Regulation of Laterally Transferred *ispD* in Meningococcal Urethral Clade US_NmUC

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Introduction

- Large urethritis clusters that emerged in the United States (US) in 2015 are caused by a novel urethral *N. meningitidis* (Nm) clade, dubbed US_NmUC
- Genome sequencing of > 200 US_NmUC isolates revealed that *Neisseria gonorrhoeae* (Ng) DNA was integrated into the Nm clade genome, including genes in an operon involved in terpenoid synthesis
- The terpenoid synthesis pathway gene *ispD* in US_NmUC isolates showed an >50-fold higher expression when compared to non-clade Nm
- *ispD* is essential in several bacteria, including *E. coli*

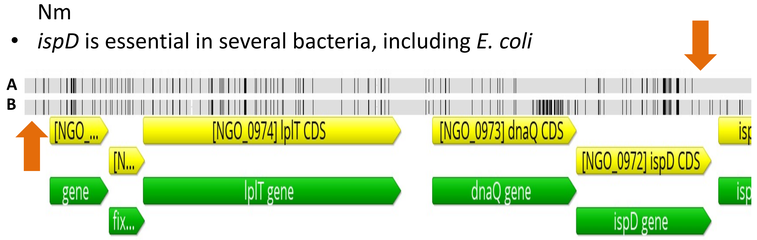


Fig 1. **US_NmUC has obtained gonococcal alleles through horizontal gene transfer**. A) US_NmUC, B) Ng. Nucleotide differences with respect to non-clade Nm are marked in black. Putative recombination boundaries are marked by orange arrows.

Methods

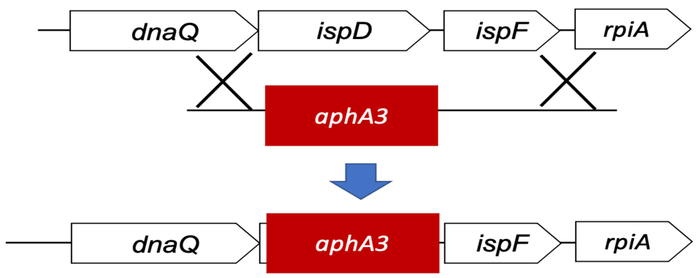


Fig 2. **Deletion of ispD.** *ispD::aphA3* nonpolar deletion-insertion constructs were generated to delete native copy of *ispD* by homologous recombination.

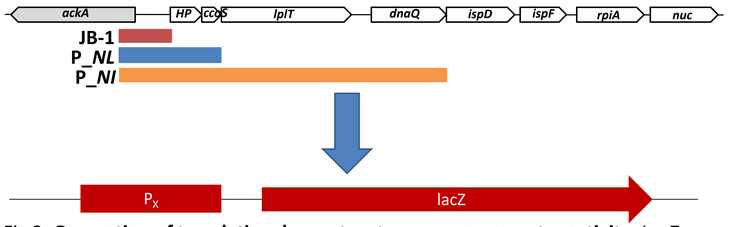


Fig 3. **Generation of translational reporters to measure promoter activity.** *LacZ* reporters with different 5' lengths of US_NmUC sequences and sequences from US_NmUC, Nm, and Ng were generated to assess potential promoter activities by β-galactosidase assay.

