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Interaction of *Agromyces ramosus* with Other Bacteria in Soil†

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Agromyces ramosus occurs in very high numbers in most soils and, based on studies of laboratory isolates, does not require host cells for growth. Nevertheless, it attacked and destroyed most of the gram-positive and gram-negative bacterial species tested as possible host organisms. *A. ramosus* also attacked and destroyed *Saccharomyces cerevisiae*. The possibility of attack on fungi was unclear. Among the bacteria serving as hosts were the important soil species *Azotobacter vinelandii, Rhizobium leguminosarum, Rhizobium meliloti*, and *Agrobacterium tumefaciens*. Dead cells were not attacked. *A. vinelandii* cysts were attacked but left unharmed. To some extent, *A. vinelandii* seemed to survive this attack by encysting. Attack by *A. ramosus* occurred in natural soil and over a broad range of nutritional levels in laboratory media. The attack did not seem to be a means for obtaining an increased supply of commonly available nutrients. Instead, it seemed to be a means of obtaining something produced, perhaps in small amounts, by a variety of organisms, but not by all organisms. Several types of culture filtrates were tested for activity. The filtrates neither stimulated nor inhibited the growth of *A. ramosus* or the host organisms. The availability of catalase activity in host organisms did not seem to be involved. It is not known whether the attack by *Agromyces ramosus* in soil can be manipulated to cause a decrease in numbers of *Agrobacterium tumefaciens* or other pathogens without simultaneously depressing the numbers of beneficial organisms in this habitat.

The soil bacterial predation system that includes *Ensifer adhaerens* (5) and a soil streptomycete (4) does not seem to attack cells of *Azotobacter* species. During a direct microscopic determination of this in soil, however, it was observed that the hyphae of some unknown indigenous actinomycete did attack *Azotobacter* cells. This actinomycete produced branched hyphae that eventually fragmented without producing conidia. Since this type of growth cycle is reminiscent of that of *Agromyces ramosus*, it was deemed advisable to test this bacterium for possible attack on other soil bacteria.

A. ramosus is a catalase-negative actinomycete that is microaerophilic to aerobic and has an oxidative metabolism (7). It produces a branched mycelium that ultimately fragments into coccoid and diphtheroid forms. Special techniques are required for its isolation from soil (2, 7, 12), and there are no methods available for enumerating it from soil. Nevertheless, the *A. ramosus* numbers in soil are greater than the total numbers for all soil bacteria that are countable or isolatable by the usual bacteriological techniques. This is known because *A. ramosus* is isolated from dilutions of soil beyond those containing other bacteria. Obviously, the occurrence of these large numbers raises a question as to the source of the nutrients required for *A. ramosus* survival in soil.

The question has also been raised as to how an aerobic, catalase-negative organism in soil protects itself from hydrogen peroxide. One answer might be that other soil organisms provide catalase activity (3, 11), although the habitat itself supplies manganese oxides that react quickly with hydrogen peroxide.

The object of this study was to determine whether *A. ramosus* might have a nonobligate ability to attack and destroy other bacteria in soil. If so, then this could provide at least some of the nutrients that would be required for the maintenance of *A. ramosus* in large numbers in soil. The relation of the catalase activity of host cells to possible attack by *A. ramosus* was also examined.

MATERIALS AND METHODS

Organisms and media. The *Agromyces ramosus* strains used were ATCC 25173 and PSU 35. These strains differ in their cell wall carbohydrate patterns

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(7). The major cell wall sugars of strain ATCC 25173 are rhamnose, galactose, mannose, and xylose; for strain PSU 35 the sugars are rhamnose and glucose. Strains of the other organisms studied were *Azotobacter vinelandii* ATCC 12837, *Proteus vulgaris* PSU 186a, *Staphylococcus aureus* PSU 240a, *Escherichia coli* PSU 106, *Pseudomonas fluorescens* PSU 192, *Pseudomonas putida* PSU 193, *Nocardia salmonicolor* ATCC 21243, and *Saccharomyces cerevisiae* PSU 321. The *Rhizobium* strains were originally obtained from the Nitragin Co. (Milwaukee, Wis.). *Rhizobium leguminosarum* (PSU 201) was Nitragin strain 128C46; *Rhizobium meliloti* (PSU 204) was Nitragin strain 102F29. *Agrobacterium tumefaciens* was supplied by F. L. Lukezic of the Plant Pathology Department of The Pennsylvania State University. It was an apple pathogen and was designated as strain 499. *Azotobacter vinelandii* was maintained on modified Burk medium which contained, per liter, K_2HPO_4, 0.64 g; KH_2PO_4, 0.16 g; $CaSO_4 \cdot 2H_2O$, 0.05 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.2 g; ferric citrate, 2 ml of stock solution (0.9 g/100 ml); $Na_2MoO_4 \cdot 2H_2O$, 1 ml of stock solution (0.25 g/100 ml); sucrose, 10 g (sterilized separately); and agar, 15 g. This medium without agar was used for broth growth of cells to be used as the inoculum in various experiments. Media for the other organisms are listed below with the turbidity experiments. *A. vinelandii* cysts (virtually 100% encystment) were produced by growth for 7 days at 27°C on modified Burk agar medium containing 0.2% *n*-butanol instead of sucrose. Heart infusion broth made up at 1/10 of the recommended strength contained 1.5% agar if the medium required solidification. Tryptone-Casamino Acids medium contained tryptone, 5 g; Casamino Acids, 4 g; $(NH_4)_2HPO_4$, 0.7 g; NaCl, 5 g; and distilled water, 1 liter (pH 7.0). Bacto medium components (Difco Laboratories, Detroit, Mich.) were used for these and other media.

