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Bacterial Succession in Necrotic Tissue of Agria Cactus (*Stenocereus gummosus*)

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The bacterial communities associated with the development of necroses in injured agria cactus tissue were examined in the field by using both human-induced injuries and cactus tissue inoculated with cactophilic bacteria. Whole-cell bacterial fatty acids were used to determine when and where each of 23 detected species occurred. This information was then used to describe successional patterns of bacterial colonization. Although the number of bacterial species in human-induced injuries reached a maximum on day 16, the Shannon-Weaver diversity index increased to a plateau, which reflects a stable bacterial community. The potential of the bacterial community to macerate the injured cactus tissue was also examined, and the results indicate that the bacteria initially colonizing the newly injured cactus tissue were more likely to contain pectinolytic, proteolytic, and lipolytic enzymes than were the bacteria entering the injuries once tissue maceration had already begun. The cactophilic fruit fly *Drosophila mojavensis* has been previously shown to carry bacteria to newly injured cactus tissue. In these studies, exclusion of these insects did not significantly affect bacterial succession or community structure. This supports our contention that bacteria colonize injured tissue primarily by passive transport, e.g., on wind-blown particles.

The columnar cacti of the Sonoran Desert occasionally develop necroses in their stems. These soft rots develop when bacteria and yeasts colonize tissue weakened by injury, environmental stress, or senescence. Often, the cactus seals off the necrotic section, limiting it to a single part of the plant and preventing destruction of the entire organism.

In a cactus necrosis, microbes lyse the plant cells, creating a wet, nutrient-rich microenvironment in the midst of the xeric Sonoran Desert. These necroses provide substrates for feeding and breeding by cactophilic *Drosophilids* species. The relationships between cacti and drosophilids in the desert have been well defined (9). In any particular locality, each fly species preferentially uses one species of cactus. For example, *Drosophila mojavensis* uses necrotic tissue of the agria cactus (*Stenocereus gummosus*) for feeding and oviposition. Where agria cacti are unavailable, *D. mojavensis* switches to organ pipe cacti (*S. thurberi*) as host plants. The drosophilids, in turn, transport microorganisms to new necroses (15, 16). *D. mojavensis* collected near agria necroses have been shown to carry as many as $\log_{10}$ 6.62 culturable bacteria and $\log_{10}$ 5.52 viable yeasts per fly (13). Thus, drosophilids serve as vectors of both yeasts and bacteria.

By comparing species associations in different rots, Starmer (24) hypothesized a temporal sequence of yeast species in agria cacti. It is likely that succession occurs within the bacterial community as well. Furthermore, bacterial colonization of human-induced injuries in agria stems has been shown to precede yeast growth (13). This previous study also demonstrated that while artificial exclusion of *D. mojavensis* (and other large insects) from newly injured agria tissue hinders colonization by yeast species, it does not affect the rate of bacterial colonization. The experiments described in this report were designed to identify the bacteria isolated from developing agria necroses, to characterize the bacterial succession in these rots, and to investigate the effects of insect vectors (primarily *D. mojavensis*) on the successionary pattern.

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MATERIALS AND METHODS

Rot production and sampling procedures. Field experiments were performed in the state of Sonora, Mexico, at a site approximately 30 km north of Kino Bay (Bahia de Kino), near the shore of the Sea of Cortez. On the basis of similarity of age, size, orientation, and general condition, one arm was chosen on each of four agria plants. All four arms were situated within 3 m of existing agria rots being used by *D. mojavensis*. Spines were removed from a 30-by-10-cm area of each arm. The surface was sterilized with ethanol, and a pair of holes (2.5 cm in diameter, 5.0 cm deep, and 20 cm apart) were drilled aseptically. A tissue sample was taken from the interior surface of each hole prior to induction of freeze damage by exposure to liquid nitrogen for a minimum of 30 s. These freeze injuries radiated approximately 2.5 cm into the tissue. To exclude drosophilids and other large insects, one member of each pair of holes was covered with sterile nylon netting that was secured with tacks. The interior surface of each hole was sampled on days 2, 4, 6, 8, and 11 postinjury. On day 12 postinjury, the arms were severed from the plants, brought back to the laboratory, and placed in an environmental chamber with naturally occurring rots which contained *Drosophila* spp. The environmental chamber was set to mimic field conditions. Additional samples were taken on days 16 and 27.

Laboratory-inoculated tissue. Since practical considerations limited the duration of field work to less than 2 weeks, an additional experiment was designed to examine later stages of rot development. In this experiment, agria tissue was prerotted in the laboratory prior to being placed in the field. Specifically, approximately 1 kg of frozen tissue chunks was thawed, peeled, cored, and homogenized in a blender. The homogenized tissue was distributed into three pairs of wide-mouth jars and sterilized by autoclaving. The tissue was then inoculated with four

fresh cultures of previously isolated cactophilic bacteria. The jars were incubated in an environmental chamber before being placed in the field. The conditions in the environmental chamber were a 12-h light-dark temperature cycle (light period, 20°C; dark period, 15°C). This technique results in tissue which approximates natural rots in the intermediate-to-advanced stage of the necrotic process. One of each pair of jars was covered with sterile nylon netting to exclude drosophilids and other large arthropods. The tissue was sampled for bacteria at the following times: (i) immediately following inoculation, (ii) when placed in the field at 12 days postinoculation, and (iii) at 18 and 23 days postinoculation. At 23 days postinoculation, the jars were covered with Parafilm, trapping larval and adult drosophilids in them. The jars were then returned to the laboratory and placed in an environmental chamber with the injured agria arms (see above) and sampled on day 30 postinoculation.

