Characterization of Bacterial Populations Colonizing the Surface of Arbuscular Mycorrhiza Spores

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Abstract

Arbuscular mycorrhizal fungi are obligate symbiotic fungi that infect the roots of many plants. Recent evidence suggests synergistic interactions between bacterial populations and mycorrhiza may play an important role in the development of the mycorrhizal symbiosis. Several researchers have noted a positive relationship between primary infection of plant roots and the presence of active microbial populations. Similar studies suggest increases in plant yield as well.

The purpose of this research was to 1) Characterize bacterial populations that colonize the surface of arbuscular mycorrhiza spores. 2) Explore the effects of vermicompost's microbiological activity on root colonization by mycorrhiza in greenhouse experiments. Vermicompost has diverse microbiological activities that may enhance plant and mycorrhizal growth.

Glomus etunicatum and *Gigosporia rosea* mycorrhiza spores were obtained from the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) for bacterial isolation. Loosely bound bacterial cells were removed by washing. Tightly bound bacterial cells were loosened by indirect sonication and then plated on rich media. Potential identification of representative isolates was completed by gas chromatograph fatty acid methyl ester analysis (GC FAME). Representatives of similar isolates were chosen for carbon compound utilization characterization. The ability of an isolate to utilize a wide selection of compounds was tested using Ecolog and Gram negative 2 MicroPlates from Biolog, Inc. The isolates were screened on colloidal chitin media for the production of chitinase enzymes. Mycorrhizal spores were also studied by scanning electron microscopy for the presence of bacterial colonies.

Medicago truncatula plants were used in the greenhouse studies. Three potting mixtures were formulated. The potting mixtures included mycorrhizal inoculum in a nutrient poor soil. The treatments included the addition of sterile vermicompost; biologically active vermicompost or sterile vermicompost colonized with selected bacterial isolates from the mycorrhizal spores. After three months, the plants were harvested and percent of root length infected with mycorrhizal fungi was counted using the gridline intersect method.

Characterization of the bacterial isolates by GC FAME analysis resulted in six clusters of closely related bacterial isolates and some other unrelated isolates. These clusters included isolates from both species of mycorrhiza suggesting the colonization of the spores was not accidental. One isolate was found only in bacterial suspensions from crushed spores suggesting a pathogenic or endosymbiotic relationship. Fifteen diverse carbon compounds were commonly utilized by the isolates. None of the isolates were capable of producing chitinase enzymes. This characterization of spore-associated bacteria is the first attempt known to me. This may contribute to understanding the role of mycorrhizal-associated bacteria in mycorrhizal symbiotic relationships.

The plant inoculation experiments did not show any significant difference in the level of mycorrhizal colonization of the plants. However, one spore-associated isolate that was introduced to the potting media was reisolated from spores collected from this treatment. I do not preclude the role bacterial activity may play in mycorrhizal symbiosis and survival. However, it is likely many factors play a role in root colonization by mycorrhiza making it hard to isolate the effects of any one element.

Introduction

Arbuscular mycorrhizal (AM) fungi are a unique group of beneficial soil fungi. AM fungi are obligate symbiotic fungi that must infect the roots of herbaceous angiosperms to survive. AM fungi are distinguished from other mycorrhizal fungi by the ability to form branched intracellular hyphal structures called arbuscules inside the host root cells. The mycorrhiza receives fixed carbon from the plant. In return, the mycorrhiza supplies the plant with necessary soil nutrients. The primary mineral transported is phosphorus (Smith and Read, 1997). Phosphorus is often available in limited amounts in natural ecosystems. Nearly all angiosperms form mycorrhizal relationships, suggesting their importance in natural ecosystems (Tinker and Gildon, 1983).

Recent research suggests bacteria may be a possible third member of this symbiotic relationship. These bacteria have been named mycorrhization helper bacteria (MHB) (Garbaye, 1994). Much of the research thus far has dealt with the differences in infection of plant roots by mycorrhiza or the differences in plant yield when bacterial isolates are included with mycorrhizal inoculum. Although the nature of bacterial-mycorrhizal relationships is not clear, the research does seem to suggest this relationship may be important in mycorrhizal symbiotic relationships.

