Bard Final Scientific Report Cover Page

BARD Project Number: US-4264-09

Institutions Identifying Number: 10011537

Date of Submission of the report: July 15, 2013

Project Title: Shaping Plant Beneficial Microbial Communities

Investigators

Institutions

Principal Investigator (PI):

David E. Crowley

University of California, Riverside

Co-Principal Investigator (Co-PI):

Dror Minz

Volcani Research Center

Collaborating Investigators:

Yitzhak Hadar

Hebrew University of Jerusalem

Keywords: microbial ecology, plant-growth promoting bacteria, rhizosphere, soil microbiology

Abbreviations : 2,4-DAPG 2,4-diacetylphloroglucinol, DGGE- denaturing gradient gel electrophoresis, IAA - indole acetic acid, PGPR- plant growth promoting rhizobacteria
PCA -principle components analyses, PLFA - phospholipid fatty acid analysis
Q-PCR- quantitative PCR, RT – PCR – reverse transcriptase Q-PCR

Budget:

IS: \$194,000

US: \$140,000

Total: \$334,000

Signature
Principal Investigator

Myrna A. Lindo
Sr. Contract & Grant Officer

Signature
Authorizing Official, Principal Institution

Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged		4	2	6
Submitted, in review, in preparation			2	2
Invited review papers				
Book chapters				
Books				
Ph.D. theses				
MS. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training:

Shajid Nadeem – University of California, Riverside
B. Shaharouna – University of California, Riverside
Maya Ofek – Hebrew University of Jerusalem
Milana Voronov-Goldman – Hebrew University of Jerusalem

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together elsewhere	Total
Short Visits and Meetings	1	0	0	1
Longer Visits (Sabbaticals)	0	0	0	0

Description of Cooperation:

Cooperation was facilitated by joint design of research project to explore different facets of the rhizosphere ecology and factors influencing the population size and activity of plant growth promoting bacteria on root surfaces. Experiments were conducted with the same plant species and used the same bacterial strains where possible. Research results were shared by email communications and by Skype meetings. At the close of the project, Crowley traveled to Israel to meet with the research groups led by Dror Minz and Yitzhak Hadar. During the two week visit, Crowley presented a seminar at the Volcani Center, had discussions with the students, evaluate results together with Dror Minz and Yitzhak Hadar and gave a 45 minute talk at the Israeli Society for Microbiology Meeting held in November 2012.

Abstract

PGPR bacteria include taxonomically diverse bacterial species that function for improving plant mineral nutrition, stress tolerance, and disease suppression. A number of PGPR are being developed and commercialized as soil and seed inoculants, but to date, their interactions with resident bacterial populations are still poorly understood, and almost nothing is known about the effects of soil management practices on their population size and activities. To this end, the original objectives of this research project were:

- 1) To examine microbial community interactions with plant-growth-promoting rhizobacteria (PGPR) and their plant hosts.
- 2) To explore the factors that affect PGPR population size and activity on plant root surfaces.

In our original proposal, we initially proposed the use of low-resolution methods mainly involving the use of PCR-DGGE and PLFA profiles for analysis of microbial community structure. However, early in the project we recognized that the methods for studying soil microbial communities were undergoing an exponential leap forward to much more high resolution methods using high-throughput sequencing. The application of these methods for studies on rhizosphere ecology thus became a central theme in this research project. Other related research by the US team focused on identifying PGPR bacterial strains and examining their effective population sizes that are required to enhance plant growth and on developing a simulation model that examines the process of root colonization.

As summarized in the following report, we characterized the rhizosphere microbiome of four host plant species to determine the impact of the host on resident versus active communities. Results of our studies showed a distinct plant host specific signature among wheat, maize, tomato and cucumber, based on the following three parameters: (1) each plant promoted the activity of a unique suite of soil bacterial populations; (2) significant variations were observed in the number and the degree of dominance of active populations; and (3) the level of contribution of active (rRNA-based) populations to the resident (DNA-based) community profiles. In the rhizoplane of all four plants a significant reduction of diversity was observed, relative to the bulk soil. Moreover, an increase in DNA-RNA correspondence indicated higher representation of active bacterial populations in the residing rhizoplane community. This research demonstrates that the host plant determines the bacterial community composition in its immediate vicinity, especially with respect to the active populations.

The results of this research provide fundamental new insight into the composition of the bacterial communities associated with plant roots, and the factors that affect their abundance and activity on root surfaces. The application of transcriptome methods pioneered in our research will ultimately lead to better understanding of how management practices such as use of compost and soil inoculants can be used to improve plant yields, stress tolerance, and disease resistance. In addition, expression of several genes in rhizospheres of both cucumber and wheat roots were identified, including mostly housekeeping genes. Denitrification, chemotaxis and motility genes were preferentially expressed in wheat while in cucumber roots bacterial genes involved in catalase, a large set of polysaccharide degradation and assimilatory sulfate reduction genes were preferentially expressed. As we look to the future, the use of metagenomic techniques combined with quantitative methods including microarrays, and quantitative PCR methods that target specific genes should allow us to better manage the plant rhizosphere to improve crop yields in agricultural ecosystems.

