Isolation and determination of specific Lethal Factor Interacting Partners Using Human Heart T7 Phage display

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Abstract

Bacillus anthracis is a spore forming pathogenic bacterium that causes anthrax disease that has been used as a biological weapon. The pathogenicity is due to two plasmids that it possesses, known as pXO1, which synthesizes an exotoxin, and pXO2, which synthesizes a capsule of poly-γ-Dglutamic acid that inhibits host phagocytosis. The lethality of the tripartite toxin secreted by the bacteria, is due the component lethal factor (LF). It is known that LF is a metallo-protease capable of inactivating regulators such as mitogen activated protein kinase (MAPK), interfering with cell cycle, leading to cell death. However, it is not clear if MAPKs are the only target for LF; therefore, it is necessary to explore novel potential ligands for LF, using different strategies to further unravel new molecular pathogenesis pathways. LF has been also associated with cardiac dysfunction, suggesting human heart (HH) cells as potential targets. The purpose of this research is to isolate and identify LF specifics interactions peptides using premade T7 Phage Display (T7PD) with cDNA HH libraries as ligands. The T7PD express the HH peptides on the capsid of the T7 phage to unravel the protein-protein interaction with the LF, using wild type (WT) and active-site mutant (MT) LF as targets. After several biopannings rounds, a total average of $3.9x10^6$ and $3.4x10^6$ pfu/mL for WT and MT LF respectively isolated. To determine the specificity of the human heart peptides with a high affinity to LF, specificity test was performed to >25 candidates. According to the results the potential T7 phages displaying HH peptides possessed affinity to the LF and blocking agents. Specificity Tests are in progress in order to map and confirm the main target and potential regions of interaction of the MT-LF. Understanding novel LF interaction ligands in the HH, have the potential of identifying targets to develop new biomarkers and therapeutics against anthrax disease in case of a bioterrorism attack.

Objectives

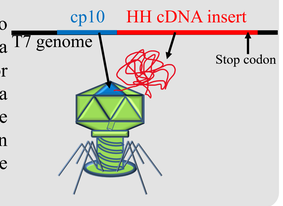
- Isolate T7 phages displaying human heart peptides having affinity to LF.
- Determine the specificity of the potential T7 phage displaying interacting partners to LF.

Introduction

Bacillus anthracis (B. anthracis), the bacterium that causes anthrax disease, is a Gram-positive spore forming bacteria [3]. In the 2001 on USA, *B. anthracis* endospores were sent by post mail, where 5 people died [3]. The endospores when in contact with the host, germinate, and in its vegetative form, cause anthrax. The pathogenicity is due to two plasmids that it possesses, known as pXO1, which synthesizes an exotoxin, and pXO2, which synthesizes a capsule of poly-γ-Dglutamic acid that inhibits host phagocytosis[3]. In this disease, pulmonary anthrax is the most lethally due to the lethal factor (LF) component of the tripartite toxin secreted by the bacteria [3]. If the patient is not treated or antibiotics are given correctly, the mortality could vary from 86-97% [6]. Antibiotic treatments act on the vegetative form of the bacteria and not on the endospores, if there are still high levels of viable endospores in the host, these can germinate and will cause the host infection again [6]. In addition, if the levels of toxins in the cells are high, treating the patient with antibiotics will not be effective, since it eradicates the bacteria, but the toxins will cause symptoms, even death [4]. On the other hand, there are vaccines against this disease, but they are not practical due to factors such as: side effects, exclusions, and others [6]. To successfully manage this disease, it is important to develop or design drugs or therapies that inhibit the steps or interactions between the components of the toxin, as well as a specific inhibitor of the effects of the toxin could be a valuable complement to existing therapy [6].