Soil and agar column slides. Soil column slides were prepared and incubated as described by Casida (4). The soil was a pH 6.1 Hagerstown silty clay loam similar to that used previously (4). A pH 5.4 soil was tested, but fungal growth sometimes interfered. Soils with pH values of 6.6 and 7.2 gave results similar to those for the pH 6.1 soil and are not reported. The preparation, incubation, and staining for agar column slides was as for the soil column slides except that an agar medium, or just 1.5% agar dissolved in water, was used in the glass cylinders in place of soil. The tempered agar or agar medium was added dropwise to the smear area within the glass cylinder, with each drop allowed to solidify before the next was added. After 4 to 5 drops had been added in this fashion, the rest of the 1.5-ml total volume was added without stopping.

The soil column and agar column slides were incubated at 27°C in petri plates placed in polyethylene bags containing an open beaker of water to retard evaporation. Slides were removed after various periods of incubation and prepared for observation (4). They were stained for 1 min with crystal violet.

Perpendicular cross-streak plates. Perpendicular cross streaks of pairs of organisms were prepared according to the method of Casida (4). Control monoculture streak plates were prepared for each organism. By comparing growth on these control plates with that on the perpendicular cross-streak plates, any possible overall growth stimulation or partial growth inhibition on the latter plates could be detected. All plates were incubated for 6 days at 27°C; periodic observations were made during incubation.

Growth determinations by turbidity. Broth medium (40 ml) was sterilized in 500-ml Erlenmeyer flasks having side arms for turbidity readings in a Klett colorimeter. Inoculum was added at 0.1 ml per organism. In some instances 0.2 ml of *Azotobacter vinelandii* inoculum was added. For trials using heat-killed *A. vinelandii* or *Agrobacterium tumefaciens* cells, the cells were added to the medium before autoclaving; *Agromyces ramosus* culture additions to these flasks were made after the sterilization was completed. *A. vinelandii* cells for use as inoculum were grown as shaken cultures for 2 to 3 days in modified Burk broth. They were washed twice by centrifugation. Inoculum for *A. ramosus* and the other organisms was prepared by quickly suspending the growth from an agar slant in 1.1 ml of sterile distilled water. This inoculum was used immediately. The agar slant media were asparagus extract-mannitol agar (1) for the rhizobia, tryptic soy agar for *S. cerevisiae*, and full-strength heart infusion agar for *A. ramosus* and other bacteria. Dilutions for *A. vinelandii* counts were plated on modified Burk agar. Counts of *A. ramosus* and *A. tumefaciens* were on full-strength heart infusion agar. Rhizobia counts were on yeast-mannitol agar (13). All counts are reported as total counts in the reaction fluids, not as counts per milliliter. Contamination checks for flasks containing monocultures of *A. ramosus* were made by streaking on full-strength heart infusion agar and testing the resulting colonies for lack of catalase activity.

Culture filtrates. Samples of broth cultures were centrifuged immediately after being taken. In one case, however, a sample was first frozen and thawed, for possible release of an active factor from the cells, and then it was centrifuged. The supernatant fluids were checked for pH values and, if necessary, adjusted to approximate neutrality. They were then passed through sterile 0.22-μm-pore-sized membrane filters (Millipore Corp., Bedford, Mass.). Sterile 13-mm-diameter paper disks (Schleicher & Schuell, Inc., Keene, N.H.), each containing 0.1 ml of sterile culture filtrate, were placed on the surfaces of inoculated agar plates. *Azotobacter vinelandii* and *Agrobacterium tumefaciens* were applied by spreading 1 drop of concentrated cell suspension across the agar surface. The other test organisms were applied by loop from agar slants. The cells were multiply streaked in three different directions across the agar surface to cover the surface. Incubation was at 27°C.

