Evaluation of bacteria for potential biocontrol of *Xanthomonas* in carrot seed crops

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Introduction

Biological control, also known as biocontrol, involves the use of living organisms to reduce pest populations. In plant pathology, microbial antagonists can be used to suppress plant diseases through hyperparasitism, the production of antagonistic antibiotics or enzymes, competition for resources or physical niches, or the induction of host resistance. Bacteria that can survive and reproduce on carrot foliage and/or seeds may be able to reduce *Xanthomonas hortorum* pv. *carotae* levels in fields and seed lots. During the course of field and seed sampling, it was observed that some bacteria appeared to inhibit the growth of *X. hortorum* pv. *carotae*. The objective of this study was to evaluate potential biocontrol activity of bacteria collected from carrot plants and seeds against bacterial blight under laboratory conditions.

Materials and Methods

A collection of 93 bacteria were collected from leaves, umbels, and seed lots of carrot seed crops grown in central OR. Bacteria were selected for biocontrol potential if: 1) they were present in high numbers on carrot leaves or seed lots and *X. hortorum* pv. *carotae* was either not detected or detected at low levels in the same assay; and/or 2) the bacteria exhibited antagonistic activity against *X. hortorum* pv. *carotae* as indicated by zones of inhibition (lack of growth) on petri plates. A subset of eight isolates representing the range of colony morphologies were selected for evaluation of biocontrol potential against *X. hortorum* pv. *carotae* using an overlay assay.

An isolate of *X. hortorum* pv. *carotae* was grown in liquid culture and inoculated into 0.7% Wilbrinks agar. Five ml of the *X. hortorum* pv. *carotae* -inoculated agar was poured into petri plates and allowed to incubate for 24 hours at 4°C. Biocontrol isolate treatments were stab-inoculated onto the center of each *X. hortorum* pv. *carotae* plate using a 10 µl pipette tip containing 10 µl of bacterial suspension. Several controls were included in this study: a non-inoculated control, a phosphate buffer stab-inoculated control, a *X. hortorum* pv. *carotae* stab-inoculated control, and a *Pseudomonas syringae* pv. *syringae* stab-inoculated control. All treatments were replicated 10 times and arranged in a randomized complete block design. After 48 hours of incubation at 28°C, the zone of inhibition was measured from digital images using ImageJ software (Schneider et al. 2012). Treatments were analyzed using analysis of variance. Isolates that exhibited inhibition against *X. hortorum* pv. *carotae* were identified using DNA sequences.

Results and Discussion

Several bacterial isolates exhibited potential biocontrol activity in the petri plate assay used in this study (Table 1). Among the eight isolates tested, six exhibited zones of inhibition that were 5 to 15 times larger than the phosphate buffer control. Two isolates and the *X. hortorum* pv. *carotae* control exhibited inhibition less than the *P. syringae* pv. *syringae* control and similar to the

phosphate buffer control.

Five of the six isolates that exhibited inhibitory effects against *X. hortorum* pv. *carotae* were identified using 16S rRNA sequences. Four isolates were identified as *Pantoea* species, including two isolates identified as *P. agglomerans*. One isolate was identified as a species of *Enterobacter*. *Pantoea* and *Enterobacter* are ubiquitous bacteria and are often found associated with plants and insects as pathogens and commensals. Certain strains of *P. agglomerans* are also used in commercially available biocontrol products for plant diseases.

Since the bacterial isolates screened in this study were isolated from carrot foliage or seeds grown in central Oregon, it would be expected that they can colonize and reproduce in central Oregon carrot seed fields; however, further testing would be required to determine their biocontrol potential under field conditions.

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References

Schneider, C.A., Rasband, W.S., Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671-675.

Tables

Table 1. Inhibition of *Xanthomonas hortorum* pv. *carotae* (*Xhc*) by various carrot-associated bacteria as determined in a petri plate stab-inoculation assay

Isolate	**Bacteria identification**	***Xhc* inhibition (diameter mm)[z]**
14-022-B	*Enterobacter* sp.	33.2 a
16-354-B	*Pantoea agglomerans*	21.0 b
16-317-B	*Pantoea* sp.	16.0 c
16-297-B	Not identified	16.0 c
16-347-B	*Pantoea* sp.	14.0 cd
14-020-B	*Pantoea agglomerans*	11.9 d
15-069-B Pss	*Pseudomonas syringae* pv. *syringae*	4.1 e
16-135-B	Not identified	2.8 f
16-024-B	Not identified	2.8 f
***Xanthomonas* control**	*Xanthomonas hortorum* pv. *carotae*	2.3 f
Phosphate buffer control	Not applicable	2.2 f
No bacteria control	Not applicable	0.0 g
	***P*-value**	< 0.0001

[z] Column means followed by the same letter are not significantly different at α=0.05 as determined by Tukey's honest significant difference test.