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Characterization of Protein Involved in Hemolysis Expressed by Sneathia amnii, a Pathogen of the Female Urogenital Tract

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Characterization of Protein Involved in Hemolysis Expressed by *Sneathia amnii*, a Pathogen of the Female Urogenital Tract

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Abstract

Sneathia amnii is a poorly characterized gram-negative anaerobe that commonly colonizes the vagina. It has been linked to many obstetric disorders, including preterm labor, preeclampsia, and chorioamnionitis. *S. amnii* lyses human red blood cells, and we aimed to identify the hemolysin. We identified two genes that appear to encode transporter and effector components of a two-partner secretion system. The putative effector, which we refer to as SaFHA, contains a domain with amino acid similarity to the filamentous hemagglutinin (FHA) of *Bordetella pertussis* and its predicted structure suggests it may form a transmembrane channel or pore. Thus, we hypothesized that SaFHA would be secreted by *S. amnii* and that it would play a role in hemoglobbin release. To test this, a portion of the gene encoding the SaFHA protein in *S. amnii* was expressed in *E. coli* and used as an immunogen in rabbits. Western analysis using anti-SaFHA revealed that the protein is secreted and localizes to the bacterial surface. Pre-treatment of *S. amnii* with anti-SaFHA blocked the hemolytic activity whereas antiserum against an irrelevant protein had no effect. We partially purified SaFHA from S. amnii using cation exchange chromatography and the partially purified protein mediated hemoglobin release from human RBC, supporting our hypothesis. Further characterization of SaFHA will help provide more insight on the virulence of *S. amnii*, and perhaps shed light on the etiology of *Sneathia*-associated vaginal conditions, as well as future treatment options.

Hypothesis

The FHA homologue of *S. amnii* plays a role in the release of hemoglobin from human RBC.

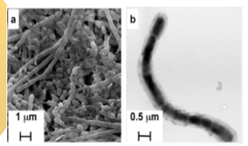


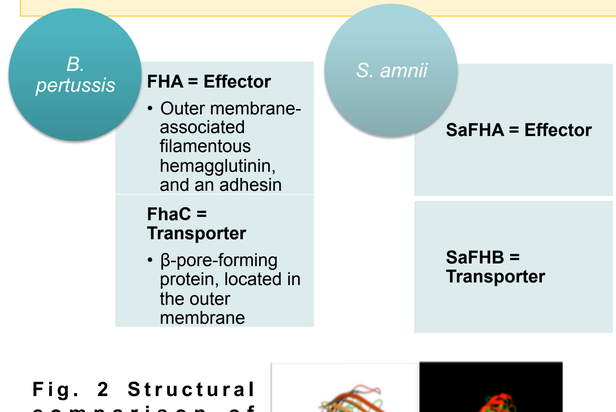
Fig. 1 (A) SEM micrograph of *Sneathia amnii* morphology. **(B)** *S. amnii* consists of long gram-negative rods and short rods and cocci.

Methods

Purification of SaFHA protein → Create an isogenic *saFHA* mutant of *S. amnii* by homolgous recombination → Characterize the role of SaFHA in hemolysis and adherence

What role does SaFHA play in *S. amnii*?

Bioinformatic analysis of Sn35 genome identified 2 genes with amino acid and structural similarity to two proteins involved in two-partner type Vb secretion (Tps) system of *B. pertussis*.



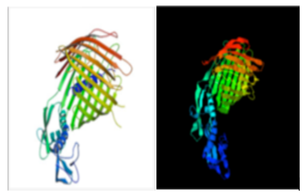


Fig. 2 Structural comparison of transporter proteins in *S. amnii* and *B. pertussis*. Left: FhaC from *B. Pertussis*. Right: SaFHB from *S. amnii*

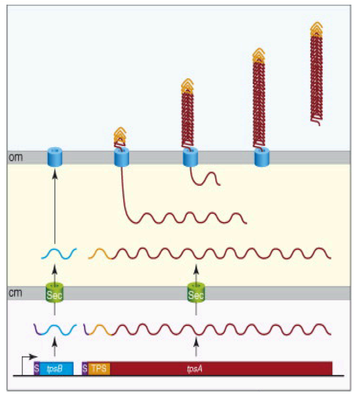


Fig. 3 General model for two-partner system (TPS) secretion. Effector (TpsA) transporter (TpsB) proteins are synthesized and translocated across the CM via the Sec system. Transporter inserts itself into the OM forming a β-barrel that mediates the secretion of effector protein. *Image adapted from Mazar, et al. (2007).*

SaFHA is localized to the outer membrane of *S. amnii.*

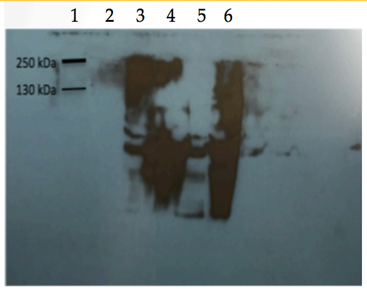


Fig. 4 Western blot of outer membrane fraction of Sn35. 1). 10-250kDa protein ladder, 2). Spent medium from first centrifugation step, 3). *S. amnii* fraction, 4). Post- French Press pellet fraction, 5). Supernatant fraction from ultracentrifuge, 6). Outer membrane fraction. *SaFHA (~200 kDa) detected in lanes 3, 4, and 6. SaFHA ~250 kDa.