RESULTS

Soil studies. *Azotobacter vinelandii* cells were added to soil in soil column slides, and the slides were incubated. Some indigenous actinomycete-like mycelium developed among these cells by 19 h of incubation, and a few of the *A. vinelandii* cells had a ghosted appearance. With further incubation, the *A. vinelandii* cells disappeared. However, this disappearance could not be definitely attributed to the mycelium, because various other soil bacteria had also developed on the

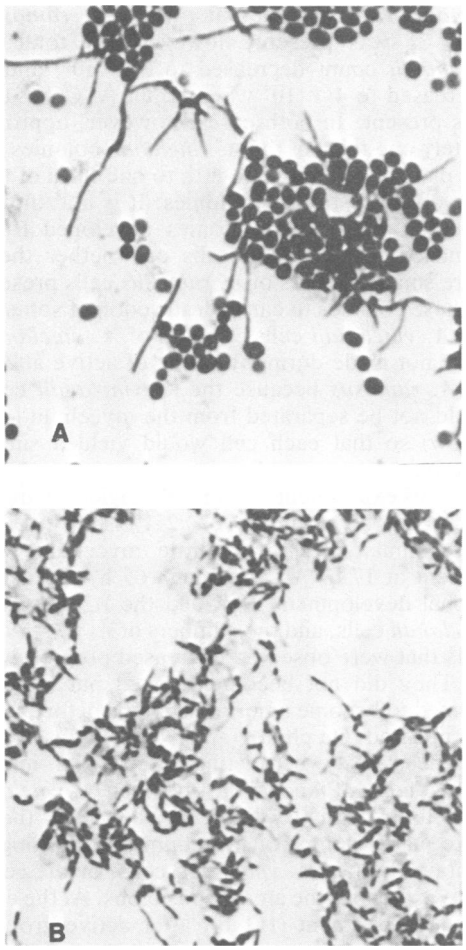

FIG. 1. Soil column slides inoculated with *Agromyces ramosus* ATCC 25173 plus *Azotobacter vinelandii* and incubated for 15 h (A) or 48 h (B). By 48 h most of the mycelium of strain ATCC 25173 has fragmented, and most of the *A. vinelandii* cells have ghosted and are barely visible. Magnification, ×1,400.

slides. When *Agromyces ramosus* cells of strains ATCC 25173 or PSU 35 were added to the soil along with the *A. vinelandii* cells, *A. ramosus* mycelium developed (Fig. 1) that appeared similar to the mycelium that developed when *A. ramosus* was not added. The mycelium occurred between the *A. vinelandii* cells and radiated from them. With continued incubation, the added *A. ramosus* cells continued to extend their hyphae, and then the hyphae fragmented. During this time, there was no development of other soil bacteria, actinomycetes, or fungi. Extensive ghosting of the *A. vinelandii* cells was present by 29 to 48 h of incubation (Fig. 1), and the mycelium had largely fragmented. Addition to soil of *S. cerevisiae* cells, without also adding *A. ramosus* cells, gave mycelial development by 18 h (Fig. 2), followed by fragmentation of the mycelium and destruction of the yeast cells by 25 h.

The "indirect phage analysis" soil percolation procedure of Germida and Casida (6) for following predatory activity of bacteria in soil was used for studying the interaction of *Agromyces ramosus* and *Azotobacter vinelandii* in soil. The soil was inoculated with *A. vinelandii* or *Micrococcus luteus*, and the percolates were tested for their content of phage that would form plaques on plates of *A. ramosus* or the nonobligate predatory bacterium *Ensifer adhaerens*. In this technique the *A. ramosus* or *E. adhaerens* cells naturally present in soil respond to added host cells by multiplying, and the soil phage naturally present in the soil then attack the multiplying cells. This phage production is monitored as

