

ENDOPHYTIC BACTERIAL POPULATIONS IN GRAPEVINES RESISTANT TO PIERCE'S DISEASE OF GRAPEVINE

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Reporting Period: The results reported here are from work conducted May 2006 to September 2007

ABSTRACT

A specific strain of the bacterium *Xylella fastidiosa* causes Pierce's Disease of grapevine (PD). This disease has caused significant disruption to the wine industry centered in the Temecula, California region; at the height of the most recent PD epidemic in the late 1990s, 25% of the grapevines in this area were lost before emergency quarantine and control measures could be instituted. Under these circumstances, the 2006 discovery of a population of apparently PD-resistant grapevines in the area was of particular interest. The vines were all located in a single vineyard, which had total PD-related losses of approximately 10%, while a neighboring vineyard suffered a nearly 100% loss of the same variety. In addition, a similar phenomenon was observed in a grapevine population located in the Agricultural Operations grounds at the University of California, Riverside. While the cause of this apparent resistance is unknown, one possible explanation for this resistance is that it is being conferred by certain endophytic bacteria present in resistant vines but not in susceptible vines. In order to test this hypothesis, cane samples from both the apparently susceptible populations and the apparently resistant populations were surface sterilized and plated onto standard microbiological media. Any observed bacterial growth was diluted into standard liquid media and then streaked out in order to obtain pure cultures, which were identified using 16S sequencing.

INTRODUCTION

Specific strains of the bacterium *Xylella fastidiosa* (Xf) cause disease in almonds, grapevines, and a variety of other economically important plants (1,2,3). Xf is spread by the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, formerly known as *H. coagulata* (4,5).

In grapevines, one strain of this bacterium is the cause of Pierce's Disease (PD). Since the preferred host of GWSS is citrus, vineyards close to a citrus grove are at increased risk for the development of PD (6). In addition, Chardonnay vines are known to be more susceptible to PD than other varieties (7). The Weaver vineyard is planted with Chardonnay vines and is immediately across from two citrus groves, meaning that it is at high risk of developing PD. However, while adjacent Chardonnay vineyards suffered catastrophic crop failure, the Weaver vineyard had a PD-related loss of far less, approximately 10%. This observation was of special interest since many of the plants in this vineyard were old enough to have survived the initial PD epidemic that occurred after the GWSS was accidentally introduced into California. The Agricultural Operations vineyard at the University of California, Riverside contains both symptomatic and asymptomatic Chardonnay vines in close proximity. These vines are younger than the ones at the Weaver vineyard.

One possible explanation for this phenomenon is that it is being conferred by bacterial endophytes that live inside the apparently resistant plants but not in the more susceptible plants. The endophytic bacterium, *Curtobacterium flaccumfaciens* has already been shown to confer resistance to Xf in sweet orange plants (8).

To test this hypothesis, cane samples from asymptomatic and symptomatic grapevines at both locations were surface-sterilized and then plated on microbiological media. The genus of any resulting bacterial growth was then identified using 16S gene sequencing. The 16S gene has been widely used to classify unknown organisms (9). Because this gene evolves very slowly, it is most useful for classifying organisms at the genus level, but not at the species or subspecies level (10). Even so, it is widespread practice to include a species name when identifying bacteria based on this sequence. These designations can be considered putative in nature.

OBJECTIVES

The primary goal of this research was and continues to be to test the initial hypothesis through isolating bacterial endophytes from asymptomatic and symptomatic grapevines at both locations and using 16S sequencing to identify them.

RESULTS

Table 1 lists currently identified endophytic bacteria isolated from symptomatic and asymptomatic vines at the Weaver vineyard and the UCR Agricultural Operations vineyard (AgOps). Two pieces of data from the BLAST results are also included with the identifications; one is the bit score and the other is the E-value. Typically, the lower the E-value, the higher the probability that the similarities in the two sequences are due to a close genetic relationship and not to random chance. All species designations included are considered putative because 16S analysis does not permit resolution below the genus level.

