Correlation of Intracellular Trehalose Concentration with Desiccation Resistance of Soil *Escherichia coli* Populations

Qian Zhang and Tao Yan

Department of Civil and Environmental Engineering, University of Hawaii at Manoa, Honolulu, Hawaii, USA

Naturalized soil *Escherichia coli* populations need to resist common soil desiccation stress in order to inhabit soil environments. In this study, four representative soil *E. coli* strains and one lab strain, MG1655, were tested for desiccation resistance via die-off experiments in sterile quartz sand under a potassium acetate-induced desiccation condition. The desiccation stress caused significantly lower die-off rates of the four soil strains (0.17 to 0.40 day^{-1}) than that of MG1655 (0.85 day^{-1}). Cellular responses, including extracellular polymeric substance (EPS) production, exogenous glycine betaine (GB) uptake, and intracellular compatible organic solute synthesis, were quantified and compared under the desiccation and hydrated control conditions. GB uptake appeared not to be a specific desiccation response, while EPS production showed considerable variability among the *E. coli* strains. All *E. coli* strains produced more intracellular trehalose, proline, and glutamine under the desiccation condition than the hydrated control, and only the trehalose concentration exhibited a significant correlation with the desiccation-contributed die-off coefficients (Spearman's $\rho = -1.0$; $P = 0.02$). *De novo* trehalose synthesis was further determined for 15 *E. coli* strains from both soil and nonsoil sources to determine its prevalence as a specific desiccation response. Most *E. coli* strains (14/15) synthesized significantly more trehalose under the desiccation condition, and the soil *E. coli* strains produced more trehalose ($106.5 \pm 44.9 \text{ } \mu\text{mol/mg of protein}$ [mean $\pm$ standard deviation]) than the nonsoil reference strains ($32.5 \pm 10.5 \text{ } \mu\text{mol/mg of protein}$).

Traditionally, *Escherichia coli* in the environment has been often considered residual cells from recent fecal inputs (46), but this has been contradicted by recent reports of the ubiquitous presence of high levels of *E. coli* cells in soil samples (5, 18, 23). Studies using molecular tools have detected unique genotypic compositions of *E. coli* populations in soil (6, 19, 23), and the persistence of certain *E. coli* strains over extended periods of time in soils further supports the notion that certain soil *E. coli* populations are naturalized members of indigenous soil microbial communities (23). Since soil-sourced *E. coli* cells may enter nearby waterways in the absence of actual fecal inputs and thus generate false signals of fecal pollution, intense debate has arisen regarding the suitability of *E. coli* as a fecal indicator in water quality monitoring.

To understand the survival and habitation of *E. coli* in soil, it is important to understand how *E. coli* resists soil environmental stresses, in particular, the common and unique soil desiccation stress caused by the natural cycles of soil wetting and drying. Studies on traditional soil bacteria have identified numerous cellular mechanisms responsible for enhanced desiccation resistance, including *de novo* trehalose synthesis (10, 29), production of extracellular polymeric substances (EPS) (36, 38), and uptake of exogenous glycine betaine (GB) (2, 4, 44). Although few studies have examined *E. coli* desiccation resistance (45), *E. coli* uses similar mechanisms to resist osmotic shock caused by increased water salinity in aquatic environments. For example, high water salinity prompted *E. coli* to synthesize trehalose and sucrose (12, 34), to take up environmental GB (7, 26), and to produce EPS (8).

De novo synthesis of trehalose is a desiccation resistance strategy commonly employed by soil bacteria, including *Bradyrhizobium* spp. (10, 40), *Rhodococcus* spp. (25), *Rhizobium* spp. (29), and *Pseudomonas* spp. (15). During desiccation, intracellular trehalose can help maintain the phospholipid bilayer of cell membrane in the liquid crystalline phase (9) and can also keep protein in a hydrated formation by hydrogen bonding and water replace-

ment (27). Consequently, trehalose is believed to be an excellent osmoprotectant under severe water activity reduction (33). The genetic capability of *de novo* trehalose synthesis is widely distributed, and *E. coli* is known to synthesize trehalose for salinity-caused osmotic stress resistance (12, 34). Previous studies have shown that overexpression of trehalose in recombinant *E. coli* strains (30) or osmotically induced trehalose accumulation in *E. coli* NCIB 9484 (45) can improve *E. coli* desiccation resistance significantly.