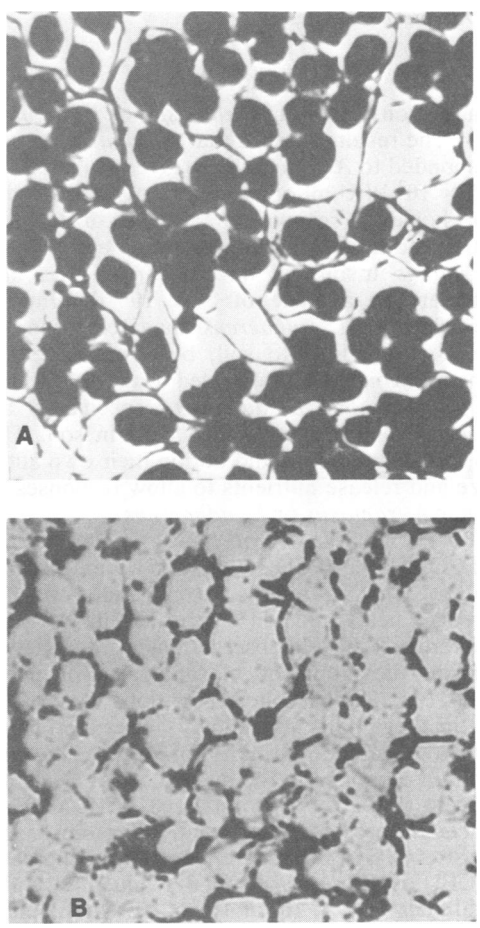

FIG. 2. Soil column slides inoculated with *S. cerevisiae*. *A. ramosus* was not added. Incubation was for 18 h (A) or 25 h (B). At 25 h fragmented mycelium is seen between the barely visible ghosted *S. cerevisiae* cells. Magnification, ×1,400.

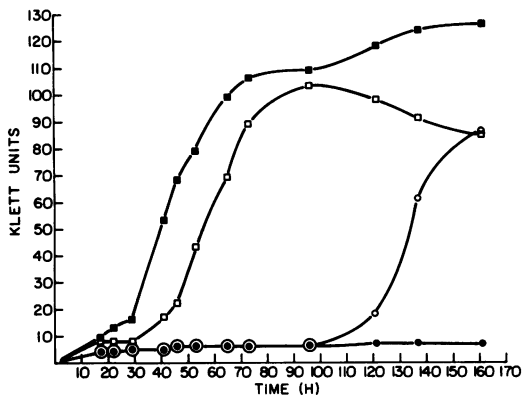

FIG. 3. Growth, based on turbidity, for interaction of *Azotobacter vinelandii* with *Agromyces ramosus* ATCC 25173 or PSU 35 shaken in 0.1-strength heart infusion broth. Symbols: ○, strain PSU 35 alone; □, strain PSU 35 plus *A. vinelandii*; ■, strain ATCC 25173 plus *A. vinelandii*; ●, strain ATCC 25173 alone or *A. vinelandii* alone.

plaques on plates of *A. ramosus* or *E. adhaerens*. The results showed that *A. ramosus* in soil responded to *A. vinelandii* to give an average of 2.5×10^2 PFU/ml of percolate. *A. ramosus* did not, however, respond to *M. luteus* or its autolytic products (assuming that some autolysis of *M. luteus* in soil may have occurred). In a like manner, an average of 5×10^3 PFU/ml of percolate for *E. adhaerens* appeared when *M. luteus* was added to soil, but none appeared when *A. vinelandii* was added to the soil. Thus, *A. ramosus* and *E. adhaerens* specifically attacked their respective host cells in soil. The host cells apparently did not on their own autolyze and release nutrients to allow responses of either *A. ramosus* or *E. adhaerens*.

Broth studies. *Agromyces ramosus* strain ATCC 25173 or PSU 35, with or without *Azotobacter vinelandii* cells, were added to 0.1 strength heart infusion broth. The flasks were shaken at 27°C, and observations were made for turbidity development, morphological changes, and CFU. The CFU determinations were made at the beginning and end of the experiment. Growth (based on turbidity) of both *A. ramosus* strains responded to the presence of *A. vinelandii* cells (Fig. 3). For strain ATCC 25173 with *A. vinelandii* present (determination was not made for strain PSU 35), this represented an increase in CFU of *A. ramosus* from 0-h value of 5×10^8 total cells to 7×10^9 at 161 h. For the control with *A. vinelandii* cells not present, the *A. ramosus* count did not change between 0 and 161 h. This is in agreement with the lack of turbidity change. The initial *A. vinelandii* count of 10^{10} total CFU decreased to 10^9 at 161 h when *A. ramosus* was not present. When *A. ramosus* PSU 35 was present, however, the total *A. vinelandii* count decreased to 3×10^7, and it decreased to 4×10^4 when strain ATCC 25173 was present. In both cases, however, approximately one-third of the *A. vinelandii* colonies on the plates were only one-fifth to one-third of the size of the rest of the colonies. It is not known whether these small colonies developed from damaged *A. vinelandii* cells or whether there were small numbers of *A. ramosus* cells present in these colonies to cause destruction of some of the *A. vinelandii* cells. Counts of *A. vinelandii* were not made during the time of active attack by *A. ramosus* because the *A. vinelandii* cells could not be separated from the mycelium (see below) so that each cell would yield a single colony.