Table 1

Code	Genus	Putative Species	Bit Score	E-value	Symptomatic	Source
45V16 2D C1	<i>Bacillus</i>	<i>niacini</i>	863	0	N	Weaver
45V16 2E C1	<i>Bacillus</i>	<i>thuringiensis</i>	2771	0	N	Weaver
46V16 2D C1	<i>Bacillus</i>	<i>cereus</i>	500	7.00E-138	N	Weaver
46V19 1C C7	<i>Bacillus</i>	<i>massiliensis</i>	2454	0	N	Weaver
46V19 2D C1	<i>Bacillus</i>	<i>cereus</i>	2736	0	N	Weaver
46V19 2F C1	<i>Bacillus</i>	M4	2605	0	N	Weaver
47V1 1B C1	<i>Bacillus</i>	LMG 20241	1844	0	N	Weaver
47V1 1C C2	<i>Bacillus</i>	<i>cereus</i>	2389	0	N	Weaver
47V1 1F C1	<i>Bacillus</i>	<i>thuringiensis</i>	2365	0	N	Weaver
47V1 2C C3	<i>Bacillus</i>	9B_1	2692	0	N	Weaver
47V3 1B C1	<i>Bacillus</i>	MB-9	2351	0	N	Weaver
47V3 1C C2	<i>Bacillus</i>	<i>drentensis</i>	1015	0	N	Weaver
47V3 1E C12	<i>Bacillus</i>	LMG 20241	401	2.00E-108	N	Weaver
47V3 2D C13	<i>Bacillus</i>	<i>gibsonii</i>	1009	0	N	Weaver
47V3 O C3	<i>Planococcus</i>	<i>maitrii</i>	2609	0	N	Weaver
47V8 1A C9	<i>Bacillus</i>	<i>cereus</i>	2561	0	Y	Weaver
47V8 R6	<i>Bacillus</i>	<i>thuringiensis</i>	2627	0	Y	Weaver
48V10 1B C1	<i>Bacillus</i>	<i>pumilus</i>	2591	0	N	Weaver
48V10 1B C2	<i>Bacillus</i>	EP23	2407	0	N	Weaver
48V15 1C C3	<i>Bacillus</i>	<i>cereus</i>	1084	0	N	Weaver
48V15 1D C2	<i>Bacillus</i>	<i>licheniformis</i>	2605	0	N	Weaver
48V15 2A C7	<i>Bacillus</i>	ge15	910	0	N	Weaver
48V19 2F C2	<i>Bacillus</i>	<i>thuringiensis</i>	2379	0	N	Weaver
49V9 1A C2	<i>Bacillus</i>	<i>subtilis</i>	979	0	N	Weaver
49V9 1C C2	<i>Bacillus</i>	<i>niacini</i>	2533	0	N	Weaver
49V9 1C C3	<i>Bacillus</i>	<i>niacini</i>	2670	0	N	Weaver
49V9 1D C3	<i>Bacterium</i>	8-gu2-10	880	0	N	Weaver
49V9 1F C1	<i>Bacillus</i>	<i>cereus</i>	1203	0	N	Weaver
49V9 1F C2	<i>Bacillus</i>	<i>cereus</i>	2660	0	N	Weaver
49V9 1F C7	<i>Bacillus</i>	<i>cereus</i>	1154	0	N	Weaver
49V9 2B C1	<i>Bacillus</i>	<i>cereus</i>	2577	0	N	Weaver
49V9 2C C1	<i>Bacillus</i>	GB02	650	0	N	Weaver
A-4 1A C1	<i>Erwinia</i>	<i>tasmaniensis</i>	722	0	N	AgOps
A-4 1E C2	<i>Bacillus</i>	<i>pumilus</i>			N	AgOps
A-4 2A C1	<i>Bacillus</i>	<i>endophyticus</i>	1352	0	N	AgOps
A-4 2A C12	<i>Bacillus</i>	<i>licheniformis</i>	2448	0	N	AgOps
B-3 1B C1	<i>Bacillus</i>	<i>thuringiensis</i>	2674	0	Y	AgOps
B-3 1D C2	<i>Bacillus</i>	<i>megaterium</i>	2750	0	Y	AgOps
B-3 2C C6	<i>Bacillus</i>	<i>megaterium</i>	2710	0	Y	AgOps
B-3 2C C7	<i>Paenibacillus</i>	<i>illinoisensis</i>	1070	0	Y	AgOps
C-1 1D C12	<i>Staphylococcus</i>	<i>epidermidis</i>	2545	0	N	AgOps
C-1 1E C1	<i>Bacillus</i>	<i>acidicola</i>	1029	0	N	AgOps
C-1 2A C6	<i>Bacillus</i>	<i>pumilus</i>	989	0	N	AgOps
C-1 2B C12	<i>Bacillus</i>	LMG 20241	946	0	N	AgOps
C-1 2B C13	<i>Bacillus</i>	<i>subtilis</i>	2441	0	N	AgOps
C-1 2D C12	<i>Bacillus</i>	<i>pumilus</i>	2753	0	N	AgOps
D-7 2D C12	<i>Bacillus</i>	<i>pumilus</i>	2640	0	N	AgOps
D-7 2F C16	<i>Erwinia</i>	<i>psidii</i>	543	6.00E-151	N	AgOps
E-1 2F C16	<i>Erwinia</i>	<i>tasmaniensis</i>	2127	0	Y	AgOps
F-15 1D C12	<i>Bacillus</i>	M31	2246	0	Y	AgOps
F-15 1D C7	<i>Pantoea</i>	MMB047	852	0	Y	AgOps
G-6 1A C2	<i>Bacillus</i>	<i>megaterium</i>	2020	0	N	AgOps

