

Selection of peptides against *Trypanosoma evansi* by *in vivo* by Phage Display

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Abstract

Surra is an animal trypanosomiasis caused by *Trypanosoma evansi*, which is transmitted by insects and bats; is lethal if not treated and affects many species worldwide. *T. evansi* causes direct and indirect economic losses on small-hold livestock and horse farms in Brazil. Focusing on the discovery of new strategies for specific detection and treatment, we explored Phage Display technology to engineer peptide libraries and select for peptides against *T. evansi*. The peptide scaffold is a small and rigid scorpion toxin (BTK-2) that forms a very stable peptide structure formed by an alpha helix linked to two beta-sheets by the presence of three disulfide bridges. BTK-2 Phage Library was produced by Kunkel mutagenesis and phages were selected against *T. evansi* by *in vivo* method, which is based on library injection on *T. evansi* infected mice, followed by animal sacrifice and *parasite* purification from animal blood. *T. evansi* bound phage were eluted and used to reinfect XL1Blue cells. A phage library with theoretical diversity of 1.3×10^5 mutations was generated and clones were sequenced, assuring correct mutation insertion in all tested clones. The first round of "*in vivo*" selection was successfully performed, since injection of phage library resulted in detection of parasite-bound phage ligands from animal serum, which did not occur when a control phage was injected. *T. evansi* enriched phage library will be used for the second round of *in vivo* selection, while twelve selected clones from the first round will be subjected to further analysis by ELISA and FACS. *In vivo* selection was successfully performed since *T. evansi* ligands were detected when the library was injected into mice as opposed to control phage injection. The scorpion toxin appears to be a good scaffold against serum proteases, since *in vivo* selection produced mutants that were stable on serum during selection.

Key words: *Trypanosoma evansi*, Phage Display, Kunitz.