Bacterial sampling and identification procedures. All tissue samples were homogenized and serially diluted. Two dilutions of each sample, separated by a factor of 100, were plated on Trypticase soy agar (TSA) supplemented with 100 mg of cycloheximide and 10 mg of amphotericin B (Sigma) per liter of medium to inhibit the growth of fungi. All plates were incubated aerobically either at ambient temperatures (in the field) or at 28°C (in the laboratory). Colonies were grouped according to morphological similarity, and representative colony morphs were streaked to single colonies three times on TSA. Stock cultures of each isolate were made by growing the isolate in Trypticase soy (TS) broth, sedimenting the cells in a tabletop centrifuge, and pouring off the spent medium. The pellet was then suspended in a TS broth-glycerol (1:1) solution and stored at −15°C until use.

Three sets of 10 *D. mojavensis* flies were caught on or near the human-induced agria injuries at 5 days postinjury. The drosophilids were ground in sterile water with a tissue grinder. Serial dilutions were plated on TSA supplemented with antifungal agents and incubated at ambient temperature. Representative colony morphs were selected and streaked to purity three times in preparation for identification. Since the flies were not surface sterilized prior to homogenization, this technique yields whole-body microbial flora.

Conspecific grouping and identification of bacteria by whole-cell fatty acid methyl ester (FAME) profiles. Stored isolates were brought out of storage by growth overnight at 28°C on TSA. The pure bacterial isolates from the selected colony morphs were subsequently grown on TSA at 28 (±1)°C for 24 (±1) h. Colonies were harvested from the quadrant containing confluent growth and spread on the bottom of a test tube (13 by 100 mm). The bacteria were saponified at 100°C for 30 min in 15% NaOH dissolved in 50% methanol-water. The saponified samples were acidified with 60% HCl in methanol, heated at 80°C for 10 min, and then rapidly cooled. The FAMEs were extracted in hexane-ether (1:1) for 10 min and then subjected to a 5-min base wash in 1.2% NaOH. The FAMEs in the organic phase were analyzed with a Hewlett-Packard 5890A gas chromatograph equipped with a fused silica capillary column (cross-linked 5% phenyl methyl silicone; 25 m by 0.2 mm with a film thickness of 0.33 μm) and a flame ionization detector. The column conditions were as follows: hydrogen carrier gas at a flow rate of 30 ml/min and an initial temperature of 170°C increasing at a rate of 5°C/min to a final temperature of 270°C. Data were processed by a Hewlett-Packard 3392A integrator and analyzed by a Hewlett-Packard 9133 personal computer.

The whole-cell FAME profile was utilized for identification of conspecific groups with the Hewlett-Packard Microbial Identification System (version 3.0) as previously described (14). Briefly, principal-component analyses and cluster analyses by the unweighted pair-group method with arithmetic averages were employed to construct the conspecific groups. Euclidean distance is used by the Hewlett-Packard Microbial Identification System software for construction of cluster dendrograms. Euclidean distance measurements represent the dissimilarity coefficients of the entire FAME profile for different samples (20). Experience with multiple analyses of many species indicates that isolates of the same species usually cluster together at a level of 10 Euclidean distances or lower (22). Here, a more conservative level of 8 Euclidean distance units was used to define a species. Colony morphologies of isolates within a cluster were identical or very similar. In ambiguous cases, the isolates were compared by reactions in physiological tests as well. A library specific to these experiments was constructed by using the FAME profiles from approximately 300 field isolates so that replicate isolates would be identified. One isolate was selected as representative of each cluster. Selection of the representative was made on the basis of the greatest similarity of its FAME profile to the largest number of isolates within the cluster. The identification of the representative of each cluster was based primarily on morphology and physiological tests.

Identification by physiological characteristics. The phenotypic characteristics of one representative isolate from each conspecific group were determined by employing the tests listed in Table 1. All of the tests were performed by using standard bacteriological procedures on overnight cultures as described by Benson (2), and the results were compared to entries in the ninth edition of *Bergey's Manual of Systematic Bacteriology* (volumes 1 and 2) for putative identification.

Statistical analyses. All statistical analyses were performed by the methods of Sokal and Rohlf (23). Shannon-Weaver diversity indices were calculated as related by Poole (21).

The potential of each of the representative isolates to macerate plant tissue (and cause rot formation) was examined by determining whether the isolate exhibited any pectinolytic, proteolytic (hydrolysis of either gelatin or casein), or lipolytic activity. The isolate was rated +1 for each type of enzyme activity expressed. The sum of the observed enzyme activity ratings was then divided by the total possible (i.e., 3) to yield a crude index of pathogenic potential.