Plant growth may be enhanced by dual inoculation with mycorrhiza and bacteria.

Azcon (1989) studied the effects of dual inoculation of AM fungi and two rhizosphere bacteria isolated from the plant root zone. Generally, inoculation of AM fungi and rhizosphere bacterial isolates enhanced plant growth. Plant growth was

enhanced the most by inoculation of two bacterial isolates on mycorrhizal plants. *In vitro* studies demonstrated an increase in mycorrhizal spore germination and growth when rhizosphere bacterial isolates where present.

Barea, et al. (1983) studied the interactions between *Azospirillum brasilense*, a nitrogen fixing bacterium, and *Glomus mosseae*, an AM fungal isolate. *A. brasilense* was found to stimulate mycorrhizal infection. This improved plant growth and nutrient uptake. The plants were of similar size, N content and higher in P content than plants grown with additional N and P supplied.

Subba Rao, et al. (1985) inoculated barley plants with five AM fungal species. *A. brasilense* was included as a variable in the inoculum. No treatments had significantly different amounts of AM root infection when *A. brasilense* was included with AM inoculum. However, the combination of some AM species and *A. brasilense* did increase grain yields.

Meyer and Linderman (1986) found dual inoculation of AM fungi and *Pseudomonas putida* increased shoot and root dry weights of subterranean clover. These increases were significantly greater than increases found with the addition of AM fungi or *P. putida* alone. The bacterial-AM fungal combination increased rhizobia nodulation, early AM root infection, and concentrations of micronutrients in the plant shoots.

Possible mycorrhiza-bacteria interactions.

Although the interactions between bacteria and AM fungi are unclear, there is a limited amount of research that suggests possible direct bacterial-AM fungal interactions. Azcon-Aguilar and Barea (1985) surface sterilized AM spores to eliminate resident

microbes. This reduced mycorrhizal growth. However, the growth was restored when a soil filtrate containing soil microbes was added to the media. Further studies suggested these microbes might be enhancing saprophytic growth of the germinating hyphae before infection of the plant roots.

Further studies by Azcon-Aguilar, et al. (1986) suggested free-living fungi might enhance germination of AM spores, enhance AM hyphal growth, and stimulate the formation of AM vegetative spores.

Mayo, et al. (1986) found that bacterial populations that colonized the surface of the AM spores seemed to enhance spore germination. Germination of surface sterilized spores was increased 1.5 to 2.2 fold by the addition of bacterial isolates recovered from unsterilized spores. The presence of bacterial populations also seemed to direct substantial differences in the morphology of the germinating hyphae. The bacterial isolates belonged to several different genera, including *Pseudomonas* and *Corynebacterium*.

Bianciotto, et al. (1996b) found rhizobia and pseudomonads are capable of adhering to the surface of AM fungal spores. The attachment material seems to be an extracellular cellulose based material produced by the bacteria. Bianciotto, et al. suggest this may mediate synergistic or antagonistic bacteria-fungal interactions.

Ectomycorrhiza work.

Much of the more recent MHB work has used ectomycorrhiza. Ectomycorrhiza is a different type of mycorrhiza that forms associations with many trees, especially conifers. One recent example is the work of Frey, et al. (1997). Frey, et al. isolated 300

fluorescent pseudomonad isolates from ectomycorrhiza hyphae and adjacent soil. These isolates where characterized according to carbon compound utilization. These isolates commonly utilized a carbon source that was found to accumulate in the mycorrhizal hyphae. Further analysis suggested the mycorrhiza might select for a particular subpopulation of fluorescent pseudomonads. This selection may be mediated by carbon utilization patterns. Genomic characterization found a high degree of polymorphism among the isolates.

Vermicompost.