Project Background.

For decades it has been recognized that plants and microorganisms have co-evolved over time through natural selection that has led to plant specific bacterial populations that enable plants to adapt to different soils and to resist environmental stress and root pathogens. This selective enrichment process is facilitated by the release of root exudates and other plant derived materials (lysates, mucigel) that are used as carbon sources for growth of mutualistic bacteria that release nutrients from organic matter, fix nitrogen, solubilize minerals, produce growth hormones, and degrade toxic substances. The zone of influence in which plant root exudates affect microbial growth and bacterial community structures is termed the rhizosphere, which is generally estimated to extend from the root surface, or rhizoplane, to about 1-3 mm distance from the root, depending on the particular root location. From prior knowledge, we know that most of the root exudates are released in the zone of elongation that occurs a few millimeters from the root tip, to a distance of about 1 cm behind the root tips. It is estimated that as much as 15% of the carbon captured by photosynthesis is released into the rhizosphere to support the commensal growth of the rhizosphere microbial community. It has been hypothesized that differences in the growth rate of various bacteria on this chemical mixture is a key driving force for the selection of the different microbial species that colonize roots. However, the extent to which plants select for different microbial communities is still poorly understood. In part this is due to the incredible complexity of the rhizosphere, in which the chemical composition of root exudates has been shown to vary for different plant species and cultivars, and in relation to the type of soil, the plant nutritional status, temperature, soil pH, and the developmental stage of the plant over time.

While early research focused on culturable bacteria that are largely comprised of a few dominant genera such as *Pseudomonas* and *Bacillus*, more recent research using molecular biology methods has suggested that the rhizosphere is actually inhabited by tens of thousands of species, the vast majority of which are nonculturable. Nonetheless, much research conducted up through the last decade has focused on those culturable bacteria that have been shown to directly influence plant root growth and architecture, and suppression of root diseases, with the aim of developing seed or soil inoculants that can be used to improve plant yields and exploit natural processes contributing to plant growth and root health. At the time this research was initiated, and continuing through today, there has been great interest in understanding how soil and environmental factors, and agricultural management practices, affect the populations and activity of plant growth promoting bacteria (PGPR) in the plant rhizosphere. Yet the answer to this question requires a fundamental understanding of the species compositions of the plant rhizosphere, which is now only becoming accessible using molecular biology methods.

Rationale for Investigating PGPR and the Plant Rhizosphere.

Virtually every soil microbiology laboratory has collections of bacteria that can stimulate root growth and suppress root disease when introduced into soils at high population densities by soil or seed inoculation. However, the practical use of PGPR bacteria has languished due to the difficulty in maintaining high population densities of single strains after their introduction into soil. New technologies are still being investigated to improve the survival and activity of soil inoculants such as the use of microalginate bead encapsulation and most recently, the use of biochar which contains a large pore space that can provide a protected habitat. To a large extent, the population size of individual strains of bacteria appears to be constrained by competition and other interactions with the

indigenous microbial community, and by predation by protozoa and nematodes. There is also recent work demonstrating that bacterial species interact with resident communities through quorum sensing which entails chemical signals that regulate stationary phase, and production of substances associated with plant growth promotion.

Still another mode of action for PGPR involves the production of an enzyme that regulates ethylene production by plant roots. Ethylene is a master hormone that interacts with auxins to control root growth. Under stress conditions, plant roots produce high concentrations of ethylene, termed stress ethylene, which inhibits further root growth. This presumably serves a protective function to limit growth during stress episodes, but also can be detrimental over the long term when restricted root growth leads to poor root system development and a decreased capacity to explore the soil for water and nutrients. Interestingly, in this case the plant allows the PGPR to intercede by secreting the precursor of ethylene, a molecule called aminocyclopropane carboxylate (ACC), into the rhizosphere in the zone of elongation. PGPR with the enzyme ACC deaminase degrade the precursor molecule and allow the plant roots to continue some degree of growth such that they are better able to grow through the stress period and take up water and nutrients, often leading to improved stress tolerance, and plant yields.

