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

**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 reservoir host.
- 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 (plasmidic) 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.

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**Figure 1.** L. interrogans proteins containing an annotated OMP85 C-terminal domain on INTERPRO database had their domain boundaries predicted by ThreeDomEx. A) L. interrogans BamA orthologue (LIC11623) possesses high conservation scores for five periplasmic domains, including non-variant POTRA 4 & 5. All OMP85 orthologues without POTRA domains present domain boundaries suggesting a novel domain of unknown function (DUF) in their N-terminal regions.

**Figure 2.** Leptospira OMP85 structural alignment. A) Structural alignment of Leptospira OMP85 intramembranous predicted by IASSER and Z. swt BamA PDB model (Salz). B) Tridimensional visualization of conserved OMP85 Lid lock motif position among all aligned structures.

**Figure 3.** The novel OMP85 domain of unknown function (DUF) is composed of a combination of alpha-helix and beta-strand structures. Consensus secondary structure prediction was performed by PRANKAL23D using PSI-PRED. Alpha-helix (α-H), and beta-strand (β-S) 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.

**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 39°C (IC250), in Dialysis Membrane Chambers (DCC), or obtained from infected mice urine. Copies were normalized against 16S rDNA. Normalized copy numbers were compared using an unpaired t test (*p<0.05).

**Figure 5.** Mice infected with *L. interrogans* lacking a single OMP85 excrete less bacteria in urine. A) Total number of leptospires recovered in infected mice urine were counted using dark-field microscopy using a Petroff-Hausser chamber. Five mice (C3H/He) per group were infected with *L. interrogans* serovar: Mannikow 1H4 Type (WT) or with the same strain containing a transposon insertion in LIC12254 gene (LIC12254). B) Western blot showing the absence of LIC12254 expression with the mutant strain.

**Figure 6.** HMRI Research results against reference proteins using L. interrogans novel OMP85 N-terminal alignment. Twenty-seven orthologues distributed into Spirochaetes, Bacteroides and Proteobacteria phyla were found by HMREI. All identified proteins have a C-terminal OMP85 domain.

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**REFERENCES**


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