Vermicompost is the name given to worm castings obtained when earthworms digest raw organic matter such as manure and plant debris. Vermicompost can be used as an amendment to potting mixes. Bachman (1998) found dry matter increases up to 40% when potting media containing up to 20% vermicompost was used. Vermicompost is known to enhance the mineral nutrition of the potting mixtures. However, sterilizing the vermicompost negated the increases in plant growth. Bachman suggests microorganisms may also be in part responsible for the increase in plant growth.

Cavender (unpublished) found significant increases in AM infection in sorghum when 5 to 20% vermicompost is added to a peat based potting mixture. Generally increases in plant mineral nutrition will limit mycorrhizal infection. Therefore, it is likely other factors such as the microbial populations in vermicompost may be influencing mycorrhizal infection.

Purpose.

The purpose of this research was to characterize the populations that colonize the surface of AM spores. If bacterial populations do play an important role in the development of plant-mycorrhiza symbiosis, then it is likely some of the closely associated bacterial isolates found on the spore walls will play a role. However, it is possible some of these bacterial isolates may also be associated in saprophytic or pathogenic relationships as well as symbiotic relationships. The second objective was to explore the effects vermicompost's microbiological activity may have on the root infection by mycorrhiza in greenhouse experiments.

Materials and Methods

Isolation of bacterial isolates from mycorrhizal spores.

Bacterial colonies were isolated from *Gigosporia rosea* and *Glomus etunicatum* spores supplied by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM). Twenty-five to 50 spores were washed with sterile water using a vacuum filter. The filter used was a Nuclepore filter with 5.0μm pores. This washing step should remove those bacteria that are loosely associated with the spore surface. The washed spores were then suspended in 1 ml of sterile water in an eppendorf tube. This suspension was indirectly sonicated for 3 minutes at 30% power output using a Heat Systems sonicator. The sonicated suspension was divided into aliquots for plating on Luria broth (LB), 1/10 LB, and 1/10 Kings media (KB). Representative isolates were selected on a basis of colony morphological differences. These isolates were purified by streak plating and stored as glycerol stocks.

Potential identification of isolates using Gas Chromatographic Fatty Acid Methyl Ester (GC FAME) analysis.

Potential identities were assigned to the selected bacterial isolates by using GC FAME analysis with a model HP6890 Microbial Identification System. This system used version 4.0 Aerobic Library database (Microbial Identification, Inc., Newark DE). Manufacturer's procedures were followed when attempting to assign a genus or species name to a particular isolate. This procedure requires growing the isolates on a Trypitose soy broth (TSA) for 24 hours. Bacterial cells were collected and lysed. Fatty acids are then extracted from the cell membranes. These fatty acids are separated using a gas chromatograph. The fatty acid profile of an isolate is compared with the profiles of known isolates in the database. From these comparisons, the software generates similarity indices to indicate the degree of homology that exists between the unknown isolate and the known isolate.

Isolates can be identified to the species level when the similarity index between an unknown and known isolate falls between 1.0 and 0.5. Identification to the genus level is possible if the similarity index falls between 0.5 and 0.3. There must be a 0.1 degree of separation between the two closest genus and species names for positive identification to the species level. A 0.1 degree of separation between the top two genus names is required for positive identification to the genus level. If an isolate did not have a similarity index greater than 0.3, or did not have the required 0.1 degree of separation, the isolate's identification remained unknown.

The fatty acid profiles were also used to group the isolates based on their similarity to each other. The software used can calculate how closely related selected isolates are based on their fatty acid profiles. Groups of closely related isolates were organized using this software. Six isolates that represented the most common groupings were chosen for further experimentation. One other isolate was also chosen for further experimentation. This isolate represented isolates that swarmed on KB. These swarming isolates, however, were unable to grow on the required TSA media for identification.

Carbon compound utilization analysis.

