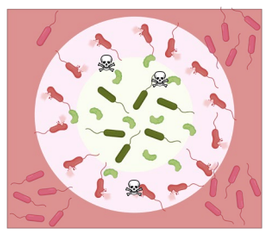
Toxic exploitation of iron uptake in *Pseudomonas*

Christine Bochynski, Dave Rogers and Paul Rainey

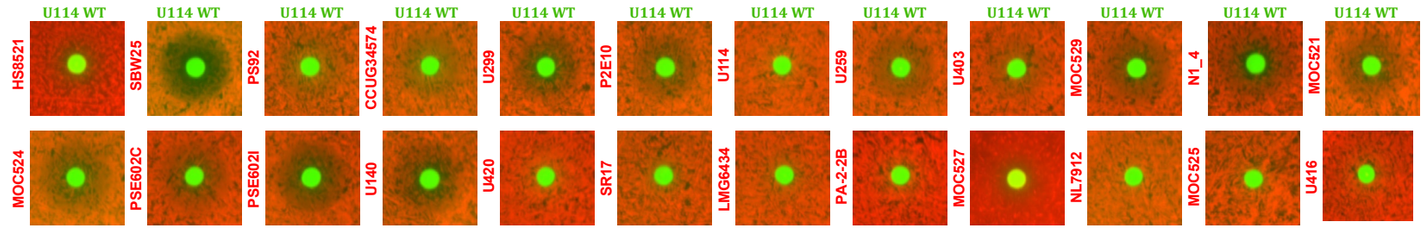
Soil environments are highly competitive due to interactions between various microorganisms and the need to acquire nutrients. Some nutrients are limited, such as oxidized (ferric) iron. Specialized iron-chelating molecules, called siderophores, facilitate the acquisition of otherwise dif﬿icult to uptake ferric iron. Primary siderophore receptors take up endogenous siderophores, while secondary siderophore receptors take up endogenous and exogenous siderophores. The ability to take up siderophores produced by other microorganisms is a huge competitive advantage as it allows sequestration of iron from the environment. However, toxins, such as S-type pyocins, can exploit this feature. Here, I present a previously unknown diffusible *Pseudomonas ﬿luorescens* toxin that is able to exploit the secondary siderophore receptor, FpvB.

1. *Pseudomonas ﬿luorescens* U114 kills closely related strains

A. Inhibition assays show variable sensitivity among close relatives

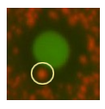


Spotting toxin producing cells on a lawn of sensitive cells generates a halo of growth inhibition where the toxin diffuses. U114 is seen in green, pyoverdine the lighter green shapes, and SBW25 is represented by red cells.

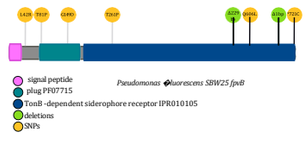


Spotting toxin producing U114 cells (green) on a lawn of close relatives (red) produces halos of inhibition with varied sensitivity.

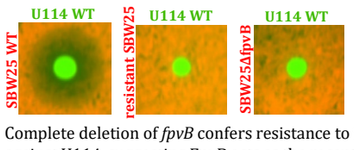
B. Spontaneous resistant types indicate FpvB is exploited by the toxin



Resistant SBW25 colonies appear spontaneously within the zone of inhibition after 120h.

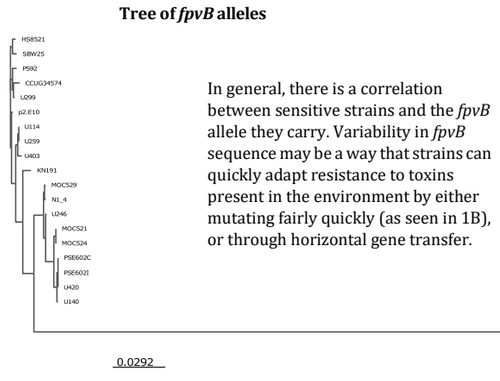


All resistant SBW25 have mutations in a siderophore receptor gene.

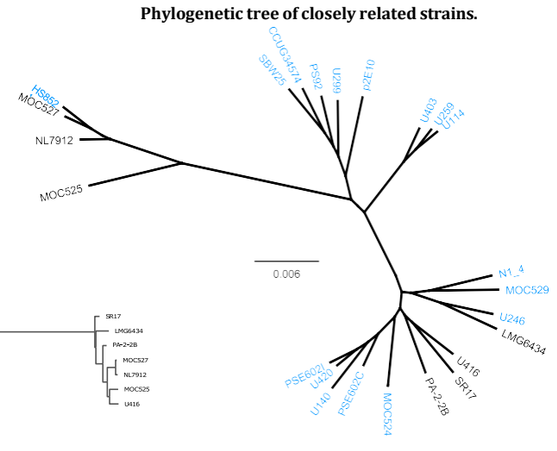


Complete deletion of *fpvB* confers resistance to SBW25 against U114, suggesting FpvB acts as the receptor for the toxin.

