Seed Treatments for Eradicating *Clavibacter michiganensis* subsp. *michiganensis* from Naturally Infected Tomato Seeds

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ABSTRACT

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Clavibacter michiganensis subsp. *michiganensis,* the causal agent of bacterial canker, was eradicated from naturally infected tomato (*Lycopersicon esculentum*) seeds that were soaked in 0.6 M HCl for 5 hr, in 0.25 or 0.50% acidified cupric acetate (ACA) for 20 min, or in water at 52 C for 20 min or at 56 C for 30 min. Other individual treatments failed to eradicate the pathogen. Most treatments reduced the saprophytic bacterial flora on tomato seeds; however, only the HCl treatment and treatment with ACA (0.25%) at 52 C for 20 min eradicated saprophytes. Germination of treated seed varied with the germination test. In the blotter test, all individual treatments that eradicated *C. m.* subsp. *michiganensis,* except water at 56 C, significantly ($P = 0.05$) reduced germination. In contrast, only the HCl treatment significantly reduced the germination of seed planted in steam-sterilized soil (UC soil mix), and seed treated with hot water (52 C for 20 min) germinated at a greater rate than the untreated controls.

Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (7), is a serious seed-transmitted disease of tomato (*Lycopersicon esculentum* Mill.) plants worldwide. Although *C. m.* subsp. *michiganensis* can survive in soil, seed is considered the most important source of inoculum (6,15,18, 19,21). The pathogen can both infest (6,17) and infect (6,14) seeds, but the relative importance of infection and contamination in seed transmission is not well understood.

Once tomato plants become infected in the field, there is no satisfactory means of control (6). The use of pathogen-free seed, whether obtained naturally or by treating seeds with chemical eradicants, would eliminate a potential source of inoculum.

A seed-extract, agar-plating technique has recently been developed for assaying tomato seed for *C. m.* subsp. *michiganensis* (9). The lack of a semiselective assay has been the main hindrance to the development of a reliable, proven seed treatment for eradicating *C. m.* subsp. *michiganensis*. Methods that have been recommended for pathogen eradication but that are not routinely used by the seed industry include fermentative extraction of seeds (4,8,18), HCl extraction followed by drying at 66 C (18,20), and soaks in hot water (5,6,8,18), HCl (17,18), NaOCl (8,17), bichloride of mercury (10), alcohol and dye (1), and antibiotics (2,13,18). Most of these recommendations were based on experiments with artificially inoculated seed, which are not representative of naturally infected seed and are not recommended for this purpose (16).

We evaluated the effectiveness of several seed treatments for eradicating *C. m.* subsp. *michiganensis* from naturally infected tomato seeds. A preliminary report has been published (11).

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MATERIALS AND METHODS

Seed and seed sampling. We randomly selected 19 samples of 100 g (about 42,000 seeds) from a naturally infected tomato seed lot supplied by J. A. Dick (Canadian Canners). The seeds had been harvested from a diseased tomato field. The level of infection of *C. m.* subsp. *michiganensis,* determined in previous assays, was approximately 1×10^5 cfu per 10,000 seeds (9).

Seed treatments. Seeds were not treated (control) or were soaked (immersed) in 600-ml aqueous solutions of 0.03 M HCl for 10 min, 0.6 M HCl for 5 hr (17), 0.5% NaOCl for 20 min, 1.05% NaOCl for 40 min, 0.5% $Ca(OCl)_2$ for 20 min, 0.1% Formalin for 20 min, 0.25% cupric acetate acidified in 0.005 N acetic acid (ACA) for 20 min, 0.5% ACA (0.5% cupric acetate) for 20 min, water at 52 C for 20 min, or water at 56 C for 30 min. All treatments except the hot water treatments were done first at room temperature and then in solutions heated to 52 C. After treatment, the seeds were rinsed twice in sterile distilled water and placed on paper towels to dry overnight at room temperature in a laminar flow hood.

Seed assay. Three subsamples of 24 g (10,000 seeds) from each treatment were removed and assayed for *C. m.* subsp. *michiganensis* by the seed-extract, agar-plating method (10). The plates were incubated for 7 days at 26 C, and colonies of *C. m.* subsp. *michiganensis* were counted. Several representative colonies were tested for pathogenicity to tomato seedlings, as described previously (14). Finally, 10,000 of the remaining 12,000 seeds in each treatment where *C. m.* subsp. *michiganensis* was not detected were rinsed twice in sterile distilled water and plated directly onto SCM (selective for *C. m.* subsp. *michiganensis*) agar medium.

