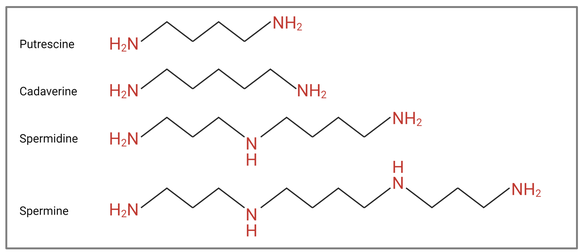
The role of polyamines in *Clostridioides difficile* pathogenesis

Werner, Bailey, Hastie, Jessica, and Carlson, Paul
Laboratory of Mucosal Pathogens & Cellular Immunology, Division of Bacterial, Parasitic, and Allergenic Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, United States Food & Drug Administration, Silver Spring, MD, USA

Introduction

Clostridioides difficile infection (CDI) affects more than half a million Americans annually, and antibiotic treatment of CDI has a low success rate that can result in recurring infections. *C. difficile* is a gram-negative, spore-forming bacterium which typically infects the gut after the microbiome is disturbed following oral antibiotic administration. *C. difficile* can survive in gut conditions with limited iron and low levels of oxygen despite the essentiality of iron for growth and its anaerobic nature. Understanding the molecular mechanisms that underlie *C. difficile* iron acquisition and resistance to oxygen will inform the development of new therapeutics to combat CDI. Polyamines have been implicated in both of these stress responses in other microbes, and previous findings suggest an increased expression of polyamine acquisition genes in *C. difficile* grown in iron-limited conditions. Polyamines are organic compounds with two or more amine groups, playing diverse, important roles in cellular processes (e.g., cell growth, proliferation, motility, and virulence). The most common bacterial polyamines are putrescine, spermidine, spermine, and cadaverine.



Objectives

- Explore a potential role of polyamine acquisition and/or synthesis in *C. difficile* iron acquisition and oxidative stress response *in vitro*
- Determine whether polyamine acquisition and/or synthesis contributes to *C. difficile* gut pathogenesis *in vivo*

Materials & Methods

Strain Generation

- Δ*potABCD C. difficile* 630 was generated via toxin-mediated allelic exchange

Growth Curves

- Wild-type and Δ*potABCD* Cd630 were grown overnight in BHIS and back-diluted in BHIS the following morning
- Upon reaching exponential growth phase, cultures were used to inoculate media in a 96-well plate to a starting OD600 of 0.01.-0.1
- Plates were incubated at 37C for 72 hours with OD600 readings taken every 15 minutes

Figure 2. Colony PCR confirming deletion of *potABCD* in mutant strain

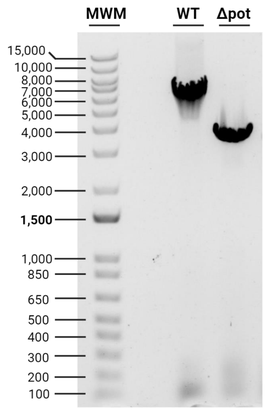
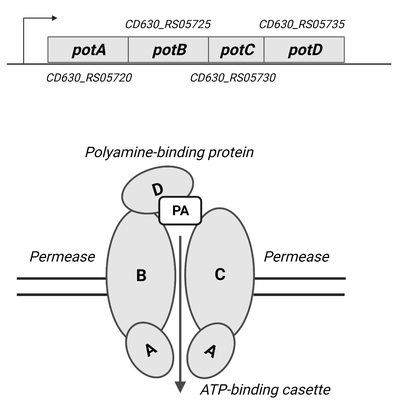