To determine the relative macerating potential (RMP) of the community at any given time, the index of pathogenic potential of each isolate present in each sample was multiplied by a fraction representing its proportion of the total community. These products were summed for each sample and averaged over replicate rots. Therefore, RMP values ranged from 0 to 1, with the latter value describing a community with maximum RMP.

RESULTS

Identification of bacteria. From these experiments, the FAME profiles of 337 different bacterial isolates were analyzed, resulting in the definition of 30 conspecific groups. Seven of the species were isolated only once and consequently eliminated from these studies because of their low frequency. The frequency of isolation of each bacterial species from the 14 experimental substrates is given in Table 1. The frequency-of-isolation data for three of the species do not include the prerotted tissue substrates, since they were used as inocula. All of the isolates of 1 of the remaining 23 conspecific groups (i.e., a *Pseudomonas* species) were lost before a physiological profile could be assayed. Its identity as a pseudomonad was therefore

TABLE 1. Characteristics of bacterial isolates

Isolate[a]	Frequency of isolation	Cell shape[b]	Gram stain	Motility	Glucose O/F[c]	Oxidase	Catalase	Lactose (acid)	MR[d]	VP[e]	Indole	Citrate	Gelatin	Casein	Starch	Pectin
Arthrobacter oxydans	0.14	R/C	+	+	−/−	−	+	−	n[f]	n	n	+	+	−	+	+
Bacillus licheniformis	0.43	R	+	+	+/+	−	+	−	n	n	n	−	+	+	−	−
Citrobacter amalonaticus	0.07	R	−	+	+/+	−	+	+	+	−	+	+	−	−	−	−
Clavibacter michiganensis	0.75[g]	R	+	−	−/−	−	+	−	n	n	n	n	−	−	−	−
Enterobacter cloacae	0.64	R	−	+	+/+	−	+	+	−	+	n	+	+	−	−	−
Enterobacter sakazakii	0.38[g]	R	−	+	+/+	−	+	+	−	+	n	+	+	+	−	−
Enterococcus faecium	0.71	C	+	+	+/+	−	−	+	n	n	n	n	−	−	−	−
Erwinia mallotivora	0.36	R	−	+	+/+	−	+	−	n	n	−	+	−	−	−	+
Erwinia stewartii	0.21	R	−	+	+/+	−	+	+	+	−	+	+	−	w[h]	−	−
Erwinia tracheiphila	0.29	R	−	+	w/w	−	+	−	−	−	+	+	−	−	−	−
Erwinia cacticida	0.88[g]	R	−	+	+/+	−	+	+	+	−	n	−	+	w	−	−
Escherichia adecarboxylata	0.57	R	−	+	+/+	−	+	+	+	−	+	−	−	w	−	−
Lactobacillus minor	0.43	PR	+	+	+/+	−	−	−	n	n	n	−	−	−	−	−
Lactobacillus plantarum	0.29	PR	+	+	+/+	−	−	−	n	n	n	+	−	−	−	−
Listeria species	0.29	PR/C	+	+	+/+	−	+	+	n	n	n	+	−	w	−	−
Pseudomonas fluorescens	0.36	R	−	+	+/−	+	+	+	n	n	n	+	+	+	−	−
Pseudomonas syringae	0.29	R	−	+	+/−	−	+	+	−	−	n	−	−	−	−	−
Xanthomonas ampelina	0.21	R	−	+	w/−	−	+	+	−	−	n	−	−	−	−	−
Xanthomonas campestris	0.07	R	−	+	w/w	+	+	−	n	n	n	+	+	w	−	+
Xanthomonas species	0.50	R	−	+	−/−	−	+	−	n	n	n	w	+	+	−	−
Unknown species 1	0.29	R	−	+	+/+	−	+	+	+	−	+	−	+	−	−	−
Unknown species 2	0.36	PR	+	+	−/−	−	+	−	n	n	n	+	−	w	−	−

[a] A *Pseudomonas* species was lost before characterization.
[b] R, rods; C, cocci; PR, pleomorphic rods.
[c] O/F, oxidation/fermentation.
[d] MR, methyl red.
[e] VP, Voges-Proskauer.
[f] n, not assayed.
[g] Calculated from field-induced injuries only.
[h] w, weak.

based only on its fatty acid profile. The physiological profiles of the rest of the representative isolates are presented in Table 1. A dendrogram of the library entries for each conspecific group is depicted in Fig. 1.

Successional patterns. The order of bacteria isolated from the human-induced injury experiment is presented in Fig. 2. The first isolation of each bacterial species demonstrates a diagonal trend representing bacterial succession. Four of the five colonizing bacterial species (isolated 2 days postinjury) were not isolated from samples after day 8. Likewise, three of the seven bacterial species isolated at 27 days postinjury had not been previously detected and represented a later successional stage. One species, *Enterobacter cloacae*, was consistently isolated from days 2 through 16 postinjury, and the unidentified *Xanthomonas* species was consistently isolated from days 2 through 27. These two species, along with several other species, were isolated throughout the course of the experiment and would be representative of generalists or nutritionally more adaptable and less exacting species of bacteria.

Figure 3 presents the order of isolation of bacteria from the covered and uncovered laboratory-inoculated tissue. Excluding the inoculum, 44% of the bacterial species were isolated on days 18 and 23 but not on day 30 or 37. Twenty-two percent of the bacteria were not isolated prior to day 30 and thus represent a later successional stage.

