Assessment of *Streptomyces* spp. from Elms for Biological Control of Dutch Elm Disease

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ABSTRACT

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A total of 433 actinomycete isolates obtained from phloem and xylem of American elm (*Ulmus americana*) were evaluated for antagonism in vitro against *Ceratocystis ulmi*, the causal agent of Dutch elm disease. Mycelial advance of a nonaggressive isolate of *C. ulmi* from Iowa or of an aggressive isolate from England was inhibited by an average of 75–76% by the actinomycetes. One isolate each of *Streptomyces albovinaceus* and *S. griseus* that inhibited various isolates of *C. ulmi* by 64–90% in vitro failed to prevent symptoms of Dutch elm disease when inoculated into elm samplings 1 wk before challenge with *C. ulmi*. *Streptomyces* isolates were recovered from 25–75% of inoculated elm saplings.

Recently, Strobel and Lanier (14) proposed a new approach to Dutch elm disease control involving injection of American elm (*Ulmus americana* L.) trees with the bacterium *Pseudomonas syringae* van Hall, a plant pathogen that inhibits *Ceratocystis ulmi* (Buism.) C. Mor. in vitro. Moderate successes in controlling Dutch elm disease in greenhouse and field inoculations have been reported (9,14,15), and large-scale testing of the efficacy of the bacterium is in progress.

Several bacteria recovered from vascular fluids of elm are antagonistic to *C. ulmi* in vitro (6). Organisms already intimately associated with tissues of American elm would probably have a better chance than *P. syringae* to establish themselves in the vascular systems of living trees. Therefore, alternative biological control agents were sought from the phloem and xylem of American elm trees.

Actinomycetes, particularly *Streptomyces* spp., often possess some potential to affect development of other microorganisms through production of antibiotics and lytic enzymes or by competition for nutrients and substrates (7). Actinomycetes have been isolated from woody tissues of trees, and some strains appear well adapted to woody substrates. Certain strains of *Streptomyces* sp. are capable of utilyzing both cellulosic and lignic components of wood tissues (3,4). *Streptomyces flavovirens* (Waksman) Waksman & Henrici has been shown to break down cell walls of Douglas-fir phloem, causing substantial weight loss in inoculated bark disks (16). Blanchette et al (2) found several *Streptomyces* spp. that colonize discolored wood of living silver maple (*Acer saccharinum* L.).

Actinomycetes have only rarely been considered as potential agents for biological control of diseases affecting aerial plant parts (13). This research was

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undertaken to determine whether actinomycetes are present in tissues of American elm, and then to determine their efficacy in controlling Dutch elm disease in elm saplings.

MATERIALS AND METHODS

Field collections. Actinomycetes were isolated from the xylem and phloem of American elm growing in east central Minnesota. Xylem and phloem were sampled together; records concerning the relative numbers of actinomycetes recovered from these tissues were not kept. Branches and stems of trees killed by *C. ulmi* but not colonized by elm bark beetles, living trees in areas with a high incidence of Dutch elm disease, and trees that apparently had recovered from infection by *C. ulmi* were sampled. "Recovered" trees were identified by the presence of discolored xylem typical of Dutch elm disease but located beneath one or more annual layers of normal sapwood.

Laboratory. A means of selectively isolating actinomycetes similar to one employed by Blanchette et al (2) was used. Chips of phloem and xylem (5 × 2 ×2 mm) were excised aseptically from the elm samples, placed in 4-dram vials containing 4 ml sterile distilled water and heated at 65 C for 60 min. Chips were then placed onto Difco actinomycete isolation agar and incubated at 25–30 C.

To evaluate the effects of actinomycete isolates on the growth of *C. ulmi* in vitro, Difco-Bacto mycological agar supplemented with 5 g Difco-Bacto agar per liter to bring the final agar concentration to 2% was suitable for growth of both organisms.

Two isolates of *C. ulmi* were employed in the initial antagonism assay: CI01 (Iowa), a moderately fast-growing isolate, and RDT2 (England, courtesy of J. N. Gibbs), a fast-growing, aggressive isolate. Both isolates were grown on mycological agar for 7 days before assay to ensure vigorous inoculum. Each actinomycete isolate was streaked across the center of a 90-mm petri dish containing mycological agar and allowed to grow in the dark at 25 C for 3 days. On the fourth day, a 5-mm disk of agar plus mycelium from the edge of a fungal colony was placed at each side of the petri dish, 40 mm from the streak. Other plates were inoculated with *C. ulmi* only. The plates were then placed in a darkened incubator at 25 C for 14 days. Growth of the fungus toward the streak was measured every second day.