The seven representative isolates were tested for their ability to utilize 102 diverse carbon compounds. Eco MicroPlates and Gram Negative 2 MicroPlates from Biolog (Hayward, CA) were used. Log phase cultures of the bacteria were suspended in sterile water. The suspensions were standardized to 8×10^8 cells per ml using optical density values at 600 nm. Growth curves and optical density values were previously obtained for each isolate. Each microtiter well was inoculated with 50 μl of this suspension. In an attempt to limit high background levels, some trials used suspensions that were starved for up to nine hours. A tetrazolium dye was used to indicate oxidation of the compounds. The microtiter plates were monitored for color changes over a period of five days.

Chitinase assay.

The seven representative isolates were tested for their ability to produce chitinase enzymes. A colloidal chitin media containing LB or KB was used in this assay. Dense suspensions of the representative isolates were streaked and stabbed on the colloidal

chitin media. *Chromobacterium*, a species known to cleave chitin, was used as a control. A mixture of all seven isolates was also streaked and stabbed to check for consortium activity. The mixture was also tested for its ability to inhibit the chitinase activity of *Chromobacterium.*

Plant inoculation experiments.

Medicago truncatula was inoculated with a mixed mycorrhizal inoculum supplied by INVAM. The difference in root infection by mycorrhiza in potting media with different microbiological actives was studied. Three different potting mixtures were used for this study. The first mixture contained 3% by weight sterile vermicompost added to a nutrient deficient subsoil. The second mixture contained 3% sterile vermicompost that had been recolonized by the addition of 10% raw vermicompost one week prior to planting. The third mixture contained 3% sterile vermicompost that had been recolonized by the addition of three selected spore-associated bacterial isolates. These isolates were inoculated as suspensions of 10^6 to 10^8 cells per g of vermicompost. All treatments were inoculated with bulk mycorrhizal inoculum at 15% by volume to the top half of each pot.

Twelve pots of 10 plants each were planted for each treatment. A control treatment included 3% sterile vermicompost, but no mycorrhizal inoculum. Four control pots of 10 plants each were planted. The pot volume was approximately one L. The vermicompost was sterilized by autoclaving at 120C, 15 psi, for 90 minutes. The vermicompost was allowed to cool for several days and autoclaved again for a total of three times. The vermicompost was derived from hog manure, and processed by

Vermicycle Organics, Inc. (Charlotte, NC). The seeds of all treatments and the control were inoculated with a dense rhizobia suspension at planting.

The mineral nutrition content of the soil mixtures was assayed by the Star Lab at the Ohio Agricultural Research and Development Center (OARDC). The phosphorus levels in the bulk soil, vermicompost and final mixtures were assayed using the Bray 1 extractant (procedure outlined by Knudsen and Beegle, 1988). Microwave digestion with a Mehlich-3 extractant (Mehlich, 1984) and P concentration determination using inductively coupled plasma spectroscopy (ICP) was also used to analyze the vermicompost (procedure outlined in Eckert and Watson, 1996). This procedure is used to obtain a representation of the P that is available and may become available from organic sources.

The plants were grown in the greenhouse for three months. At this point, 1/3 of the pots were harvested. Subsets of the root ball were taken and stained using trypan blue dye. The percentage of root length infected with mycorrhizal fungi was measured using the gridline interest method (Giovannetti and Mosse, 1980). Plant shoot dry weight was also recorded.

Results

Isolation of bacterial isolates from mycorrhizal spores.

Several batches of bacterial isolates were plated on LB, 1/10 LB, and 1/10 KB. Representatives of different colony morphologies were selected for further study. Eighty-two bacterial isolates were selected from *G. rosea* and *G. entunicatium* spores. Most of the isolates were recovered from *G. rosea* spores. The number of bacteria recovered from the spores varied from tens to hundreds per spore. Each spore seemed to be colonized by several different colonial morphotypes. The variation of the colonial morphotypes found on each spore seemed to be representative of the variation found on the spores in general. One unique isolate was found only when the spores were crushed before indirect sonication.

Identification of spore-associated bacterial isolates.

