CURE Antimicrobial Discovery: Using undergraduate coursework to identify a novel antifungal compound in Pseudomonas

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Tiny Earth
studentsourcing antibiotic discovery

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INTRODUCTION

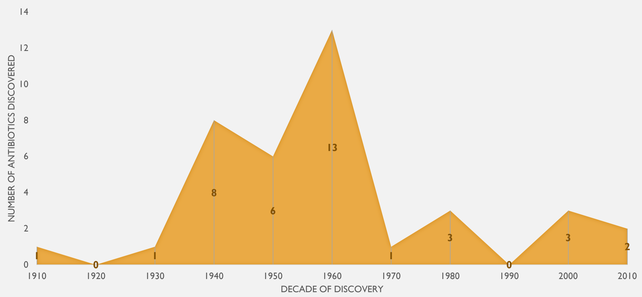


Figure 1: Antibiotic discovery over time. Antibiotic discovery has declined over the past decades due to low incentive for pharmaceutical companies to discover novel antibiotics. Biology 3130 and 4260 uses a CURE format to engage students in science while performing drug discovery research.

- This research is the result of an antibiotic discovery Course-based Undergraduate Research Experience (CURE)
- BIOL 3130, the first course in the series, has students identify, isolate, and perform experiments with *Pseudomonas* strains from soil samples to identify antibiotic producing strains
- In BIOL 4260, the second course, students use bioinformatics to characterize these antibiotic producing strains

METHODS (BIOL 3130)

- *Pseudomonas* strain TE3-3-F2023 was isolated from a soil sample collected in downtown Bowling Green, Ohio
- This strain was found to inhibit the growth of the opportunistic fungal pathogen *Candida parapsilosis*
- Transposon mutagenesis (*Figure 2*) and genome annotation were used to identify the biosynthetic gene cluster (*Figure 3*) responsible for antagonistic activity against *C. parapsilosis*

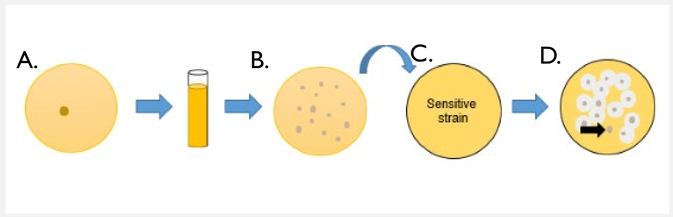


Figure 2: Transposon mutagenesis. (A) The transposable element pBAM1 was randomly inserted into the genome of strain 3-3. (B) *Pseudomonas* mutants were selected on a kanamycin and cetrimide plate. (C) Mutants were replica-plated onto a lawn of *C. parapsilosis*. (D) A mutant unable to inhibit the pathogen was identified and used for linker-mediated PCR to identify the BCG responsible for antagonistic activity.

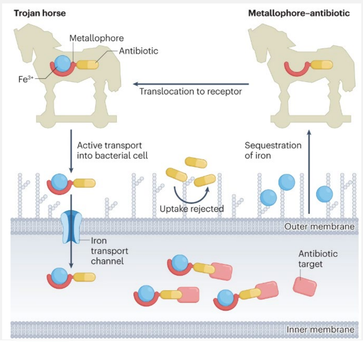
RESULTS (BIOL 4260)

Table 1: Strain 3-3 Genome Statistics	Number of bases	Total gene #	% of total
Total number of bases	5725836		100%
DNA coding number of bases	5128960		89.58%
Protein coding genes		5160	96.72%
RNA genes		104	1.95%
Protein coding genes with function predicted		4334	81.16%

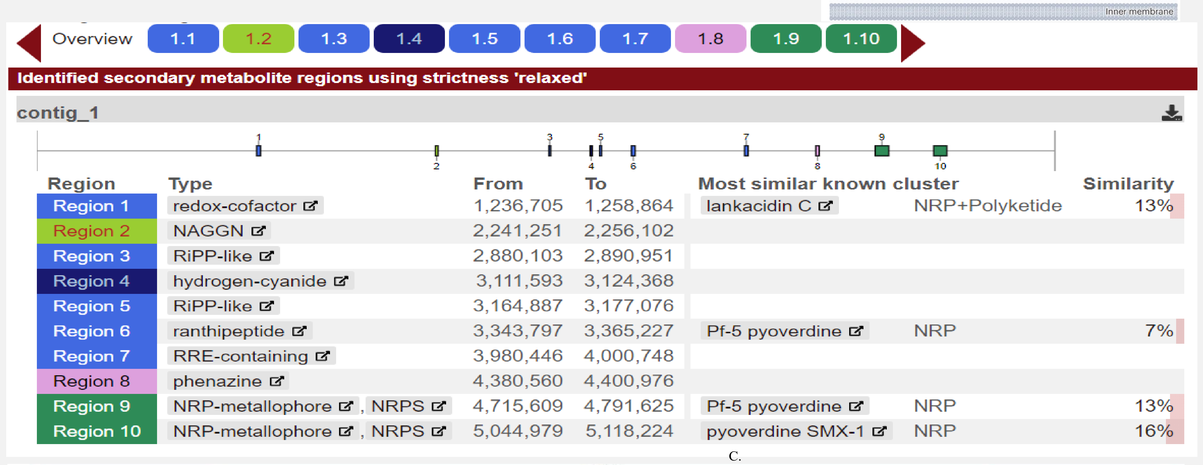
Table 2: Species most similar to strain TE3-3-F23 (query strain)

Subject strain	dDDH	Confidence Interval	G+C content difference
Pseudomonas asiatica JCM 32716T	61.9%	[59.0% -64.7%]	0.3%
Pseudomonas inefficax Pseudomonas sp. JV551A3	55.8%	[53.0% -58.5%]	0.02%
Pseudomonas oryzicola RD9SR1	41.7%	[39.2% -44.2%]	0.07%

C.