Two actinomycete isolates (A67 and A472) that strongly inhibited *C. ulmi* and had stable growth and sporulation characteristics were tested further with seven local isolates of *C. ulmi* that grew at different rates. The same actinomycete isolates were also used in an experiment with elm saplings in a greenhouse.

Greenhouse. Forty samplings of American elm in their third growing season were inoculated by infusion with *Streptomyces* isolate A67, and 40 were inoculated with isolate A472. Inoculation procedures were similar to those employed by Semer (M.S. thesis, Ohio State University). A rubber collar was formed by punching a hole with a cork borer in the base of a serum-bottle stopper. The stoppers were reglued around the stems of the saplings with Goodyear Pliobond cement. A 3-ml spore suspension of one of the actinomycetes ($>10^7$ cells per milliliter) was introduced into the reservoir formed by the serum-bottle stopper, and small wounds into xylem were made with a sterile scalpel on either side of the stem below the level of the liquid. The reservoirs were then covered with aluminum foil to retard evaporation.

Two weeks after inoculation with actinomycetes, half of the trees treated with each actinomycete were inoculated with a virulent isolate of *C. ulmi* (82C18, Minnesota). A small wound was made in each sapling with a sterile scalpel about 5 cm above the previous inoculation wound. About 0.5 ml of a spore suspension of *C. ulmi* (2×10^7 cells per milliliter) was introduced into the wound with a syringe, and the wound was closed by wrapping laboratory film around the stem. After 6 wk, the plants were harvested. Six serial sections (1 mm thick) were removed from each stem beginning about 5 cm above the site of inoculation of *C. ulmi*. Three sections from each sapling were placed onto 2% malt extract agar for recovery of *C. ulmi* and three sections were processed for actinomycete isolation.

RESULTS

Four hundred thirty-three actinomycete isolates were recovered from phloem and xylem of American elm and tested for antagonism against two isolates of *C. ulmi*. The average radial growth (after 12 days) of *C. ulmi* isolates CI01 and RDT2 from the edges of petri dishes was 4 and 5.5 cm, respectively. Most of the actinomycetes suppressed radial growth of *C. ulmi*. The average inhibition of the Iowa isolate of *C. ulmi* (CI01) in the presence of actinomycetes was 75%; and the English isolate (RDT2) was inhibited by 76%.

Actinomycete A67 was identified as *S. albovinaceus* (Kudrina) Pridham et al (11). It produced a faint pink diffusible pigment on glycerol-asparagine and yeast-malt agars (10). Isolate A67 grew in media containing 4%, but not 7%, NaCl (10). Isolate A472 was identified as *S. griseus* (Krainsky) Waksman & Henrici. It conformed to the description in Shirling and Gottlieb (12) except that it apparently used sucrose and failed to produce melanin in tyrosine agar. Isolate A472 grew poorly on Czapek's solution agar (10).

S. albovinaceus A67 inhibited seven isolates of *C. ulmi* by 64–80% (Table 1). *S. griseus* A472 inhibited the same isolates by 80–90%. Unchallenged isolates of *C. ulmi* grew an average of 0.4–0.5 cm/day after an initial lag, whereas in the presence of either actinomycete, the average rate of growth declined rapidly as the leading edge of the mycelium approached the actinomycete streak (Fig. 1). *S. griseus* exerted a greater inhibitory effect than *S. albovinaceus*. Inhibition by *S. griseus* was also apparent sooner than that by *S. albovinaceus*.

All *C. ulmi* isolates initiated growth when placed at 4.5 or 2 cm from either actinomycete streak; however, growth usually ceased after 2–3 days. Mycelium growing in the presence of actinomycetes

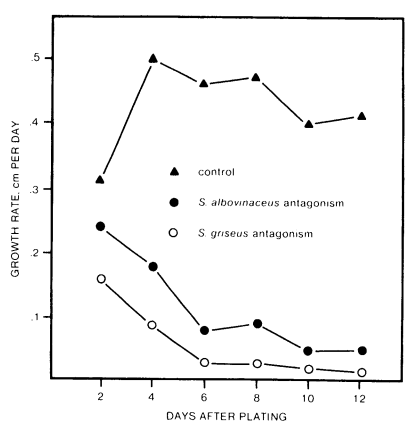

Fig. 1. Average growth rate of seven isolates of *Ceratocystis ulmi* (four replicates) in axenic culture (control) and in the presence of *Streptomyces albovinaceus* or *S. griseus*.