GC FAME analysis was used to determine the similarity between the unknown spore-associated bacterial isolates and known bacterial isolates. This analysis procedure requires growing the bacterial isolates on full strength TSA. Full strength TSA is a rich media. Some isolates were unable to adjust to this media. These 12 isolates therefore could not be characterized using this identification system. Those isolates recovered from 1/10 KB seemed poorly adapted to growing on full strength TSA media in comparison to those isolated on LB. Seventy bacterial isolates were characterized using the GC FAME system. Isolates were identified to the genus and species level if possible.

These isolates were grouped into clusters based on their similarity in the cell membrane fatty acid profiles. Nine clusters were formed. Twenty-six isolates remained as unique isolates that did not have fatty acid profiles closely matching the profiles of other spore-associated bacterial isolates. Many isolates did not have fatty acid profiles that matched any known isolates in the database. The unique isolate found only in a crushed spore suspension did not have a fatty acid profile that matched to any isolates in the database or any other spore-associated isolates. The six general clusters shown in Table 1 encompassed many of the isolates. Representative isolates of these six clusters

Table 1. GC-FAME identification of spore-associated bacterial isolates.

Isolate	Similarity Index	Isolate	Similarity Index
Isolate 1		**Isolate 5**	
No growth on TSA.		Clavibacter	0.942
Isolate swarms on KB.		**C. michiganense**	0.942
		Corynebacterium	0.596
Isolate 2		C. aquaticum	0.596
Staphylococcus	0.797		
S. epidermidis	0.797	**Isolate 6**	
S. warneri	0.751	**Micrococcus**	0.514
		M. luteus	0.514
Isolate 3		M. lylae	0.420
Ralstonia	0.888	**Bacillus**	0.504
R. pickettii	0.888	B. psychrosaccharolyticus	0.504
Isolate 4		**Isolate 7**	
Arthrobacter	0.739	**Brevundimonas**	0.344
A. crystallopoietes	0.739	B. diminuta	0.344
A. protophormiae/ramosus	0.683	B. vesicularis	0.269
Paenibacillus	0.651	Sphingomonas	0.221
P. polymyxa	0.651	S. paucimobilis	0.221

Tentative identifications are shown in bold. Isolates can be identified to the species level when similarity indices are between 1.0 and 0.5. Isolates can be identified to the genus level when similarity indices are between 0.5 and 0.3. In both cases a 0.1 degree of separation is required between the top two taxa names to be considered a potential match.

were selected for further study. One additional isolate was added to this group to represent the isolates that swarmed on 1/10 KB, but could not be cultured on TSA.

Carbon Compound Utilization analysis.

The ability of the seven selected isolates to utilize 102 diverse carbon compounds was tested using Biolog plates. Most of the selected isolates utilized a limited number of the carbon compounds. However, 15 diverse compounds were commonly utilized by the selected isolates. Table 2 shows a list of the commonly utilized carbon compounds. There does not appear to be any clear trends in these utilization patterns. Isolates 4, 5 and 7 utilized less than 10% of the compounds. Only isolates 3 and 6 utilized more than about one-fifth of the compounds. Isolates 3 and 6 utilized many of the same compounds not listed in Table 2.

Table 2. Substrates commonly utilized by mycorrhizal spore-associated bacterial isolates.

	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7
Pyruvic Acid Methyl Ester	+	-	+	+	+	(+)	+
Tween 40	+	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	+
D-galacturonic acid	+	-	+	+	+	+	+
D-mannitol	+	(+)	+	+	+	+	+
D-xylose	+	-	-	+	(+)	+	-
L-asparagine	+	(+)	+	+	+	+	+
L-threonine	+	(+)	+	-	+	+	-
D-galactonic acid g-lactone	+	-	+	+	-	(+)	(+)
N-acetyl-D-glucosamine	+	-	+	+	+	+	+
Glycyl-L-Glutamic acid	(+)	-	+	-	-	+	(+)
a-keto butyric acid	(+)	(+)	(+)	-	-	-	-
Glycogen	(+)	(+)	(+)	-	-	-	-
a-D-glucose	-	(+)	+	-	-	+	-
D-trehalose	-	(+)	+	-	-	+	-
Other substrates used	8	7	40	1	0	27	0
Total from 102 used	21	16	54	10	9	40	9

