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Introduction of plasmid DNA into Sneathia vaginalis; the first step to genetic manipulation

Rea Mansukhani
Virginia Commonwealth University

Cathyrn O'Brien
Virginia Commonwealth University

Zion McCoy
Virginia Commonwealth University

Kimberly K. Jefferson
Virginia Commonwealth University

Introduction of plasmid DNA into *Sneathia vaginalis*; the first step to genetic manipulation

Rea Mansukhani[1]; Cathyrn O'Brien[2]; Zion McCoy[2]; Kimberly K. Jefferson, PhD[2]

Department of Biology, College of Humanities and Sciences[1];Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine[2]

Introduction

The World Health Organization estimates nearly 15 million preterm births annually worldwide[1]. Oftentimes, the cause remains unknown. Recent advancements in DNA sequencing and genomic analysis, have led to discoveries of microorganisms that are associated with, and may contribute to preterm birth. *Sneathia vaginalis* (*S. vaginalis*) was one of those microorganisms[2].

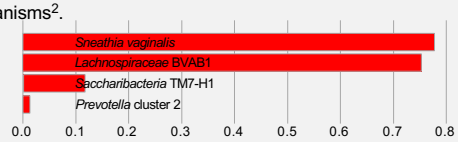
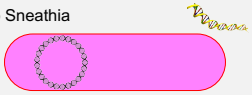


Fig1. *S. vaginalis* is associated with PTB. LefSe, a linear model that produces a score based on weighted log(abundances), detected four taxa in vaginal 16S rRNA profiles in the 6- to 24-week gestational age range that were associated with PTB. Of these, *S. vaginalis* had the highest score.

Sneathia vaginalis is a fastidious gram-negative anaerobe that requires human blood for growth. We have demonstrated that *S. vaginalis* is able to cross the fetal membrane and forms pores in human cells due to the production of the cytopathogenic toxin A (CptA)[3]. In order to further characterize the role of CptA in pathogenesis, we need to genetically manipulate *S. vaginalis* and delete the *cptA* gene. To delete *cptA*, we first need to optimize conditions to introduce foreign DNA into *S. vaginalis*. Results from this study are critical to the characterization of the role of CptA (and other virulence factors) in the pathogenesis of *S. vaginalis*.

Methods

1. Get DNA into Sneathia



2. Avoid restriction digestion

3. Express antibiotic resistance

Electroporation:
Log-phase *S. vaginalis* was washed repeatedly in 10% glycerol. Electrocompetent cells were incubated on ice with plasmid DNA and pulsed at 1.8 kV in 0.1 cm cuvettes.

Plasmid maxi-prep:
Concentrated plasmid DNA was purified using Qiagen Maxiprep kit. DNA was methylated with Taq methyltransferase.

Cloning:
We cloned homologous regions from the *cptA* gene and different antibiotic resistance cassettes including ermB, ermC, and Gm.

Results

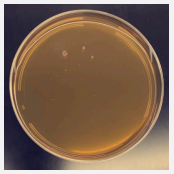


Figure 1. Erythromycin resistant colonies following transformation of *S. vaginalis* with the construct shown in Figure 2.

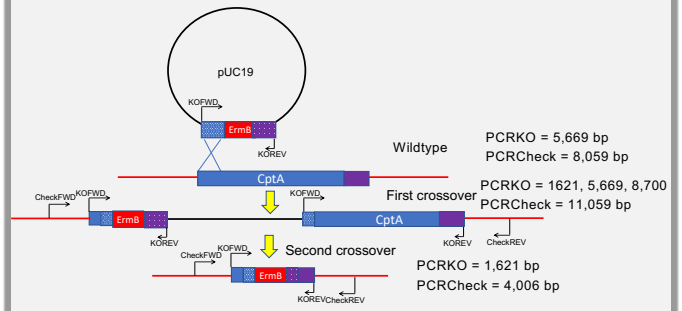


Figure 2. Sample strategy for inactivation of *cptA* by homologous recombination

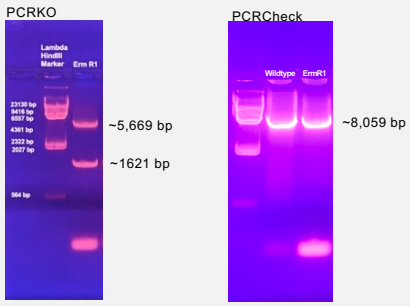


Figure 3. Agarose gels showing PCR products yielded from amplification of erythromycin resistant mutant DNA using the KOFWD and KOREV primers (PCRKO) or the CheckFWD and CheckREV primers (PCRCheck). The PCRKO amplification demonstrates that the construct is present in the ermR mutant, but the PCRCheck amplification demonstrates that the chromosomal DNA is at the *cptA* locus is still wildtype, suggesting that the plasmid is episomal.

Discussion

- Spontaneous resistance to erythromycin and gentamicin was more common than plasmid-mediated resistance.
- Methylation with Taq methyltransferase did not increase the rate of plasmid integration.
- The *ermB* resistance gene conferred erythromycin resistance to *S. vaginalis*. We did not isolate antibiotic-resistant isolates using the gentamicin resistance cassette or the *ermC* gene.
- Erythromycin resistant mutants containing the construct shown in Figure 2 were isolated, but retained the wildtype cptA locus, suggesting that the plasmid remained episomal.
- This is the first successful genetic manipulation of the emerging pathogen, *Sneathia vaginalis*.
- Future plans include optimizing the introduction of DNA into *S. vaginalis* so that insertional inactivation of genes is possible and confirming that the plasmid in the ermR mutant obtained is episomal.

References

1. *Preterm Birth*. (2018). World Health Organization. https://www.who.int/news-room/fact-sheets/detail/preterm-birth

2. Fettweis JM, Serrano MG, Brooks JP, et al. The vaginal microbiome and preterm birth. *Nat Med*. 2019;25(6):1012-1021.

3. Gentile, G.L.; Rupert, A.S.; Carrasco, L.I.; Garcia, E.M.; Kumar, N.G.; Walsh, S.W.; Jefferson, K.K.. Identification of a cytopathogenic toxin from *Sneathia amnii*. *J Bacteriol*. Published online April 14, 2020.

Acknowledgments

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