Tropical soils in Hawaii have been reported to contain high levels of *E. coli* (5, 16, 20), and recent sampling efforts in the Manoa watershed on the Island of Oahu, HI, also detected high levels of *E. coli* (603 to 1,820,000 CFU/100 g of soil) during a 9-month sampling period (18). By constructing a soil *E. coli* library for the Manoa watershed, numerous *E. coli* strains were frequently found at different sampling locations and on different sampling dates (19). These soil *E. coli* strains are thus considered naturalized soil *E. coli* populations and are expected to have higher desiccation resistance than *E. coli* populations from other sources. In this study, desiccation-contributed die-off levels of four representative soil strains and the K-12 strain MG1655 were investigated under a desiccation condition in sterile quartz sand. The *E. coli* strains' cellular responses to desiccation, including exogenous GB uptake, EPS production, and synthesis of compatible organic solutes, were quantified and correlated with their respective desiccation-contributed die-off patterns. Finally, additional *E. coli*

Received 13 June 2012 Accepted 5 August 2012

Published ahead of print 10 August 2012

Address correspondence to Tao Yan, taoyan@hawaii.edu.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AEM.01904-12

TABLE 1 Isolation patterns of select *E. coli* strains from soil in the Manoa watershed

Strain	Detection date(s) (mm/dd/yy)	No. of sites ^a	Frequency ^b
S-A34	5/15/09, 6/8/09	2	12
S-B31	5/15/09, 6/8/09, 8/31/09	3	16
S-B32	6/8/09, 10/14/09, 12/21/09	2	10
S-B54	5/15/09, 8/31/09, 12/21/09, 2/24/10	3	12
S-A18	5/15/09	1	9
S-A37	2/24/10	1	8
S-B35	5/15/09	1	8
S-E52	10/14/09, 2/24/10	2	7
S-F13	8/31/09	1	7
S-F18	5/15/09	1	7

^a Number of sites at which the strain was found, among a total of six sampling sites.

^b Number of isolates identified as the indicated strain, from among the total of 630 isolates from soil.

strains from soil and nonsoil sources were tested for the production of trehalose as a specific and major desiccation response.

MATERIALS AND METHODS

***E. coli* strains.** A previous study isolated 234 unique *E. coli* genotypes from 630 *E. coli* isolates obtained from soil samples collected from the Manoa watershed on the island of Oahu, HI (19). Ten *E. coli* strains were selected to represent soil *E. coli*, based on their repetitive detection in different sampling sites and at different sampling times (Table 1). Four *E. coli* isolates were isolated from wastewater collected at the Sand Island Wastewater Treatment Plant of Honolulu, HI, and considered representative of *E. coli* strains from human fecal sources. The sewage *E. coli* isolates obtained were verified and characterized using the same procedures as for the soil *E. coli* isolates (19). One additional reference *E. coli* strain was the common lab K-12 strain MG1655. A subset of the *E. coli* strains (S-A34, S-B31, S-B32, S-B54, and MG1655) were tested for cell die-off under desiccation stress and investigated for their specific cellular responses to desiccation, while the whole strain set was tested specifically for the most prevalent desiccation resistance mechanism identified (i.e., *de novo* trehalose synthesis).

Incubation of *E. coli* cells in quartz sand. Die-off experiments for exponential-phase cells of *E. coli* strains (S-A34, S-B31, S-B32, S-B54, and MG1655) in sterile quartz sand were conducted under a desiccated condition and a fully hydrated condition. The fully hydrated condition was used to determine the natural endogenous decay of the *E. coli* cells. Fresh overnight single colonies of the *E. coli* strains were used to inoculate 5 ml of mineral salts medium plus glucose (36), and these cells were subsequently incubated in a shaking incubator at 200 rpm and 37°C to reach mid-exponential stage (optical density at 600 nm, 0.4 to 0.7). Cells were harvested by centrifugation at 10,000 × g for 10 min and resuspended in phosphate-buffered saline (PBS) to make cell stock solutions. To establish the experimental microcosms, 2.6-ml volumes of the freshly prepared cell stock solutions, equivalent to ca. 10⁹ cells, were spiked into 10 g sterile, acid-washed quartz sand placed in Pyrex petri dishes.

