# Genome-wide association and dissociation studies in *Pantoea ananatis* reveal potential virulence factors affecting in *Allium porrum* and *Allium fistulosum*

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**Introduction**:

*Pantoea ananatis* is a part of a Pantoea species complex that causes center rot of onions as well as causing infection in other Allium crops (leeks (*Allium porrum*), chives (*A. schoenoprasum*), bunching onion or Welsh onion (*A. fistulosum*), garlic (*A. sativum*)). This pathogen lacks type II, III, or IV secretion systems but relies on a chromosomal phosphonate biosynthetic gene-cluster and the plasmid-borne alt cluster for disease in onion. Pathogenicity and virulence factors associated with other Allium species remain unknown. We used phenotype-dependent genome-wide association (GWAS) and phenotype-independent gene-pair coincidence (GPC) analyses on 106 *P. ananatis* strains inoculated on *A. porrum* and *A. fistulosum* x *A. cepa* to compare with onions.

**Methods**:

*P. ananatis* strains (n=106) used in this study were isolated from diverse sources; weeds, thrips, onion foliage and bulbs and seeds in Georgia from 1992-2019. Visual breakdown of strain diversity is shown in figure 1B. Pathogenicity and aggressiveness of the strains were determined on *A. porrum* (cv. King Richard) and *A. fistulosum x A. cepa* (cv. Guardsman) under controlled greenhouse conditions. Pathogenic potential of *P. ananatis* strains were phenotyped on onion scale using the red onion scale necrosis (RSN) assay. Phenotype-dependent GWAS was conducted utilizing Roary-Scoary pipeline. Phenotype-independent GPC was conducted utilizing Coinfinder. To explore the role of pepM gene in the pbg cluster an unmarked deletion of pepM gene in *P. ananatis* PNA 02-18 was made using the pR6KT2GW allelic exchange vector.

**Results**:

• Phenotyping of 106 *P. ananatis* strains displayed variability in level of pathogenicity and aggressiveness in both Allium spp. Visual representation of strain aggressiveness is shown on figure 1A. The results of the foliar necrosis assay are visualized in figure 1B. The results of the RSN assay are visualized in figure 1B. Among the 61 scale positive strains, 70.5% (43/61) of strains were pathogenic on both A. porrum and A. fistulosum x A. cepa whereas 0% (0/61) and 22.9% (14/61) of strains were only pathogenic on either on A. porrum or on A. fistulosum x A. cepa, respectively.

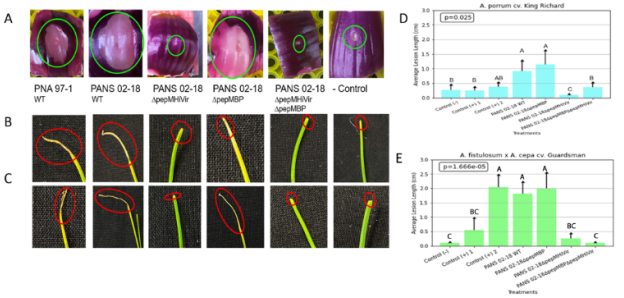
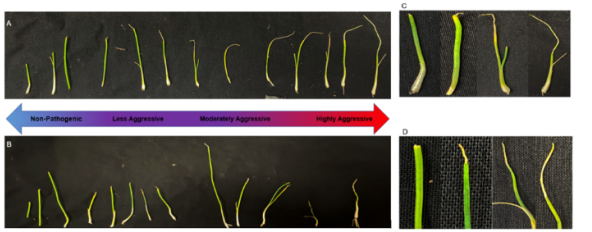



Figure 2. Results of the *pepM* pgb deletion experiments. Illustrative example of the results of gene deletion on RSN (A). Illustrative example of the results of gene deletion on foliar necrosis on *A. porrum* (B) and *A. fistulosum* x *A. cepa* (C). Statistical analysis of gene deletion mutants on *A. porrum* (D) and *A. fistulosum* x *A. cepa* (E).



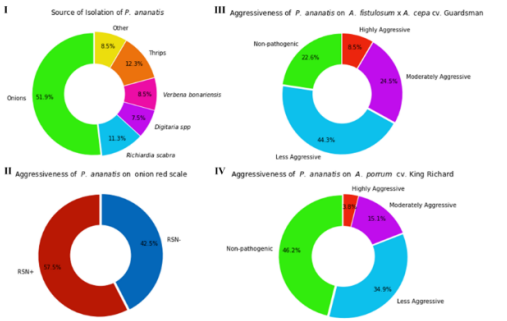




Figure 1A (top) and figure 1B (right). Figure 1A showcases foliar lesion severty and strain agressiveness on *A. fistulosum* x *A. cepa* (A, C) and *A. porrum* (B, D). Figure 1B describes the proportion of strain isolation sources (I), aggressiveness on *A. fistulosum* x *A. cepa* (II), the results of the RSN (III), and the aggressiveness on *A. porrum* (IV).

