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Determining Rate of Change in Cucumber Rhizosphere Microbial community composition in response to soil pH , Salinity, and Boron

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1 INTRODUCTION

In the San Joaquin Valley (SJV) of Californian, the problem of increasing salinity and consequently, decreasing crop productivity, are major concerns. The salinity of the soil plays a prominent role in the microbial selection process as environmental stress has been shown to reduce bacterial diversity (Borneman et al., 1996). Environmental stress in soil gains importance, especially in saline agricultural soils, where high salinity results from irrigation practices and application of chemical fertilizer. The concentration effect is always more pronounced in the rhizosphere as a result of mass flow and water uptake by plants due to transpiration. Other factors such as soil composition, organic matter, pH, heavy metals, water and oxygen availability, along with the host plant, also play a major role in the selection of the natural flora (Ross et al. 2000). San Joaquin Valley soils are alkaline in nature (pH range of 7.5 to nearly 9.0) with high salinity usually correlated with high boron. Very little is known about how soil pH influences salinity-boron effects on cucumber production. To the best of our knowledge, nothing has been reported about how these variables affect microbial composition and functions in the rhizosphere of vegetable crops. The objective of this study was to use the polymerase chain reaction (PCR) to determine how the incorporation of pH into the experimental variables may provide insights into how salinity-boron interactions may affect rhizosphere microbial population.

The polymerase chain reaction is a powerful tool for amplifying and detecting specific nucleic acid molecules present at low levels in the environment. The dynamics of the dominant bacterial communities inhabiting the rhizosphere of cucumber were examined using the PCR approach of analyzing 16S ribosomal RNA (rRNA) profiles generated by denaturing gradient gel electrophoresis (DGGE). The resulting DNA band pattern provided a fingerprint of the microbial community structure, in which each band represented a group of bacteria having 16S rRNA sequences with a similar melting temperature (Muyzer et al., 1993). This approach provided evidence of microbial development dynamics with plant maturity in a way that the culture dependent method lacked. As a result the distribution, diversity, and composition of microbial communities associated with developing cucumber plant grown under different treatment regime were determined.

2 MATERIALS AND METHODS

Plant growth. The experiment was conducted in a closed, recirculating volumetric lysimeter system (VLS) consisting of 24 experimental plant growth units at the George E. Brown Jr. Salinity Laboratory in Riverside, CA to determine the interactive effects of salinity, boron

and pH on the rhizosphere microbial composition of cucumber (*Cucumis sativus* L. cv. Seminis Turbo hybrid). Planting and different treatments were added as described by Grattan et al. (this volume). In short, the VLS were irrigated from individual reservoirs containing a modified half-strength Hoagland's nutrient solution combined with various salinity, boron, and pH treatments. Crop water use was determined volumetrically by reservoir water depletion. Treatments included irrigation waters with two salinity levels, 3 and 8 dS/m; three boron concentrations of 0.7, 5, and 8 mg/L; and two pH levels where reservoir solutions were frequently adjusted to 6.5 and 8. Treatments were replicated twice. Plants were routinely observed for foliar injury and fruit development. Data were collected and analyzed for various ions to determine their distributions within the plant and ion interactions.

DNA extraction and PCR-DGGE analysis. Community DNA was extracted from plant samples with the Ultra Clean Soil DNA Kit (MoBio Laboratories, Solana Beach, CA) and stored at –20^{o} C. A 236-bp DNA fragment in the V3 region of the small subunit ribosomal RNA genes of eubacteria was amplified by using primer set PRBA338f and PRUN518r (Øverås et al 1997). For the purpose of separating the 16S rRNA bacterial communities in a DGGE gel, a GC clamp was added at the end of primer PRBA338f. Ready-To-Go PCR beads from Amersham Pharmacia Biotech (Piscataway, N. J) and 5 pmol of primers in a total volume of 25 ml were used in the PCR reaction. PCR amplifications were done under the following conditions: 92°C for 2 min; 30 cycles of 92°C for 1 min, 55°C for 30 s, 72°C for 1 min followed by a final extension at 72°C for 6 min.

