptospira* spp. They can be further divided into three subgroups, mainly pathogenic, intermediate and non-pathogenic (saprophytic) species. Pathogenic species cause acute disease to susceptible hosts or can serve as renal carriers, while saprophytic spirochetes live freely in the environment and never cause diseases (Adler et al., 2011). Leptospires can occupy diverse environments, showing various life cycles, where almost every mammalian and amphibian species act as a

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maintenance host (Adler et al., 2011), and humans are exploited as incidental hosts (Lehmann et al., 2014). The common natural habitat of pathogenic *Leptospira* spp. is represented by the proximal renal tubules of reservoir animals, such as rats (Adler et al., 2011), which spread them into the environment through their urine.

Leptospira transmission occurred via direct contact with the infected urine or infected animal tissues, or even indirectly through contact with water and soil contaminated by the infected host's urine (Lehmann et al., 2014). Leptospires can enter into the host via cuts, abrasions in the skin, through mucus membranes, like nose and eyes or through prolonged immersion in contaminated water (Lehmann et al., 2014). Leptospirosis infection in humans causes a range of symptoms, even if some infected patients may have no symptoms at all. The disease usually onsets with fever accompanied by chills, intense headache, severe myalgia, abdominal pain, conjunctiva suffusion and, occasionally, skin rashes. In critical cases, patients may develop Weil's disease, the renowned "yellow fever", in which a person turns yellow, accompanied with kidney failure and bleeding (hemorrhage) that may result fatal (Evangelista and Coburn, 2010). Other possible manifestations may include pulmonary hemorrhage, meningitis, jaundice and encephalitis (Guerra, 2013).

The detection of *Leptospira* in infected patients is challenging due to the similarity in the clinical symptoms with other tropical diseases like influenza and dengue (Khodaverdi Darian et al., 2013; Priya et al., 2017); this can lead to the misdiagnosis that cause fatal cases. Additionally, the early detection of *Leptospira* is still considered a major problem due some concerns related to laboratory assays currently used. Available diagnostic techniques, including microscopic agglutination test (MAT), polymerase chain reaction (PCR) and IgM enzyme-linked immunosorbent assay (ELISA), are not completely satisfactory. MAT needed pair sera and living *Leptospira* strains as antigens for the detection purposes, which commonly end up with cross agglutination (Plank and Dean, 2000). The main constraint in PCR method is the selection of specific primers to amplified all the infected strains; in addition, the PCR method is quite expensive as it needs specific equipment and reagents (Khaki, 2016). ELISA method cannot indicate infecting serovars and the confirmation of leptospirosis cases must be supported by PCR results (Picardeau, 2013). The biphasic lifecycle of *Leptospira,* composed by the spirochetemic phase and an immune phase, also represents one of the factor leading to failure of the performance of rapid detection kit, where the rapid tests are based on IgM detection assays and IgM is not detectable until the second week after onset symptom (Musso and La Scola, 2013).

In this scenario, the identification of the target genes present in all phases can facilitate the early diagnosis of leptospirosis. In the disease progression, the identification of target markers represents a crucial step, since it would give proof for the current condition of the patient compared to the past or post-infection (Fernando et al., 2016). Moreover, the identification of highly conserved genetic regions of *Leptospira* that can easily be detected by immune system, may lead to the development of successful diagnostic techniques (Khodaverdi Darian et al., 2013). Therefore, recent research focused on molecular studies to identify up-regulated and down-regulated target gene expression during leptospirosis infection in mammals. The genomic studies improve our understanding about the mechanisms of virulence and pathogenesis of leptospirosis (Fouts et al., 2016).

The leptospiral outer membrane proteins (OMPs) and lipopolysaccharide (LPS) are the major antigens that confer immunity to leptospires and are thought to be involved in host–pathogen interactions. The identification of virulent OMP components has represented an important step in the understanding on the pathogenesis of leptospirosis as in presence in both pathogenic and intermediate *Leptospira* species (Vedhagiri et al., 2009). One of the genes that have been studied is LipL32 (Hap1); LipL32 is an outer membrane protein (lipoprotein) with ~32 kDa (Murray, 2013). The gene responsible for the production of LipL32 is exclusive of pathogenic *Leptospira*, becoming a major target gene to develop antigens for detection of specific antibodies produces during the infection (Boonyod et al., 2005). In additional, the LipL32 protein has been widely used as a primer in PCR (Yaakob et al., 2015), where the PCR approach is known for its sensitivity and capacity to give an early diagnosis of leptospirosis.

The aim of this study was to mine comprehensive information on LipL32 and to evaluate its potency as a novel target virulent gene in early diagnosis of leptospirosis. In this research, we investigated the presence of LipL32 in fourteen *Leptospira* pathogenic strains. We analyzed the LipL32 position in their genomes and the pairwise alignment of *Leptospira* strains, constructing a phylogenetic tree based on LipL32 differences among the studied strains. Furthermore, the physicochemical properties of LipL32 in different strains were studied, shedding light on the identity of signal peptides, as well as on the secondary and tertiary structure of the LipL32 protein, supported by 3D modelling assays.

2. Material and methods

2.1. Leptospira strains and genome annotation

Sixteen complete whole genomes of *Leptospira* strains were selected and compiled from the NCBI database. Replicons for all compiled genomes were downloaded and annotated using the Rapid Annotation,

Table 1
List of *Leptospira* strains used for this study.

Taxonomy ID	Species	Serovar	Strain	Cluster	References
189518	*L. interrogans*	Lai	56601	Pathogen	Ren et al. (2003)
573825	*L. interrogans*	Lai	IPAV	Pathogen	Zhong et al. (2011)
1279460	*L. interrogans*	Hardjo	Norma	Pathogen	Cosate et al. (2015)
1395589	*L. interrogans*	Linhai	56609	Pathogen	Zhu et al. (2015)
338215	*L. interrogans*	Bratislava	PigK151	Pathogen	Pigk et al. (2015)
214675	*L. interrogans*	Manilae	UP-MMC-NIID LP	Pathogen	Satou et al. (2015)
214675	*L. interrogans*	Manilae	UP-MMC-NIID HP	Pathogen	Satou et al. (2015)
44275	*L. interrogans*	Copenhageni	FDAARGOS_203	Pathogen	[a]
267671	*L. interrogans*	Copenhageni	Fiocruz L1-130	Pathogen	Zhong et al. (2011)
355276	*L. borgpetersenii*	Hardjo-bovis	L550	Pathogen	Bulach et al. (2006b)
355277	*L. borgpetersenii*	Hardjo-bovis	JB197	Pathogen	Bulach et al. (2006b)
280505	*L. borgpetersenii*	Ballum	56604	Pathogen	Wang et al. (2015)
758847	*L. santarosai*	Shermani	LT 821	Pathogen	Chou et al. (2012)
28452	*L. alstonii*	Room22	GWTS#1	Pathogen	Gwts (2016)
456481	*L. biflexa*	Patoc	Patoc 1 (Paris)	Saprophyte	Picardeau et al. (2008)
355278	*L. biflexa*	Patoc	Patoc 1 (Ames)	Saprophyte	Picardeau et al. (2008)

[a] Genome directly submitted to NCBI Prokaryotic Genome Annotation Pipeline and the reference journal was unpublished.