Bacteria carried by *D. mojavensis*. Table 2 lists the bacteria which were isolated from 30 *D. mojavensis* flies captured on or near the human-induced injuries and their respective frequencies of isolation from the experimental substrates. Six species of bacteria were carried at a detectable level by the 30 *D. mojavensis* flies sampled. Of these six bacterial species, only

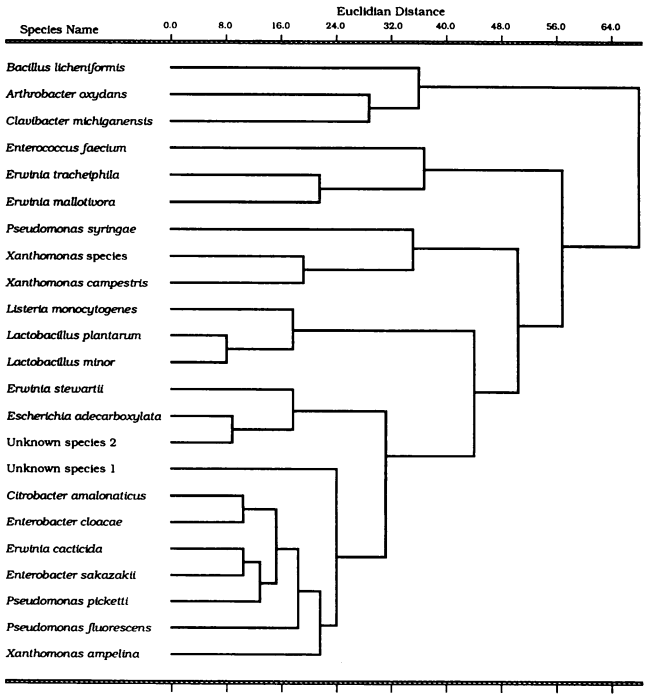

FIG. 1. Dendrogram of the 23 conspecific groups isolated from necrotic agria tissue.

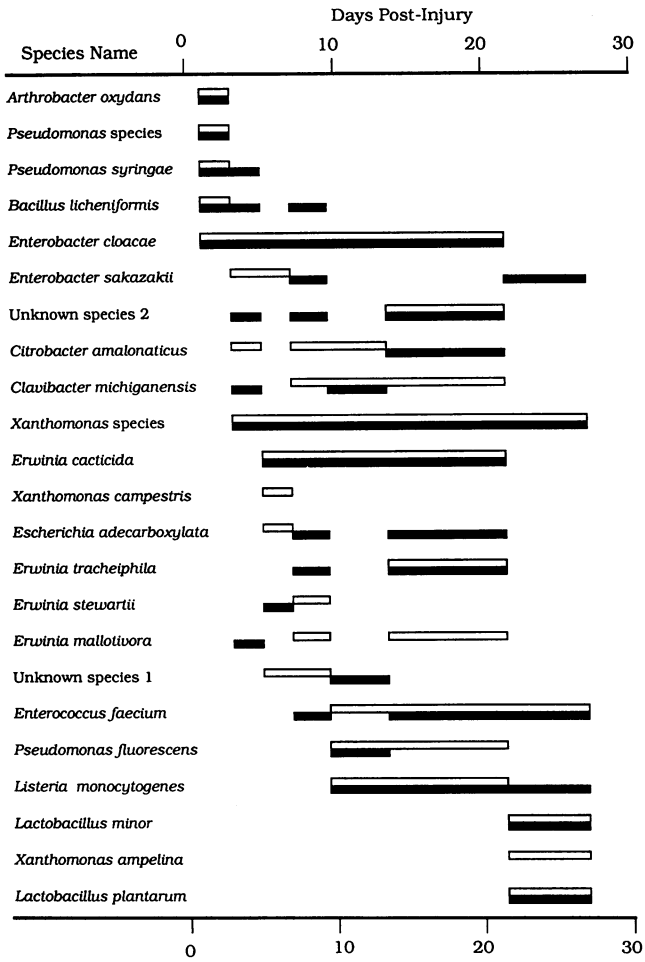

FIG. 2. Order of occurrence of bacteria in injured agria tissue. The open bars represent bacterial isolations from uncovered injuries, and the filled bars represent isolations from covered injuries.