Table 1. Inhibition of seven isolates of *Ceratocystis ulmi* in vitro by *Streptomyces albovinaceus* A67 and *S. griseus* A472

C. ulmi isolate no.	Growth of unchallenged isolate (cm)[a]	Percent inhibition by[b]	
		S. albovinaceus	*S. griseus*
A304	4.9	74	86
B210	4.7	70	87
VMD 01	4.8	75	85
C5	2.5	64	80
C6	4.9	76	86
C7	8.0	80	90
C11	6.3	70	86

[a] Average radial mycelial growth on four plates after 12 days.
[b] Average of four replicates after 12 days. Inhibition was determined by comparing growth of challenged isolates with growth of unchallenged isolates.

Table 2. Recovery of *Ceratocystis ulmi* and *Streptomyces* spp. from inoculated elms

Organisms used for inoculations[a]	No. of saplings from which organisms were recovered	
	Streptomyces sp.	*C. ulmi*
S. griseus	15	0
S. griseus and *C. ulmi*	8	20
S. albovinaceus	5	0
S. albovinaceus and *C. ulmi*	7	20
C. ulmi alone	0	20

[a] Twenty saplings per treatment.

showed increased vacuolation and transparency of the hyphae compared with axenic cultures, apparently indicating processes leading to lysis. Sterile agar disks obtained adjacent to *Streptomyces* colonies also inhibited growth of *C. ulmi* in vitro.

Greenhouse. Each sapling inoculated with *C. ulmi* developed symptoms of Dutch elm disease, whether or not previously inoculated with *S. albovinaceus* or *S. griseus*. Symptoms included extensive vascular discoloration, wilting, and death of the foliage. No symptoms were observed in saplings inoculated with either actinomycete isolate alone.

Attempts to recover actinomycetes from inoculated saplings 10 cm above the point of inoculation were successful in 25–75% of the trials (Table 2). Culture morphology and color of isolates recovered were characteristic of the original isolates used in the inoculations. No further tests were made to confirm that the original isolates were those recovered. *C. ulmi* was recovered from every sapling inoculated with the fungus, regardless of prior treatment with *S. albovinaceus* or *S. griseus*.

DISCUSSION

Actinomycetes, particularly *Streptomyces* spp., appear to be common inhabitants of the phloem and xylem of American elm. Some *Streptomyces* spp. are able to grow in the presence of relatively high concentrations of phenolic compounds (2) and thus may be primary colonizers of the bark, which normally serves as a physical and chemical barrier to fungal colonization to the xylem. The number of actinomycete isolates obtained and the extent of inhibition of fungal growth in vitro indicates that actinomycetes may play a significant role in the succession of bark microflora in American elm.

The reasons for antagonism by the actinomycetes against *C. ulmi* in vitro were not determined; mycolytic enzymes or antibiotics may be involved. *S. griseus* strains produce a wide variety of antibiotic compounds (1). Certain *S. griseus* strains are known to produce an antibiotic (candicidin), which is effective against yeasts and dimorphic fungi (7). Although *C. ulmi* was the assay organism that led to discovery of candicidin (8), few studies of the effects of candicidin on Dutch elm disease development have been published (5).

Attempts to control Dutch elm disease in the greenhouse with inoculations of *Streptomyces* spp. failed (Table 2); however, both *Streptomyces* isolates were recovered from xylem 8 wk after inoculation. Production of antibiotics or mycolytic enzymes by the actinomycetes in vitro may have been blocked in vivo because of a lack of certain required nutrients; alternatively, the titer of any fungitoxic substances produced may have been too low to effectively inhibit growth of *C. ulmi*.

Various eubacteria have been recovered from vascular fluids of American elm (5); some have been shown antagonistic to *C. ulmi* in vitro but none have controlled Dutch elm disease. Such findings seem to indicate that the in vivo situation is not analogous to in vitro assays for antagonism. These "antagonistic" bacteria would seem to have a competitive advantage in elm vascular fluids compared with such alien organisms as *P. syringae*.

The vascular system of American elm is a complex environment to which *C. ulmi* is particularly well suited. Antagonistic microorganisms may affect the saprophytic survival of *C. ulmi* (17) but appear unlikely to exert significant effects on Dutch elm disease in the living tree.

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