In this project summary, now being prepared for publication, we report the findings of our research on the bacterial community within the bulk soil and rhizoplane of 12-day-old cucumber, tomato, wheat and maize seedlings. Following normalization and subsampling of the data, (from a total of 296,905 high quality bacterial 16S rRNA gene /transcript sequences), a table of OTU frequencies containing 1,102 OTUs was obtained. Normalized pair-wise similarities between all samples (32 from roots rhizoplane and 30 from soil rhizosphere), were used to examine the effects of root proximity (soil vs. rhizoplane), molecule type (DNA vs. RNA) and plant species, as individual factors using ANOSIM and AMOVA, with three different similarity indices (Bray-Curtis index, Jaccard coefficient, and Yuan & Clayton theta). These analyses revealed significant effects ($P < 0.01$) for all three factors

Significant plant host signature effect observed for both bulk soil and rhizoplane communities

The effect of plant species on the community of bulk soil was best resolved at the 16S rRNA level, while DNA based 16S rRNA profiles of the different plant species did not uniquely cluster (Figs. 1 and 2). A similar trend was also observed in the structural parameters of these communities. Both richness and evenness could not be distinguished among plant species in bulk soil DNA samples, but significantly differed for the rhizosphere soil RNA (active communities). This demonstrates that although a similar bacterial pool resided in the bulk soil of all four plants, each plant species stimulates the activity of different subsets of populations.

To examine the role of plant species in stimulation of activity in the rhizosphere, we compared the bacterial profiles between DNA and RNA samples. Using a stringent false-discovery-rate (FDR) value of < 0.001 (Benjamini adjusted), a total of 35 OTUs were verified as enriched in RNA compared to DNA profiles of the different plant species' bulk soil samples (Fig. 3a). The majority of those (30 of the 35 OTUs) showed plant species-specific pattern and comprised significant percentages of the respective active communities (Fig. 3a). Differences in taxonomic composition of the plant species-enriched OTUs were notable, with Betaproteobacteria characterizing the maize-enriched OTUs, Bacteroidetes and Actinobacteria in the cucumber rhizosphere, and Planctomyces and Cyanobacteria

for wheat and tomato respectively. Furthermore, for each plant species, the most significantly stimulated OTUs belonged to different phyla (Fig. 3b-e). Our results demonstrate a clear plant effect and a unique plant species signature on the active community within the bulk soil. The dominance of unidentified cyanobacterial rRNA in tomato bulk soil is surprising.

Figure 1: Composition of bulk soil (a) and rhizoplane (b) bacterial communities of four plant species. Compositions were compared based on rarified OTUs table, defined at 97% sequence similarity threshold. Non-metric multidimensional scaling analyses was performed based on pairwise, normalized Bray-Curtis distances between samples. The stress values indicate the degree of fit between the original distances in the matrix and the reproduced distances within the ordination plot.

Figure 2: Neighbor-joining hierarchical clustering of UniFrac metrics between bulk soil (squares) and rhizoplane (triangles) DNA (a) or RNA (b) samples. The UniFrac distances were calculated pairwise based on OTUs table (defined at 97% sequence similarity threshold) after sub sampling. Color coding is the same as in Figure 1. The scale bar represents UniFrac distance metric. Triangles and squares represent soil and root samples, respectively.

Figure 4: Relative abundance of dominant classes (>2% of total reads) in active bacterial communities of the rhizosphere of four plant species. Means and standard deviations of four replicates are presented. For each presented class, different letters indicate significant difference based on non-parametric Kruskal-Wallis ANOVA ($P < 0.05$), followed by multiple comparisons of mean ranks.

In detail, activity of Gammaproteobacteria was stimulated in the rhizosphere of maize and wheat; Stimulation of Alphaproteobacteria, Sphingobacteria and Flavobacteria characterized the cucumber rhizosphere, and in tomato, members of Betaproteobacteria class were highly stimulated (Fig. 5). Other classes, including Actinobacteria and Deltaproteobacteria, although displaying significant variation among plant species rhizosphere communities, contributed less to the plant species-specific signature (*i.e.*, the variance was not linked to a single plant species).

Many of the stimulated groups found in the current study are generally classified as copiotrophs (*e.g.*, Gamma- and Betaproteobacteria, Flavobacteria and Sphingobacteria). The rhizosphere of the four plant species could clearly be characterized by high dominance of very few active populations defined as OTUs of 97% sequence similarity (Fig. 6, appendix). The cumulative relative abundance (RA) of the four most abundant OTUs in rhizosphere communities accounted for 78%, 69%, 55% and 25% of the active community in (respectively) maize, wheat, tomato and cucumber rhizosphere. This level of dominance demonstrates the highly selective, specific impact of roots. Nevertheless, as DNA based studies demonstrated that community complexity increase with plant age (Gomes *et al.*, 2001; Ibekwe and Grieve 2004), it may represent an early, transient stage in root bacterial community succession, governed by high root growth and rhizodeposition rates.

