Research in Microbiology 164 (2013) 83–89

www.elsevier.com/locate/resmic

Colorimetric microbial diversity analysis and halotolerance along a soil salinity gradient at the Great Salt Plains of Oklahoma

Mark A. Schneegurt

Department of Biological Sciences, Wichita State University, 1845 Fairmount, Wichita, KS 67260, USA

Received 3 July 2012; accepted 18 September 2012
Available online 12 October 2012

Abstract

Microbial diversity was measured along a salinity gradient at the Great Salt Plains of Oklahoma using colony color quantified as RGB components of microbial isolate streaks. Numerical taxonomy was performed using a UPGMA method to create trees of relatedness, define OTUs, and calculate diversity indices. Surface soil samples along a 6-m salinity gradient (from hypersaline soil with 7.5% salinity to oligohaline rangeland soil) at WP68 were dilution-plated on SP medium of various salinities and hundreds of random colonies were collected. The salinity tolerance of isolates along the gradient was determined. From the 1364 colonies examined, 338 OTUs were defined by colony color and their distribution statistically analyzed by soil type and the salinity of enrichment media. Most colonies were shades of cream that became distinguishable based on RGB color components. Diversity indices were high overall and it is likely that the OTUs defined by colony color are below the species level, at the strain level, where the greatest diversity lies in this environment. These results are complementary to previous molecular genetic analyses of 16S rRNA clone libraries from soils at the Great Salt Plains. Great diversity at lower taxonomic levels supports the suggestion that gene flow is not highly fragmented, a result of less specialization, as expected given the highly variable salinity observed at the salt flats with rain events.

Keywords: Ecology; Extreme environment; Halophile; Pigment; Tolerance

1. Introduction

The Great Salt Plains (GSP) of Oklahoma comprise a 65-km^2 expanse of unvegetated mudflats covered with a thin evaporite crust of mainly NaCl. Groundwater beneath high-salt soils within the salt flats is a nearly saturated brine (Johnson, 1980; Major et al., 2005; Reed, 1982). At the margins of the salt flats, soil salinity drops over 5–10 m, leading to oligohaline rangeland. Rain events wash away the evaporite crust leaving transient pools of nearly fresh water that can persist for several days. High-salt soils experience greater salinity shifts with rain events than more oligohaline soils.

Microbes living in hypersaline environments experience a high degree of selection pressure and isolates from the GSP are mainly from genera associated with hypersaline systems (Caton et al., 2004, 2009; Caton and Schneegurt, 2012). However, few of the bacterial isolates are halophilic specialists that require high salt concentrations for growth. In fact, nearly all (84%) of the bacterial isolates are euryhaline, exhibiting broad salinity tolerance ranges of greater than 15%, with a third of the isolates having permissible salinity ranges of 20% or more. Culture-based and culture-independent (using 16S rRNA gene sequences) microbial diversity indices in high-salt GSP soils are relatively low (Caton et al., 2004, 2009; Caton and Schneegurt, 2012), suggesting that temporal salinity variations in high-salt soils lead to reversible responses from ecological generalists (with respect to salinity tolerance). Genetic evidence suggests that there is a great deal of subspecies level genetic variation that is not reflected in rRNA gene diversity and that this variation is being driven by high rates of recombination and lateral gene transfer (Rudrappa and Miller, 2009).

E-mail address: mark.schneegurt@wichita.edu.

0923-2508/$ - see front matter © 2012 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.resmic.2012.10.004

The current report measures two phenotypic characteristics, salinity tolerance and colony color, across a large random microbial isolate collection from soils obtained along a salinity gradsect at the edge of the GSP. More isolates from high-salt soils, experiencing the greatest salinity shifts, were euryhaline and thus were less specialized with respect to salinity tolerance than were isolates from soils of lower salinity. Numerical taxonomy based on RGB color analysis of isolate colony color was used as a measure of diversity and should be seen as complementary to previous molecular genetic studies of GSP soils using rRNA gene sequences (Caton et al., 2009; Caton and Schneegurt, 2012). While the distribution of colonies into color classes varied along the gradsect, diversity indices based on colony color were consistently high. Finally, there is a discussion of colony color assessment with respect to the curation and description of environmental isolate collections. A brief account of this work was presented previously (Schneegurt, 2009).