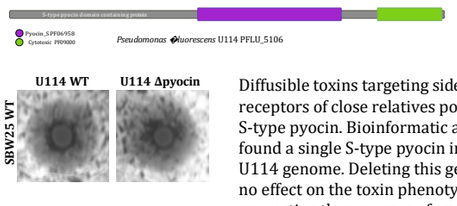
C. *fpvB* allele aids in determining susceptibility to U114, not phylogeny



In general, there is a correlation between sensitive strains and the *fpvB* allele they carry. Variability in *fpvB* sequence may be a way that strains can quickly adapt resistance to toxins present in the environment by either mutating fairly quickly (as seen in 1B), or through horizontal gene transfer.



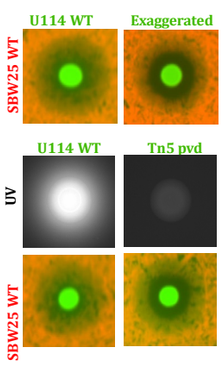
D. S-type pyocin is not responsible for toxin positive phenotype



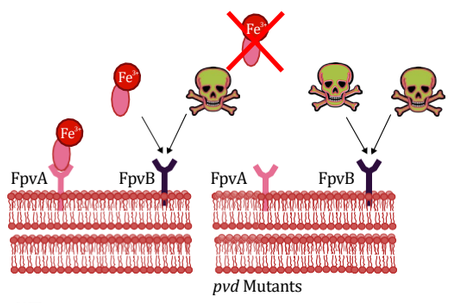
Diffusible toxins targeting siderophore receptors of close relatives point to an S-type pyocin. Bioinformatic analysis found a single S-type pyocin in the U114 genome. Deleting this gene had no effect on the toxin phenotype, suggesting the presence of a previously unknown diffusible toxin.

2. Transposon mutagenesis reveals two different toxin related phenotypes:

A. Exaggerated toxin phenotype

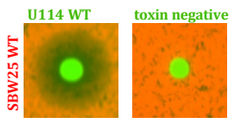


Exaggerated phenotype mutants have transposon insertions in pyoverdine biosynthesis genes. These mutations result in a complete loss of UV ﬿luorescent siderophore production.



Competition for the FpvB receptor between the toxin and pyoverdine might explain the exaggerated phenotype.

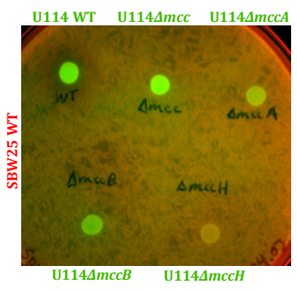
B. Toxin negative phenotype



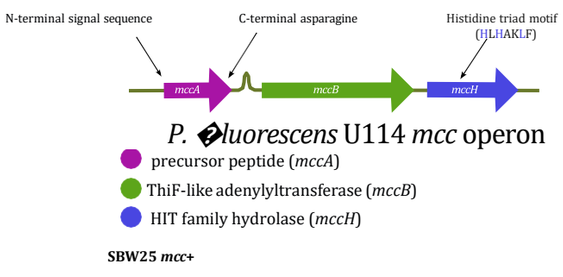
Tn5 insertion in a HIT family hydrolase gene resulted in a toxin negative phenotype. A BLAST search for *mccB* showed similarity to an *E. coli* microcin toxin.

3. ~~Mcc-like operon encodes tox~~icity and immunity

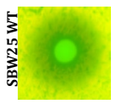
A. *mcc* operon needed for toxicity



Deletion of the entire *mcc* operon results in a no toxin phenotype on a lawn of SBW25. The same no toxin phenotype is generated by deleting *mccA*, *mccB*, and *mccH* individually.

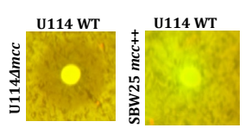


SBW25 *mcc+*



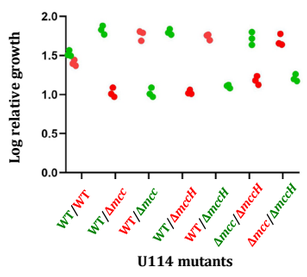
Overexpressing *mcc* in SBW25 confers the ability to produce the toxin, inhibiting growth of SBW25 WT.

B. *mcc* operon needed for immunity



Deleting the *mcc* operon makes U114 sensitive to its own toxin (left). Similarly, SBW25 *mcc* overexpression confers resistance to U114 (right).