Figure 1. *potABCD* encodes a putative polyamine transporter

A



B

ATP-binding casette

	E. coli	
C. diff	PotA	PotG
PotA	**51.85**	42.04

Permeases

	E. coli			
C. diff	PotB	PotC	PotH	PotI
PotB	**40.16**	22.66	37.93	24.29
PotC	21.33	34.45	22.30	**34.47**

	C. diff
C. diff	PotB
PotC	51.85

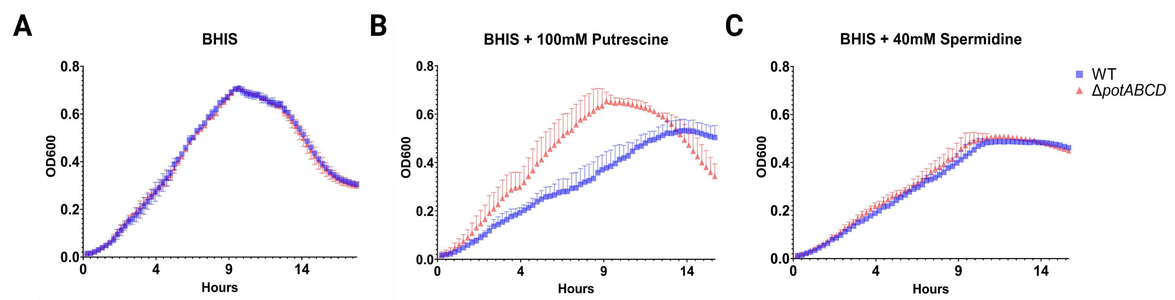
Polyamine-binding protein

	E. coli	
C. diff	PotE	PotD
PotD	30.79	**40.12**

The *C. difficile* genome contains a *potABCD* operon (*CD630_RS05720-RS05735*) encoding a putative polyamine ABC transporter (Fig. 1A). This includes an ATP-binding cassette, two permeases, and a polyamine-binding protein. PA = polyamine. Some microbes have multiple transport systems, including *E. coli* with PotABCD and PotFGHI. *E. coli* PotABCD is spermidine-preferential but does import putrescine, while PotFGHI is putrescine-specific. The *C. difficile* PotABCD protein sequence more closely aligns with *E. coli* PotABCD (using Clustal Omega alignment with default parameters), but *C. difficile* permeases PotB and PotC align well with both *E. coli* permeases (Fig. 1B). Bold font indicates proteins with highest identity percentage between *C. difficile* and *E. coli* Pot transporters.

Results

Figure 3. potABCD contributes to putrescine sensitivity



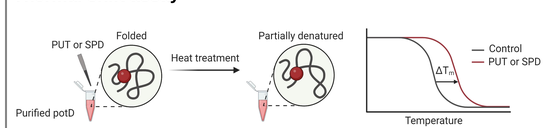
Wild-type and Δ*potABCD* Cd630 exhibit comparable growth in BHIS (Fig. 3A). Supplementation of 40mM spermidine similarly inhibits wild-type and Δ*potABCD* Cd630 growth, both peaking at ~0.5 OD600 in spermidine compared to ~0.7 in BHIS alone (Fig. 3C). However, growth of Δ*potABCD* Cd630 was less inhibited by 100mM putrescine than wild-type (Fig. 3B). This suggests that the PotABCD transporter may exclusively contribute to putrescine sensitivity, which would indicate putrescine-specific transport by PotABCD. This data is preliminary (n=2), but polyamine transporters in other microbes such as *E. coli* also exhibit specificity or preference for certain polyamines. Complementation strains have also been prepared for future use to confirm phenotypes are specific to the lack of the *pot* operon.

Next Steps

Polyamine-specificity of PotABCD

- Use complementation strains to confirm putrescine-specific phenotype is due to the lack of the *pot* operon
- Probe the specificity of *C. difficile* PotABCD transport capabilities using purified PotD for thermal shift assays to determine binding preference

Thermal shift assay



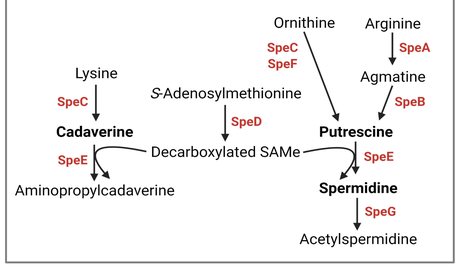
Polyamine Acquisition & Synthesis in Stress Responses

While PotABCD is implicated in polyamine acquisition, *C. difficile* also encodes polyamine synthesis genes, specifically a *spe* gene operon *(CD630_RS05025-RS05040*). These genes encode enzymes responsible for spermidine synthesis. Similar to *potABCD*, *spe* gene homologs in other microbial species are implicated in stress responses to nutrient limitation and oxygen exposure, making this operon an additional set of genes to explore.

- Generate Δ*speADEB* Cd630 via allelic exchange
- Test Δ*speADEB* Cd630 and Δ*speADEB* Δ*potABCD* Cd630 in oxidative stress (e.g., 2% oxygen, hydrogen peroxide, reactive oxygen species) and iron-limited conditions

This data will further elucidate whether polyamines contribute to *C. difficile* stress response, informing our understanding of its pathogenesis and the development of therapeutics to combat CDI.

Spermidine synthesis pathway in *E. coli* K12



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