In comparing the different communities, the dominance of *Pseudomonas* in both maize and wheat rhizosphere communities was most striking. *Pseudomonas* formed 67% and 48% of the active bacterial community of maize and wheat rhizosphere, respectively (Fig. 7, appendix). Bacteria of this genus are renowned for their plant growth-promoting properties and are often shown to be enriched in the rhizosphere of many plant species. Only few reports provide assessment of the taxonomic composition of wheat or maize rhizosphere bacterial communities, and the richness of indigenous *Pseudomonas* in particular. Another interesting association was that of *Cellvibrio* spp. (members of Pseudomonadaceae)

and cucumber roots (Fig. 7, appendix). While accounting for 11.5% of the dominant active populations, their relative abundance of the total bacteria based on DNA data in cucumber rhizoplane, reached 20%. Members of this genus are known for their ability to degrade plant cell wall components and other complex polysaccharides. We have previously reported high RA of *Cellvibrio* spp. colonizing root surface of cucumber seedling after 48h of germination in disease suppressive compost amended potting medium (Ofek *et al.*, 2011). This evidence points to a possible general plant-beneficial effect of *Cellvibrio* members and further stress the importance of the particular enrichment in the cucumber rhizoplane. The most dominant active bacterial population in the tomato rhizoplane, with RA of 37%, was classified as related to the order Burkholderiales.

Gene expression in root microbial communities

mRNA of the root community in cucumber and wheat was extracted, purified and reverse transcribed. The resulting cDNA was sequenced by Illumina and the resulting metatranscriptome was analyzed. The results are presented in figures 8 and 9. The major findings of this analysis were that in both roots the microbial community expressed housekeeping genes. Genes expressed preferentially to one of the roots were those responsible for denitrification, chemotaxis and motility (wheat roots) and genes involved in catalase, a large set of polysaccharide degradation and assimilatory sulfate reduction (cucumber). These results are currently still analyzed bioinformatically.

Figure 5: Average relative abundance of dominant OTUs in active rhizoplane bacterial communities of four plant species (including the five most dominant OTUs for each plant species). Each bar represents a single OTU defined at 97% sequence similarity threshold.

Figure 8. Genes highly expressed in both cucumber and wheat roots

Figure 9. Genes preferentially expressed by bacterial communities associated with wheat or cucumber roots.

Returning to other themes laid out in the original proposal, research conducted at UCR during the last year of the project examined the effects of organic amendments that enhance the population size and activity of PGPR in the rhizosphere, using cucumber as a model plant species. Experiments conducted on this final topic examined the use of compost or pyrolyzed carbon (biochar). Compost provides a slowly labile source of carbon, whereas biochar is a highly stable carbon material that is now being promoted as a means to store carbon in soils and to enhance soil physical, chemical, and biological properties. In experiments conducted in the final year of the project, we examined the effects of these amendments on the population size of indigenous rhizosphere bacteria with ACC deaminase activity, and on the effects of soil inoculants derived from the 11,700 year old King Clone of *Larrea tridentata*. The isolates selected for use as soil inoculants were prescreened for their temperature tolerance, with the idea in mind that introduction of heat tolerant PGPR into the rhizosphere may help to protect plants against thermal stress as well as the other types of environmental stresses that have been demonstrated in previous work (drought, heavy metals, pH, hypoxia). Our screening of the King Clone isolates showed that many of the bacterial strains we isolated, including gram-negative bacteria, have extreme temperature tolerance that exceeds the previously described temperature limit of 42°C for pseudomonads. As shown in Table 1, isolates from the *L. tridentata* rhizosphere from the Mohave desert were able to grow at temperatures of 50°C. Experiments are still in progress with these bacteria to determine their ability to increase plant tolerance to high soil temperatures and will be reported in an article that will be submitted after the close of this grant.

Results of the experiments that were conducted to examine biochar and compost effects on the population size and activity of PGPR with ACC deaminase activity and the effects of these treatments on plant growth are presented in Figure 10 and Table 2 (appendix). Cucumber plants were grown in a sandy loam soil with complete fertilizer to supply adequate nutrients. Inoculation of plants with PGPR and use of either compost or biochar all significantly increased plant root and shoot biomass as compared to control plants grown in unamended soils, with compost plus inoculant yielding plants with the highest biomass. This increase in plant biomass was associated with increased populations of PGPR that were obtained by use of compost or soil inoculation with ACC degrading bacteria (Fig 10, appendix). Plants produced in the control treatment had a biomass of 22 g with approximately 10^3 cfu of ACC degrading bacteria in the rhizosphere. Use of compost stimulated an increase in population density of indigenous ACC degrading bacteria to approximately $10^{4.5}$ cfu g root, which was associated with a 30% increased biomass. Inoculation of seeds with ACC degrading bacteria resulted in population densities of 10^7 to 10^8 cfu g root. Plants receiving both the compost and PGPR inoculant had 10^8 cfu g root of ACC degrading bacteria, and had the highest biomass at 38.3 g (~75% greater biomass than the control plants). Use of biochar alone or in combination with compost also significantly increased plant biomass, but was not associated with large increases in ACC degrading bacteria, suggesting that the increase in population densities of PGPR is associated with the increase in labile carbon that occurs with use of compost as a soil organic amendment. The mechanisms behind plant growth promotion with the use of biochar are still not understood, but are being investigated by us and other researchers who are now studying this phenomenon.