In this experiment, the morphological changes undergone by strains ATCC 25173 and PSU 35 were similar. Relatively little mycelium was present at 17 h. At 29 through 65 h, extensive hyphal development entwined the *Azotobacter vinelandii* cells, and the numbers of *A. vinelandii* cells that were observed decreased progressively. They did not become ghosted but instead seemed to become amorphous blobs before they disappeared. On photographs, these blobs could not necessarily be distinguished from intact cells. Figure 4B shows the entwined *A. vinelandii* cells at 53 h. By this time, however, there were many clumps of mycelium that no longer contained intact *A. vinelandii* cells, or the cells were present as the amorphous blobs. At the end of the experiment (161 h), after active growth had ceased, the mycelium appeared partially or extensively broken apart with some fragmentation into bacillary elements. Most of the *A. vinelandii* cells had disappeared. When *A. vinelandii* cells were not added to the flasks, *Agromyces ramosus* produced short, branched, hyphal extensions which promptly fragmented into coccoid-rod elements that did not yield further growth (Fig. 5).

When the above-described experiment was carried out in a nutritionally richer medium (full-strength brain heart infusion broth), the picture was approximately the same. *Azotobacter vinelandii* by itself did not grow. Strain PSU 35 did not grow in the absence of *A. vinelandii*, but reached 438 Klett units in its presence. Strain ATCC 25173 reached 380 Klett units with *A. vinelandii* present. In the absence of *A. vinelandii* cells, a delayed growth initiation followed by a slowed growth rate allowed strain ATCC 25173 finally to reach 216 Klett units. This delayed growth did not occur when the experiment was repeated. Heat-killed *A. vinelandii* cells, tested in this medium, did not allow the growth responses of strain ATCC 25173 or PSU 35.

Other possible host organisms for strains ATCC 25173 and PSU 35 were tested, as described above, in shaken flasks of 0.1 strength heart infusion broth. There was no response to *M. luteus* or *E. adhaerens*. Growth of strains ATCC 25173 and PSU 35 did respond, however, to the presence of cells of *S. aureus*, *E. coli*, *Agrobacterium tumefaciens*, *R. leguminosarum*, *R. meliloti*, *P. fluorescens*, *P. vulgaris* (but minimal for strain PSU 35), *N. salmonicolor*, and *S. cerevisiae*. The responses to *S. cerevisiae* are shown in Fig. 6. The total counts for *A. tumefaciens* cells while in the presence of *Agromyces ramosus* decreased from an initial value of 1×10^6 to $<4 \times 10^3$ by the end of the experiment. A culture filtrate prepared at the start of maximum stationary growth for strain ATCC 25173 interacting with *A. tumefaciens* showed no inhibition or stimulation of growth of *A. tumefaciens* or of *A. ramosus* strain ATCC 25173 or PSU 35. During the interaction of strains ATCC 25173 and PSU 35 with *R. meliloti*, the *R. meliloti* total counts decreased from an initial value of 4×10^9 to a value of $<4 \times 10^3$ at the end of the experiment. Differential final counts for the other potential host organisms proved difficult or were not made. With *N. salmonicolor* there appeared to be a distinct inverse relation of *N. salmonicolor* pigment density to the amount of growth of strain ATCC 25173 or PSU 35, but this was not quantitated.

Other culture filtrates, in addition to the *Agrobacterium tumefaciens* filtrate noted above, were tested for activity. None of them inhibited or stimulated the test organisms. The filtrates were prepared from the following cultures. *Agromyces ramosus* strains ATCC 25173 and PSU 35 as monocultures in full-strength heart infusion broth were shaken for 7 days then left stationary for 1 additional day; the filtrates were tested against *Azotobacter vinelandii* and *M. luteus*. Monocultures of strains ATCC 25173 and PSU 35 were incubated for 5 to 7 days as both shaken and stationary cultures in full-strength heart infusion broth, 0.1% glucose nutrient broth, and tryptone-Casamino Acids broth; the filtrates were tested against *A. tumefaciens* and *A. vinelandii*. Strains ATCC 25173 and PSU 35, with and without added *A. vinelandii* cells present, were incubated stationary (with intermittent hand shaking) in 0.1 strength heart infusion broth. Samples taken at 5 and 7 days were tested against strains ATCC 25173 and PSU 35, *A. tumefaciens*, *P. putida*, *E. coli*, *R. legumino-*