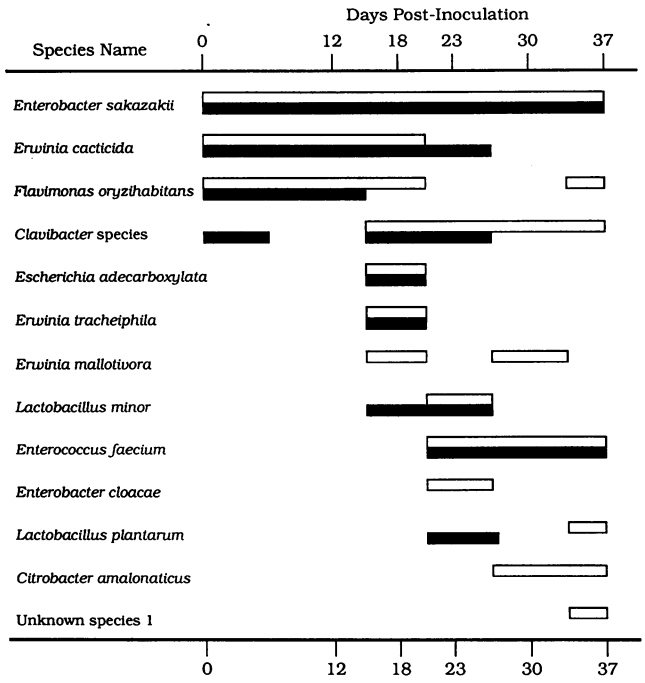

FIG. 3. Order of bacterial occurrence in laboratory-inoculated tissue. The open bars represent bacterial isolations from uncovered injuries, and the filled bars represent isolations from covered injuries.

Xanthomonas ampelina was isolated solely from the uncovered injuries and not in the injuries covered to exclude drosophilids and other large insects. *X. ampelina* was not detected in laboratory-inoculated tissue. The remaining five species of bacteria were detected in both uncovered and covered injuries. *E. adecarboxylata* and *E. sakazakii* were detected in uncovered injuries prior to isolation from covered injuries. *E. adecarboxylata* was detected simultaneously in both covered and uncovered laboratory-inoculated tissues. Detection of *E. sakazakii* in the laboratory-inoculated tissue experiment cannot be used to interpret the role of *D. mojavensis* as a bacterial vector, since *E. sakazakii* was used to inoculate the tissue.

Bacterial species numbers and diversity during colonization of human-induced injuries. The number of bacterial species detected in each sample of injured agria tissue averaged two or fewer during days 2 to 6 postinjury (Fig. 4). The number of bacterial species isolated from the injuries increased to an obvious maximum on day 16 and significantly declined by day 27. The maximum average number of species isolated was 4.75 from the uncovered samples taken on day 16. Paired *t* tests comparing covered and uncovered injuries on a per-day basis did not show a significant difference in the numbers of species between treatments on days 2 to 8, 16, and 27. Only on day 11 was there a significantly greater number of bacterial species in the uncovered injuries at the 0.05 level.

Shannon-Weaver diversity indices for the human-induced injury experiment are graphed in Fig. 5. Microbial growth patterns resulted in a patchiness of the bacterial community which is reflected in these data. A curve-smoothing function using a three-sample running average was employed for these data to dampen some of the variation. The general trend for both treatments was an increase in the Shannon-Weaver diversity index for days 2 to 11. The species diversity remained relatively constant for days 11 to 27 postinjury, reflecting a relatively stable bacterial community.

The relative macerating potential for the entire bacterial community was calculated on a per-sample basis for the human-induced injury experiment. The results were averaged for each treatment and are graphed in Fig. 6. Although these enzymes may be inducible and there is considerable variation in this parameter over the temporal samples, it is noteworthy that the bacterial communities which initially colonized the injured tissues had the highest macerating potential. All of the bacteria initially isolated from the injuries demonstrated at least some macerating potential. More than 70% of the

TABLE 2. Bacteria isolated from *D. mojavensis* adults and frequency of isolation from experimental cactus substrates

Bacterial species	Frequency of isolation (%)
Erwinia cacticida	79
Enterococcus faecium	71
Enterobacter cloacae	64
Enterobacter sakazakii	64
Escherichia adecarboxylata	57
Xanthomonas ampelina	21

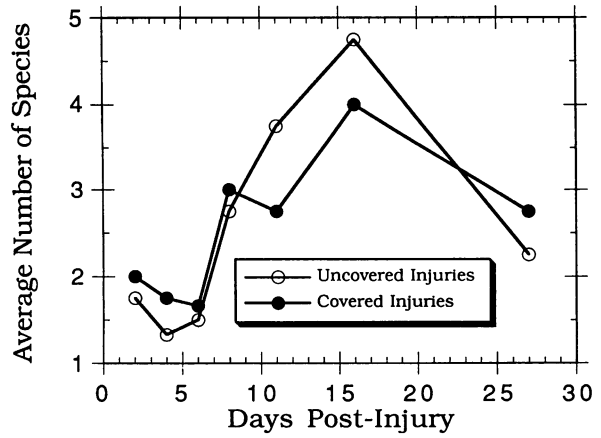

FIG. 4. Average number of species in each of the four pairs of human-induced injuries.

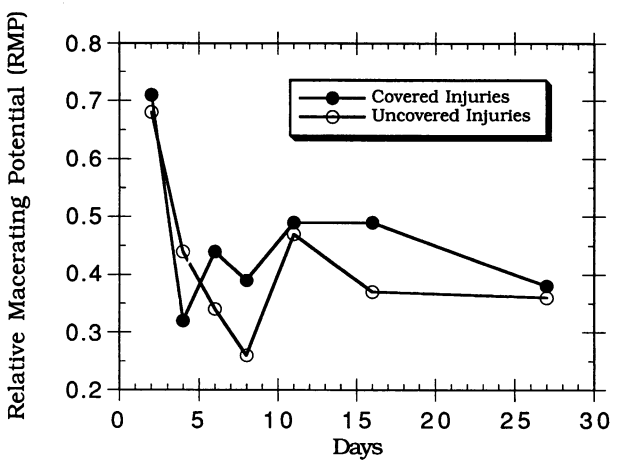

FIG. 6. Average RMP of bacterial communities in injured agria tissue over time.

bacterial species isolated from the day 2 injuries had a minimum of two of the three macerating enzymes present. Later in rot development, an increasing proportion of the bacterial communities comprised bacteria lacking macerating ability; e.g., more than 20% of the species isolated on day 8 lacked all three enzymes.