Six identical microcosms were established for each of the five *E. coli* strains: three microcosms for the desiccation experiment and the other three for the hydrated control. The desiccated microcosms were placed in desiccators using a saturated potassium acetate (KOAc) solution as the desiccant, which produces 27% relative humidity at equilibrium (10). The hydrated control microcosms were incubated in desiccators containing sterile double-distilled water to maintain 100% relative humidity. Water content in the microcosms was monitored daily by weighing the microcosms. The microcosms in the desiccators were incubated at room temperature in the dark. Sand samples collected from the microcosms were first extracted using PBS to release *E. coli* cells (3) and then enumerated on tryptic soy agar plates.

Incubation of *E. coli* cells on membranes. *E. coli* cells were also incubated on polycarbonate membranes under both the desiccated condition and the hydrated condition to quantify the production of EPS, uptake of exogenous GB, and the production of organic compatible solutes, including trehalose. The membrane-based desiccation experiments, in contrast to the sand-based desiccation experiments, facilitated the extraction and quantification of EPS, GB, and intracellular organic solutes by avoiding interference from sand. The mid-exponential-stage cells of the *E. coli* strains were filtered onto polycarbonate membranes (0.2-μm pore size, 47-mm diameter; Whatman, Springfield Mill, United Kingdom). The experimental setup and incubation conditions were otherwise exactly the same as for the sand microcosm experiments.

The cell-bearing membranes were incubated for 4 days for quantification of EPS production and the synthesis and accumulation of intracellular compatible organic solutes. For the quantification of exogenous GB uptake, the cells were incubated under the desiccated or the hydrated conditions for 48 h, and then 800 μl glycine betaine (100 μM) was added to each membrane and incubated for 15 min, which allowed the uptake of GB by *E. coli* cells to reach a plateau, according to Perroud and Le Rudulier (32). The membranes were immediately vacuumed and washed twice with 20 ml of a 0.9% NaCl solution to remove residual liquid.

EPS extraction and quantification. *E. coli* cells on the membranes were washed off with 1 ml of 0.15 M NaCl solution and collected in glass test tubes. The test tubes were incubated at 100°C for 10 min, cooled to room temperature, and then centrifuged at 10,000 × g for 15 min. Supernatants of the cell extracts were subjected to the phenol-sulfuric acid test (12). Briefly, cell extracts (500 μl) were first mixed with 12.5 μl of 80% phenol and then swiftly amended with 1,250 μl of concentrated sulfuric acid to initiate reaction at high temperature. The reactions were allowed to progress for 10 min before brief shaking to mix. After approximately 20 min of further incubation at room temperature, absorbance measurements at 485 nm were taken, which were used to calculate the EPS concentration based on a five-point calibration curve.

Intracellular organic solute extraction. For the intracellular compatible organic solute synthesis and the exogenous GB uptake experiments, the cell-bearing membranes were extracted following a procedure described by Higo (21). Briefly, the membranes were submerged in 800 μl 80% ethanol, incubated at 65°C for 3 h, and finally centrifuged at 10,000 × g for 10 min. The supernatants were separated from the pellets and then vacuum dried. The pellets were resuspended in 0.5 ml sterile deionized water and centrifuged at 10,000 × g for 10 min, and the supernatants were pooled and vacuum dried for subsequent solute quantification by different methods.

Quantification of compatible organic solutes. Nuclear magnetic resonance (NMR) quantification of compatible organic solutes was conducted by the NMR lab at the University of Hawaii at Manoa. The cell extract pellets were first dissolved in 250 μl D₂O, and ¹H NMR spectra were obtained using a Varian Unity Inova 500 spectrometer. The acquisition parameters used for ¹H (500-MHz) NMR were 40.5° pulse width, 3.5-s acquisition time, 0.5-s relaxation delay, and 64 repetitions. Chemical shifts were expressed in parts per million downfield from sodium 3-(trimethylsilyl) sulfonate (DSS) (an example proton NMR spectrum and the identification of organic solutes based on chemical shifts is shown in Fig. 1), and quantification was achieved by comparing the integrated peak areas of the organic solutes with that of DSS, which was introduced as the internal quantification standard.

Trehalase assay. The intracellular trehalose amount in the cell extracts was also quantified using a trehalase assay (21). Briefly, aliquots of cell extracts (100 μl) were mixed with 100 μl of reaction mixture that contained 100 mM morpholinoethanesulfonic acid (MES)-KOH (pH 6.0) and 5 × 10⁻³ U/ml trehalase. The reaction mixtures were incubated at 37°C for 1 h to convert trehalose into glucose before reactions were terminated by the addition of 50 μl of 500 mM Tris-HCl (pH 7.5). The resulting glucose was subsequently quantified using a glucose kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's procedure.