Major conclusions, solutions, achievements.

To the best of our knowledge, this is the first study to employ high-throughput sequencing technology for a coupled DNA and RNA analysis of soil and rhizoplane bacterial community composition. This analysis enables issuing of data regarding the contribution of residing bacterial populations (DNA-

based analysis) to the active community (RNA-based analysis). This task was mainly addressed in the past employing comparative methodologies, such as PCR-DGGE with relatively low resolution. In order to examine DNA-RNA correspondence, the correlation coefficient between DNA and RNA profiles were calculated for each plant species in each compartment (Fig. 6, appendix). Means were then compared by non-parametric Kruskal-Wallis test, which verified significant differences ($P < 0.001$). For all plant species (although to a lesser degree in cucumber), the DNA-RNA mean correlation coefficients were higher in rhizoplane samples compared to soil samples. This indicates higher representation of residing bacterial populations in the active community at the rhizoplane, *i.e.*, the rhizoplane bacterial community may be composed mainly of active cells. However, the rhizoplane correlation coefficient values varied greatly among plant species, with maize presenting the highest mean, followed by wheat and lowest were those of tomato and cucumber samples (Fig. 6, appendix). This, again, emphasizes the different nature of the rhizoplane fraction of different plant species.

By the combination of simultaneous data collection from RNA and DNA, some clues can be found for the different physiological states of dominant bacterial populations in association with the host plant roots. In order to improve the ability to compare trends among different OTUs, the rarified reads counts were normalized to the average number of rRNA of known affiliated species, according to the ribosomal RNA database (rrnDB, Michigan State University). For most of these dominant active populations, the RNA-DNA ratio was >1 , indicating that most cells in the population were highly active. In contrast, one of the two populations of *Cellvibrio* that dominated the cucumber rhizoplane had RNA-DNA ratio <1 (Fig. 7, appendix). This situation could reflect technical limitations (*e.g.*, variability in cell lysis during nucleic acids extraction), but could indicate different population dynamics scenarios for this group: 1) the physiological state of individual cells in the population is highly variable; 2) following a peak in activity and abundance, the population decline, due to shift in niche conditions or due to competitive exclusion. The latter could be related to a natural age-related process of succession, characterizing rhizosphere communities.

Achievements

One of the major challenges in exploiting PGPR for agricultural biotechnology has been the lack of methods to monitor the population size and activity of specific bacterial strains that are used as soil inoculants, and a general lack of understanding of microbial ecology and the interactions within bacterial communities. Using selected marker gene sequences, the population densities of specific bacterial strains or taxonomic groups can be monitored over time and in specific root locations, but population size alone does not reveal their actual level of activity. In this case, it is necessary to also use cDNA methods to determine differences between the abundance and levels of expression of particular genes. Other difficulties in dissecting microbial interactions include a general lack of knowledge on the functional traits of the vast majority of root colonizing microorganisms, which may employ different genes and pathways to produce substances that affect root growth, or that contain variations in the gene sequences that are used to monitor these processes. Ultimately, the only way to get to the heart of microbial ecology appears to be the use of high throughput sequencing and metagenomic and transcriptomic analyses of the rhizosphere community, under highly defined conditions that take into account the plant species, root zone location, plant age, soil texture, and the soil organic matter content.

To this end, the research conducted here examined the complexity of bacterial communities in high detail in terms of resident *vs.* active components. Use of high throughput technology enabled the detection and taxonomic identification of distinct bacterial classes, stimulated by different plant hosts, thus pointing towards a host-linked variation in soil microbiome functions. Unlike the bulk soil, though under strong soil influence to which it is exposed, the rhizoplane presents a high degree of selectivity towards both its residing and active colonizers with a significant plant host signature. This selectivity was indicated by a dramatic loss of diversity, a strong shift towards a more structured community, high correspondence between prevalence and activity of populations and major deviations in composition among hosts. It is thus suggested that the root has a major effect on its microbiome that is manifested in its immediate vicinity. Although DNA evidence point to a short range influence on soil communities, the root effect on the active fraction prevails even to the more distant zone of the surrounding soil. The major influence of the plant host on the composition of active populations suggest there may be significant differences in the functional services rendered by different host associated microbiomes that remains to be addressed.