DISCUSSION

Environmental microbiology studies are recognized as limited because there is no medium which is appropriate for all of the microbes in a given sample (19). It has been estimated that only 1% of the bacteria from many natural environments are cultivable (10). Our isolation procedures could have been similarly limited by the selectivity of the medium that was used. Because agria cactus tissue is a relatively nutrient-rich substrate, containing approximately 12% (dry weight) soluble carbohydrates (11), TSA, a complete medium, was chosen for isolation. Some of the early colonizing bacteria might not have been detected on this medium, but once tissue maceration had begun, the developing necroses would have represented a nutrient-rich environment and TSA should be a suitable medium for most species. In addition, some of the bacteria inhabiting the necroses may not have been isolated because of the dilution process employed, since species with a density of less than 1% of the most prevalent bacterial species could have been either overgrown or missed. It is reasonable to assume, however, that rare bacteria make a proportionately smaller contribution to the microbial community and to the developing necroses. Finally, these experiments were limited to isolation of aerobic and facultative anaerobic bacteria. Previous experiments with bacteria from developing agria necroses indicated the presence of few obligate anaerobes. For this reason, no attempt was made to isolate strict anaerobes.

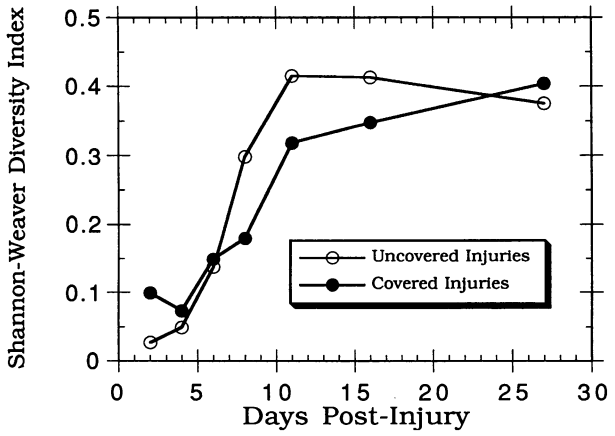

FIG. 5. Shannon-Weaver diversity indices over time for bacterial communities in human-induced injuries.

In these experiments, cluster analysis of the fatty acid profiles of bacterial isolates resulted in the construction of 23 conspecific groups of bacteria (Fig. 1). The use of fatty acid profiles (analyzed as FAMEs) to identify bacterial species (1, 6–8) or characterize bacterial communities (3, 14) has been increasing. Many of the species descriptions in the ninth edition of *Bergey's Manual of Systematic Bacteriology* (18) contain information on major fatty acids. Although the use of 8 Euclidian distance units to separate the species in Fig. 1 is somewhat arbitrary, the fact that the conspecific groups were also distinguishable on the basis of physiological characteristics (Table 1) lends support to this method.

The development of a necrosis in a cactus stem represents a case of degradative succession. Bacterial species colonize the cactus tissue and are subsequently replaced by other species as resources change (some disappear while others become available). Additionally, changes in physical conditions favor some species over others. Examples of these changes in cactus rots have been described (12, 17) and include significant reductions in soluble carbohydrates, increases in certain lipophilic compounds, and a change from acidic to alkaline pH.

A successional pattern in the bacterial community would appear as a diagonal trend when the presence of individual species versus the temporal isolation record was plotted. Some species would be present in the early time stages and not in the later time periods, whereas other species would be detected only in the later time periods. In the successional environment, the bacterial species with the highest reproductive rate under the current growth conditions would be expected to outgrow other species and thus dominate.

A diagonal pattern of population colonization and extinction was observed in both human-induced injury (Fig. 2) and laboratory-inoculated tissue (Fig. 3) experiments. Figure 2 shows that three species of bacteria (*Arthrobacter oxidans*,

Pseudomonas species, and *P. syringae*) were isolated only from the earliest colonizing stage. Likewise, three bacterial species (*Lactobacillus minor*, *L. plantarum*, and *Xanthomonas ampelina*) were not detected prior to 27 days postinjury and represent a later successional stage. Fourteen of the 23 bacterial species detected in the human-induced injuries were isolated from at least three different sampling periods. These bacteria may be tolerant of a broader range of environmental conditions than the bacteria isolated only from a single sampling period.

The bacteria isolated from the intermediate and late sampling periods were predominantly facultative anaerobes. In the laboratory, 76% of the isolates from days 6 to 27 postinjury grew in a GasPak (BBL Microbiology Systems) anaerobic jar (Table 1). Thus, as the lesions become fluid filled and the microbial communities deplete the available dissolved oxygen, fermentative metabolism occurs, resulting in accumulation of volatile compounds such as acetic acid, ethanol, and methanol (13).