DGGE was performed with a Dcode Universal Mutation Detection System (Bio-Rad, Hercules, CA). Twenty microliters of the PCR product was loaded onto an 8% (wt/vol) acrylamide gel (acrylamide/bis solution, 37.5:1) containing a linear chemical gradient ranging from 30% to 70% denaturant [7 M urea and 40% (vol/vol) formamide]. The gels were run for 3 h at 200 V in 1X TAE electrophoresis buffer (0.04 M Tris-acetate 1 mM EDTA, pH 8.5). The gels were stained in ethidium bromide solution for 15 min, rinsed for 5 min in water, and photographed.

Statistical Analysis. DNA fingerprints obtained from the 16S rRNA banding patterns on the DGGE gels were photographed and digitized using ImageMaster Labscan (Amersham-Pharmacia Biotech, Uppsala. Sweden). The lanes were normalized to contain the same amount of total signal after background subtraction. The gel images were straightened and aligned using ImageMaster 1D Elite 3.01 (Amersham-Pharmacia Biotech, Uppsala, Sweden) and analyzed to give a densitometric curve for each gel. Band positions were converted to R_f values between 0 and 1 and profile similarity was calculated by determining Pearson's coefficient for the total number of lane patterns from two gels (Ibekwe et al., 2001). A second approach was used to determine community structure based on peak height from the Excel files for the different bacterial groups (16S rRNA bands) and was analyzed to generate diversity index (*H*). The peak height values generated from the sampling points were integrated and analyzed using the Excel program. Data obtained were used to integrate the area under each peak for each lane in every treatment. For this analysis, each band was presumed to represent the ability of that bacterial species to be amplified. The Shannon index of diversity (*H*) was used to compare changes in diversity of microbial communities within the four treatments at each time (Shannon and Weaver**,** 1963) by using the following function:

$H = - \{P_i \log P_i\}$

$P_i = n_i/N$, n_i = height of peak, N = sum of all peak heights in the curve.

3 RESULTS AND DISCUSSION

The results indicated that salinity and pH were the most influential factors determining yield???, both of which were highly significant (P< 0.01). Mean comparison with the Tukey's test showed that the low EC (3) and pH (6.5) produced significant higher plant yield than the high EC (8) and pH (8.0) (Smith et al, this publication).