Seed germination. We tested 300 seeds from each treatment for germination in the wet blotter test as recommended by the Association of Official Seed Analysts (3). In addition, four replicates of 50 seeds each were planted in a steam-sterilized UC soil mix. Temperature in the greenhouse varied from 13 C (at night) to 25 C (during the day). After 14 days, seedlings with fully expanded cotyledons were recorded as germinated. Four tomato cultivars commonly grown in California—FM 785 (Ferry Morris), Alta (Campbell Soup Co.), VF 6203, and UC 204-C—were used in further tests of the effects of hot water (52 C), ACA (0.25%), and ACA (0.25%) at 52 C on germination. Results were recorded after 14 days.

RESULTS

C. m. subsp. *michiganensis* could not be detected in extracts of seed soaked in 0.6 M HCl, 0.25% or 0.5% ACA, hot water, or the heated solution of several chemicals (Table 1). Moreover, the pathogen was not detected when samples from these treatments were directly plated on SCM. Most of the treatments reduced saprophytic bacteria associated with tomato seeds (Table 1). However, only treatment with 0.6 M HCl or 0.25%

ACA at 52 C eradicated saprophytes.

Some of the treatments adversely affected seed germination (Table 2). In general, more of the untreated seeds germinated in the blotter test than in the soil test. With several of the treatments, however, germination was greater in soil. For the treatments that eradicated *C. m.* subsp. *michiganensis* from infected seeds, mean germination ranged from 0.3 to 92.0% in the blotter test and from 5.0 to 95.5% in the soil test (Table 2). The 0.6 M HCl and ACA treatments significantly ($P = 0.05$) reduced germination in the blotter test. In contrast, when seeds were planted in soil, the germination of seeds treated with ACA did not differ significantly from that of the control seeds. Results were similar with seeds treated with water alone at 52 C: germination was significantly less than the control in the blotter test but significantly greater than the control in the soil test. In further tests, treatment with hot water or with ACA either at room temperature or at 52 C did not reduce the germination of seeds of four cultivars in soil.

Table 1. Effect of tomato seed treatments on *Clavibacter michiganensis* subsp. *michiganensis* and seed-associated saprophytes[a]

Treatment	*C. m.* subsp. *michiganensis*	Seed-associated saprophytes
Untreated	1.75×10^5	4.76×10^6
0.29% HCl, 10 min	2.38×10^4	2.95×10^5
5.0% HCl, 5 hr	0.0	1.33×10^2
0.5% NaOCl, 20 min	8.72×10^4	2.86×10^5
1.05% NaOCl, 40 min	1.87×10^3	1.61×10^5
0.5% $Ca(OCl)_2$, 20 min	3.27×10^3	4.72×10^4
0.1% Formalin, 20 min	4.67×10^3	9.65×10^4
0.25% ACA,[b] 20 min	0.0	4.67×10^3
0.5% ACA, 20 min	0.0	1.10×10^3
Water at 52 C, 20 min	0.0	3.34×10^4
Water at 56 C, 30 min	0.0	1.62×10^3
0.29% HCl at 52 C, 10 min	0.0	2.56×10^2
5.0% HCl at 52 C, 5 hr	0.0	0.0
0.5% NaOCl at 52 C, 20 min	0.0	4.12×10^4
1.05% NaOCl at 52 C, 40 min	0.0	3.17×10^3
0.5% $Ca(OCl)_2$ at 52 C, 20 min	0.0	2.68×10^3
0.1% Formalin at 52 C, 20 min	0.0	2.10×10^3
0.25% ACA at 52 C, 20 min	0.0	0.0
0.5% ACA at 52 C, 20 min	0.0	3.5×10^2

[a] Data are colony-forming units and are the means of three replicates of 10,000 seeds. Seeds were assayed by plating liquid extracts onto SCM agar medium (9).
[b] Acidified cupric acetate.

