Exploring the Role of a DUF262 Domain-Containing Protein in Antibiotic Cross-Resistance in *Pseudomonas fluorescens*

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Introduction

Antibiotic resistance is a growing issue, limiting the efficacy of current treatments. A contributing factor is pleiotropic resistance in which a single mutation or mutations allow resistance to structurally and functionally unrelated toxic compounds. This research explores pleiotropic resistance between diacetyl and antibiotics.

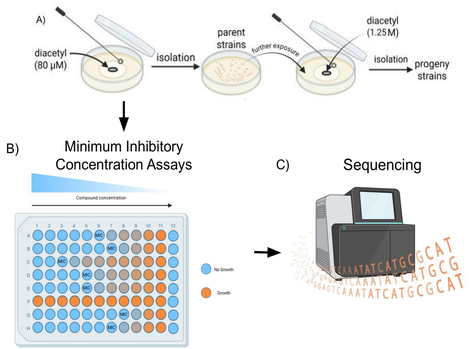
Diacetyl, a byproduct of bacterial fermentation, is found in food products such as cheese, wine, and beer. It also has biocidal properties, with potential mechanisms of action including:

- the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which damage cellular structures
- acetylation of arginine, inactivating enzymes and other proteins.[2]

Hypothesis: Mutations allowing improved survivability in the presence of diacetyl may also cause less sensitivity to antibiotics.

Studying these mutations can reveal new mechanisms by which bacteria adapt to environmental stressors and resist the effects of antibiotics.

Methods



A) Diacetyl-adapted strains were isolated by sequential exposure of wild-type *Pseudomonas fluorescens* 225 to increasing concentrations of the biocide to generate "parent" and "progeny" strains.

B) Minimum inhibitory concentration assays (MICs) test bacterial response to several dilutions of an antibiotic.[3]

C) *Pseudomonas* strains that exhibited pleiotropic resistance were sequenced alongside the parent strains.

The effect of mutations on DUF 262 protein structure were predicted using AlphaFold Server and visualized in PyMOL.

Unique mutations will be generated in the wild-type strain through a gene editing process known as multiplex automated gene engineering (MAGE) which requires an accessory plasmid. The plasmid was transformed into *E. coli* for amplification and confirmed with a restriction digest.

Results

MIC assays: *P. fluorescens 225*, and the 1.2C and 3.2C parent strains were inhibited by 32 µg/mL chloramphenicol. Three of the 24 tested progeny strains exhibited increased resistance to chloramphenicol as noted by the higher MIC.

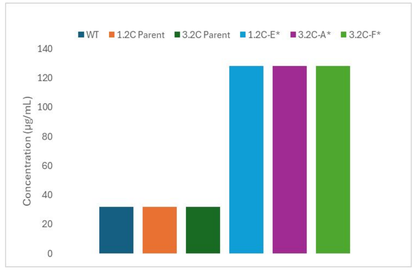


Figure 1. Identification of chloramphenicol resistant strains. Concentration range of chloramphenicol was from 0.25-128 µg/mL. Asterisk (*) indicates growth in highest concentration of antibiotic tested.

DUF262: The DUF262 gene, which has homology to a Type IV restriction endonuclease, was mutated in both progeny lines (1.2C=L189P; 3.2A/F =F193L). This suggests that the protein and the specific region play an important role in pleiotropic resistance.

Structural predictions of the protein/DNA structure indicated:

- Protein dimerization
- Ability to bind DNA.
- Differential DNA binding of wild-type and mutant proteins

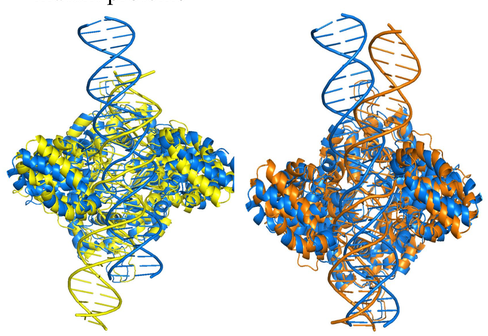


Figure 2. Mutant DUF262 protein binds DNA differently than the wild-type protein. The wild-type protein is shown in blue, 1.2C–E progeny proteins are shown in yellow, and 3.2C-A/F progeny proteins are shown in orange. All proteins were bound to the same random 28 base pair DNA fragment.

Sequencing: Among many mutations in the progeny strains, a few were considered:

Strain	2- amino-thiazoline-4-carboxylic acid hydrolase (GOF: CT→C)	DUF262 domain-containing protein (SNP: T→C, **F193L**)	DUF262 domain-containing protein (SNP: T→C, **L189P**)
1.2C	X		
3.2C	X		
1.2C-E	X		X
3.2C-A	X	X	
3.2C-F	X	X	

Table 1. Nonsynonymous mutations in parent strains and three antibiotic resistant progeny strains. Not all mutations are shown; 1.2C-E had a total of 25 mutations, 3.2C-A with 8 mutations, and 3.2C-F with 29 mutations.

Restriction Digest: MAGE will be used to generate the F193L mutation in a wild-type *P. fluorescens 225* to assess its role in the pleiotropic resistant phenotype. The EVA2514-R9-mutLE36KPP plasmid was transformed into *E. coli* for amplification. To confirm plasmid identification, plasmid DNA was extracted and subject to restriction digest analysis.

- BamHI produced the expected 680 bp fragment
- EcoRI produced the expected 3 fragments of 400–500 bp.

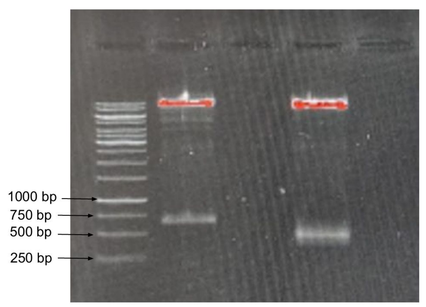


Figure 3. Restriction digest confirms successful plasmid identification Gel electrophoresis shows the expected fragment sizes for EVA2514-R9-mutLE36KPP following restriction enzyme digestion. Lane 1: 1 kb DNA ladder. Lane 2: BamHI digest. Lane 3: EcoRI digest

Conclusions

1) Some level of pleiotropic resistance can occur between food preservatives like diacetyl and antibiotics.
2) Independent appearance of mutations in the DUF262 gene suggests a functional role of this protein in the pleiotropic resistance phenotype. However, other genetic changes in the mutant strains could contribute to the phenotype. Future steps will include generating the single F193L mutation in the *P. fluorescens 225* background using multiplex automated gene engineering.[5]

Overall, the results suggest that the mechanisms that allow bacteria to adapt to non-antibiotic biocides may also contribute to a loss of antibiotic sensitivity; these may involve genes not yet known to be involved in antibiotic cross-resistance.

References

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