Excluding the inoculum, all of the bacteria detected in the laboratory-inoculated tissue experiment were also detected in the human-induced injury experiment, and 70% of the species were isolated from only one or, at most, two sample periods. This successional pattern roughly corresponds to the one observed in the human-induced injury experiment. In laboratory-inoculated tissue, 66% of the species appeared at a point in the successional pattern similar to that observed in the human-induced injury experiment. However, three of the species in the laboratory inoculation experiment deviated from this pattern. For example, *L. minor* was detected during the first sampling period after being placed in the field rather than late in the experiment, as was observed in the human-induced injury experiment. Likewise, *Citrobacter amalonaticus* and unknown species 1 were both detected late in the laboratory-inoculated tissue experiment but relatively early in the human-induced injury experiment. These discrepancies may reflect an effect of the inoculum used or the fact that the laboratory-inoculated cactus tissue was mechanically macerated in a blender and autoclaved.

The *D. mojavensis* visiting the human-induced injuries carried an average of greater than 100,000 bacteria per fly (13). The density of the bacteria carried by drosophilids does not indicate whether or not the flies are the primary source of the inoculum. The most direct indication of whether or not *D. mojavensis* acts to spread bacteria to newly injured agria tissue is the differences in the appearance of the individual bacterial species between covered and uncovered tissues (Fig. 2 and 3), especially of those bacteria detected on or in the resident drosophilids. The bacterial distribution patterns indicate that most species are not dependent on drosophilids for transportation. Of the species isolated from human-induced injuries, 26% were isolated from net-covered tissue prior to detection in the developing necroses accessible to large insects. Forty-eight percent of the bacteria were first isolated simultaneously from both covered and uncovered injuries. Only 26% of the bacteria were recovered either solely from uncovered tissue or from uncovered injuries prior to detection in tissue covered with nylon netting to exclude drosophilids. Of these six species, only *E. adecarboxylata*, *E. sakazakii*, and *X. ampelina* were detected on or in drosophilids. Examination of laboratory-inoculated tissues (Fig. 3) showed that *E. adecarboxylata* appeared simultaneously in covered and uncovered tissues, suggesting that this species is not dependent solely on drosophilids for transportation. The other two species are not useful for addressing this question, since *E. sakazakii* was used as an inoculum for laboratory-inoculated tissue and *X. ampelina* was not isolated from these tissues. Their dependence on drosophilid vectors remains unclear. Overall, the hypothesis that the initial source of bacterial inocula is dust (13) appears intact.

The average number of bacterial species within human-induced necroses increased with time (Fig. 4). Initially, there were few species present. This may be partially due to a small initial inoculum. With time, more bacteria would be blown in on dust or aerosols, increasing the number of species entering the injuries. In addition, the first colonizing species would be those best adapted to utilize newly injured tissue, i.e., those capable of lysing plant cells, causing tissue maceration. These initial conditions are both acidic and relatively nutrient poor. As tissue maceration progressed, additional nutrients would be released, the pH would increase, and consequently a greater number of bacterial species would be capable of growth in the developing rot. This process was reflected in an increase in the number of species over time, reaching a maximum on day 16. By day 27 postinjury, as the resources declined, the number of species also decreased.

Species diversity is an ecological parameter commonly used by macroecologists to represent both the number of species present and the evenness of distribution of organisms between species. The greater the number of species and the more even the population sizes, the higher the species diversity. High species diversity is maintained by population interactions and represents a stable community. The pattern of species diversity in agria injuries, as indicated by the graph of Shannon-Weaver diversity indices (Fig. 5), followed the pattern expected for macrobiota. During succession, the number of species should increase until a community stabilized by population interactions is established. The species diversity should remain relatively constant as long as the community remains stable (4). In our experiments, species diversity did not decrease significantly between days 16 and 27, as did the average number of species (Fig. 4). This demonstrates that as the bacterial community developed, there was a more even distribution of organisms among the different species.

Beyond the basic ecology of these bacterial populations, it is interesting to study their role in necrosis formation. The production of pectinolytic, lipolytic, and proteolytic enzymes by bacteria is known to be involved in the maceration of plant tissue (5). Pectin is a plant cell wall component that binds adjacent cells together. Proteins are components of both the walls and membranes of agria cells, and lipids are a major component of cell membranes. Hydrolysis of these components by bacteria contributes to the maceration of agria tissue. The role of bacterial populations during maceration can be indicated by in vitro enzyme assays on pure cultures of the bacterial isolates. The RMP of every bacterial community sampled during the human-induced injury experiment is graphed in Fig. 6. At least one member (species) of the bacterial community during every sampling period was capable of producing a macerating enzyme. Furthermore, the initial bacteria which colonized the injured but not yet macerated cactus tissue had the greatest macerating potential. As the necroses progressed, saprophytic bacteria lacking all three macerating enzymes were able to invade the soft rots, apparently utilizing the nutrients released by the other hydrolytic bacterial species. After an initial drop in RMP (Fig. 6), the average RMP appeared to stabilize.