During the course of our studies, we also investigated the effects of compost, salinity, and the presence of a plant root pathogen on the population sizes and activity of selected bacterial strains. The research culminated with the application of a transcriptome analysis that allowed a comprehensive description of the taxonomic structure of the rhizosphere community associated with cucumber plants, and a means to differentiate between active and dormant populations over time and in relation to specific changes in soil conditions. As this represents a somewhat limited scenario, we also were interested in developing simulation models and artificial neural network models that allow the analysis of nonlinear interactions among all of the variables that affect PGPR populations and the structure of microbial communities. The latter effort is expected to provide a conceptual foundation and extrapolation of our findings to other plant-bacterial-soil combinations as this research continues in future research by our group and others.

In addition to our main research project, we also took advantage of opportunities arising from other ongoing projects to carry out complimentary research that may provide avenues for direct application of PGPR based agricultural technology. This included the analysis of the rhizosphere of one of the world's oldest living plants, the 11,700 year old King Clone of *Larrea tridentata*, from which we obtained bacterial isolates that were used to inoculate cucumber plants. From this study we established the effective population size for PGPR, which we estimated at 10^5 cfu per gram rhizosphere soil in the zone of elongation. We further demonstrated that individual isolates varying in their production of auxin and ACC activity had dramatically different effects on root growth and architecture (branching patterns). Likewise, taking advantage of another ongoing study examining salinity tolerance in avocado, we enumerated the populations of PGPR with ACC deaminase activity that were associated with different rootstocks of avocado in soils that varied in salinity.

Rhizosphere bacteria that suppress stress ethylene production via 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity play an important role in alleviation of plant stress caused by salinity and drought. While there has been considerable research on development of soil inoculants that carry this trait, relatively little is known about the population dynamics and functional diversity of indigenous ACC-degraders in field soils. Here, we surveyed plant growth promoting rhizobacteria (PGPR) that are associated with avocado trees under different degrees of stress resulting from irrigation-induced soil salinity. Variations in PGPR populations were examined for 4 rootstocks at 3 locations with levels of salinity ranging from 0.91 to 3.90 dS m⁻¹. To characterize the culturable ACC degrading bacteria, soil water extracts from rhizosphere samples were streaked on to media containing

ACC as sole nitrogen source. 10^5 colonies were isolated from which 20 were selected and identified by 16S rRNA gene sequence analysis. These strains were further characterized with respect to indole acetic acid (IAA) production, phosphate solubilization, and siderophore and cyanide production. Results of the survey revealed that all of the rootstocks supported indigenous ACC-degrading rhizobacteria at population densities that ranged from 1.5×10^4 to 8.5×10^6 cells g⁻¹ rhizosphere soil, with the highest population densities occurring on the fine roots in the zone of elongation behind the root tips. The culturable ACC-degrading PGPR included species of *Pseudomonas*, *Bacillus* and *Variovorax*. All of the cultured isolates had different combinations of PGPR traits and varied substantially in their maximum activity levels as measured under laboratory conditions. PGPR population densities varied for the different rootstocks but were not significantly influenced by differences in soil salinity. The results suggest that indigenous PGPR are common in orchard soils, and that soil management practices that increase the population and activity of indigenous ACC degrading bacteria may serve as an alternative to soil inoculation for enhancing the stress tolerance of salt sensitive crops in saline soils. These studies suggested that in typical orchard soils, the PGPR populations associated with fruit tree roots were just at or below the threshold densities that are required to improve plant tolerance to saline soils. We also evaluated the effects of these bacteria on cucumber root growth. These extensions of our primary research suggests that we are now at a stage where practical applications of our research can be realized by applying the newly developed tools to monitor PGPR under field conditions as this technology becomes more routine and affordable.

Another component of our research was the development of automata cellular and agent-based models that were developed to model vertical and horizontal colonization of the rhizoplane by bacteria under different numerical scenarios. The formation of bacterial aggregates on the rhizoplane as well as increased abundance of bacteria on the rhizoplane zone with increased nutrient availability was also simulated and predicted by using several bacterial (feeding, starvation and reproduction) and environmental (space, nutrient content and pH) parameters. The present model is the first approach to analyzing experimental and theoretical parameter values in the rhizosphere, but is still based on only one bacterial species. Future research should be focused on evaluating the competence and ecology of various bacterial species in the rhizoplane microsites and developing more accurate predictive models that can stimulate new investigations with practical applications for agricultural biotechnology. While some (mainly housekeeping related) genes are expressed in both cucumber and wheat roots, other genes were specifically preferentially expressed in one of the two plant root systems.

Details of cooperation: whether and how project objectives were promoted as a result of the cooperation.