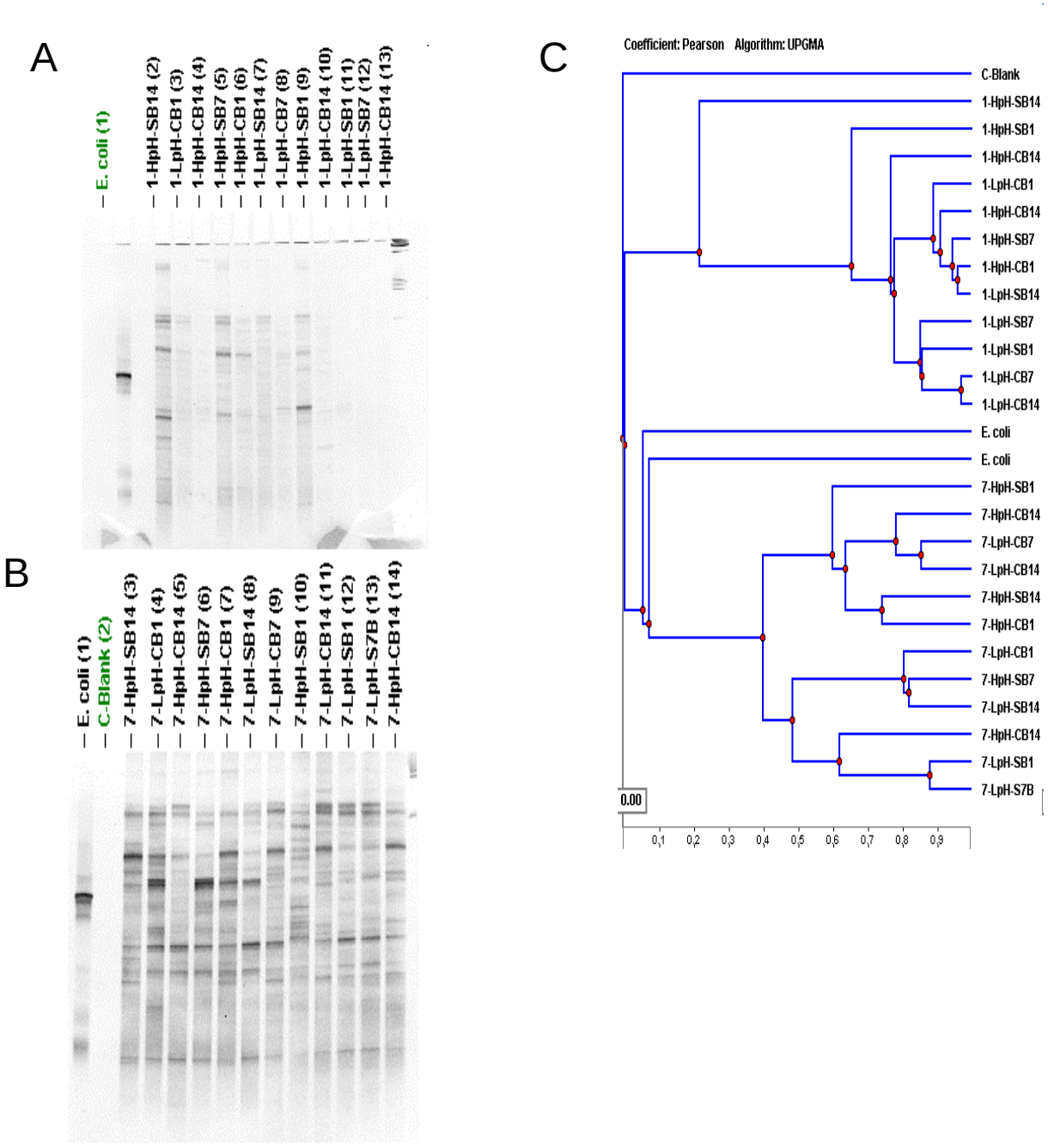

Figure 1. DGGE patterns of the 16S rRNA fragment (primers P338f and P518r) amplified from cucumber rhizosphere grown at high and low pH (HpH/LpH) and salinity (S/C) at three B concentrations (0.7 ppm (B1), 5 ppm (B7) and 8 ppm (B14) from week 1 (A) and week 7 (B) and a UPGMA dendrogram describing pattern similarities (C).

Analysis of soil microbial community structure by PCR-DGGE. DGGE analysis of 16S rRNA fragments was used to examine the effects of the pH, salinity, and B on soil microbial communities. Figures A and B show DGGE patterns of the 16S rRNA fragment (primers P338f and P518r) amplified from the rhizosphere of cucumber from week one and seven after planting. More bands were observed during week seven than week one. To compare DGGE patterns for the two sampling points, Pearson's coefficients were determined for comparisons of all profiles, and UPGMA was used to create a dendrogram describing pattern similarities (Fig. 1 C).

4 CONCLUSIONS

This analysis clearly showed the impact of low pH on microbial community during the first week than week seven. The effects of salinity and B were mixed. The second method for determination of the structural diversity was the calculation of the Shannon index of diversity *H* from the DGGE banding pattern of the samples. *H* was calculated on the basis of the number and relative intensity of bands on a gel strip. This section of the results will be presented.

5 REFERENCES

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