Diverse Environment Related (Der) protein is a novel OMP85 subfamily present in free-living bacteria and pathogenic *Leptospira* spp.

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BACKGROUND

Leptospira and Leptospirosis

- ✓ Incidence of acute leptospirosis is estimated to be >1 million cases annually, with more than 59,000 deaths, most caused by *Leptospira interrogans*.
- ✓ Infection begins when a naïve host, including humans, comes into direct contact with water or soil contaminated with urine from an infected reservoir host.
- ✓ Like their non-pathogenic saprophytic counterparts, pathogenic leptospires may survive for weeks outside of the host in water and soil.
- ✓ Little is known about the survival programs and gene products required for environmental adaptation and how they differ between saprophytic and pathogenic *Leptospira* spp.

OMP85 Proteins

- ✓ D15/Oma87/Omp85-like (Omp85) proteins are outer membrane β-barrels widely distributed in Gram-negative bacteria.
- ✓ The hallmark feature of Omp85 is the presence of a conserved C-terminal membrane-embedded β-barrel domain.
- ✓ The Omp85 superfamily can be further divided into at least 10 subfamilies based on the domain architecture of their N-terminal (periplasmic) regions.
- ✓ Omp85 proteins have functions in the assembly of other outer membrane proteins (e.g., BamA) and protein translocation (e.g. FhaC).

OBJECTIVE

This study aimed to understand the distribution and function of OMP85 in *Leptospira* spp., and to describe a novel identified subfamily.

Leptospira interrogans has four OMP85 paralogs

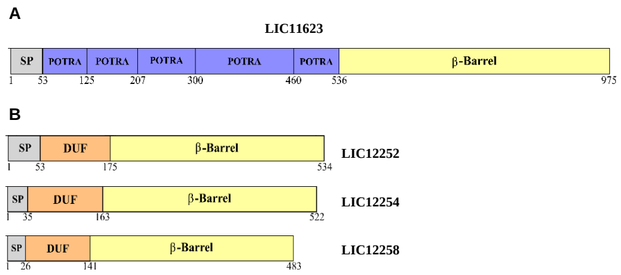

Figure 1. ***L. interrogans*** **proteins containing an annotated OMP85 C-terminal domain on INTERPRO database had their domain boundaries predicted by ThreaDomEx.** A) *L. interrogans* BamA orthologue (LIC11623) possess high conservation scores for five periplasmic domains, including a non-annotated POTRA 4. B) All OMP85 orthologues without POTRA domains present domain boundaries suggesting a novel domain of unknown function (DUF) in their N-terminal regions.

Identified *Leptospira* proteins display characteristic structural features of prototypical OMP85s

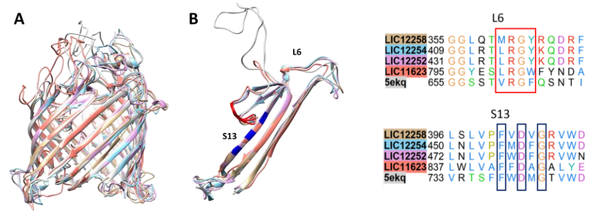

Figure 2. ***Leptospira*** **OMP85 structural alignment.** A) Structural alignment of *Leptospira* OMP85 tridimensional structures predicted by ITASSER and *E. coli* BamA PDB model (5ekq). B) Tridimensional visualization of conserved OMP85 Lid lock motif position among all aligned structures.

Predicted DUF secondary structure is unique compared to previously reported OMP85

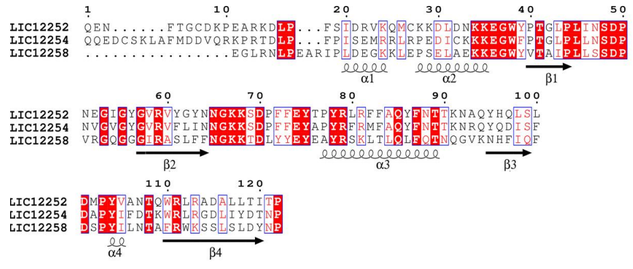

Figure 3. The novel OMP85 domain of unknown function (DUF) is composed by a combination of alpha-helix and beta-strand structures. Consensus secondary structure prediction was performed by PROMALS3D using PSI-PRED. Alpha-helix (α1-α4) and Beta-strand (β1- β4) structures were predicted on the same regions for all *L. interrogans* novel OMP85. No previously reported OMP85 N-terminal have the same combination of secondary structures.

