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Bacterial diversity in cucumber (*Cucumis sativus*) rhizosphere in response to salinity, soil pH, and boron

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ABSTRACT

Soil salinity is a major factor relating microbial communities to environmental stress in the microbial selection process as stress can reduce bacterial diversity. In the San Joaquin Valley (SJV) of California, the problem of increasing salinity and consequently, decreasing crop productivity, due to reuse of saline drainage water are major concerns. An experiment was conducted in a closed, recirculating volumetric lysimeter system (VLS) consisting of 24 experimental plant growth units to determine the interactive effects of salinity, boron and pH on rhizosphere and non-rhizosphere microbial composition of cucumber (*Cucumis sativus* L. cv. Seminis Turbo hybrid). Plants in the VLS were irrigated from individual reservoirs containing a modified half-strength Hoagland's nutrient solution combined with salinity, boron (B), and pH treatments. The results indicated that salinity and pH were the most influential factors affecting the growth of plants and the effect of boron on the plant was more severe under slightly acidic conditions. Total bacterial DNA was extracted from rhizosphere and non-rhizosphere samples, and a 236-bp DNA fragment in the V3 region of the small subunit ribosomal RNA genes of eubacteria was amplified. The 16S rRNA and the products were subjected to denaturing gradient gel electrophoresis (DGGE) and sequencing. Analyses of bacterial diversity showed that the effects of salinity, boron, and pH were more severe on the rhizosphere bacterial population during the first week of growing cucumber, with decreasing impacts with plant growth. However, there was no salinity–B–pH interaction effects on plant biomass, but the effects were seen in the number of heterotrophic bacteria in the rhizosphere and on species richness and diversity during week seven of the study. These suggest that the effects of salinity–B–pH interactions may influence microorganisms first before plants and may pose long term effects on soil quality.

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1. Introduction

Reuse of saline drainage water is a necessary management practice for reducing the volume of drainage produced in the Westside of California in USA. Increasing soil salinity due to the reuse of saline drainage water is a major concern for sustainable agriculture in the San Joaquin Valley (SJV) in California. Another concern with this practice is the extent to which B, a naturally occurring element in drainage water, will affect crop growth and yields. Numerous studies have demonstrated the effects of either salinity or B on growth and yield responses on crops (Eaton, 1944; Ehret and Ho, 1986; Maas and Grattan, 1999). Other studies have addressed the effects of both with mixed conclusions (Holloway and Alston, 1992; El-Motaium et al., 1994; Grattan et al., 1996; Grieve and Poss, 2000; Alpaslan and Gunes, 2001; Ferguson et al., 2002; Wimmer et al., 2003). Soil solution pH is known to affect B availability in soils, B-ion reactions and ion interactions with other trace elements such as As, Se, etc in the drainage water. The pH conditions recorded in salinity and B experiments (Sternberg et al., 2001; Ben-Gal and Shani, 2002,) were either slightly acidic (pH 6.5) or alkaline (pH 7.5–8.5) but pH was not used as an experimental variable. It has also been reported that trace elements can move up the aquatic food chain and become more concentrated, resulting in high levels of exposure for those animals near the top of the food chain (Fan et al., 1988). In some habitats, reproductive failures and deformities were observed in waterfowl (Tanji et al., 1986; Letey et al., 2002). Currently, there is no information available about the microbial communities as affected by the interaction among salinity, pH, and B content in the SJV soils. This information is needed as microorganisms maintain the whole ecosystem health and functioning.

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Salinity is a major factor in controlling microbial abundance, diversity, composition and functions (Borneman et al., 1996). Salinity has been shown to have significant effects on microorganisms (Polonenko et al., 1981; Omar et al., 1994; Matsuguchi and Sakai, 1995). Environmental stress in soil gain importance, especially in saline agricultural soils, where high salinity results from irrigation practices and application of chemical fertilizer. This effect is always more pronounced in the rhizosphere pursuant to increased water uptake by the plants due to transpiration. The simple explanation for this is that life in high salt concentrations is bioenergetically taxing because microorganisms must maintain an osmotic balance between their cytoplasm and the surrounding medium while excluding sodium ions from the cell interior, and as a result, sufficient energy is required for osmoadaptation (Oren et al., 2002; Jiang et al., 2007). 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 in the soil during salinity (Ross et al., 2000).

It has been shown that the accumulation of B may be more of a limiting factor to plant growth than the total salt concentration (Ayars et al., 1993). Boron is an essential micronutrient for plants, but it is toxic to many plants at higher concentrations. The optimum concentration range of plant-available B is very narrow for most crops (Grattan and Grieve, 1999). The B tolerance of crops is species dependent and can vary widely among cultivars within a given species from <6 to 10 g m^{-3} (Benlloch et al., 1991). In arid and semiarid irrigated areas, high B concentrations in soils are often associated with high salt concentrations (Grieve and Poss, 2000). This observation was further shown by a linear relationship ($r^2 = 0.81$) between soil B content and soil salinity in a three year study in the San Joaquin Valley (Shouse et al., 2006). The authors concluded that the correlation between salinity and B in the field probably exists because they share a common origin, namely the alluvium derived from sedimentary marine deposits of the Coast Range Mountains on the western side of the San Joaquin Valley (Letey et al., 2002). The effect of B on microorganisms is largely unknown; however, studies with pure cultures have shown than B can inhibit growth of bacteria (Bringmann and Kuhn, 1980; Butterwick et al., 1989) at high concentrations.