Table 2. Effect of physical and chemical seed treatments on germination of tomato seeds

Treatment	Mean germination (%)[w]	
	Blotter[x]	Soil[y]
Untreated	94.0 abc	79.5 bcde
0.29% HCl, 10 min	88.0 de	86.0 b
5.0% HCl, 5 hr	0.3 i	5.0 h
0.5% NaOCl, 20 min	96.0 ab	76.0 cdef
1.05% NaOCl, 40 min	96.7 a	72.5 ef
0.5% $Ca(OCl)_2$, 20 min	94.3 abc	56.5 g
0.1% Formalin, 20 min	90.0 cd	87.5 ab
0.25% ACA,[z] 20 min	15.0 g	83.0 bcd
0.5% ACA, 20 min	9.0 h	71.5 ef
Water at 52 C, 20 min	83.0 e	95.5 a
Water at 56 C, 30 min	91.0 bcd	89.0 ab
0.29% HCl at 52 C, 10 min	75.0 f	76.0 cdef
5.0% HCl at 52 C, 5 hr	2.0 i	5.0 h
0.5% NaOCl at 52 C, 20 min	91.7 abcd	86.0 b
1.05% NaOCl at 52 C, 40 min	83.7 e	87.5 ab
0.5% $Ca(OCl)_2$ at 52 C, 20 min	92.0 abcd	84.5 bc
0.1% Formalin at 52 C, 20 min	92.0 abcd	69.5 f
0.25% ACA at 52 C, 20 min	13.3 gh	84.0 bc
0.5% ACA at 52 C, 20 min	13.0 gh	74.0 def

[w] Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[x] Three replicates of 100 seeds each were tested following rules of the Association of Official Seed Analysts (3).
[y] Four replicates of 50 seeds each were planted in a steam-sterilized UC soil mix. Temperature in the greenhouse varied from 13 C (at night) to 25 C (during the day). Seedlings with fully expanded cotyledons were considered normal and germinated.
[z] Acidified cupric acetate.

DISCUSSION

Although several seed treatments are available for eradicating seedborne bacteria (1,2,4,8,12,13,17,18,20), none has been effective for eradicating *C. m.* subsp. *michiganensis*. An effective seed treatment must destroy the bacteria beneath as well as on the seed coat (6,15). In our tests, soaking seeds in NaOCl destroyed surface populations of the pathogen but apparently did not eliminate internal populations. Although NaOCl treatments have been recommended in the past (8,17), they did not appear to be effective in our study. In their tests with NaOCl, Shoemaker and Echandi (17) inoculated seed in the laboratory and used a grow-out assay to evaluate the effectiveness of the treatment. They also recommended treating seed with about 0.6 M HCl for 5–10 hr to eradicate the pathogen. We agree that such acid treatments eradicate the pathogen, but they may severely reduce germination.

Our study confirms that soaking seed in water at 56 C is an effective treatment (6,17,18). In this case, germination is not adversely affected. However, a temperature of 56 C may be difficult to maintain accurately under commercial conditions, and some seed lots could be injured.

Treatment with 0.25 or 0.5% ACA at room temperature eradicated *C. m.* subsp. *michiganensis* from the seed, and soaking seed in heated solutions of 0.25 or 0.5% ACA virtually eliminated all external and internal microbes. The effects of these treatments on germination varied with the test. Germination of the treated seeds did not differ significantly from that of the untreated control seeds in the soil test but was significantly reduced in the blotter test (Table 2). The reason for this discrepancy is unknown. However, the severe reduction in germination in the blotter test may have resulted from the failure of cupric acetate residues to disperse. In the soil test, the cupric acetate could have reacted with or bound to anionic components within soil and therefore would not have been available.

Use of 0.25% ACA at 52 C, in addition to eradicating *C. m.* subsp. *michiganensis,* resulted in the best reduction of saprophytic bacteria associated with tomato seed (Table 1). Use of 0.5% ACA at 52 C for 20 min gave similar results but was associated with a significantly lower germination percentage than the 0.25% treatment (Table 2).

We recommend assaying all tomato seed lots for *C. m.* subsp. *michiganensis* by the seed-extract, agar-plating assay (9). Any seed lot that tests positive should then be treated with water at 52 C or 0.25% ACA at 52 C for 20 min. Because commercial tomato seed lots may differ in their response to these treatments, a sample should be tested first to ensure that germination is not adversely affected. All treated seed lots should be

reassayed for *C. m.* subsp. *michiganensis* to verify the effectiveness of the treatment.

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