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Dissecting the *Pyrenophora tritici-repentis* (tan spot of wheat) pangenome

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Introduction

The necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Ptr) is a major foliar wheat pathogen with annual global losses estimated at ~5%[1]. The Ptr-wheat interaction is used as a model system to help understand the evolution of virulence in necrotrophic pathogens. In this work, we sequenced a global collection of 40 Ptr isolates with representatives from each of the eight established races and a novel phenotype recently identified in North Africa[2]. Ptr races are defined by their ability to produce different combinations of three effectors (host-selective toxins): ToxA, ToxB, and ToxC. Additionally, two isolates were sequenced with long-reads and annotated to near chromosomal level. These were compared to previously sequenced Ptr isolates (Pt-1C-BFP and M4) to infer chromosomal rearrangements and structural organizations, particularly around the effector genes *ToxA* and *ToxB*.

Materials and Methods

Fungal isolates, sequencing, assembly, & annotation

Ptr isolates in this study were collected from Canada (21), Algeria (3), Azerbaijan (8), Syria (3), and Tunisia (5) (Table 1). DNA for Illumina HiSeq X (all isolates, 150bp paired-end) and PacBio RS II sequencing was extracted using 'Genomic-tip 100/G Kit' (Qiagen). Sequencing was performed by Genome Quebec. Illumina reads were assembled with Shovill/SPAdes[3,4] and PacBio reads assembled with Flye[5] and polished with Pilon[6]. Genomes were annotated with FunGAP[7] (genes) and EDTA[8] (transposons).

Table 1. Races of Ptr and number sequenced (+ = present; - = absent)

Race	PtrTox			Number sequenced
	A	B	C	
1	+	-	+	10
2	+	-	-	6
3	-	-	+	4
4	-	-	-	3
5	-	+	-	8
6	-	+	+	3
7	+	+	-	2
8	+	+	+	3
novel	-	+	-	1
Total				40

Pangenome, phylogeny, & *ToxA* translocation

Pangloss[9] was used to perform pangenome analysis using the default settings. We included a reference genome of a race 1 isolate (Pt-1C-BFP) retrieved from GenBank. The binary matchtable output was used to generate figures with R. An alignment of all concatenated Core proteins was used as input for RAxML[10] to generate a maximum likelihood tree and gene prence/absence used for Hierarchical sets[11]. Syntenic blocks of the *ToxA* containing contigs of I-73-1 and Pt-1C-BFP were aligned then visualized with Circos[12].

Results

Pangenome

The average genome size was 34.8 ± 2.1 Mb with 13,071 predicted genes. Approximately 43% of genes are conserved across all isolates (core). The remaining 57% of genes were present in a subset of isolates, with 56% of these accessory genes present as singletons (Figure 1a). Most CAZymes (cell wall degrading enzymes) were present in all isolates while the majority of effectors were accessory genes. The high accessory count and continually increasing gene count per genome added (Figure 1b) indicate Ptr has an open pangenome.

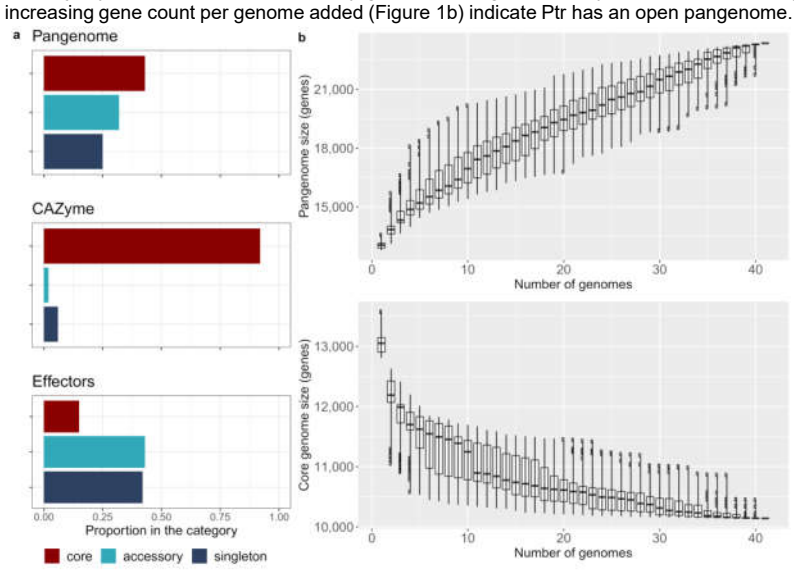

Figure 1. The pangenome of *Pyrenophora tritici-repentis*. **a** Proportion of genes present in core, accessory (excluding singletons), and singleton sets for the pangenome, CAZymes, and effectors. **b** Total number of unique genes and core genes in the pangenome of *Pyrenophora tritici-repentis* as genomes are added. Starting genome was always Pt-1C-BFP with subsequent genomes added randomly (x100).

Results

Core protein phylogeny and hierarchical sets of accessory genes

The maximum-likelihood tree of all core genes produced three major branches (Figure 2). The first contained 20 isolates and is dominated by Canadian ToxA producing isolates. The few isolates not from Canada in this branch produce ToxB. The second branch contains 19 isolates, most of which do not produce ToxA and are from the Fertile Crescent and nearby regions. The final branch, which acts as an outgroup, contains non-pathogenic (race 4) isolates and a weakly virulent race 5. The hierarchical set construction of accessory genes (Figure 2; bordered inset) showed a similar evolutionary structure based solely on gene gains and losses.