Table 1 (continued)

Code	Genus	Putative Species	Bit Score	E-value	Symptomatic	Source
G-6 1B C16	<i>Bacillus</i>	LMG 20241	385	3.00E-103	N	AgOps
G-6 1E C13	<i>Bacillus</i>	<i>subtilis</i>	1076	0	N	AgOps
G-6 1F C12	<i>Bacillus</i>	<i>licheniformis</i>	2561	0	N	AgOps
G-6 2D C2	<i>Bacillus</i>	M31	2246	0	N	AgOps
G-6 2E C8	<i>Bacillus</i>	<i>pumilus</i>	722	0	N	AgOps
G-6 2F C6	<i>Bacillus</i>	<i>oleronius</i>	2629	0	N	AgOps
G-6 2F C16	<i>Staphylococcus</i>	<i>caprae</i>	562	9.00E-137	N	AgOps
H-11 1D C2	<i>Bacillus</i>	PC1	2658	0	N	AgOps
H-11 1E C2	<i>Bacillus</i>	<i>benzoevorans</i>	2520	0	N	AgOps
H-11 2C C7	<i>Bacillus</i>	<i>benzoevorans</i>	1061	0	N	AgOps
I-6 1C C12	<i>Bacillus</i>	<i>herbersteinensis</i>	1110	0	Y	AgOps
J-9 1A C2	<i>Bacillus</i>	<i>pumilus</i>	2789	0	Y	AgOps
J-9 1A C7	<i>Bacillus</i>	<i>pumilus</i>	997	0	Y	AgOps
J-9 1C C1	<i>Brevibacillus</i>	<i>laterosporus</i>	2678	0	Y	AgOps
J-9 2A C16	<i>Erwinia</i>	<i>tasmaniensis</i>	2452	0	Y	AgOps

CONCLUSIONS

Members of the genus *Bacillus* were most frequently isolated from both symptomatic and asymptomatic plants at the Weaver Vineyard and the Agricultural Operations Vineyard. Bacteria tentatively identified as *Bacillus thuringiensis* were isolated slightly more frequently from asymptomatic vines than from symptomatic vines at both locations. Other bacteria much less commonly isolated from these populations included members of *Staphylococcus*, *Pantoea*, *Brevibacillus*, and *Planococcus*.

There also appeared to be several location-specific effects. For example, bacteria that were tentatively identified as *Bacillus cereus* were isolated solely from asymptomatic vines found at the Weaver Vineyard but were not recovered from any vines in the Agricultural Operations Vineyard. In addition, bacteria tentatively identified as *Bacillus pumilus* were much more frequently isolated from asymptomatic and symptomatic vines at the Agricultural Operations Vineyard than from either symptomatic or asymptomatic vines at the Weaver Vineyard. Finally, members of the genus *Erwinia* were also commonly isolated from symptomatic and asymptomatic vines growing at the Agricultural Operations Vineyard. However, members of this genus have not yet been isolated from any vines in the Weaver Vineyard. The reasons for these differences remain unclear.

REFERENCES

1. Davis, M. *et al.* 1980. *Phytopathology* 70: 472-475
2. Davis, M. *et al.* 1978. *Science* 199: 75-77
3. Purcell, A. *et al.* 1999. *Phytopathology* 89: 53-58
4. Redak, R. *et al.* 2004. *Annu. Rev. Entomol.* 89: 243-270
5. Takiya, D. *et al.* 2006. *Ann. Entomol. Soc. Amer.* 99(4):648-655
6. Perring, T. M. *et al.* 2001. *Calif. Agric.* 55:13-18.
7. UC IPM Online. <http://www.ipm.ucdavis.edu/MG/r302101211.html>
8. Lacava, P. *et al.* 2004. *Lett. Appl. Microbiol.* 39: 55-59.
9. Turner, S. (1997). Molecular Systematics of Oxygenic Photosynthetic Bacteria. *Origin of Algae and Their Plasmids*. D. Bhattacharya. New York, Springer-Verlag.
10. Weisburg, W. *et al.* 1991. *Journal of Bacteriology* 173: 697-703.
11. Zchori-Fein, E. *et al.* 2004 *Int. J. Syst. Evol. Microbiol.* 54: 961-968.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.