

Examining Virulence Evolution of *Bordetella bronchiseptica* using comparative genomics

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Abstract:

We examine the underlying factor(s) that cause some strains of bacteria to be more pathogenic than others using *Bordetella bronchiseptica* as our model system, as strains of this clonal species can cause different severities of respiratory disease. By comparing two *B. bronchiseptica* strains (strain RB50, isolated from an asymptotically infected host and strain 1289, isolated from a host with *B. bronchiseptica*-induced disease) using a murine model of infection, transcriptomics, functional and mutational analyses, we determined that the type III secretion system (TTSS) was partially responsible for the increased virulence of *B. bronchiseptica* strain 1289. Additionally, these studies showed that another, unidentified factor was also responsible for the increased virulence strain 1289, as well as other strains in its lineage. Since other virulence factors were similarly expressed and genetic differences were not detected via comparative microarray analyses between strains RB50 and 1289, we resequenced strain 1289 to identify the novel genetic factors that may contribute to this strain's increased virulence. Thus far, we've identified a ~5.9KB extrachromosomal plasmid, which may be involved in the increased virulence of this strain. We utilized optical mapping during our sequence assembly, a tool that allowed us to rapidly disentangle contig misassemblies and identify differences in the genomic structure between strain 1289 and our reference strain, RB50. We identified four misassemblies, two due to repeat regions and two caused by a 1.5MB genome inversion. This result was confirmed by completing an optical map of the reference genome, strain RB50, which showed that the genetic structure of this strain aligns with the published genome sequence. As far as we're aware, this is the largest single inversion in a microbial genome to date, and suggests that microbial inversions may be much larger than previously realized.

Figure 3. The TTSS partially contributes to the increased virulence of strain 1289, as well as other closely related strains.

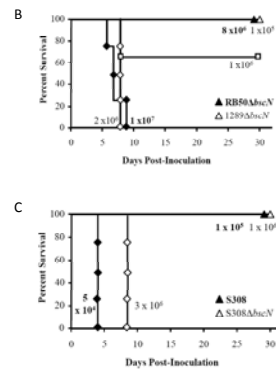
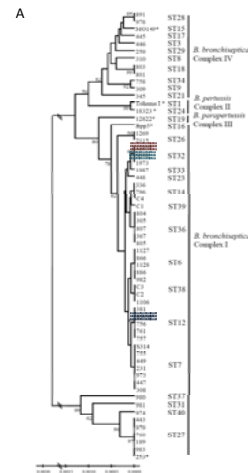


Figure 1. Strain RB50, isolated from an asymptomatic host, is less virulent than strain 1289, isolated from a host with *B. bronchiseptica*-induced disease.

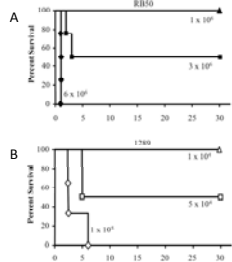


Figure 2. The TTSS genes are upregulated in strain 1289.

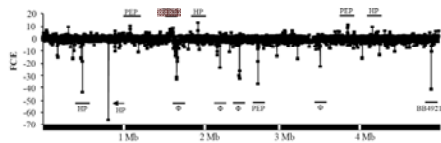


Figure 4. Metagenomic analysis of the 454 sequencing reads of the 1289 genome and identification of a plasmid in strain 1289.

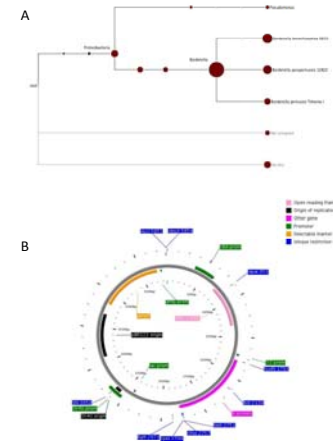


Figure 5. Use of Optical Mapping in genome assembly.

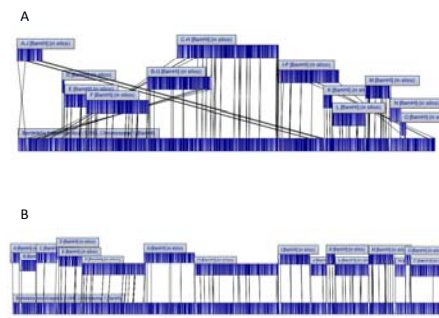


Figure 6. Genome-wide comparative genomics between strains 1289 and RB50.

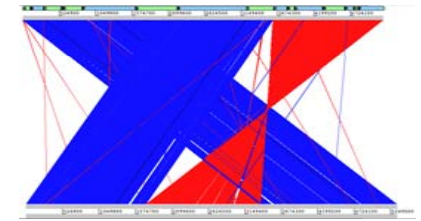
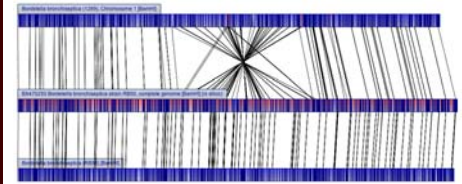


Figure 7. Comparative genomics using an optical map validates 1.5MB genome rearrangement.



Future Avenues of Research:

1. The genome of *B. bronchiseptica* strain 1289 is nearly closed. We will then identify novel and deleted genes, SNPs, and small rearrangements, which will help us determine how the genomes of *B. bronchiseptica* strains evolve.
2. Identify genes that may be involved in the increased virulence of strain 1289. Using traditional bacterial genetic approaches, we can then determine their contribution to increased virulence of the strain.
3. Examine whether other *B. bronchiseptica* strains and *Bordetella* species harbor the novel plasmid identified in strain 1289 and the relation between plasmid presence and bacterial phylogeny and virulence.
4. Determine if this plasmid enhances virulence by introducing the plasmid into other *B. bronchiseptica* strains.