Characteristics of Mutants of *Pseudomonas fluorescens* Pf-5 Deficient in Production of the Antibiotic Pyoluteorin

OSU
SBI Internship

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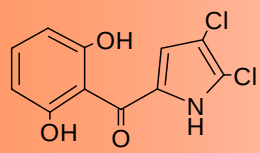
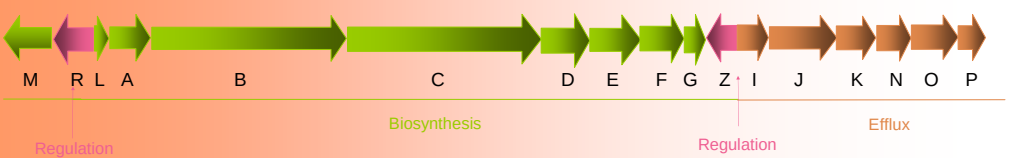
Introduction

The rhizosphere bacterium *Pseudomonas fluorescens* Pf-5 is a biological control agent that suppresses plant disease caused by soil fungi and Oomycetes. For example, Pf-5 suppresses Pythium damping off caused by the Oomycete pathogen *Pythium ultimum*.

Biological control of Pythium damping-off. An equal number of cotton seeds were planted in both flats, but the seeds on the right were treated with the *Pseudomonas fluorescens* Pf-5 whereas the seeds on the left were untreated. Pf-5 protects the seeds from infection by *P. ultimum*, resulting in greater numbers of healthy seedlings in the flat on the right.



Pf-5 produces many secondary metabolites, including the antibiotic pyoluteorin, which is toxic to *Pythium ultimum* and other Oomycetes.



A 33kb gene cluster (*pltA-pltR*) is responsible for pyoluteorin biosynthesis and efflux (Thompson et al., 1999).

Table 1. Mutants deficient in pyoluteorin production were obtained by transposon Tn5 mutagenesis in an earlier study (Kraus and Loper, 1992), but several of the mutants (highlighted in Table 1) were not characterized previously.

Tn5 linkage Group	Strain	Insertion Location	Size (kb) of Tn5 containing fragment	
			*Eco*RI	*Bam*HI
Plt-III	JL4171	*liuE**	12.7	9.4, 6.2
Plt-IV	JL4175	*pltC*	18.3	22.5, 7.8
	JL4274	*pltC*	18.3	18.3, 12.1
	JL4211	*	18.3	22.3, 9.9
Plt-V	JL4293	*	24.8	10.9, 7.2
	JL4134	*	24.8	12.1, 7.2
	JL4139	*sucC*	24.8	12.1, 7.2

* Mutants evaluated by this study

Objective

Characterize mutants of *P. fluorescens* Pf-5 deficient in pyoluteorin production.

Results

Mutant JL4211

- The genomic DNA flanking the Tn5 insertion in mutant JL4211 was cloned previously.
- I sequenced the cloned DNA using primers internal to Tn5.
- Sequence analysis showed that the Tn5 was in *pltC*, and that a 9 nucleotide region of *pltC* was duplicated at the borders of the Tn5 insertion (Figure 1).

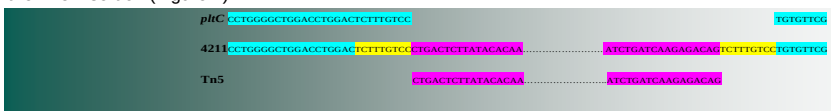


Figure 1. Sequence analysis of the Tn5 insertion in JL4211. The sequence of *pltC* is shown in blue (top row). The sequence of Tn5 is shown in fuchsia (bottom row). A 9 nucleotide region of *pltC* that is duplicated at the borders of theTn5 insertion in JL4211 is shown in yellow (center row).