C. MccH may prevent self-intoxication

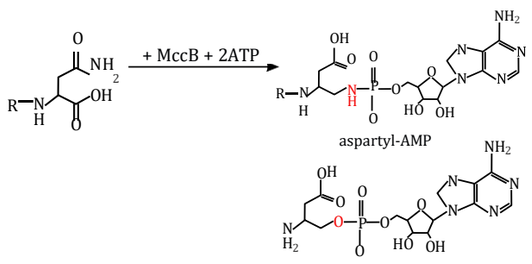


A comparison between WT/Δ*mcc* and WT/Δ*mccH* ﬿itness competition shows that both mutants strains are inhibited by U114 WT similarly. Competing Δ*mcc* and Δ*mccH* shows that Δ*mccH* is inhibited. As Δ*mcc* does not have a functional toxin, this shows that Δ*mccH* producing MccA and MccB has a ﬿itness cost.

4. The toxin targets AspS

C-terminal asparagine

Toxic analog of aspartyl-AMP



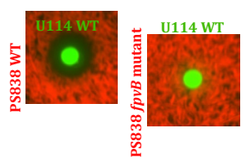
The C-terminal asparagine from MccA is processed by MccB to form a toxic analog of aspartyl-AMP that inhibits protein synthesis by interfering with aspartyl-tRNA-synthetase (AspS). While the P-O bond of aspartyl-AMP is hydrolyzable by AspS, the P-N bond of the toxin is only hydrolyzable by the MccH HIT family hydrolase putative immunity protein.



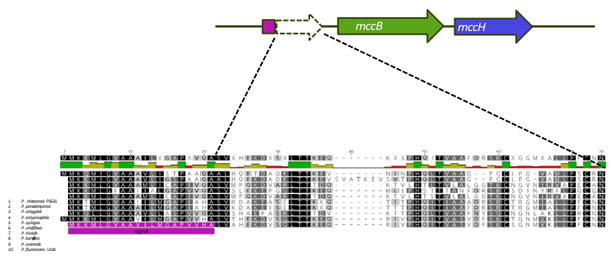
SBW25 WT is sensitive to U114. However, when overexpressing the toxin targets *aspS* in SBW25, the inhibitory effect can be overcome. Overexpressing other tRNA synthetases (like *proS*), leaves SBW25 susceptible to the toxin.

5. Some environmental strains are susceptible to U114

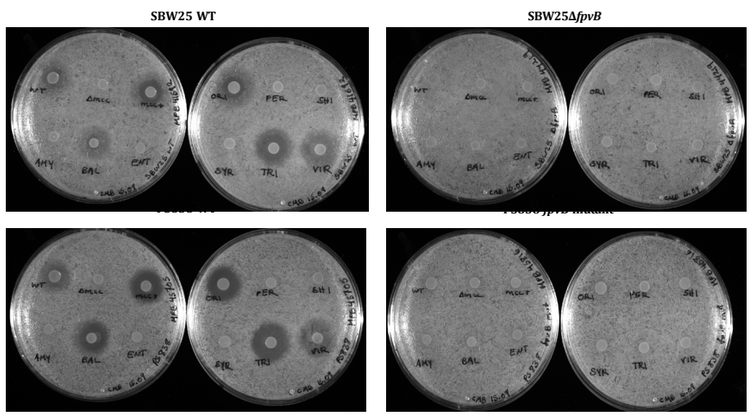
More distantly related environmental *Pseudomonas* strains were tested to see if U114 can target more disparate strains. Among the approximately 60 strains tested, only ﬿ive of the environmental strains are inhibited by U114 (shown in blue). This shows that although this toxin can target distant relatives it is predominantly effective against close relatives. One environmental strain, PS838, showed a very large inhibitory zone. Evolving this strain to be resistant to U114 showed that the entry mechanism of the toxin still relies on an allele of *fpvB*. Strikingly, the nucleotide sequence similarity between the *fpvB* of SBW25 and PS838 is only around 69%. This shows that sensitivity to the toxin cannot be predicated based of off *fpvB* allele sequence similarity, but rather that the tertiary structure of FpvB must play a deciding role in toxin-receptor interaction.



6. The precursor peptide is interchangeable with other *Pseudomonas* microcin-like toxins



Bioinformatic analysis revealed microcin-like toxins widespread in the *Pseudomonas* genus with largely similar operon structure but very high variability in *mccA* sequence similarity. This raises the question of whether *mccA* can frequently aquire mutations to target the wide array of FpvB alleles present in soil environments. For this *mccA* from other *Pseudomonas* strains was put in place of the native U114 *mccA,* but keeping the native *mccB* and *mccH* in place (*mccA* "swap").



Some *mccA* swaps still produce a toxin that exploits FpvB. The phenotypes between the expression plasmids are not identical, suggesting variation in toxin-receptor af￿inity.