NOTE: In Vitro Antagonism of Actinomycetes Isolated from Fungus-Growing Ants against Plant Pathogenic Fungi


Fungus-growing ants have been found recently to be symbiotic with actinomycetes living on the ant’s cuticle; these bacteria are inhibitory to soil fungi that are detrimental to the ants’ fungus gardens. In order to investigate whether actinomycetes found on the cuticle of attine ants also had inhibitory properties against plant pathogenic fungi, we isolated 32 strains of actinomycetes from fungus-growing ants (Atta, Trachymyrmex, and Cyphomyrmex), from the Mexican states of Coahuila, Nuevo León and Tamaulipas. Of the actinomycetes tested against selected plant pathogenic fungi (Alternaria solani, Aspergillus flavus, Colletotrichum lindemuthianum, Rhizoctonia solani, Sclerotium sp.) on Czapek-Dox agar medium, 13 isolates inhibited at least one of the fungi. C. lindemuthianum was inhibited by 11 actinomycetes, and Rhizoctonia by three. An actinomycete strain isolated from Cyphomyrmex rimosus inhibited all five fungi tested.

KEY WORDS: Atta sp.; attine ants; Cyphomyrmex sp.; mutualism; plant pathogen; Tra-chymyrmex sp.

Synthetic chemicals are the most commonly used tools for control of plant diseases. Several strategies can complement chemical control for management of plant pathogens in agroecosystems. Among these, the utilization of microbes antagonistic to plant pathogens is a promising biological control alternative that already includes a number of successes (9,14). One of the main and initial challenges to this alternative is the detection of microbes with strong antagonistic properties. In this respect, natural ecological situations where antagonism and antibiotic properties play important roles could be sources of promising biocontrol microbes. Analysis of the fungus-growing ant mutualism has unveiled intricate associations involving several antagonisms (5,10,13). Briefly, fungus-growing ants in the tribe Attini (Hymenoptera: Formicidae) cultivate specific fungi (Agaricales: Agaricaceae) for food. They use plant material and other organic substrates to cultivate their fungal ‘gardens’, where the substrate is inoculated with and colonized by special symbiotic fungi that the ants feed upon. However, contaminant fungi and bacteria readily colonize the fungus garden. In particular, species of Escovopsis (Ascomycota: Hypocreales) are specialized parasites and exploit the symbiotic fungi of attine ants as a source of nutrients (10). In several genera of attine ants, the workers harbor Actinomycetes on their body surface (several strains of Pseudonocardia, formerly in Streptomyces); these produce metabolites that specifically inhibit the growth of Escovopsis (4,5,10). These bacterial partners of the ants are presumably transmitted vertically to the newly formed ant colonies. Actinomycetes are well known as versatile antibiotic producers in several ecological situations besides the fungus-growing ant symbioses. Free-living actinomycetes from soil are the source of most of the antibiotics humans use in a completely different setting: medicine and the human body.

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In view of the recently found antmycotic properties of the actinomycetes of attine ants, we wanted to test whether ant cuticle-associated actinomycetes possessed in vitro inhibitory properties also against selected, economically important fungal plant pathogens (Ascomycota and Basidiomycota). Our goal was to screen for antifungal metabolites active against these fungi. Novel antifungal metabolites can be expected from interesting ecophysiological interactions, i.e., from actinomycetes isolated from fungus-growing ants. We isolated actinomycetes from the body of three genera of fungus-growing ants from Mexico, and tested their inhibitory activity against four plant-pathogenic fungi in vitro.

Fungus-growing ants were collected in the northeastern Mexican states of Coahuila, Nuevo León and Tamaulipas (Table 1). Live ants were collected by digging in their nests, attempting to gather fungus gardens, workers, brood, males and females (6,14). Ants and fungal gardens were maintained in the laboratory for up to 157 days in artificial nests (10). Ants were identified using the keys in Creighton (3) and Longino (7).

For isolation of actinomycetes, five live worker ants of each of the smaller-sized ant genera (Cyphomyrmex and Mycocepurus) were placed in Eppendorf tubes with 1 ml of sterile distilled water, and thoroughly ground with a sterile glass rod. Loopfuls of the supernatant were transferred with a sterile loop to starch-casein agar (8,12) supplemented with glycerol (SCA-G) (2). Larger-sized ants of the genus Trachymyrmex harbored filamentous bacterial growth ('bloom') on their ventral thoracic sclerites (sterna) (4). These bacterial cells were scraped with a sterile needle and transferred to SCA-G medium. Ten Atta mexicana workers (which do not show visible actinomycete growth on their body surface) were individually rolled on SCA-G. Plates were incubated at 30°C for 5 days, under 10 h per day of diffuse fluorescent light. After incubation, isolated colonies were picked up from the agar with a sterile needle; polvose (powdery) growth from these plates was transferred to standard Czapek-Dox agar (C-D). Colonies were identified as Actinomycetes by microscopic observation (8). Individual colonies were transferred to SCA and C-D; each colony thus transferred from plates with insect material constituted a strain.

The pathogens tested include morphospecies responsible for serious diseases of crops: Colletotrichum lindemuthianum (Sacc. & Magnus) Briosi & Cavara (anthracnose fungus) and Alternaria solani (Ell. & Mart.) Jones & Grout (early blight) cause important foliar and fruit lesions of beans, tomato, potato, mango, and other crops. Rhizoctonia solani Kühn and Sclerotium rolfsii Sacc. are soil plant pathogens causing seedling death (damping-off) and also produce lesions on potato tubers. Aspergillus flavus Link infections reduce the quality of many kinds of seeds worldwide (1). The plant pathogens were transferred and maintained on PDA plates.

To detect antagonism, three 4-mm-diam agar disks (explants) with bacterial growth (same strain) from C-D plates were placed equidistant (120°) onto fresh C-D plates (100 mm) at 20 mm from the plate edge. These plates were incubated at 25°C for 72 h in the dark. Then, a fungal explant of the fungus to be tested growing on PDA was individually placed on the center of the C-D dish with the three bacterial explants. C-D medium is a poor substrate for growth of these plant pathogens (unpublished observations). However, the PDA disks with fungus growth on them supported enough residual growth of the fungi onto the underlying C-D medium for evaluations to be made. Three petri dishes per fungus were prepared this way. Plates were incubated at 25°C for 5 days under diffuse fluorescent light. Control plates consisted of fungal explants placed on C-D dishes without explants of actinomycetes as described. Radial fungal growth was measured on a straight line between explants of fungus and actinomycetes with a vernier (Mitutoyo Corp., Aurora, IL, USA). Inhibition was determined by comparison of the fungal growth on agar plates with and without bacterial explants. For each fungus/actinomycete comparison, three petri dishes with three explants were used for a total of nine growth measurements. Mean growths for contrasts were compared statistically using Tukey's test (11). Thirty-two strains of actinomycetes were isolated from...