(+) shows weak utilization

Some of the isolates consistently showed high levels of background oxidation. In an attempt to limit the background levels, periods of starvation in sterile distilled water were used. These starvation periods were not successful in reducing high background levels. Other isolates had very limited levels of activity on the Biolog plates. These isolates may need some other compounds for active growth or may require different growing conditions than what is offered on a Biolog plate.

Chitinase assay.

One of the commonly utilized substrates was N-acetyl-D-glucosamine, the building monomer of chitin. Colloidal chitin plates were used to test for chitinase activity. Although most of the isolates were capable of using the monomer, none of the isolates were capable of cleaving the chitin themselves. These isolates are often found together on the same spores. Therefore, consortium activity was assayed using a mixture of the isolates. No chitinase activity was observed after one week. The isolate mixture was not able to inhibit the activity of *Chromobacterium*, an active chitinase producer.

Plant inoculation experiments.

The percent of root length infected with mycorrhizal fungi was measured after three months of growth. There was not any significant difference between the treatments at this time point (Table 3). It is important to note the large standard deviations found in the infection percentages. The level of infection was highly variable both between pots in the same treatment and between subsamples within the same pot. Four pots of each

Table 3. Percent root length colonized by mycorrhizal fungi.

Treatment	Average percent root length colonized	Standard deviation
Sterile vermicompost	52%	27%
Self recolonized Vermicompost	53%	17%
Vermicompost Recolonized with Spore-associated isolates	51%	23%
No mycorrhiza Control	0%	

treatment were sampled. Three subsamples were taken from each pot. There was not any observable trends found in the shoot dry weights.

Several of the pots in each treatment were affected by Pythium damping off at emergence. The bulk subsoil used likely was the source of the Pythium infestation. Pythium isolation media was used to confirm the presence of Pythium. The early stages of the disease seemed to affect the sterile vermicompost and control treatments the most. However, the differences in the level of infestation leveled out by two weeks after emergence. The heavily infected pots were removed from the experiment. The pots were planted with excess seeds to account for lower germination percentages noticed in the *M. truncatula* seed batch. All of the remaining pots had at least ten healthy seedlings when the Pythium infestation subsided.

To reduce the inhibitory effects often seen with high phosphorus soils, attempts were made to limit the P levels in the soil. The bulk soil contained very little P. Bray 1 tests showed 2 ppm P was present. Organic P was not important in this soil.

Calculations using a Bray 1 test on the vermicompost was used estimate the amount of P added by the vermicompost. Soil tests taken from the final mixture showed unexpected P levels about 1.5 times field conditions (Table 4). Further testing using Microwave digestion with a Mehlich3 extractant showed a much larger than expected reservoir of organic P in the vermicompost. The vermicompost was about 4% organic P by weight. This is more than enough to supply the plants with all the needed P. Therefore mycorrhizal infection was not likely to be in response to limited soil P. Although other soil nutrients were greatly enhanced by the addition of vermicompost, none were likely to negatively influence plant growth.

Table 4. Potting mixture nutrient analyses.

Standard analyses (Bray 1 P extractant)

Mixture	pH	P (ppm)	K(ppm)	Ca (ppm)	Mg (ppm)	% N
3% sterile verm.	7.8	63	104	3085	419	0.124
3% recolonized verm. with raw verm.	7.8	66	102	3275	422	0.118
3% recolonized verm. with bacterial isolates	7.8	61	113	3120	439	0.124
100% sterile verm.	6.91	2057	1420	2860	1336	
Bulk soil (Organic matter=1.4%)	8.1	2	51	3490	199	

Microwave Digestion with Mehlich-3 extractant for 100% vermicompost.