FIG 1 An example of the ^1H NMR spectra of intracellular organic solutes of soil *E. coli* strain S-B54 under the desiccation stress.

Data analysis. The die-off of *E. coli* cells was modeled using first-order decay kinetics ($\ln C_d - \ln C_0 = -(k_d + k_w)t$, and $\ln C_w - \ln C_0 = -k_w t$), based on the desiccation-contributed cell die-off coefficient k_d and cell natural decay coefficient k_w (t represents time, in days). The remaining cell concentrations under the desiccation and the hydrated control conditions are C_d and C_w , respectively. The initial cell concentrations for each strain (C_0) for the desiccated experiment and the hydrated control were the same. The desiccation-contributed cell die-off can be mathematically represented by the following equation: $\ln(C_d/C_w) = -k_d t$.

The concentrations of EPS, trehalose, and other organic solutes were expressed in reference to the cellular protein concentration, which was determined using a bicinchoninic acid assay (BCA) kit (Thermo Scientific, MA) (1). Student's t test was used to compare the mean values of two different treatments. Spearman's ρ values were calculated to determine the rank correlation between desiccation-caused die-off coefficients and the accumulated intracellular organic solutes. Unless otherwise stated, the default statistical significance level is $P \leq 0.05$. Statistical analyses were performed using a StatistixXL add-in package in Microsoft Excel.

RESULTS

Desiccation resistance of soil *E. coli* strains. The cell die-off patterns of *E. coli* strains S-A34, S-B31, S-B32, S-B54, and MG1655 in quartz sand under the desiccated condition and the hydrated control condition were determined. Cell die-off as a result of desiccation (C_d/C_w) showed a significant difference between the soil *E. coli* strains and MG1655 (Fig. 2). Under the hydrated control condition (100% relative humidity), the majority of *E. coli* strains maintained relatively stable populations without apparent cell die-off, and cell concentrations of strains MG1655 and S-A34 actually increased by approximately 10-fold, indicating cell growth (data not shown). Under the desiccation condition, the water content in the microcosms gradually decreased from 27% and eventually stabilized at approximately 5% at day 5. A clear reduction in viable cell counts was observed for MG1655 on day 2, when the water content was 13%, while for the four soil *E. coli* strains viable cell count reduction only occurred on day 4, when the water content was 5%. No significant difference in cell die-off under the desiccated condition was detected among the four soil *E. coli* strains toward the end of the experiment.

FIG 2 Reduction of culturable *E. coli* cells as a result of desiccation in quartz sand microcosms over time. C_d represents the *E. coli* concentration under the desiccation condition, and C_w represents the *E. coli* concentration under the hydrated control condition. The bar graph shows the change of water content in the desiccated microcosms. Error bars show standard errors of the means from triplicate microcosms.

Exogenous GB uptake. To determine whether or not the uptake of exogenous GB is an important mechanism for *E. coli* desiccation resistance, the soil *E. coli* strains and MG1655 were allowed to take up exogenous GB under both the desiccation condition and the hydrated control condition. The amount of GB uptake by the soil *E. coli* strains was not significantly higher than that of MG1655 (Fig. 3). In fact, one soil *E. coli* strain (S-B32) exhibited significantly less GB uptake ($1.28 \mu\text{mol/mg}$ of protein) than MG1655 ($4.51 \mu\text{mol/mg}$ of protein), despite exhibiting significantly higher desiccation resistance than the latter (Fig. 2). Furthermore, the amounts of exogenous GB taken up by MG1655 and three of the soil *E. coli* strains (S-A34, S-B31, and S-B32) under the hydrated condition were higher than under the desiccated condition, indicating that desiccation stress might actually limit the uptake of exogenous GB by these strains.

EPS production. The production of EPS by the *E. coli* strains under the desiccated condition and the hydrated condition was

FIG 3 Concentration of GB taken up by the *E. coli* strains under the desiccated condition (solid bars) and the hydrated condition (open bars). Error bars are the standard errors of the means from triplicate tests.