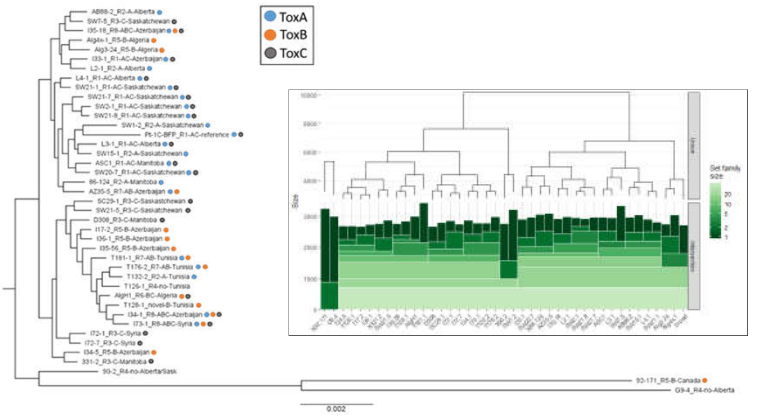

Figure 2. Maximum-likelihood phylogenetic tree of *Pyrenophora-tritici repentis* based on aligned and concatenated genes proteins in the core genome (total 10,159 proteins); **bordered inset:** hierarchical set of accessory genes, size represents number of genes shared by isolates in each cluster, where inclusion in a cluster is based on any given isolate overlapping with the horizontal bars.

Novel transposon associated with *ToxA*

The *ToxA* containing chromosome 6 of the reference isolate Pt-1C-BFP fully aligned with the homologous contig 17 of I-73-1, with the notable exception of *ToxA* which aligned to contig 3. In-depth analysis of the 143 kb region surrounding the single copy *ToxA*, revealed short-direct repeats on the edges suggesting that this is a transposon. Functional annotations of the genes in this region revealed the presence of a tyrosine recombinase and other proteins within the transposon (e.g. NACHT, FRE, HET, etc.). The large size, short-direct repeats, and the presence of a tyrosine recombinase are strong evidence that this transposon is part of a new class of called *'Starships'*[13]. We have named this novel transposon 'Horizon'. Additionally, we found multiple copies of *ToxB* on a putative 294 kb transposon with terminal inverted repeats (not shown), but final transposon classification remains.

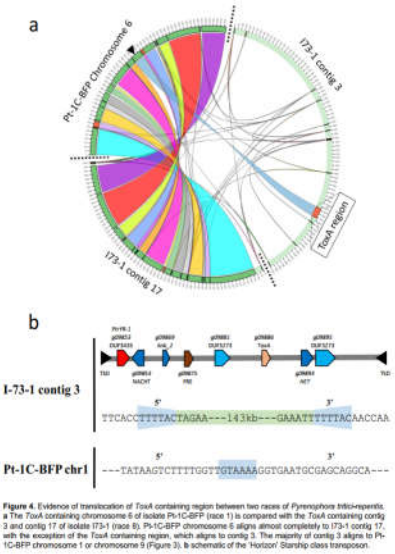

Figure 4. Evidence of translocation of *ToxA* containing region between two races of *Pyrenophora tritici-repentis*. **a** The *ToxA* containing chromosome 6 of isolate Pt-1C-BFP (race 1) is compared with the *ToxA* containing contig 3 and contig 17 of isolate I73-1 (race 8). Pt-1C-BFP chromosome 6 aligns almost completely to I73-1 contig 17, with the exception of the *ToxA* containing region, which aligns to contig 3. The majority of contig 3 aligns to Pt-1C-BFP chromosome 1 or chromosome 9 (Figure 3). **b** schematic of the 'Horizon' Starship class transposon.

Conclusions

- 40 new high quality short read assemblies
- First long-read assemblies of race 3 (ToxC) and race 8 (ToxA, ToxB, and ToxC)
- Ptr has an 'open' genome and is highly adaptable
- Ptr has a highly plastic genome which is likely 'one-compartment (not shown)
- Distinct phylogenetic clustering by ability to produce certain efffectors
- Confirmation of ToxA translocation within Ptr via nested transposons on a Starship TE
- Putative transposon associated with ToxB (not shown)
- bioRxiv preprint available doi: 10.1101/2022.03.07.483352

References

[1]Savary et al., 2019. *Nature Ecology & Evolution*, 3(3), 430; [2]Kamel et al., 2019. *Frontiers in Plant Science*, 10, 1562; [3]Bankevich et al., 2012. *Journal of Computation Biology*, 19(5), 455-477; [4]Seemann, 2019. github.com/tseemann/shovill; [5]Kolmogorov et al., 2019. *Nature Biotechnology* 37(5), 540–546; [6]Walker et al., 2014. *PloS One*, 9(11), e112963; [7]Min et al., 2017. *Bioinformatics* 33(18), 2936-2937; [8]Ou et al., 2019. *Genome Biology* 20(1), 1; [9]McCarthy & Fitzpatrick, 2019. *Genes* 10(7), 521; [10]Stamatakis, 2014. *Bioinformatics* 30(9), 1312; [11]Pedersen 2016. github.com/thomasp85/hierarchicalSets; [12]Krzywinski et al., 2009. *Genome Research* 19(9), 1639; 13 Gluck-Thaler et al., 2021. *bioRxiv* https://doi.org/10.1101/2021.12.13.472469

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