The effector gene *ToxB* is present on a putative *Starship* transposon in *Pyrenophora tritici-repentis (tan spot of wheat)*

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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 ~4.3% (Savary et al., 2019). The Ptr-wheat interaction is used as a model system to help understand the evolution of virulence in necrotrophs. Ptr races are defined by their ability to produce different combinations of three effectors (host-selective toxins): ToxA, ToxB, and ToxC. Due to it's more damaging symptoms, the most extensively studied effector to-date has been *ToxA* which is a necrosis inducing toxin. Previously we have shown that the *ToxA* carrying transposon *ToxhAT* (McDonald et al., 2018; 2019) is itself nested within a larger *Starship* class transposon (*Horizon*) (Gourlie, et al., 2022). ToxB which causes chlorosis symptoms has been less well studied, perhaps due to its multi-copy nature. In this work, we used long-read assemblies to identify the genomic position of *ToxB*-copies and found ToxB to be present within a different putative Starship transposon (*Icarus*) (Gourlie et al., 2022). Additionally, we've created the first haplotype network of *ToxB* and identified homologs in additional classes of fungi. Taken together, we provide the first evidence that *ToxB* may be, or once have been, mobile.

Results and Discussion

Starship *Icarus:* BLAST results revealed four copies of *ToxB* in the race 8 (produces ToxA, ToxB, ToxC) isolate I-73-1. Each copy appeared to be identical. Three copies were located within a 12-kb region on contig 12 (chromosome 4), and the fourth copy was present on a very small 6-kb contig (contig 13) which failed to assemble within the chromosome-sized contigs. The Australian isolate M4 (race 1; ToxA and ToxC) was used to examine the 12-kb ToxB region by alignment of I-73-1 contig 12 to M4 contig NQIK01000005. The alignment revealed a larger 294-kb gap around the region of *ToxB* (Figure 1, black arrow). Examination of the edges of this gap revealed the presence of terminal inverted repeats (TIRs) in I-73-1 and a potential target site duplication in M4 indicating the presence of putative transposon (Figure 2). Looking into another isolate, D308 (race 3; ToxC), BLAST reveal a single copy of the inactive *ToxB* homolog dubbed *toxb*. This same 294-kb transposon with the same TIRs was present in D308 on contig 9 (chromosome 3) (Figure 1, grey arrow). A schematic of the putative transposon was created to show the presence of genes contained within the region (Figure 2). Genes annotated as DUF3435 tyrosine recombinase are known to be transposases involved with *Starship* transposons (Gluck-Thaler et al., 2021). This 'captain' gene was absent in both I-73-1 and D308, other known cargo genes such as the heterokaryon incompatibility gene was present in I-73-1 while DUF3273 and patatlin-like phospholipase genes were present in D308. Additionally, DDE endonucleases (D:aspartic acid; E:glutamic acid) commonly present in class II DNA transposases were present in both isolates along with many other genes associated with class I RNA intermediate transposons. These findings taken together suggest this region may be either disabled or a type of *Starship* which makes use of something other than a tyrosine recombinase. We have called this putative *Starship* '*Icarus*'.

