**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.

**Methods**

**A)** Deletion of ispD. ispD::aphA3 nonpolar deletion-insertion constructs were generated to delete native copy of ispD in 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, US_NmUC::ispD was grown with IPTG 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)** US_NmUC::ispD was grown with IPTG and without IPTG. Mutation was confirmed by PCR to only have the complement copy of ispD. 

**Results**

**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::ispD, Nm::US_NmUC::ispD.
- 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.

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