Anthrax LF is a metallo-protease capable of inactivating regulators such as mitogen activated protein kinase (MAPK), interfering with cell cycle, leading to cell death [2]. It is not clear if MAPKs are the only target for LF; therefore, it is necessary to explore novel potential ligands for LF in eukaryotic cells, using different strategies to further unravel new molecular pathogenesis pathways. LF has been also associated to causing ventricular dysfunction and cardiomyocytes, correlated with cardiac dysfunction, suggesting human heart (HH) cells as potential targets [1], but it's unknown how it interacts. Therefore, it's why we are interested in **isolating and determining specific interactions between displayed human heart peptides and the anthrax toxin LF, wild-type and active-site mutant, utilizing the T7 Phage Display (T7PD) system.**

The T7PD system have premade T7 phages (virus who infects bacteria) with a modified genome, where a complementary deoxyribonucleic acid (cDNA) coding for HH protein, is fused with the *cp10* gene [5]. This gene has a role in the structure and assembly of T7 phages [5]. When the phage is assembled, it is terminated with the cDNA protein fused to the phage capsid, remaining towards the outside environment, to achieve protein-protein interaction [5].



Methods

Biopanning/Bioamplification

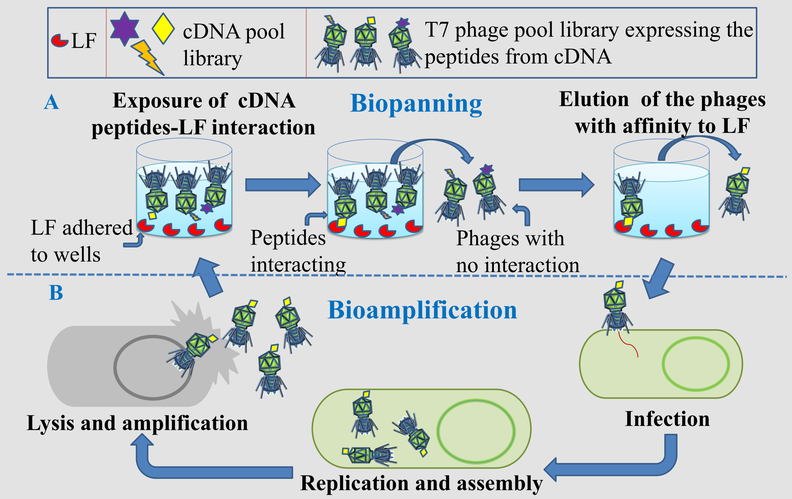


Figure 1: Isolation of the T7 phages displaying human heart peptides with affinity to LF, by the biopanning (A) and the bioamplification (B).

Specificity Test

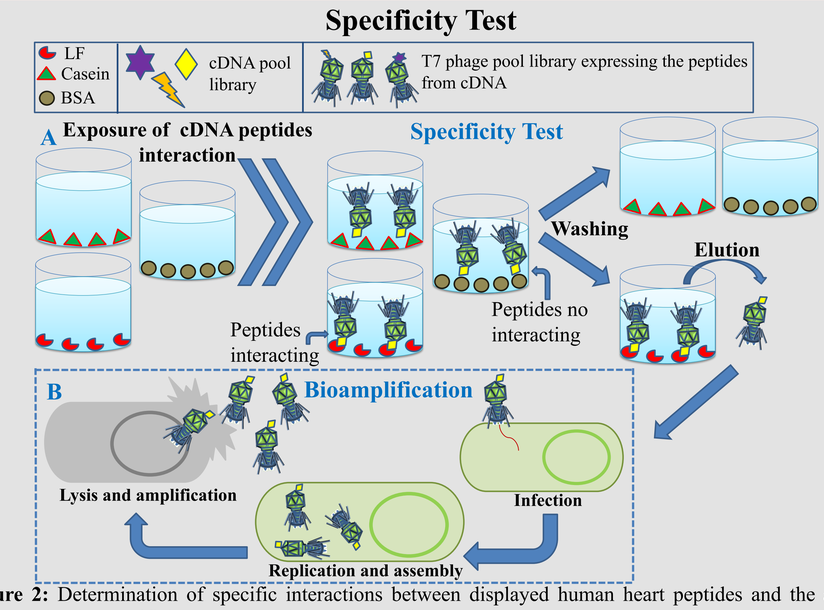


Figure 2: Determination of specific interactions between displayed human heart peptides and the anthrax toxin LF, by the specificity test (A) and the bioamplification (B).