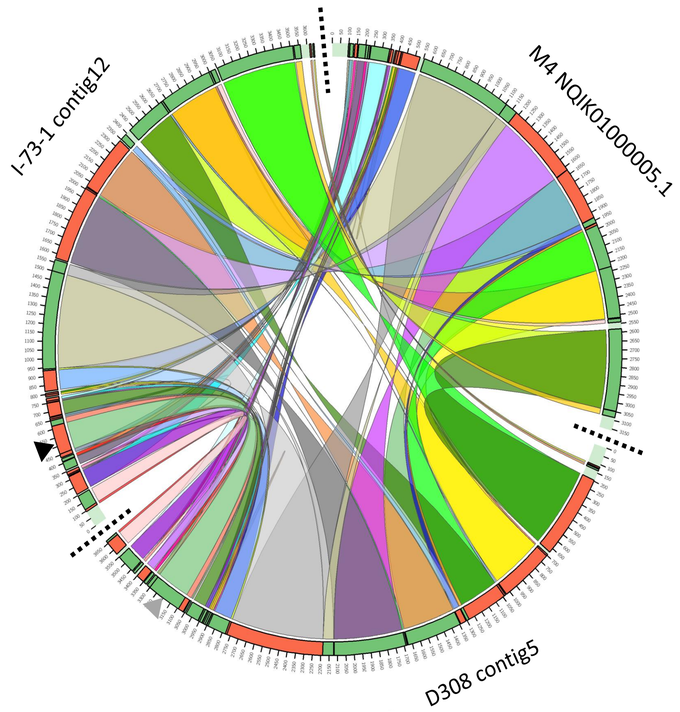

Figure 1: Evidence of putative *Starship* transposon '*Icarus*' associated with *ToxB* in *Pyrenophora tritici-repentis*. Contig 5 from race 3 isolate D308, contig 12 from race 8 isolates I-73-1, and contig NQIK01000005 from race 1 isolates M4 aligned. A large 294 Kb region which contains three copies of the *ToxB* (black arrow) is visible which aligns with a section in D308 containing a single copy of the inactive *toxb* (grey arrow).

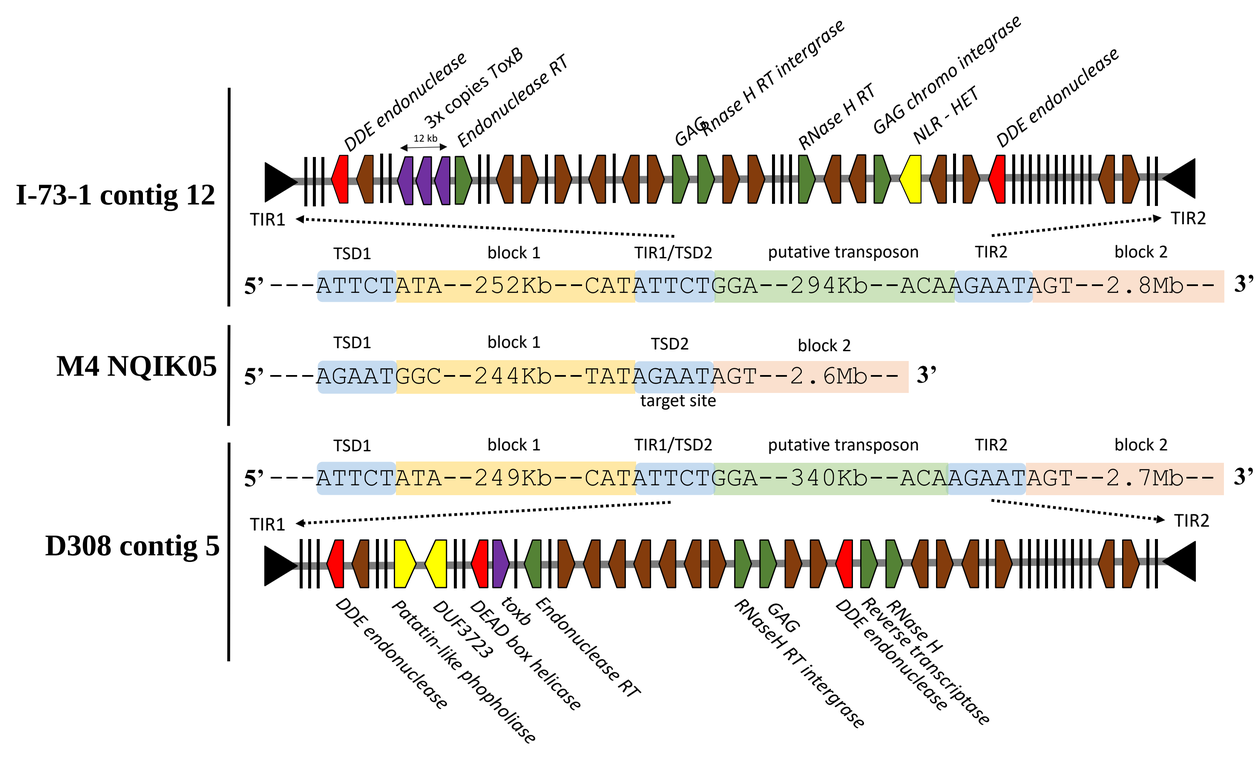

Figure 2: Schematic of '*Icarus*' transposon including possible terminal inverted repeats and target site duplication suggest transposon activity. (red genes = associated with type II DNA transposons; green = associated with type I RNA transposons; yellow = known Starship cargo genes; purple = *ToxB/toxb*; brown = annotated gene; black bar = gene with no known function)