Our original project plan involved a close coordination of experiments in which we set out to examine a broad range of questions as laid out in our original proposal. One important aspect of our collaboration was the use of the same PGPR strain *V. paradoxus* and cucumber plant model, which provided complimentary information on the interactions of inoculated PGPR with indigenous PGPR and with the larger bacterial community that inhabits the rhizosphere. To this end, the US team provided a strain of the PGPR bacterium *Variovorax* to the Israeli team, which was used in their initial research studies. We also designed our experiments to use the same plant species and soil textural class, and each team carried out research examining the effects of soil organic matter amendments on survival and activity of the selected PGPR inoculant. Nonetheless, as the research progressed, each team increasingly focused on the most productive avenues of research by their students and postdoctoral associates to bring the work forward for publication. This was especially the case with the transcriptome component of the project, which was carried out by the Israeli team.

One component of this research that we were hoping to complete during the three year duration of this grant was the exchange of reporter strains that were planned for use in experiments in both countries. Due to difficulties in completing that portion of the project in time for the third year, we were not able to carry out those experiments. However, we view our cooperation as highly successful, mainly in the way in which both teams sought to provide complimentary information on fundamental processes governing the ecology of the plant rhizosphere, the interactions between specific populations and the indigenous soil communities, and the effects of soil organic matter and other environmental factors on shaping plant beneficial microbial communities.

While we had an ongoing communication throughout the project, in retrospect it would have been desirable to have additional travel opportunities for exchange of information. In the last year of the project, Crowley visited the Israeli research group and was able to spend a full 10 days interacting with the students and postdocs who were working on the project, and gave reports and seminars summarizing the advances from the US side. This time was especially valuable for brainstorming and was a highlight of the project. We would encourage BARD to enable a greater number of travel opportunities to facilitate these types of personal interactions.

List of Publications: Include only reviewed publications reporting on work at least partially supported by BARD and which includes an acknowledgement to BARD.

- Martinez, O., M. Jorquera, D.E. Crowley, G. Gajardo, and M.L. Mora. 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.* 10:293-319.
- Jorquera, M.A., B. Shaharoona, S.M. Nadeem, M. Arshad, and D.E. Crowley. 2012. Rhizosphere bacteria associated with the 11,700 year-old “King Clone” of *Larrea tridentata*. *Microbial Ecology*. 64:1008-1017.
- Nadeem, S.M., B. Shaharoona, M. Arshad, and D.E. Crowley. 2012. Population density and functional diversity of plant growth promoting rhizobacteria associated with avocado trees in saline soils. *Appl. Soil Ecol.* 62:147-154.
- Muci, A.L., M.A. Jorquera, A.L. Avila, Z. Rengel, D.E. Crowley, and M.L. Mora. 2012. A combination of cellular automata and agent-based models for simulating root surface colonization by bacteria. *Ecol. Modeling*. 247:1-10.
- Ofek, M., Y. Hadar, D. Minz. 2011. Colonization of cucumber seeds by bacteria during germination. *Environ. Microbiol.* 13:2794-2807.
- Ofek, M., Y. Hadar, D. Minz. 2012. Ecology of root colonizing Massilia (Oxalobacteraceae). *PLoS One* 10.1371/journal.pone.0040117
- Ofek, M., M. Voronov-Goldman, Y. Hadar, D. Minz. Host signature effect on plant root-associated microbiomes revealed through analyses of resident vs. active communities. In review.

Appendix (technical information supporting the research findings): Provide a table of contents and include the following: Published papers. (One copy of each ‘in press’, ‘accepted’ or ‘submitted’ paper.)

Table of contents:

Figure 6. Correspondence between composition of resident (DNA) and active (RNA) bulk soil and rhizoplane bacterial communities. Correlation coefficients were calculated between all possible pairs of DNA and RNA samples for each plant species within each compartment. Means and standard deviations are presented. Different letters indicate significant difference based on non-parametric Kruskal-Wallis ANOVA ($P < 0.05$), followed by multiple comparisons of mean ranks.

Figure 7. Correspondence between the relative abundance of dominant active OTUs relative abundance in DNA and RNA samples. Values were normalized based on numbers of rRNA operons of closest known related taxa. Each symbol represents the mean of a single OTU defined at 97% sequence similarity threshold. For each plant species, the three most dominant OTUs are presented. Horizontal and vertical bars represent the range of values for each OTU on each axis.

Table 1. Temperature tolerance of selected PGPR with ACC deaminase activity isolated from the rhizosphere of the 11,700 year old King Clone of *Larrea tridentata*.

Table 2. Effects of soil inoculation with an ACC degrading strain of *P. fluorescens* and PGPR compost and biochar soil amendments on growth of cucumber plants in sandy loam soil.