Mutants JL4134 and JL4293

- Mutants JL4134 and JL4293 were known to be in the same class as the *sucC* mutant JL4139, based on the size of *Eco*RI and *Bam*HI genomic fragments containing the Tn5 insertion (Table 1).
- I used PCR to determine if the Tn5 insertions in JL4134 and JL4293 were in the *sucC* gene.
- The PCR analysis demonstrated that mutants JL4134 and JL4293 have a Tn5 insertion in *sucC*, but the Tn5 insertion in JL4293 is at a different site within *sucC* than the Tn5 insertion of JL4139 and JL4134 (Figure 2).
- I then sequenced the PCR products from JL4134 and JL4293 to determine the precise sites of the Tn5 insertions.
- Sequence analysis showed that the Tn5 insertion in JL4293 is 212 bp from the Tn5 insertions in JL4139 and JL4134 (Figure 3).

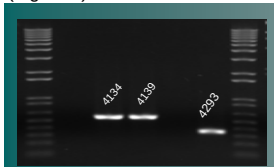


Figure 2. PCR analysis of mutants JL4134 and JL4293. Using one primer complementary to *sucC* and a second primer complementary to Tn5, PCR products were obtained from the genomic DNA of mutants JL4134, JL4293, and JL4139. Differences in sizes of the PCR fragments indicate that the Tn5 insertions in JL4293 is at a different location within *sucC* than the Tn5 insertion in JL4139.

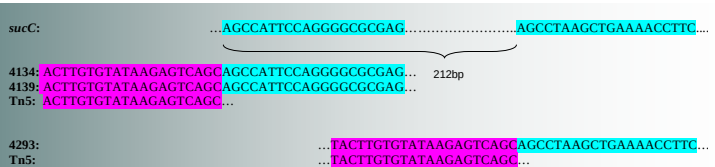


Figure 3. Sequence analysis of the Tn5 insertions in JL4134, JL4293, and JL4139. The sequence of two portions of *sucC* is shown in blue (top row). The sequence of the terminus of Tn5 is shown in fuchsia. Analysis of the sequences of the borders between the Tn5 insertions and genomic DNA for the three mutants show that the insertion sites are 212 bp apart.

Mutant JL4171

- Previously, the Tn*5* insertion in mutant JL4171 was mapped to *liuE.*
- Recently, *liuE* was shown to be essential for the catabolism of leucine and cyclic terpenes (such as citronellol and isovalerate) in *Pseudomonas aeruginosa* (Förster-Fromme et al., 2006).
- I tested JL4171 for growth on these compounds.
- Pf-5 grew on leucine, citronellol, and isovalerate whereas the *liuE* mutant JL4171 did not grow on these compounds (Table 2).

Table 2: Use of carbon sources by Pf-5 and JL4171.

Strain	Growth On			
	Succ (0.6%)	Leu (1%)	CL (0.6%)	IsoV (0.06%)
Pf-5	++	++	+	+
JL4171	++	--	--	--

Bacteria were incubated on solid 925medium with the following carbon sources at 27C for 48 hours: Succinate 0.6% (Succ), Leucine 1% (Leu), Citronellol 0.6% (CL), and Isovalerate 0.06%, Good growth (++) growth (+) and no growth (--) as indicated.

Conclusions

- Mutant JL4211 has a Tn5 insertion in *pltC.*
- Mutants JL4134 and JL4139 each have Tn5 insertion in *sucC.*
- Mutant JL4171 does not grow on leucine, citronellol or isovalerate, due to a mutation in *liuE.*

References

Förster-Fromme, et al. 2006. Applied and Environmental Microbiology. 72:4821-4823.

Kraus, J. and Loper, J. 1991. Molecular Plant Pathology. 82:265-266.

Thompson, et al. 1999. Journal of Bacteriology. 18:2169.

Acknowledgements

This project was funded by the Oregon State University Subsurface Biology Initiative through an undergraduate internship to Megan Kelly. Special thanks to the Loper lab, Joyce Loper, Brenda Shaffer, Marcella Henkels, Virginia Stockwell, and Marci Sams.