FIG 4 Production of EPS by the *E. coli* strains under the desiccated condition (solid bars) and the hydrated condition (open bars). Error bars are the standard errors of the means from triplicate tests.

quantified to determine the involvement of EPS in *E. coli* desiccation resistance. Under the desiccated condition, all four soil *E. coli* strains produced significantly more EPS than MG1655 (Fig. 4), which corresponds to their higher desiccation resistance than the latter and suggests the involvement of EPS in the high desiccation resistance expressed by the soil *E. coli* strains. The majority of the soil *E. coli* strains, including S-A34, S-B32, and S-B54, produced significantly more EPS under the desiccation condition than under the hydrated condition, while no statistical differences were detected for strain S-B31 and MG1655 under the two experimental conditions.

Major intracellular organic solutes. Major organic solutes present in *E. coli* cells under the desiccation and hydrated conditions were identified and quantified using ¹H NMR. Trehalose, proline, glutamine, acetate, valine, and glucose were identified as major organic solutes in the desiccated and hydrated *E. coli* cells by ¹H NMR. The intracellular concentration differences between the desiccated condition and the hydrated control were calculated and correlated with their desiccation-caused die-off coefficients (Table 2). Among the major intracellular organic solutes, trehalose, proline, glutamine, and glucose consistently showed elevated intracellular concentrations under the desiccated condition for all strains. Spearman's rank correlation coefficients between the elevated intracellular concentrations of organic solutes under the desiccated condition and the desiccation-contributed die-off coefficients were calculated (Table 2). Among the major organic solutes, a strong ($\rho = -1.0$) and significant ($P = 0.02$) correlation

FIG 5 Intracellular concentrations of trehalose in 10 soil *E. coli* strains (designated with an S), four *E. coli* isolates from municipal wastewater (designated by WW), and the lab strain MG1655 under the desiccation condition (solid bars) or the hydrated condition (open bars).

was observed only for trehalose, suggesting that *de novo* trehalose synthesis is a universal and specific response to desiccation stress by the *E. coli* strains.

Prevalence of trehalose as a desiccation response. A total of 15 *E. coli* strains, including 10 soil *E. coli* strains, 4 *E. coli* isolates from municipal wastewater, and 1 lab reference *E. coli* strain, were used to investigate the prevalence of elevated intracellular trehalose production as a specific response to desiccation stress. The intracellular concentrations of trehalose of *E. coli* cells incubated under the desiccated condition and the hydrated condition were determined (Fig. 5). After 4 days of incubation under the fully hydrated control condition, the *E. coli* cells accumulated small amounts of trehalose (4.4 ± 5.4 μmol/mg of protein [mean $\pm$ standard deviation]), except for soil strain S-A37 (97.1 μmol/mg of protein). All *E. coli* strains produced significantly more trehalose under the desiccated condition than under the hydrated control condition ($P \leq 0.05$), except for strain S-A37, indicating that *de novo* trehalose synthesis is a common and specific desiccation response by *E. coli*. Under the desiccation condition, different *E. coli* strains produced different levels of intracellular trehalose (20.9 to 260.5 μmol/mg of protein). The 10 soil *E. coli* strains synthesized and accumulated significantly more trehalose (106.5 ± 44.9 μmol/mg of protein) than the wastewater *E. coli* isolates and MG1655 (32.5 ± 10.5 μmol/mg of protein).

TABLE 2 Desiccation-contributed die-off constants and concentration differences of major organic solutes between desiccated and hydrated cells							
Strain	k_d (day ⁻¹)	Concn difference (μmol/mg of protein)					
		Trehalose	Proline	Glutamine	Acetate	Valine	Glucose
MG1655	0.85	18.9	8.3	24.7	2.4	−9.0	4.5
S-A34	0.40	27.2	1.0	13.6	11.8	−26.7	9.3
S-B31	0.35	41.3	6.9	12.3	−0.2	−24.4	8.7
S-B32	0.27	55.5	12.8	13.9	2.0	−11.1	8.1
S-B54	0.17	121.5	47.1	7.3	4.7	−6.0	59.3
Spearman's ρ^a		−1.0 (0.02)	−0.7 (0.23)	0.7 (0.23)	0.1 (0.95)	−0.4 (0.52)	−0.6 (0.35)

^a Spearman's rank correlation coefficient between the k_d and the concentration of the compatible solute. P values are shown in parentheses.