A.



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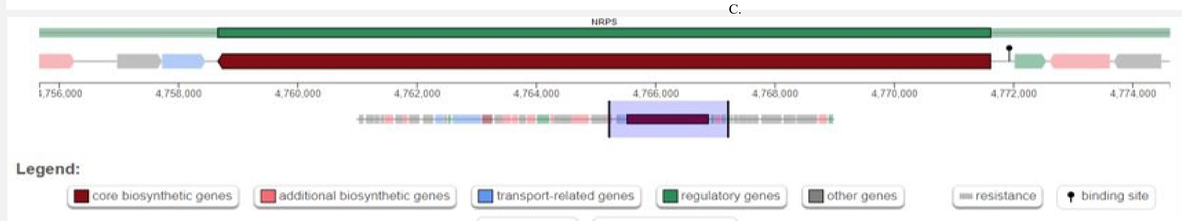

Figure 3: Biosynthetic gene cluster (BCGs) and predicted products involved in antagonistic activity of *Pseudomonas* strain sp. TE3-3-F2023. (A) Overview of predicted BCGs identified using antiSMASH. A total of 10 gene clusters were identified. (B) Specific BCG involved in activity was identified by linker-mediated PCR, which aligned to genome coordinates 4770426-4770798, of Region 9. (C) An NRP-metallophore was predicted to be the product. Metallophores are produced by cells to collect metals from surrounding environments. It is predicted that these can be paired with an antibiotic compound and act as a 'Trojan horse', inhibiting microbes.

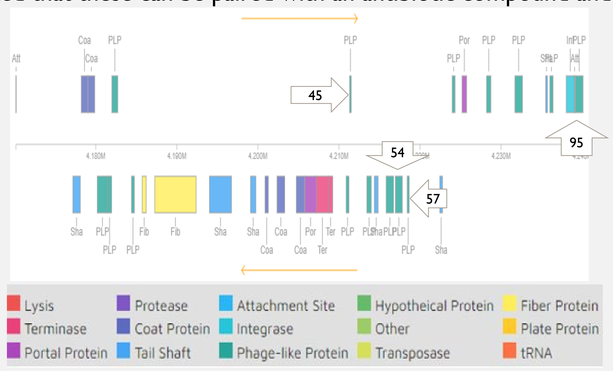

Figure 4: Prophage genome within the TE3-3-F2023 genome using PHASTER. One predicted intact prophage was identified. Open reading frame (ORF) of interest include region 45 identifies a toxin-antitoxin system. ORP 54 identifies a phage antirepressor protein. ORP 57 identifies a Cro receptor. ORP 95 identifies PBP 6B.

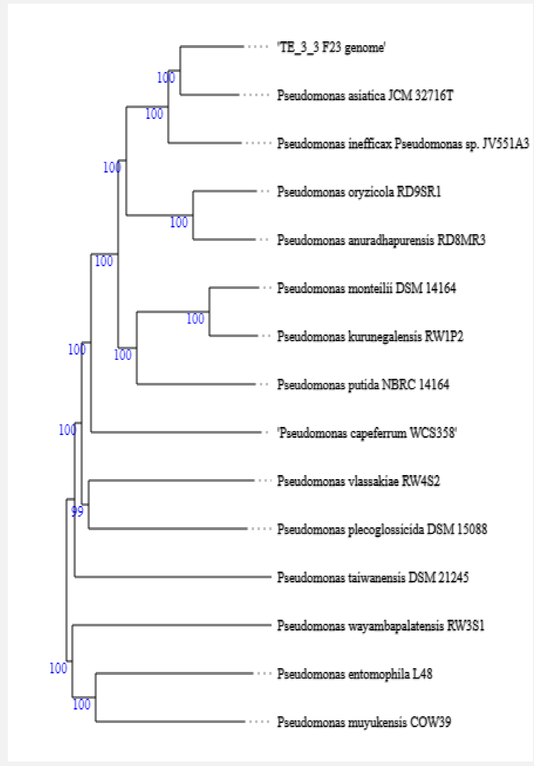

Figure 5: A phylogenetic tree of strain TE3-3-F2023 and similarity to related species. *Pseudomonas asiatica* is the most similar type strain. However, strain TE3-3-F2023 may be novel species (Table 2).

CONCLUSIONS

- From BIOL 3130 and 4260, strain TE3-3-F2023 was characterized
- Antifungal activity was noticed against *Candida parapsilosis* during BIOL 3130
  - This opportunistic pathogen is a major cause of tissue infections and sepsis in humans.
  - Only 10 antifungal drugs exist on the market
- Antifungal compounds are difficult to develop, as their anti-eukaryotic cell nature is often harmful to humans
- Antifungals which use metallophore transport have not yet been explored
  - This warrants additional research into strain 3-3's antifungal properties for potential human use.