• Post-sequencing, 87% of the total reads (1,413,144,772 quality reads) were retained. An overview of the final pangenome shows a core genome (occurs in 99% or more genomes, N >= 105) of 3,192 genes, a soft-core (occurs in 95% to 99% of genomes, N = 101 to 105) of 364 genes, a shell genome of 2,288 genes (occurs in 15% to 95% of the genomes, N = 16 to 101), and a cloud genome (occurs in 0% to 15% of genomes, N = 0 to 16) of 43,307 genes for a total of 49,151 genes.

• The GWAS on *A. fistulosum x A. cepa* resulted in 2,731 genes that are significantly associated (naïve significance of p = 0.05) with the pathogenic phenotype, whereas the GWAS with phenotypes obtained on *A. porrum* resulted in 232 genes that are significantly associated (naïve significance of p = 0.05) with the pathogenic phenotype. We also found 166 genes in GWAS results that were shared between two hosts.

• In 8/106 of *P. ananatis* strains (PANS 02-12, PANS 99-31, PANS 200-2, PANS 2-5, PANS 2-7, PANS 2-8, PANS 99-11, PANS 99-12) we detected the pbg cluster mentioned in Pollidore et al., (2021), which was found to be not important for the onset of onion-bulb rot. Deletion of the annotated *pepM* in the pbg cluster did not affect symptoms (RSN and foliar necrosis).

• Gene-pair association of the *P. ananatis* pangenome resulted in a total of 952 nodes separated into 159 components.

• Gene-pair dissociation of the *P. ananatis* pangenome resulted in a total of 415 nodes separated into 64 components. Some clusters of interest are visualized in figures 3 and 4.

• Comparison of three strains pathogenic on only the *A. fistulosum x A. cepa* and three strains only pathogenic on *A. porrum* led to the finding of a type-III secretion system in PNA 15-3. However, the strain fails to present a hypersensitive response on tobacco plants.

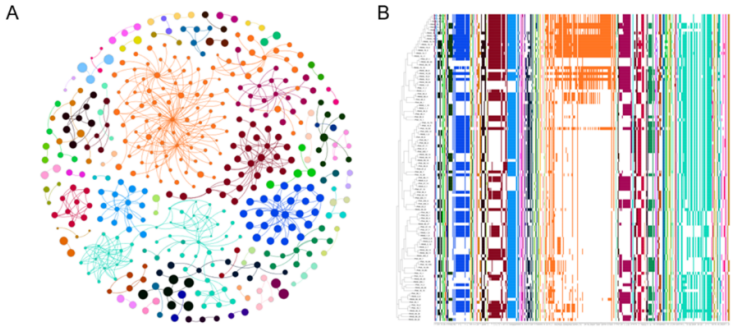



Figure 3A is an overview of coinfinder output for gene-pair dissociation analysis of the *P. ananatis* pangenome. 3B is a heatmap representation of dissociative gene pairs.

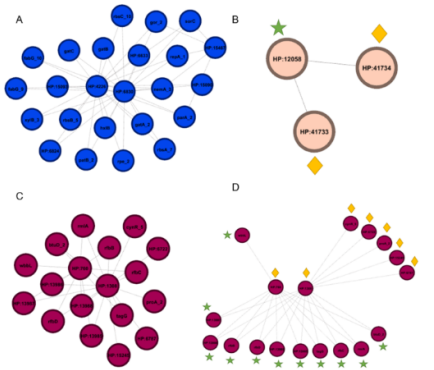



Figure 4 emphasizes three gene-pair networks of interest. 4A emphasizes an alt gene dissociation network, 4B is a small gene network showcasing genes associated with both pathogenicity phenotypes (green star) dissociating with genes associated with only *A. fistulosum x A. cepa* (gold star). 4C and 4D is similar to 4B.

**Conclusions**:

• *P. ananatis* uses a common set of virulence factors that results in foliar necrosis among in multiple Allium species (*A. cepa, A. fistulosum* x. *A.cepa*, and *A. porrum*). Some strains are pathogenic on one host but non-pathogenic on others, indicating there are diverse sets of virulence factors.

• Some strains seem capable of causing foliar necrosis despite lacking the well-characterized HiVir phosphonate biosynthetic gene cluster.

• The pgb gene cluster is not important for inducing symptoms on foliage and *A. cepa* bulbs and also is not important in Allium species (*A. cepa, A. fistulosum* x. *A.cepa*, and *A. porrum*)

• Phenotype-dependent gene association and phenotype-independent gene-pair coincidence analysis were able to predict similar list of genes for most part, indicating that gene-pair coincidence can potentially be a supporting methodology to typical GWAS analysis. However, well-characterized virulence factors failed to be detected via the phenotype-independent strategy.

• Comparative genome analysis revealed a presence of a type-III secretion system in *P. ananatis*, but more work needs to be done to determine its relevance *A. cepa* pathogenesis.

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