Results

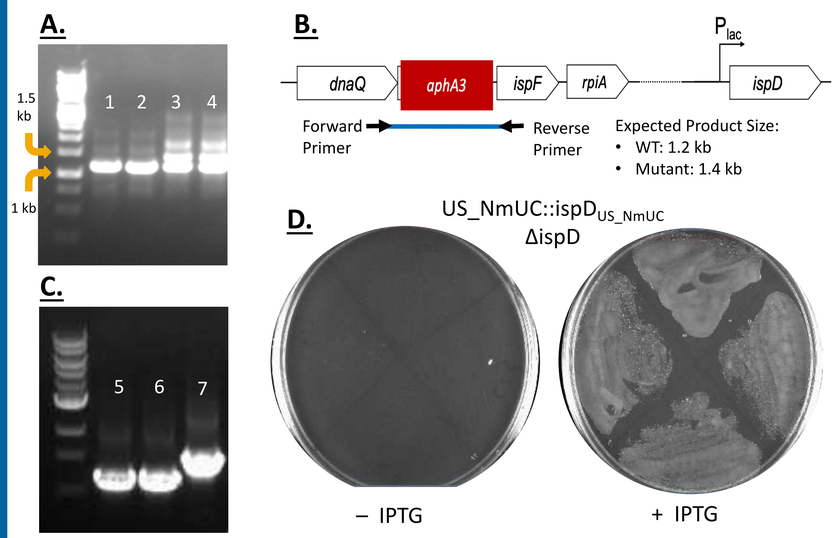


Fig 4. ***ispD* is essential in US_NmUC**. A) 1/2) US_NmUC WT, 3/4) ΔispD US_NmUC. PCR across *ispD* deletion region showed that generated "deletion mutants" contained an additional copy of *ispD.* B) To determine if *ispD* is essential in US_NmUC, *ispD* under the control of a *lac* promoter was inserted into the genome. Gene expression is induced by the addition of IPTG. *ispD* complement US_NmUC transformants were generated, and then the native *ispD* was deleted. C) 5) US_NmUC WT, 6) US_NmUC::$ispD_{US\_NmUC}$ 7) US_NmUC::$ispD_{US\_NmUC}$ Δ*ispD.* Deletion mutant was confirmed by PCR to only have the complement copy of *ispD*. D) US_NmUC::$ispD_{US\_NmUC}$ Δ*ispD* was grown with *ispD* expression induced and uninduced.

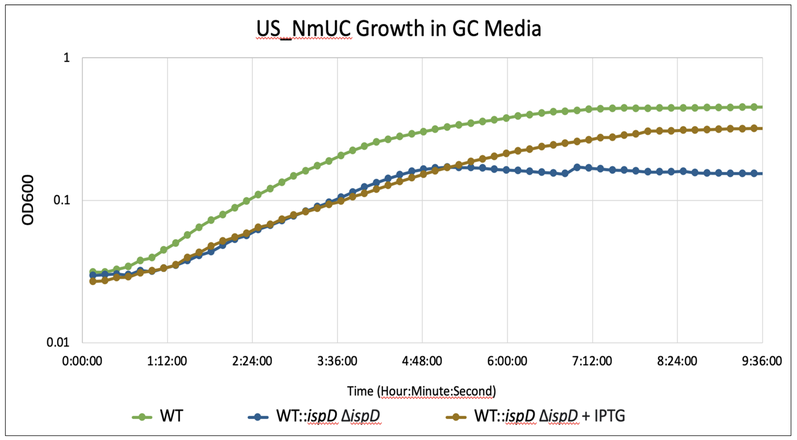


Fig 5. **IspD is involved in meningococcal growth**. US_NmUC strains were grown in a 96 well plates for 24 hours at 37ºC. When the native copy of *ispD* is deleted and the *lac* promoter is uninduced, US_NmUC growth decreases. When the *lac* promoter is induced, US_NmUC reaches a similar OD600 to the WT at log phase.

Results

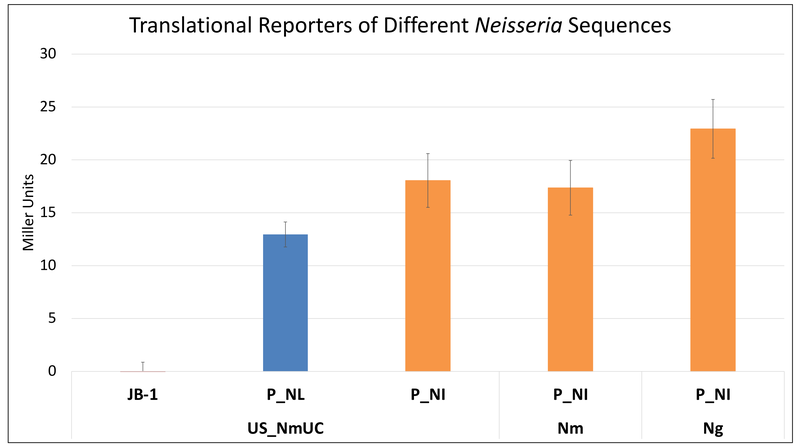
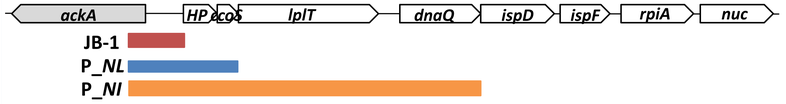


Fig 6. **Increased *ispD* expression in US_NmUC is not due to changes in promoter**. Promoter activity of the P_*NI* regions of US_NmUC, non-clade Nm, and Ng was compared by β-galactosidase assay. P_*NI* activity is comparable between clade and non-clade Nm.



Conclusions & Future Directions

Conclusions

- A mutation in the native *ispD* can only be made in a strain carrying a complemented copy of *ispD*, suggesting that *ispD* is essential in Nm.
- Reducing *ispD* expression decreases growth in the clade
- Comparable activities of P_*NI* reporters between clade and non-clade Nm sequences suggest that the increased *ispD* expression in US_NmUC isolates is not due to newly created promoters

Future Directions

- Generate mutants with different strain's *ispD* complemented into genome, i.e. US_NmUC::$ispD_{Nm}$, Nm::$ispD_{Nm}$, Nm::$ispD_{US\_NmUC}$
- Measure complement strains' growth over time and the effect of different strains' *ispD* on growth
- Perform mRNA decay assay to measure different strains' *ispD* decay rate

Acknowledgements

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