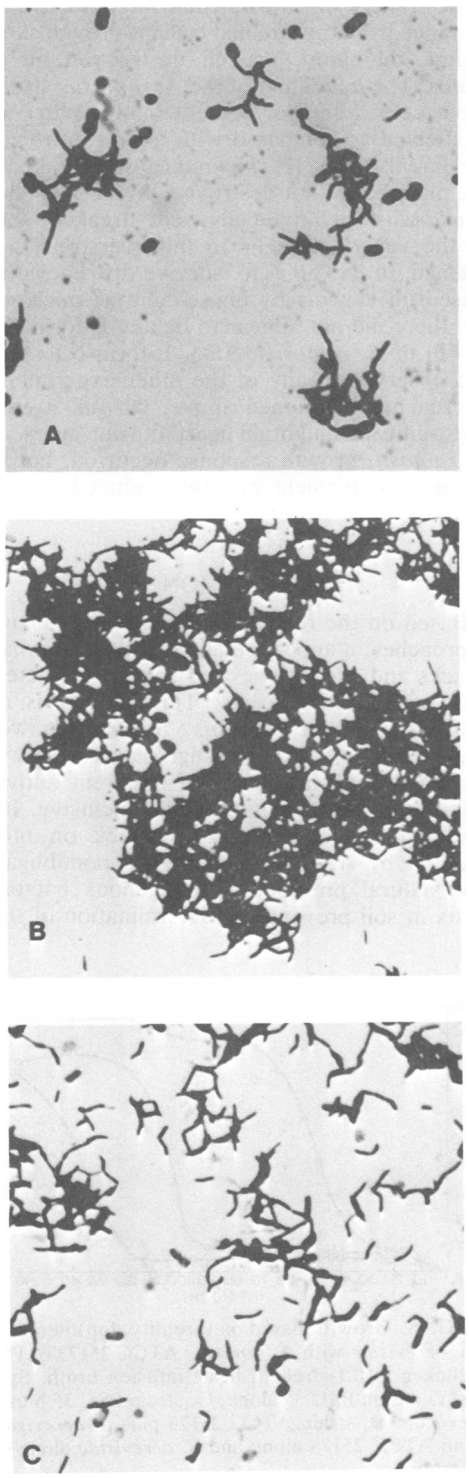

FIG. 4. Interaction of *Agromyces ramosus* ATCC 25173 with *Azotobacter vinelandii* in shaken 0.1-strength heart infusion broth. (A) Hyphal extensions to *A. vinelandii* cells at 29 h. (B) *A. vinelandii* cells entwined in *A. ramosus* mycelium at 53 h. (C) Fragmented *A. ramosus* mycelium at 161 h. Only a few *A. vinelandii* cells were still present at 161 h. Magnification, ×1,120.

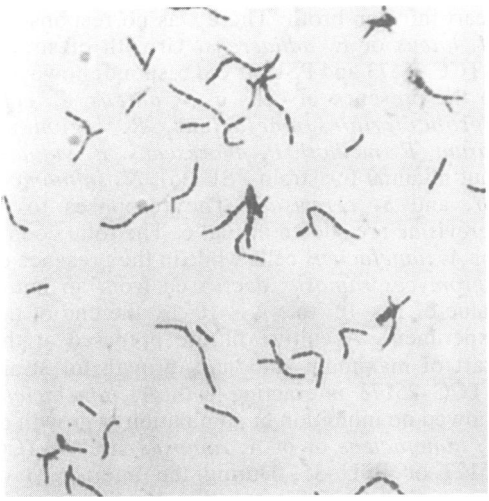

FIG. 5. *Agromyces ramosus* ATCC 25173 shaken for 161 h in 0.1-strength heart infusion broth in the absence of *Azotobacter vinelandii*. Magnification, ×1,120.

sarum, P. vulgaris, and *A. vinelandii*.

Agar column slides and perpendicular streak plates. Various results obtained in situ in soil and in broth cultures were confirmed by use of agar column slides and perpendicular cross-streak plates. Agar column slides containing either 1.5% Bacto agar dissolved in water or 0.1 strength heart infusion agar showed *Azotobacter vinelandii* destruction by *Agromyces ramosus* in a manner similar to that seen in Fig. 1 and 4. The *A. ramosus* cells that were near *A. vinelandii* cells sought out the *A. vinelandii* cells by producing mycelium. The *A. vinelandii* cells were then entwined and destroyed. *A. ramosus* cells not in the immediate vicinity of *A. vinelandii* cells did not multiply. *A. vinelandii* cells in contact with or entwined by *A. ramosus* mycelium became ghosted and then disappeared. *A. vinelandii* cells that were not in contact with the mycelium or that were studied as monoculture control slides did not undergo ghosting, and there was no apparent decrease in their numbers. There was no *A. ramosus* multiplication in the presence of, and no attack on, heat-killed *A. vinelandii* cells. Use of the Bacto agar column slides showed that *A. vinelandii* cysts became surrounded with extensive mycelial development, but this did not result in ghosting or destruction of the cysts.