Novel leptospiral OMP85s are upregulated in infected mice urine

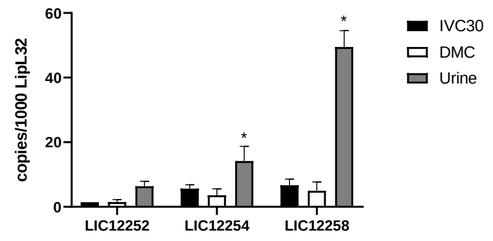

Figure 4. **Reverse transcription (RT)-PCR of** ***L. interrogans*** **novel OMP85 under different conditions.** Total RNA was isolated from leptospires (four biological replicates per condition) cultivated in vitro at 30ºC (IVC30), in Dialysis Membrane Chambers (DMC), or obtained from infected mice urine. Copies were normalized against lipL32. Normalized copy numbers were compared using an unpaired *t* test (*p<0.05).

Mice infected with *L. interrogans* lacking a single OMP85 excrete less bacteria in urine

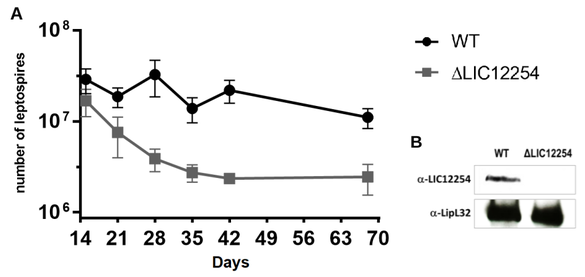

Figure 5. **Mice infected with a** ***L. interrogans*** **ΔLIC12254 mutant excreted less bacteria in urine.** A) Total number of leptospires recovered in infected mice urine were counted under dark-field microscopy using a Petroff-Hausser chamber. Five mice (C3H/HeJ) per group were infected with *L. interrogans* serovar Manilae Wild Type (WT) or with the same strain containing a transposon insertion in LIC12254 gene (ΔLIC12254). B) Western blot showing the absence of LIC12254 expression by the mutant strain.

The novel DUF is conserved in OMP85 orthologues encoded by diverse bacteria

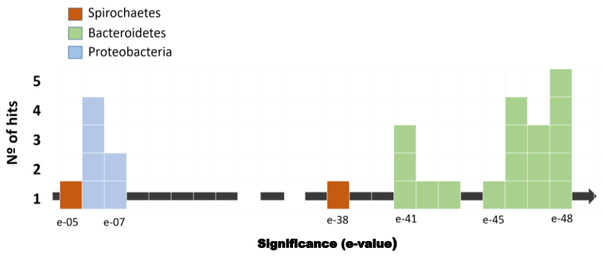

Figure 6. HMMsearch results against reference proteomes using ***L. interrogans*** **novel OMP85 N-terminal alignment.** Twenty-seven orthologs distributed into Spirochaetes, Bacteroidetes and Proteobacteria phyla were found by HMMER. All identified proteins have a C-Terminal OMP85 domain.

Diverse Environment Related (Der) proteins are present in free-living bacteria and pathogenic *Leptospira* spp.

- ✓ Apart from *Leptospira* spp., species harboring novel OMP85 genes are free-living with majority of them isolated from diverse/harsh environments.
- ✓ The novel OMP85 subfamily was named Diverse Environment Related (Der) protein.
- ✓ Based on phylogenetic analysis five variants of *Leptospira* Der proteins (Ldp) were found in *Leptospira:*
  - Named Ldp1A, Ldp1B, Ldp2, Ldp3 and Ldp4.
- ✓ Ldp1A and 1B share high sequence similarity.
- ✓ Only Ldp1A is present in all four *Leptospira* clades.
- ✓ Ldp1B (LIC12254) is exclusive from Pathogenic spp.
- ✓ Ldp2 and Ldp4 are exclusive from Saprophyte spp.
- ✓ Despite the observed relation between Der proteins and environment fitness, its function remains to be understood.
- ✓ Diversity in Omp85 composition could have allowed *Leptospira* spp. to evolve a more diverse outer membrane proteome and to adapt to its changing environments.

CONTACT

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