2017

Characterization of a Putative Hemolysin Expressed by Sneathia amnii, a Preterm Birth-associated Pathogen

Lizette Carrasco
Virginia Commonwealth University

Kimberly Jefferson
Virginia Commonwealth University

Characterization of a putative hemolysin expressed by *Sneathia amnii*, a preterm birth-associated pathogen

Lizette I. Carrasco[1], Kimberly K. Jefferson[2]

[1]Department of Biology, Virginia Commonwealth University

[2]Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University

Abstract

The gram-negative bacteria *Sneathia amnii* is a poorly-characterized commensal of the female urogenital tract frequently associated with adverse clinical outcomes such as bacterial vaginosis (BV), amnionitis, and preterm labor. To investigate its potential role in virulence, we sought to identify and characterize virulence determinants produced by *S. amnii* in an effort to better understand the pathogenesis of infectious preterm birth. Through sequencing of the Sn35 genome (type strain of *S. amnii*), we identified two genes with amino acid sequence similarity and structural similarity to the filamentous hemagglutinin (FHA) protein of *Bordetella pertussis* and its Type Vb transporter. Because *S. amnii* requires human blood components for growth and lyses human red blood cells, we hypothesized that this two-partner system was involved in hemolysis. To characterize the function of the FHA-like protein, a purified, recombinant peptide was used to induce an antibody response. The polyclonal rabbit serum against the antigenic peptide was incubated with *S. amnii* to block the FHA-like protein prior to the addition of red blood cells. Pre-treatment with the antiserum inhibited hemolytic activity against human erythrocytes suggesting that the FHA-like protein is somehow involved in hemolysis. Additionally, we found that the hemolytic activity of S. amnii was highly specific against human red blood cells; it did not lyse horse or rabbit red blood cells and only minimally lysed sheep red blood cells. Further research efforts will focus on purifying functional FHA-like protein for further characterization and to determine whether it is sufficient to induce hemolysis.

Introduction

Sneathia amnii (Sn35) is a gram-negative anaerobic bacterium isolated from the vagina of an African American woman presenting with symptoms of preterm labor at 26 weeks gestation. *Sneathia* species have been isolated as part of the normal reproductive microflora; however, *Sneathia* species are frequently associated with clinical conditions such as bacterial vaginosis (BV), amnionitis, and preterm labor and thus, ***Sneathia* species have emerged as a potential pathogen of the female reproductive system.** Currently, very little is known about the role *S. amnii* has in bacterial vaginosis or the pathogenesis of infectious preterm birth.

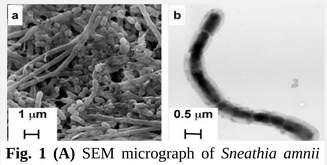

Fig. 1 (A) SEM micrograph of *Sneathia amnii* morphology. **(B)** *S. amnii* consists of long gram-negative rods and short rods and cocci.

Hypothesis: Sn35 is equipped with factors that facilitate invasion of the fetal-maternal environment
Project aim: Identification and characterization of Sn35 virulence determinants to better understand the pathogenesis of infectious preterm birth

Methods

Bioinformatic analysis of Sn35 genome → Purify recombinant peptide from antigenic region within Sn35 FHA → Polyclonal rabbit antiserum production

Determination of human serum component required for growth → **Red blood cell hemolysis assay** → **Purification of functional Sn35 FHA-like protein**

Putative virulence determinant encoded by *Sneathia amnii*

Bioinformatic analysis of Sn35 genome

Identified two genes with amino acid sequence similarity and structural similarity to

- FHA/FhaC two-partner type Vb secretion system of *Bordetella pertussis* where
  - **FHA** (filamentous hemagglutinin) analogous to Sn35 **TvbA**
  - **FhaC** (transporter) analogous to Sn35 **TvbB**
- Hemagglutinins and hemolysins

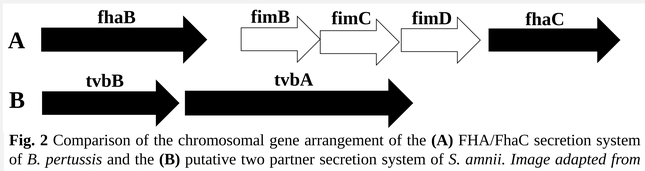

Fig. 2 Comparison of the chromosomal gene arrangement of the **(A)** FHA/FhaC secretion system of *B. pertussis* and the **(B)** putative two partner secretion system of *S. amnii*. *Image adapted from Willems, et al. (1994).*

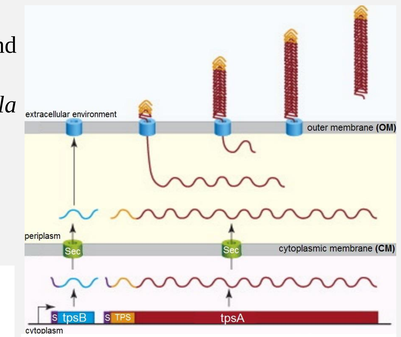

Fig. 3 General model for two-partner system (TPS) secretion. TpsA and TpsB proteins are synthesized and translocated across the CM via the Sec system. TpsB inserts itself into the OM forming a β-barrel that mediates the secretion of TpsA protein. *Image adapted from Mazar, et al. (2007).*

