UNIVERSITÄT ZU LÜBECK

FACH HOCHSCHULE LÜBECK
University of Applied Sciences

Master's Program: Biomedical Engineering

Master's Thesis Title: "The Characterization and Application of a cDNA Library from the Skin of an Axolotl (Ambystoma Mexicanum) with Respect to its Wound Healing and Immune Defense Abilities"

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Project Summary:

The axolotl (Ambystoma Mexicanum) is a type of salamander known for its regenerative abilities. Amphibians in general are also known for their method of immune defense, in the form of antimicrobial peptides. The peptides are released from their skin and being able to isolate them was the focus of this project. By creating a cDNA library from the skin of an axolotl, experiments could be performed in which a characterization of this library could be performed. From one sample of skin three libraries were created from fractionated cDNA that ranged in size from largest to smallest ~200 – 2000 bp (library 5 – 8), ~200 – 1000 bp (library 9 – 11), and ~180 – 500 bp (12 – 15). To get a visual confirmation of the individual library complexities a series of plaque lifts were performed that were subsequently tested via gel electrophoresis against a known ladder.

To isolate the antimicrobial peptides, an experiment known as biopanning/ phage display was attempted. This technique involved the use of a ligand (lipopolysaccharide) bound to an immobile surface (polystyrene dish) whereon the target (antimicrobial peptides) can be bound to the ligand and all unbound elements can be washed away and the desired target can then be eluted. The eluted product can then be tittered and amplified to be used in 3 – 5 more rounds. In this case several different strains of lipopolysaccharide were used as well as a variety of cell culture dishes/ plates, and the antimicrobial peptides within the 12 – 15 library were used as the target. This method proved to be ineffective, and additionally, as a positive control this was performed using antibodies from axolotls, which was also unsuccessful. Due to this fact several positive control experiments were conducted to prove the presence of genes expressed in the axolotl skin as well as the presence of peptides.

Using six different axolotl primers, real time polymerase chain reactions (PCR) were tested against the 12 – 15 library to prove the presence of genes that were expressed in axolotl skin, and was effective in doing so. In addition to this, several axolotl antibodies were tested with the cDNA library via western blotting and immunoprecipitation. Both of these methods resulted in bands that were evidence of peptides in the library.

For future studies, optimization of the biopanning method will be required for complete antimicrobial isolation following which the peptides can be tested against microbes for antimicrobial properties and then sent in for deep sequencing.