Recently, Nelson and Mele (2007) studied the subtle changes in rhizosphere microbial community structure in response to increased B and sodium chloride concentrations. They concluded that B and sodium chloride are more likely to affect rhizosphere microbial community structure indirectly through root exudates quantity and/or quality than directly through microbial toxicity, and that plant health is a major determinant in rhizosphere microbial community structure and normal N cycle. However, this study did not include pH as a variable. San Joaquin Valley soils that have the combination of high salinity and B are alkaline in nature (pH range of 7.5 to nearly 9.0). Very little is known about how pH influence salinity–B interactions in this soil, and how they affect plant growth. To the best of our knowledge, nothing has been reported about how these variables affect soil microbial composition and functions in the rhizosphere of vegetable crops in this region. Incorporation of pH into the factorial design of the experiment may provide insights into salinity–B interactions and the modified tolerances of B in the presence of salinity observed in many other studies. The objectives of this study were study the effects of soil salinity, B, and pH on rhizosphere and non-rhizosphere microbial composition by using both culture dependent and independent approaches. To achieve these goals polymerase chain reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE) and sequencing of 16S rRNA genes were used to explore how the incorporation of pH into the experimental variables may provide insights into how salinity–B interactions affect rhizosphere microbial population.

2. Materials and methods

2.1. Plant growth

The experiment was conducted in a closed recirculating volumetric lysimeter system (VLS) (Poss et al., 2004) consisting of 24 experimental plant growth units (81.5 cm wide $\times$ 202.5 cm long $\times$ 85 cm deep) at the U.S. Salinity Laboratory in Riverside, CA to determine the interactive effects of salinity, B and pH on the rhizosphere and non-rhizosphere microbial composition of cucumber (*Cucumis sativus* L. cv. Seminis Turbo hybrid). The lysimeters were constructed in 1995. Between 1995 and 2004, many types of plants had been grown. Crops grown in the model soil whose physical properties were found to be comparable to field soils by Wang (2002) have included many different type of crops such as Poplar trees, Paspalum, Bermudagrass, Saltgrass, Pistachio rootstocks, leafy vegetables, and two years of alfalfa and tall wheatgrass. The year prior to the current cucumber study, a floral crop, was grown. Each crop was irrigated with solutions having a minimum electrical conductivity of 2 dS m^{-1}. The lysimeters were filled with sand (particle size distribution ranging from 0.09 mm to 4 mm) that resulted in a medium with volumetric water content of 0.1–0.3 $cm^3\ cm^{-3}$ and similar thermal conductivities and heat capacities as field soil (Wang, 2002). This medium had a high saturated soil hydraulic conductivity (400 cm day^{-1}) and provided limited exchange of soil water inorganic constituents with the solid phase, thus simplifying control of soil water chemistry. Twenty-four sand tanks, arranged in a randomized complete block design, were irrigated from individual reservoirs containing a modified half-strength Hoagland's nutrient solution combined with various salinity, B, and pH treatments. Each tank was plumbed with 5.1 cm PVC pipes, one for irrigation to the sand tank, and one for return flow to a 1740-L reservoir in the basement below. The pH adjustments on the reservoirs were performed most week days and salinity and B concentrations were periodically monitored. Crop water use was determined volumetrically by reservoir water depletion. The rhizosphere samples were collected after shaking loosely held soil on the roots into the stomacher bags and weigh and 10 g of non-rhizosphere samples were collected at least 10 cm away from plants. All samples were collected weekly for five weeks (weeks 1, 3, 4, 5, and 7). Samples were also collected for heterotrophic plate counts and analyzed by serial dilution as well as samples for total community DNA extraction.

2.2. Treatments

Treatments included a concentration of two salinity levels, 3 and 8 dS m^{-1}; three B concentrations of 0.7, 5, and 8 mg L^{-1}; and two pH levels where solutions were frequently adjusted to 6.5 and 8. Treatments were replicated twice. The concentration of B was selected based on the B concentrations in drainage waters in SJV (Letey et al., 2002). Plants were routinely observed for foliar injury and fruit development. Data collected included fresh and dry weights of plants, leaves, stems, and roots as well as cucumber fruit weight and quantity. Collected tissues were analyzed for various ions to determine their distributions within the plant and ion interactions.

2.3. DNA extraction, PCR-DGGE, and phylogenetic analysis

Community DNA was extracted from rhizosphere and non-rhizosphere samples with the Ultra Clean Soil DNA Kit (MoBio Laboratories, Solana Beach, CA) and stored at $-20\ ^\circ C$ after further cleanup steps. A 236-bp DNA fragment in the V3 region of the small subunit ribosomal RNA genes of eubacteria was amplified by using