The perpendicular cross-streak plates on 0.1 strength heart infusion agar showed growth stimulation (Fig. 7) and yellow pigment formation for *Agromyces ramosus* in the part of the *A. ramosus* streak (the overstreak part) that had *Azotobacter vinelandii* or *Agrobacterium tumefaciens* cells mixed in. There was no response to *M. luteus* or *E. coli*, nor was there a response to heat-killed *A. vinelandii* cells (dead *A. tumefaciens* cells were not tested) streaked on the plates. There was no diffusible factor (active at a distance from the streaks) causing growth stimulation, inhibition, or cell destruction on the plates. The initial half of the *A. ramosus* streaks (host cells were not present in this part) was unaffected as compared with monoculture control streak plates. *A. vinelandii* cysts streaked on the plates were not destroyed. In fact, when *A. vinelandii* vegetative cells were streaked, some of the vegetative cells in the overstreak area seemed to be able to survive by encysting. Based on viewing by phase-contrast microscopy, there did not appear to be any L-form cells (8–10) of *A. ramosus*. Also, L-form cells were not observed in any of the other experimental approaches mentioned above. On full-strength heart infusion and brain heart infusion agars, the *A. ramosus* growth response occurred, but the *A. ramosus* pigment was not produced.

DISCUSSION

Based on the results of several experimental approaches, it appears that *Agromyces ramosus* attacks and destroys several species of bacteria and even attacks yeasts. This activity is not obligatory for *A. ramosus*. Some tests were made for activity against fungi (data not shown). Although some instances of apparent activity were noted, the results were inconclusive. It is assumed that in nature the attack on other bacteria by *A. ramosus* really is nonobligate. The natural presence of indigenous bacterial hosts in soil prevented a determination of this.

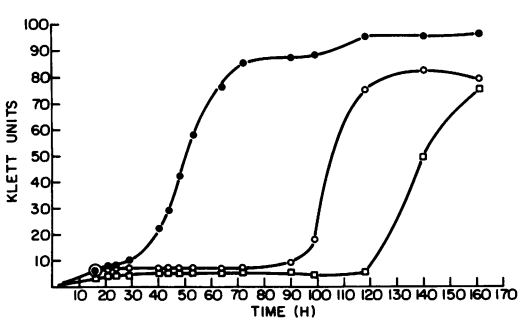

FIG. 6. Growth, based on turbidity, for interaction of *S. cerevisiae* with *A. ramosus* ATCC 25173 or PSU 35 shaken in 0.1-strength heart infusion broth. Symbols: □, strain PSU 35 alone; ○, strain PSU 35 plus *S. cerevisiae*; ●, strain ATCC 25173 plus *S. cerevisiae*. Strain ATCC 25173 alone and *S. cerevisiae* alone did not grow.

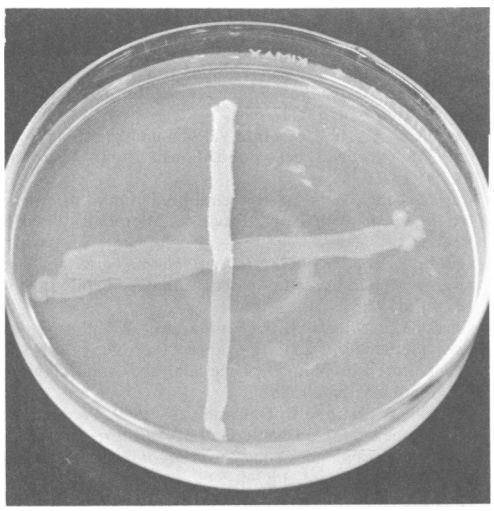

FIG. 7. Growth stimulation of *Agromyces ramosus* PSU 35 in response to *Agrobacterium tumefaciens* on a perpendicular cross-streak plate of 0.1-strength heart infusion agar. *A. tumefaciens* was streaked first as a horizontal streak on the plate; this was followed immediately by a vertical streak of strain PSU 35, with the streak direction from bottom to top. The stimulation occurs only in the upper overstreak portion that contains both *A. tumefaciens* and *A. ramosus*.

