



Transgenic disease resistance in *Vitis vinifera*: potential use and screening of antimicrobial peptides

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Background. Anti-microbial peptides (or antimicrobials) are naturally occurring, small proteins produced by plants and animals to fight off bacterial and fungal infections, much like antibiotics do in humans. Grapes are susceptible to a host of bacterial and fungal pathogens, including those that cause powdery mildew, bunch rot, and crown gall disease. One option for improving disease resistance is through transgenic grapevines, but first the most effective antimicrobials must be identified. Here we report on testing in the lab of nine antimicrobials on key pathogens of grape: powdery mildew (*Erysiphe necator*), bunch rot (*Botrytis cinerea*), and crown gall (*Agrobacterium vitis* and *Agrobacterium tumefaciens*). In addition, we report on crown gall resistance in transgenic Chardonnay containing two antimicrobial genes.

Experimental design. Nine antimicrobials were selected for testing. To evaluate their effectiveness against crown gall, crown gall bacteria and antimicrobials were placed together with growth medium in the wells of small volume plates (100 ul, i.e., less than 1/8 teaspoon; shown in Figure 1) in the Bioscreen C Workstation to measure bacterial growth over time. A parallel experiment was performed with bunch rot spores. In addition, to determine if an antimicrobial affected spore germination versus fungal growth, we placed antimicrobials on clean glass slides with spores and photographed them at regular intervals. For powdery mildew,

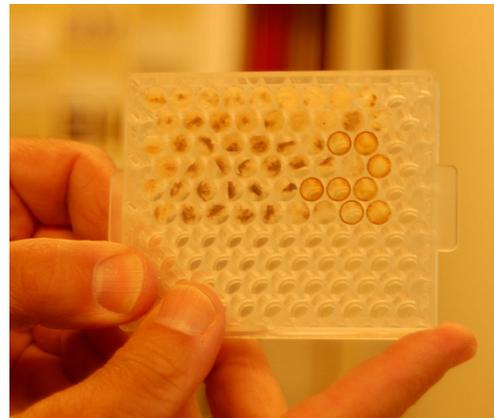


Figure 1. An example of the small volume plate used to test antimicrobials.

antimicrobials and powdery mildew spores were placed on detached leaves in a petri dish and evaluated after 21 days. In addition, we inserted two antimicrobial genes into the genome of Chardonnay (clone 95) and tested the transgenic plants for resistance to powdery mildew and crown gall in the greenhouse.

Results and conclusions:

- Two antimicrobials were effective against crown gall. Called Cecropin B and MSI-99, they are based on naturally-occurring antimicrobials from the giant silk moth and the African clawed frog.
- Overall, the fungus that causes bunch rot was much less sensitive to the antimicrobials, but three antimicrobials effectively reduced growth (called alpha-PTH, PGL, and ESF39). They have different modes of action (inhibiting spore germination versus stunting the fungal growth).
- None of the antimicrobials were effective against powdery mildew.
- In the test of the transgenic Chardonnay plants expressing two antimicrobial genes, crown gall size was reduced compared to the controls. There was no difference in resistance to powdery mildew.

The bottom line. Like antibiotics to fight human diseases, antimicrobial peptides can be used to combat disease in grapevines. We have identified five with promising effectiveness against bunch rot and crown gall, but none that are effective against powdery mildew. In related work, antimicrobials that control Pierce's disease of grapevines are being tested by researchers from the University of Florida. Ongoing field trials show promise.