SaFHA antiserum, but not pre-immune serum or antiserum against an irrelevant protein, blocks hemolytic activity against human erythrocytes, suggesting SaFHA is involved in hemolysis.

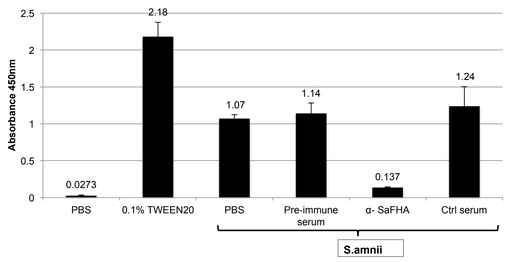


Fig. 5 Antiserum inhibition of Sn35 hemolytic activity. *S. amnii* strain Sn35 was pre-treated with either PBS, pre-immune rabbit serum, antiserum against SaFHA, or antiserum against an irrelevant protein. An erythrocyte lysis assay was performed by incubating 200µL of human erythrocytes with 50µL PBS (negative control), 0.1% TWEEN 20 (positive control) or 10^6 pre-treated bacteria for 2hr at 37˚C. The cells were removed by centrifugation and the absorbance of the supernatant at 450 nm was measured.

Purification Schematic of SaFHA Through Affinity Chromatography

SaFHA has a predicted pI of 9.4. Cation exchange was used to purify SaFHA from outer membrane extracts of *S. amnii.*

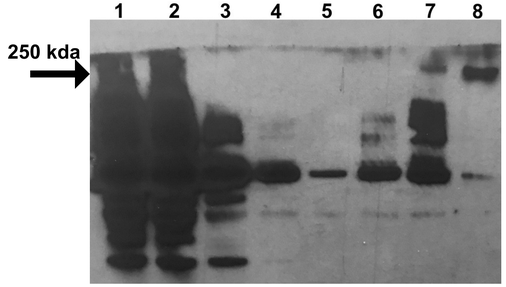


Fig. 6 Purification of SaFHA using cation exchange. OM extracts were suspended in 20mM Tris pH8 + 0.5% Triton-X, sonicated, and filter sterilized. The OM was applied to a cation exchange column, and passed through a series of wash steps. Western analysis was performed on the flow through from each wash step. 1). OM Before filtering, 2). OM After filtering, 3). OM cation flow through, 4). Tris pH 8, 5). Tris pH 8 + 100mM NaCl, 6). Tris pH8 + 200mM NaCl, 7). Tris pH 8 + 500mM, 8). Tris pH 8 + 1M. SaFHA ~250 kDa.

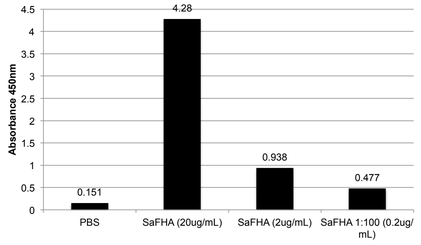


Fig. 7 Hemolytic activity of SaFHA. Purified SaFHA was added to human erythrocytes at increasing concentrations and allowed to incubate for 2 hrs. at 37˚C. Remaining whole cells were removed by centrifugation and the amount of hemoglobin released into the supernatant was quantified by measuring absorbance at OD450nm.

Scheme for Production of Isogenic *saFHA* Deletion Mutant

In order to characterize the role of the SaFHA in the lysis of human red blood cells, an isogenic *saFHA* mutant of Sn35 will be constructed through homologous recombination. This mutant strain, in addition to purified protein (explained above), will be used in adherence and hemolysis assays to assess the role of SaFHA in adherence and hemolysis.

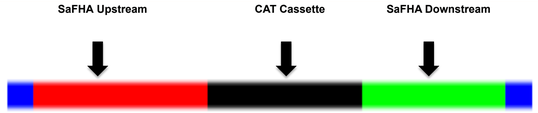


Fig. 8 Scheme for production of isogenic *saFHA* deletion mutant. A small portion of the upstream and downstream region of the gene remains intact, while the middle is knocked out and replaced with a CAT antibiotic resistance cassette, obtained from *E. coli* pKD3 plasmid.

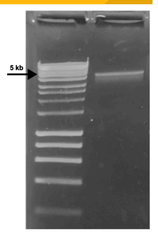


Fig. 9 Agarose gel of linearized DNA fragment, constructed through SOE PCR. 1). 1 kb DNA ladder, 2). SOE PCR product.

Acknowledgements

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