Characterization of the Bacterial Stem Blight Pathogen of Alfalfa, *Pseudomonas syringae* pv. *syringae* ALF3

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ABSTRACT

Bacterial stem blight of alfalfa occurs sporadically in the central and western U.S. Yield losses of up to 50% of the first harvest can occur with some cultivars. Developing resistant cultivars is hampered by lack of information on the pathogen and a standard test for evaluating plant germplasm. Bacteria producing a fluorescent pigment were isolated on King's B agar from alfalfa with symptoms of bacterial stem blight from near Cheyenne, WY. The strain ALF3 was tentatively identified as *Pseudomonas syringae* pv. *syringae* based on 16S rDNA sequence and PCR amplification of *syrB* for lipodepsinonapeptide toxin production. Multilocus sequence analysis indicated that ALF3 falls within a clade containing strains of *P. syringae* pv. *syringae* with closest affinity to FF5 from pear. Comparison of a draft whole-genome sequence of ALF3 further confirmed that ALF3 most closely resembles FF5 (~96% sequence identity) and *P. syringae* pv. *aptata* DSM50252 from beet. ALF3 was highly pathogenic to snapbean pods but caused only mild symptoms on leaves of snapbean, pear, and sugarbeet. Alfalfa cultivars with fall dormancy ratings of 1 and 2 had higher percentages of resistant plants than cultivars with fall dormancy ratings of 8-11.

INTRODUCTION

- Bacterial stem blight is caused by *Pseudomonas syringae* pv. *syringae*, one of the best characterized plant pathogens. However, little is known about the strain that infects alfalfa.
- The disease is widespread in the central and western US.
- First harvest losses of 40-50% have been reported from western mountainous regions.
- The disease follows late spring freezes. The bacterium enters through frost cracks.
- Yield loss is usually blamed on frost injury.
- Diseased plants are stunted with brittle stems.
- Stem lesions are amber and glisten from dried bacterial exudate.
- Leaves become water-soaked and yellow.

MATERIALS AND METHODS

Isolation of the Bacterium

- Bacteria were isolated on King's B, produced a fluorescent pigment, and were ice nucleation active.
- Bacteria were positive by PCR for *syrB* (2) and were confirmed to be *P. syringae* by 16S DNA sequence.
- Multilocus sequence analysis using seven housekeeping genes (1) was used for cluster analysis.
- DNA was purified and used for whole genome sequencing and compared to previously sequenced strains (3).

Plant Inoculation

- Alfalfa plants: Plants were grown to the 3-leaf stage. Stems were wounded with a 22 gauge needle then a bacterial suspension (OD_{600}=0.1) was applied with a sponge. Symptoms were rated 7 days post-inoculation.
- Bean leaves: Leaves were dusted with carborundum powder then swabbed with a bacterial suspension (OD_{600}=0.1).
- Bean pods: Pods were surfaced disinfested and placed in a moist chamber. Wounds were made with a 22 gauge needle and a 10 µl drop of bacteria (OD_{600}=0.1) placed on the wound.
- Pear leaves: Leaves were surfaced disinfested and placed in a moist chamber. Wounds were made on the midrib with a scalpel blade and a 10 µl drop of bacteria (OD_{600}=0.1) placed on the wound.
- Beet seedlings: Wounds were made with a 22 gauge needle and a 10 µl drop of bacteria (OD_{600}=0.1) placed on the wound.

RESULTS

Multilocus sequence analysis

Sequences were obtained for *acn1*, *cts*, *gapA*, *pgi*, *rpoD*, *gyrB* and *pfk*. The ALF3 strain clustered with in the *P. syringae* 2b clade and was most closely related to *P. syringae* pv. *syringae* FF5 (pear pathogen).

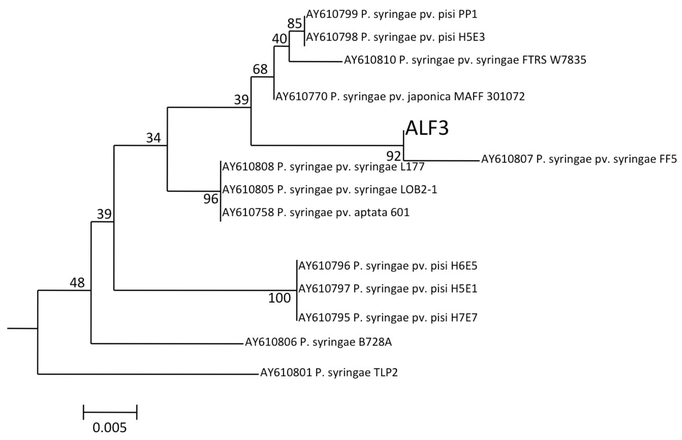

Figure 1. Cluster analysis of *gryB* sequences.

Whole genome comparisons

ALF3 is most closely related to *P. syringae* pv. *syringae* FF5 and *P. syringae* pv. *aptata* DSM50252 (beet pathogen). Approximately 60 genes are unique to ALF3 and are not found in the closely related strains. Many of these genes appear to be phage-associated. ALF3 has several predicted genes in the T3SS cluster that are not conserved in the other strains.

Disease symptoms

- ALF3 caused mild disease symptoms on bean leaves, beet leaves and pear leaves.
- Significant water soaking on bean pods occurred 2 days post-inoculation.
- Chlorosis of alfalfa leaves above the inoculation site occurred 2 days post-inoculation followed by necrosis of apical leaves at 5 days. Necrosis at the site of inoculation was visible at 2 days post-inoculation.

Figure 2. Symptoms on beet (left), bean pods (center), and pear leaves (right). M= Mock-inoculated. Pss=*P. syringae* pv. *syringae* 35 (bean pathogen). ALF3=*P. syringae* pv. *syringae* ALF3.

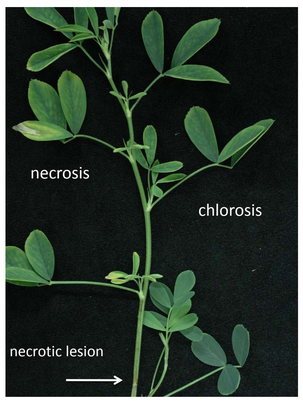

Figure 3. Symptoms on alfalfa.

Table 1. Average disease severity and percent resistant plants in check cultivars for fall dormancy (FD) and winter survival (WS). Disease was rated on a 1 to 5 scale. A rating of 1 or 2 was considered resistant.

Check	Cultivar	Avg. Disease Severity	Percent Resistant Plants
FD1	Maverick	2.3	60
FD2	Vernal	2.7	36
FD3	5246	3.2	0
FD5	Archer	3.1	11
FD6	ABI700	3.7	0
FD7	Dona Ana	3.5	10
FD8	Pierce	3.5	18
FD9	CUF101	3.9	0
FD10	UC1887	4.1	0
FD11	UC1465	4.2	0
WS1	ZG9830	2.2	60
WS2	5262	2.8	33
WS3	WL325HQ	3.0	17
WS4	G-2852	3.6	0

CONCLUSIONS

- ALF3 is most closely related to *P. syringae* pv. *syringae* FF5 that causes disease on pear and *P. syringae* pv. *aptata*, that causes disease on beet.
- In controlled conditions, ALF3 causes mild symptoms on beet, bean pods, and pear leaves.
- ALF3 is highly pathogenic on alfalfa with symptoms developing 2 days after inoculation.
- Cultivars with greater fall dormancy have greater resistance to ALF3 than cultivars with lesser fall dormancy.

REFERENCES

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