A previous report presented the results of a survey of bacteria found in rots of other columnar cacti in the Sonoran Desert, i.e., organ pipe, senita, and saguaro cacti (14). Only five of the bacterial species named in this report have been isolated from rots of other cactus species. In the previous report, it was hypothesized that the differences in the observed

bacterial distribution patterns were primarily due to differences in the chemical composition of the cactus species. It is, therefore, not surprising that the bacterial community of agria cactus only overlaps those of other cacti to a limited extent since agria is chemically distinct. The results reported here suggest an additional source of variation in the bacterial communities of cactus rots, i.e., temporal variation due to community succession.

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REFERENCES

1. **Alcorn, S. M., T. V. Orum, A. G. Steigerwalt, J. L. M. Foster, J. C. Fogleman, and D. J. Brenner.** 1991. Taxonomy and pathogenicity of *Erwinia cacticida* sp. nov. Int. J. Syst. Bacteriol. **41**:197–212.
2. **Benson, H. J.** 1990. Microbiological applications. W. C. Brown, Dubuque, Iowa.
3. **Bobbie, R. J., and D. C. White.** 1980. Characterization of benthic microbial community structure by high-resolution gas chromatography of fatty acid methyl esters. Appl. Environ. Microbiol. **39**:1212–1222.
4. **Brewer, R.** 1979. Principles of ecology. The W. B. Saunders Co., Philadelphia.
5. **Chatterjee, A. K., and M. P. Starr.** 1980. Genetics of *Erwinia* species. Annu. Rev. Microbiol. **34**:645–676.
6. **DeBoer, S. H., and M. Sasser.** 1986. Differentiation of *Erwinia carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atorseptica* on the basis of cellular fatty acid composition. Can. J. Microbiol. **32**:796–800.
7. **Dees, S. B., and C. W. Moss.** 1975. Cellular fatty acids of *Alcaligenes* and *Pseudomonas* species isolated from clinical specimens. J. Clin. Microbiol. **1**:414–419.
8. **Dees, S. B., and C. W. Moss.** 1978. Identification of *Achromobacter* species by cellular fatty acids and by production of keto acids. J. Clin. Microbiol. **8**:61–66.
9. **Fellows, D. P., and W. B. Heed.** 1972. Factors affecting host plant selection in desert-adapted cactiphilic *Drosophila*. Ecology **53**:850–858.
10. **Ferguson, R. L., E. N. Buckley, and A. V. Palumbo.** 1984. Response of marine bacterioplankton to differential filtration and confinement. Appl. Environ. Microbiol. **47**:49–55.
11. **Fogleman, J. C., and J. R. Abril.** 1990. Ecological and evolutionary importance of host plant chemistry, p. 121–143. *In* J. S. F. Barker, R. J. MacIntyre, and W. T. Starmer (ed.), Ecological and evolutionary genetics of *Drosophila*. Plenum Press, New York.
12. **Fogleman, J. C., S. M. Duperret, and H. W. Kircher.** 1986. The role of phytosterols in host plant utilization by cactophilic *Drosophila*. Lipids **21**:92–96.
13. **Fogleman, J. C., and J. L. M. Foster.** 1989. Microbial colonization of injured cactus tissue (*Stenocereus gummosus*) and its relationship to the ecology of cactophilic *Drosophila mojavensis*. Appl. Environ. Microbiol. **55**:100–105.
14. **Foster, J. L. M., and J. C. Fogleman.** 1993. Identification and ecology of bacterial communities associated with necroses of three cactus species. Appl. Environ. Microbiol. **59**:1–6.
15. **Ganter, P. F.** 1988. The vectoring of cactophilic yeasts by *Drosophila*. Oecologia **75**:400–404.
16. **Ganter, P. F., W. T. Starmer, M.-A. Lachance, and H. J. Phaff.** 1986. Yeast communities from host plants and associated *Drosophila* in southern Arizona: new isolations and analysis of the relative importance of hosts and vectors on community composition. Oecologia **70**:386–392.
17. **Kircher, H. W., and B. Al-Azawi.** 1985. Longevity of seven species of cactophilic *Drosophila* and *D. melanogaster* on carbohydrates. J. Insect Physiol. **31**:165–169.
18. **Krieg, N. R., and J. G. Holt.** 1984. Bergey's manual of systematic bacteriology, vol. I–IV. The Williams & Wilkins Co., Baltimore.
19. **Leadbetter, E. R., and J. S. Poindexter.** 1985. Methods and special applications in bacterial ecology. Bacteria in nature, vol. 2. Plenum Press, New York.
20. **Pielou, E. C.** 1977. Mathematical ecology. John Wiley & Sons, Inc., New York.
21. **Poole, R. W.** 1974. An introduction to quantitative ecology. McGraw-Hill Book Co., New York.
22. **Sasser, M.** 1990. Tracking a strain using the microbial identification system. MIDI technical note no. 102. MIDI, Newark, Del.
23. **Sokal, R. R., and F. J. Rohlf.** 1981. Biometry (2nd ed.). W. H. Freeman & Co., New York.
24. **Starmer, W. T.** 1982. Analysis of community structure of yeasts associated with the decaying stems of cactus. I. *Stenocereus gummosus*. Microb. Ecol. **8**:71–81.