Figure 10. Population densities of ACC-deaminase bacteria in the rhizosphere of cucumber grown in soil amended with compost or biochar, and inoculated or noninoculated treatments with an ACC degrading strain of *Pseudomonas fluorescens*.

Published Papers (reprints follow)

1. Ofek, M., Y. Hadar, D. Minz. 2011. Colonization of cucumber seeds by bacteria during germination. *Environ. Microbiol.* 13:2794-2807.
2. Martinez, O., M. Jorquera, D.E. Crowley, G. Gajardo, and M.L. Mora. 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.* 10:293-319.
3. Jorquera, M.A., B. Shaharoona, S.M. Nadeem, M. Arshad, and D.E. Crowley. 2012. Rhizosphere bacteria associated with the 11,700 year-old “King Clone” of *Larrea tridentata*. *Microbial Ecology.* 64:1008-1017.
4. Nadeem, S.M., B. Shaharoona, M. Arshad, and D.E. Crowley. 2012. Population density and functional diversity of plant growth promoting rhizobacteria associated with avocado trees in saline soils. *Appl. Soil Ecol.* 62:147-154.

5. Muci, A.L., M.A. Jorquera, A.L. Avila, Z. Rengel, D.E. Crowley, and M.L. Mora. 2012. A combination of cellular automata and agent-based models for simulating root surface colonization by bacteria. *Ecol. Modeling.* 247:1-10.
6. Ofek, M., Y. Hadar, D. Minz. 2012. Ecology of root colonizing Massilia (Oxalobacteraceae). *PLoS One* 10.1371/journal.pone.0040117
7. Ofek, M., M. Voronov-Goldman, Y. Hadar, D. Minz. Host signature effect on plant root-associated microbiomes revealed through analyses of resident vs. active communities. In review.

Figure 6. Correspondence between composition of resident (DNA) and active (RNA) bulk soil and rhizoplane bacterial communities. Correlation coefficients were calculated between all possible pairs of DNA and RNA samples for each plant species within each compartment. Means and standard deviations are presented. Different letters indicate significant difference based on non-parametric Kruskal-Wallis ANOVA ($P < 0.05$), followed by multiple comparisons of mean ranks.

Figure 7. Correspondence between the relative abundance of dominant active OTUs relative abundance in DNA and RNA samples. Values were normalized based on numbers of rRNA operons of closest known related taxa. Each symbol represents the mean of a single OTU defined at 97% sequence similarity threshold. For each plant species, the three most dominant OTUs are presented. Horizontal and vertical bars represent the range of values for each OTU on each axis.

Table 1. Temperature tolerance of selected PGPR with ACC deaminase activity isolated from the rhizosphere of the 11,700 year old King Clone of *Larrea tridentata*.

Isolate	Growth at 28°C		Growth at 35°C		Growth at 40°C		Growth at 45°C		Growth at 50°C	
	Without G plus MS	With G plus MS	Without G plus MS	With G plus MS	Without G plus MS	With G plus MS	Without G plus MS	With G plus MS	Without G plus MS	With G plus MS
<i>Bacillus megaterium</i>	++	++	++	++	++	++	+	++	-	++
<i>Bacillus licheniformis</i>	++	++	++	++	++	++	++	++	+	++
<i>Pseudomonas putida</i>	++	++	++	++	++	++	+	++	-	+
<i>Pseudomonas fluorescens</i>	++	++	++	++	+	++	-	+	-	-
<i>Variovorax paradoxus</i>	++	++	+	++	+	+	-	-	-	-
2KC <i>Pseudomonas sp.</i> HQ396625	++	++	++	++	++	++	+	++	+	++
2KC <i>Pseudomonas sp.</i> HQ396627	++	++	++	++	++	++	++	++	-	+

Table 2. Effects of soil inoculation with an ACC degrading strain of *P. fluorescens* and PGPR compost and biochar soil amendments on growth of cucumber plants in sandy loam soil

	Control		Compost		Biochar		compost+ biochar	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Root weight (g)	1.2 d	6.1 ab	3.2 bcd	7.9 a	4.8 abc	3.8 bcd	5.3 abc	4.2 bc
Shoot length (cm)	17.3 b	25.1 a	23.3 ab	27.5 a	23.3 ab	23.7 ab	24.9 ab	25.1 a
Shoot weight (g)	20.6 b	30.7 a	27.6 a	30.4 a	28.7 a	28.7 a	29.8 a	28.9 a
Plant biomass (g)	21.8 d	36.7 ab	30.8 c	38.3 a	33.6 abc	32.5 bc	34.4 abc	34.6 ab

Figure 10. Population densities of ACC-deaminase bacteria in the rhizosphere of cucumber grown in soil amended with compost or biochar, and inoculated or noninoculated treatments with an ACC degrading strain of *Pseudomonas fluorescens*.