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Shotgun phage display for the identification of new adhesins from *Leptospira interrogans*

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Background: Pathogenic species of *Leptospira* are etiologic agents of leptospirosis. Most of these species, mainly *L. interrogans*, have countless specific genes encoding proteins with unknown functions, suggesting that leptospires have unique virulence factors. Bacterial adhesins are important exposed virulence factors, potential vaccine antigen candidates and the identification of conserved adhesins in pathogenic *Leptospira* species from techniques such as shotgun phage display, can reveal new strategies for leptospirosis treatment and prevention.

Methods: Construction of bacteriophage libraries for shotgun phage display using fragmented genomic DNA of *L. interrogans* and pG8SAET phagemid vector. Selection of new possible adhesins was performed by biopanning of the libraries in eukaryotic cells.

Results: The most successful biopanning was performed

in Vero cells using the library with the highest proportion of valid clones (called BBT2, with 3x10⁷ primary clones), resulting in eleven proteins fused to protein VIII and/or signal peptide. In silico analysis revealed three hypothetical proteins that would be secreted by the bacteria, bearing a signal peptide or transmembrane region in their structures. Those proteins possess further characteristics, including presence only in pathogenic species and selection in more than one phage by biopanning; one of them is a hypothetical lipoprotein.

Conclusion: The phage display technique has proved successful in identifying possible adhesins that may have a role in bacteria-host interaction and infection. Recombinant portions of these proteins are being currently expressed and purified to be further analyzed for conservation, binding domains and antigenic potential.

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