P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Al (ppm)	B (ppm)	Cu (ppm)
39386	2676	84859	4385	3924	34.84	364.57

Fe (ppm)	Mn (ppm)	Mo (ppm)	Na (ppm)	S (ppm)	Zn (ppm)
7554	855.03	5.10	1774.70	6257	715.00

Visual observation of bacteria on the spore surface.

Scanning electron micrographs were taken using a low pressure SEM. A Hitachi S3500N SEM (Hitachi Scientific Instruments) was used with the help of the Molecular and Cellular Imaging Center (MCI) at OARDC. This SEM can be used to view hydrated samples without fixation or preparation. This allowed direct viewing of the spore surface. Images of both washed and unwashed *G. rosea* spores were studied. The unwashed spore surface is quite cluttered with fragments that are likely organic and inorganic. Washing removed nearly all of these fragments. A very limited number of structures were seen that appeared bacterial. The washed spores contained fewer than five potential bacterial cells. This is in contrast with the bacterial counts found in sonicated spore suspensions. A limited amount of fluorescent microscopy was also used to observe potential bacterial cells at the spore surface. The spores were stained with acridine orange. This stain only stains live bacterial cells with a fluorescent dye. Stained spores did not show more than a few bacterial cells per spore as well.

Conclusions

Several groups of spore-associated bacteria were recovered by indirect sonication from the surface of washed arbuscular mycorrhizal spores. Similar groups of spore-associated bacteria were found on spores from two different mycorrhiza genera. Similar groups of spore-associated bacteria were also found on the spores recovered from the pots of mycorrhizal plants growing in several different potting mixtures. Recovering consistent groups of spore-associated bacterial isolates suggests these isolates may be uniquely or specially adapted to colonization of arbuscular mycorrhizal spores.

One unique isolate was found only in bacterial suspensions from crushed mycorrhizal spores. This isolate must be located inside the spore wall or deeply lodged in the spore wall itself, suggesting a pathogenic or endosymbiotic relationship. Bianciotto, et al. (1996a) discovered an obligate endosymbiotic bacteria-like organism in the spores and hyphae of mycorrhiza. This supports the possibility of an endosymbiotic relationship.

These spore-associated bacterial isolates utilized a limited number of carbon compounds. Fifteen of the 102 compounds were utilized by most of the selected isolates. This suggests these bacterial isolates may depend on specific carbon sources that are found on the mycorrhizal spore surface.

Mycorrhizal spores walls contain a high proportion of chitin in a thick composite wall (Smith and Read, 1997). None of these selected spore-associated isolates were found to produce chitinase enzymes, alone or in consortium. Bonfante-Fasolo and Vian (1984) suggest bacteria may enhance spore germination by partly digesting the cell wall. However, my results suggest these isolates do not actively degrade spore walls.

No significant differences in root colonization were observed when spore-associated bacterial isolates were introduced in the soil. Several researchers have found significant differences in root colonization by mycorrhiza when selected bacterial isolates are added to the mycorrhizal inoculum (Azcon, 1989 and Meyer and Linderman, 1986). However, the selected spore-associated bacteria I introduced may not contribute to host infection by mycorrhiza. These isolates may instead represent species that are saprophytic specialists or pathogens of the spores. If they are symbiotic, they must act via a different mechanism that was not observed in this study.

One of the introduced isolates, a bacillus, was recovered from the spores collected from the pot of mycorrhiza infected plants in this treatment. This bacillus isolate is at least capable of finding and colonizing the spores from generation to generation.

No significant differences in root colonization were observed when the microbial activity of vermicompost was introduced to the soil. The biological activity of vermicompost must not be responsible for the increased mycorrhizal infection observed by Cavender (unpublished). However, counting infection percentages at three months may have masked early differences in root infection by mycorrhiza. The high phosphorus levels found in vermicompost may have affected mycorrhizal infection as well.

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