Results

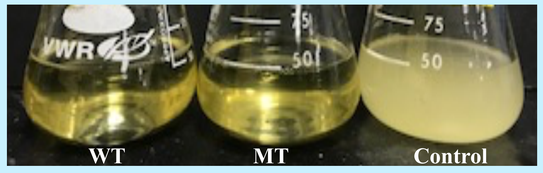


Figure 3: Bioamplification of the potential phages expressing peptides from the human heart cDNA, with a potential affinity to the LF, isolated from the biopanning 1. **C: negative control. WT: wild-type LF protein. MT: active-site mutant LF protein.**

Results

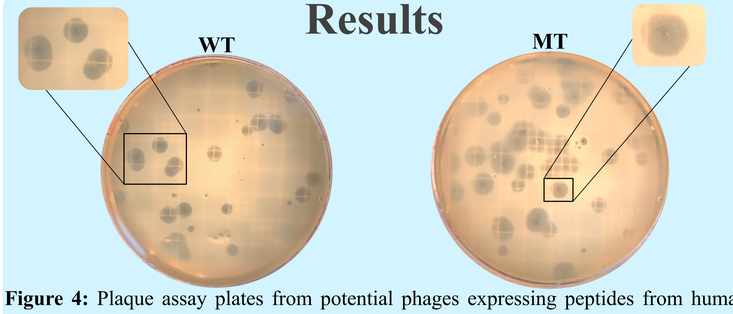


Figure 4: Plaque assay plates from potential phages expressing peptides from human heart cDNA, with a potential affinity to the LF protein, isolated in the biopanning. **WT: wild-type LF protein. MT: active-site mutant LF protein.**

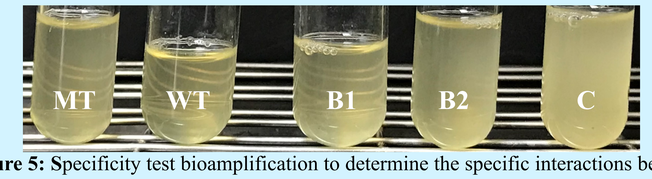


Figure 5: Specificity test bioamplification to determine the specific interactions between displayed human heart peptides and the anthrax toxin LF. **WT: wild-type LF protein. MT: active-site mutant LF protein. B1: Bovine serum albumin. B2: Casein. C: Control.**

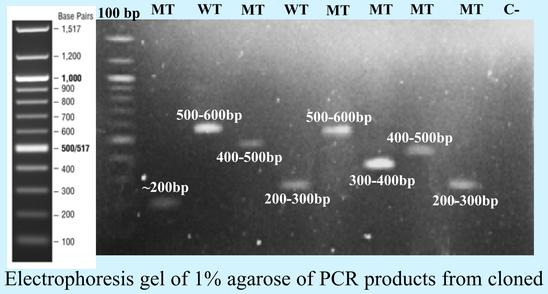


Figure 6: Electrophoresis gel of 1% agarose of PCR products from cloned fragments of human heart cDNA in T7 phage that encode for potential peptides with affinity to wild-type (**WT**) and active-site mutant (**MT**) LF. **M 100bp**: 100 bp ladder from New England Biolabs.

Summary

- Potential phages expressing human heart peptides with affinity for wild-type (WT) and active-site mutant (MT) LF, were isolated.
- After several rounds of biopannings, using WT and MT LF as targets, a total average of $3.9x10^6$ amplified interacting T7 phage display mixture for WT and $3.4x10^6$ pfu/mL MT were obtained respectively.
- Specificity tests have been performed on >25 human heart peptides candidates where they showed non-specific interactions to the anthrax toxin LF.
- DNA extractions of individuals plaques was performed to 26 plaques, cloned human heart cDNA was amplified by PCR (amplicons ranged from 100 to 700bps), 12 for WT LF and 14 to MT LF as targets, and 20% of the amplicons were sent to be sequenced, to further perform *in silico* analysis.

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