However, if the attack in nature were obligate, or nearly so, it could help to explain the fact that *A. ramosus* is so difficult to isolate from soil. The modified dilution-frequency procedure for isolation (2, 7) used soil dilutions beyond the dilution endpoint for other organisms in the soil. The *A. ramosus* cells in these dilutions (no other organisms present) required special conditions and a prolonged period for their adaptation to cultivation on laboratory media. Another isolation procedure, the agar plating isolation method of Labeda et al. (12), yielded *A. ramosus* isolates only on those soil dilution plates that had colonies of other soil bacteria on them. It is possible, therefore, that *A. ramosus*, as it is isolated from soil, has at least minimal dependence on other soil bacteria.

The mechanism by which *A. ramosus* destroys other bacteria could not be determined. No inhibitory or stimulatory activity was found when a variety of culture filtrates was tested. Also, no diffusible factor was seen during growth on agar plates. *A. ramosus* did, however, approach, attach to, and entwine its host cells with its mycelium before destroying the host cells. It is assumed that the ghosting of *Azotobacter vinelandii* cells seen in some instances and the protoplasmic blob seen in other instances relate to the rate at which the cells were lysed under the prevailing conditions.

The precise nature of the benefit derived by *Agromyces ramosus* through attack on other bacteria is unclear. The attack by *A. ramosus* and the resultant growth stimulation of *A. ramosus* occurred over a broad range of nutritional levels provided in its environment. This seems to rule out attack brought on by a major requirement of *A. ramosus* for one or more of the commonly available nutrients. Instead, it would seem that *A. ramosus* needs, but does not have an absolute requirement for, some factor produced in living host cells but not present in dead cells. Several, but not all, of the bacteria tested produced this factor. This factor was not restricted to bacterial host cells, however, since it was present in *S. cerevisiae*. Only a relatively small amount of this factor was required by *A. ramosus* under a given set of environmental conditions, or, alternatively, the relative amount required was not critical. For example, the maximum growth rate portions of the curves for *A. ramosus* ATCC 25173 attack on *Azotobacter vinelandii* and *S. cerevisiae* in Fig. 3 and 6, respectively, can be superimposed. The slopes of these curves were determined. The slopes were also determined for strain ATCC 25173 interactions with three other host species that, as monoculture controls, did not produce even minimal growth in this medium. The slopes for *A. ramosus* interaction with *A. vinelandii, S. cerevisiae, R. leguminosarum*, and *R. meliloti* averaged 3.3, but with a standard deviation of only 0.25. With *Agrobacterium tumefaciens* as host, the slope was 2.3.

Agromyces ramosus is catalase negative although it can grow as a microaerophile or as an aerobe. Nevertheless, the beneficial factor(s) provided by the host cells does not seem to be catalase activity. The attack on other bacteria by *A. ramosus* occurred in shaken cultures, but it also occurred under microaerophilic conditions, i.e., under a 4-mm depth of agar in the column slides. Previously (12) it was shown that 0.02% catalase in the medium did not improve the isolation of *A. ramosus* from soil. In the present study, *A. ramosus* on perpendicular cross-streak plates did not respond to the presence of *M. luteus* or *E. adhaerens* cells, which are catalase positive, nor did it respond to the presence of several of the strongly catalase-positive fungi that were tested. In experiments not reported here, *A. ramosus* growth as perpendicular streaks on 0.1 strength heart infusion agar responded to *Azotobacter vinelandii* even when 0.07% beef liver catalase had been incorporated in the agar or when excess catalase (2 mg) was added as an aqueous spot to the agar surface. The added catalase retained its activity during the experiment. *A. ramosus* as a monoculture did not respond to the catalase.

The ecological significance of the attack of *Agromyces ramosus* on other bacteria in soil is difficult to assess. The fact that this ability to attack other bacteria is available to *A. ramosus* but is not obligatory could help to explain the ability of *A. ramosus* to maintain very high numbers in a nutritionally poor (but nutritionally variable) environment such as soil. However, another factor in the maintenance of high *A. ramosus* numbers is that it does not seem to be destroyed by *E. adhaerens*, which is a bacterial predator in soil. The attack by *A. ramosus* on *Azotobacter* and *Rhizobium* species could help explain the low numbers, or even absence, of these organisms in some soils. However, encystment by azotobacters should protect them so long as the cyst does not germinate. My results predict that *A. ramosus*, to some extent, may be holding in check the plant pathogen *Agrobacterium tumefaciens*. It is not known whether adjustment of the soil environment to the benefit of *A. ramosus* (e.g., fewer of its cells in a dormant state) could increase its activity against *A. tumefaciens* without simultaneously increasing its activity against beneficial soil organisms.

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