2. Materials and methods

2.1. Soil sample collection

Surface grab samples (top 4 cm) were cleanly taken using sterile tools along a salinity gradient near the edge of the salt flats at WP68 (N 36° 42.856′ W 93° 15.725′). Each bulk sample was mixed by hand in sterile Whirl-Pak bags, transported at 25 °C, and processed within 1–2 h of collection. For salinity measurements, soils were dried to constant weight at 110 °C, diluted 1:10 with distilled water, agitated for 1 h, and allowed to settle. The liquid phase was filtered (0.22 μm; Millipore) and examined with a handheld salinity refractometer with automatic temperature compensation (Fisher).

2.2. Microbial collection and salinity tolerance

Soil samples from along the salinity gradient at WP68 were dilution-plated on SP medium (Caton et al., 2004) with NaCl concentrations of 1, 10, and 20% and grown at room temperature. Ten grams of soil were added to 90 ml of brine at 1, 10 or 20% salinity, shaken at 150 rpm on a rotary shaker (1-in stroke dia) for 20 min, serially diluted using the corresponding brine and plated for counting of CFUs on media having the same salinity as the dilution brine. At least 100 widely separated colonies from each set of enrichment plates were randomly collected by position on the plate and each was transferred to SP test plates at 1, 10, 20 or 30% salinity, and visually scored for growth at appropriate times after inoculation (higher salt plates needed longer incubations).

2.3. Diversity analysis by colony color

Microbial streaks on the plates used for measuring salinity tolerance were digitally photographed (Nikon E3200; 300 dpi) under diffuse cool white fluorescent light for analysis of colony color. The RGB color components of each microbial streak were averaged over four five-pixel areas using Photoshop (Adobe). Colony color frequency distributions were statistically treated using chi-squared analysis. Numerical taxonomy clustering was performed with NTSYS pc v. 2.1 (Applied Biostatistics, Inc.) as interval data employing a UPGMA method (SAHN) to create trees of relatedness. Broad phenoms (color classes) were defined using a coefficient threshold of 16.0, while OTUs for diversity analysis were defined using a coefficient threshold of 2.7. Diversity indices were calculated using Estimates 8.0.0 (Colwell, 2006).

3. Results

3.1. Salinity tolerance of random isolates along a salinity gradsect

Along the western margin of the GSP, salt-crusted mudflats meet oligohaline grasslands (WP68; Fig. 1). The salinity gradsect studied here covered 6 m from mud flat soils with 7.5% salinity (high-salt soil) to sandy soils with 0% salinity (low-salt soil) that supported grasses. An intermediate soil, 2 m from the low-salt soil, was barren and had a salinity of 2% (medium-salt soil). High-salt soils at the GSP typically have crusts of mainly NaCl evaporites, 15% water content, and overlay shallow groundwaters with salinities of 20% or more (Major et al., 2005). Samples were collected when there had been no rainfall or flooding for at least two days, leaving the flats covered with an evaporite crust.

Soils from each of the three surface samples were used for dilution plating experiments at 1, 10 and 20% salinity (Table 1). For all three soils, the highest plate counts were

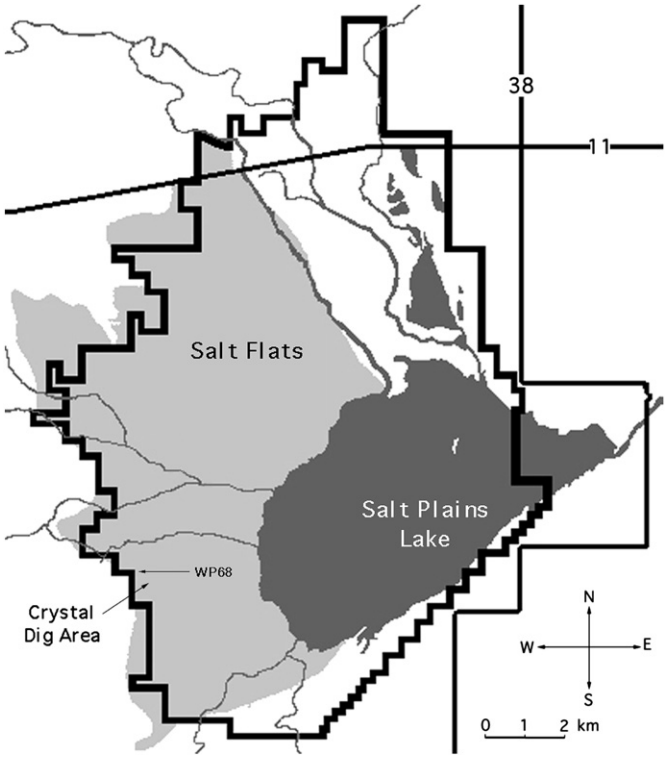

Fig. 1. Map of the Great Salt Plains showing sampling location.