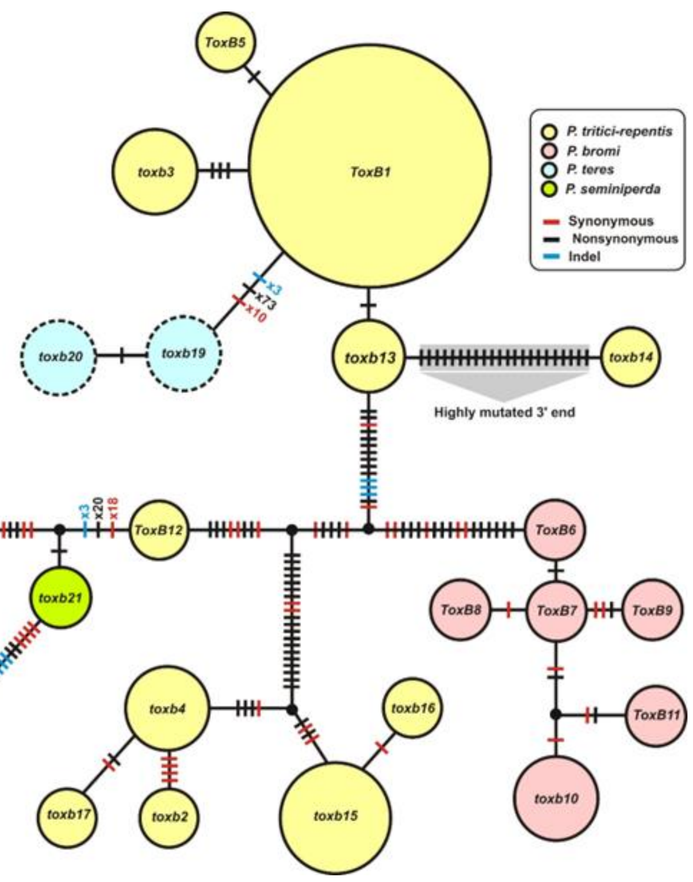

Figure 3: Global *ToxB* haplotype network in *Pyrenophora tritici-repentis* and other *Pyrenophora* spp. Circles represent unique *ToxB* haplotypes, circle size indicates relative frequency (smallest circle, 1 sequence; largest circle, 105 sequences). Hatch marks along branches individual mutations (red = synonymous; black = nonsynonymous; blue = indel). Dashed circles are *in silico* identified haplotypes. Haplotypes named according to Aboukhaddour et al. 2023.

Materials and Methods

Sequencing, assembly, annotation: High-molecular weight DNA extracted with 'Genomic-tip 100/G Kit' (Qiagen) and gDNA sequenced with PacBio RS II at Genome Quebec. Long-reads assembled with Flye (Kolmogorov et al., 2015) and polished with Pilon (Walker et al., 2014). Gene annotations were done with FunGAP (Min et al., 2017).

Transposon identification: Linear alignments between sequenced isolates and reference M4 (v2.2) done with Mauve (Darling et al., 2010). Followed by circular alignments of *ToxB* carrying chromosomes with Sibelia (Minkin et al., 2013) and Circos (Krzywinski et al., 2009). TSD and TIR identified manually. Schematic created in PPT using data generated during annotation and with BLAST outputs.

Haplotype network: A total of 154 *ToxB/toxb* sequences were analyzed, 111 from PCR products and 43 from GenBank. PopART (Bryant 2015) was used to construct a TCS haplotype network which was curated with CorelDraw x4 Graphic.

Haplotype network: Based on sequenced PCR products we identified 22 haplotypes of *ToxB/toxb* within four species of the *Pyrenophora* genus. Out of 72 polymorphic site, the prevalence of nonsynonymous mutations (76.4%) was higher than synonymous mutations (19.4%). Indel mutations were found at three different positions in the network. Most haplotypes are inactive forms producing no symptoms on wheat. However, some haplotypes appear as though they should still produce active forms as the protein product should not be altered. Further analysis of the upstream regions revealed promoter mutations such as missing TATA-box or variable numbers of repetitive elements which may affect transcription. Additional *in silico* analysis revealed putative homologs in other fungi from the Dothideomycetes, as well as the Sordariomycetes and Leotiomycetes. It has been suggested that *ToxB*, unlike *ToxA*, was acquired by Ptr vertically from a common ascomycete ancestor (Andrie et al. 2008; Ciuffetti et al. 2014). The irregular distribution of *ToxB* and its homologs in certain Ascomycete species, as well as the absence of *ToxB* in certain races of Ptr, could be explained by gene loss or mobility in the evolutionary history of *ToxB*.

References

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