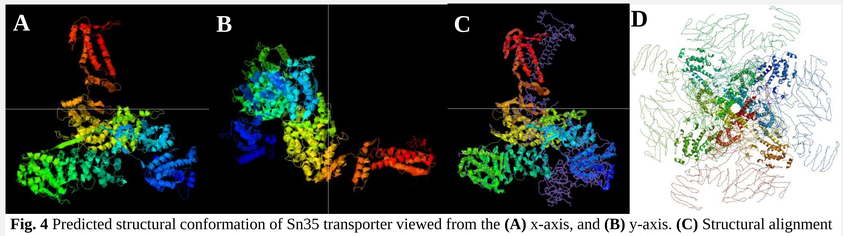

Fig. 4 Predicted structural conformation of Sn35 transporter viewed from the **(A)** x-axis, and **(B)** y-axis. **(C)** Structural alignment of the predicted protein structure of Sn35 transporter (shown in cartoon) to the known structure of 3JAVA (shown as purple backbone structure), which shares 97.8% structural similarity to target protein. **(D)** Structure of full length 3JAV, a rat inositol triphosphate receptor type 1 channel in the apo-state. 3JAV is classified as a transport protein and consists of 4 homologous protein subunits. *Images generated by I-TASSER protein structure prediction software.*

S. amnii requires human serum components for growth

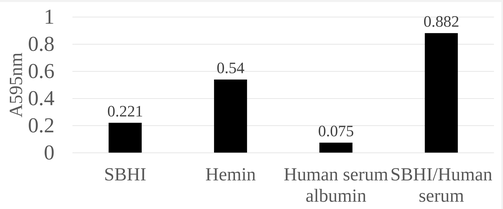

Fig. 5 Sn35 growth after 48h. in SBHI, hemin, human serum albumin, or SBHI supplemented with 10% human serum.

- In human hosts, most iron is contained within RBCs as hemoglobin
- A small percentage of iron can be found circulating within blood serum. Serum iron is normally bound to iron-binding proteins such as transferrin.
- Upon lysis of hemoglobin, hemin is released and is rapidly bound by albumin.
- Human serum albumin is a highly abundant protein in serum.
- Internalized hemin can serve as a source of iron for pathogenic bacteria.

Sn35 hemolytic activity specific to human erythrocytes

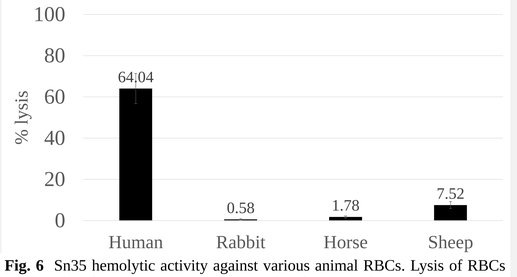

Fig. 6 Sn35 hemolytic activity against various animal RBCs. Lysis of RBCs incubated with 0.1% Tween was adjusted to be 100% lysis. Lysis of RBCs incubated with PBS adjusted to be 0% lysis

Antiserum inhibition of hemolytic activity

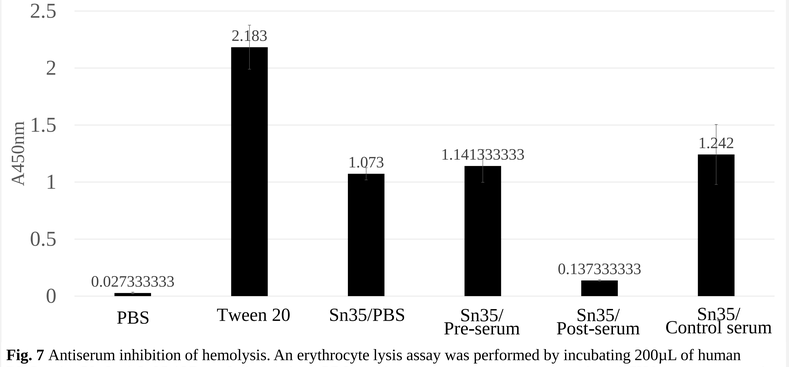

Fig. 7 Antiserum inhibition of hemolysis. An erythrocyte lysis assay was performed by incubating 200μL of human RBCs with 50μL of Sn35 (OD_{600}=0.1) and either PBS, pre-immune serum, polyclonal rabbit anti-FHA serum, or negative control serum in a 1:1 ratio for 2h. at 37˚C. 0.1% TWEEN 20 serves as a positive control.

Conclusion

- *S. amnii* produces a virulence determinant that is exported by a two-partner type Vb secretion system with sequence similarities to genes involved in the export of *Bordetella pertussis* FHA.
- *S. amnii* requires components of human serum for growth.
- Hemolytic activity of *S. amnii* is highly specific against human red blood cells.
- Antiserum pre-treatment inhibited hemolytic activity against human erythrocytes suggesting that Sn35 FHA-like protein is somehow involved in hemolysis.
- ***Sneathia amnii* likely secretes hemolytic toxins as a mechanism to access host iron for nutrient acquisition during growth and infection.**

Acknowledgements and References

- Financial support for this project was provided by the VCU IMSD Program (NIH grant 2R25GM090084-5) and the Jefferson Lab (NIH P60 grant 4P60MD002256-10)
- Data from Fig. 6 (Sn35 hemolytic activity against various animal RBCs) provided by Erin Garcia (Jefferson Lab).

References:

- Harwich, M., *et al.* (2012). Genomic sequence analysis and characterization of *Sneathia amnii* sp. nov. *BMC Genomics.* 13(Suppl 8)
- Mazar, J., *et al.* (2007). New insight into the molecular mechanisms of two-partner secretion. *Trends Micro.* 15, 508-515
- Willems, R., *et al.* (1994). Mutation analysis of the *Bordetella pertussis fim/fha* gene cluster: identification of a gene with sequence similarities to haemolysin accessory genes involved in export of FHA. *Mol. Micro.* 11, 337-347
- PDB ID: 3JAV. Fan, G., *et al.* (2015). Gating machinery of InsP3R channels revealed by electron cryomicroscopy. *Nature.* 527, 336-341.