DISCUSSION

Recent studies have provided strong evidence indicating that *E. coli* can exist as an autochthonous component of soil microbial communities (5, 6, 16, 20, 23). This recognition questions the reliability of conventional water quality monitoring approaches, which use *E. coli* as a fecal indicator. At geographic locations where environmental conditions favor the habitation of *E. coli* in soil, the soil-sourced *E. coli* cells can be transported into water under various conditions (e.g., rainfall runoff), resulting in high *E. coli* counts in water in the absence of actual fecal pollution. Even for situations with actual fecal pollution, the capability of soils to support *E. coli* growth may significantly prolong cell survival in the environment, thus making soils lasting secondary reservoirs of fecal *E. coli* cells. The former scenario would invalidate the specificity of water quality monitoring, while the latter would undermine the time-sensitive qualities of the monitoring efforts.

To inhabit and maintain a population in soil, *E. coli* must be able to resist soil environmental stresses, one of which is the desiccation stress associated with natural soil wetting-drying cycles. Desiccation is a common and unique environmental stress in soil, and soil-dwelling bacteria are generally equipped with mechanisms for desiccation resistance (33, 41). Since habitat stresses affect bacterial adaptation and divergence within the same species (35, 39), one may expect that the *E. coli* strains that inhabit soil generally exhibit higher desiccation resistance than strains occupying other niches. The present study showed that four soil *E. coli* strains survived better than the common lab strain MG1655 in desiccated sterile quartz sand. The soil strains were observed to maintain relatively stable population sizes until the water content in the quartz sand dropped from 26.8% to 9.3% in 3 days, exhibiting considerable desiccation resistance (Fig. 2). It is worth noting that the desiccation die-off experiments were conducted in the absence of other environmental stresses (such as sunlight irradiation, temperature fluctuations, and competition/antagonism from indigenous soil microbial communities) that also affect *E. coli* survival (13, 18). In natural soil environments, the soil desiccation stress, together with other environmental stresses, determines the fate of soil *E. coli* populations.

The five *E. coli* strains used in the desiccation die-off experiments were further investigated to identify major desiccation resistance mechanisms of the strains, which is important to the understanding of *E. coli*'s ecology and adaptation in soil environments. A reasonable hypothesis is that soil *E. coli* strains utilize the mechanisms used by typical soil bacteria, such as *Rhizobium/Bradyrhizobium* (10, 29) and *Pseudomonas* (36, 38), for desiccation resistance. Numerous mechanisms are employed by typical soil bacteria to resist desiccation (33), with the common ones being exogenous GB uptake (2, 4, 44), EPS production (36, 38), and *de novo* trehalose synthesis (10, 29). In this study, the involvement of certain mechanisms in *E. coli* desiccation was identified by quantifying multiple *E. coli* strains' specific cellular responses to the desiccation stress, which were then correlated with their respective desiccation resistance levels. This approach differs from conventional mechanistic studies, which have used single *E. coli* strains, and draws its strength from the involvement of multiple strains.

Among the different mechanisms for the observed desiccation resistance is the uptake of exogenous GB, which is a common osmoprotectant responsible for desiccation resistance in soil bac-

teria (33). Although *E. coli* lacks the capability of synthesizing GB *de novo*, *E. coli* was shown to take up exogenous GB from aquatic environments to enable survival and growth under high-salinity conditions (7, 26, 32). However, GB did not appear to be an effective osmoprotectant under desiccation, likely due to the severe water activity reduction associated with desiccation (33). A previous study showed that the salinity-induced intracellular GB accumulation did not increase the desiccation resistance of *E. coli* strain NCIB 9484 (45); this finding differs from results for lactic acid bacteria, which showed increased desiccation resistance due to salinity-induced GB uptake (24). The noneffectiveness of GB to *E. coli* desiccation resistance is also supported by the observed nonspecific GB uptake in response to desiccation stress by the five *E. coli* strains in the present study; four out of five *E. coli* strains tested actually accumulated less exogenous GB under the desiccation condition than under the hydrated control condition (Fig. 3). Furthermore, the amount of GB uptake did not correlate with the desiccation-contributed die-off of the *E. coli* strains.

Comparatively, EPS production appeared to be more involved in soil *E. coli* desiccation resistance than exogenous GB uptake, and this finding was supported by the significantly higher EPS production by the soil *E. coli* strains than MG1655 under the desiccation stress (Fig. 4). Since EPS is essential to the formation of biofilms, which confer desiccation resistance to soil bacteria (14), the elevated EPS concentrations corresponded well with the higher desiccation resistance of the soil *E. coli* strains. Among the four soil *E. coli* strains tested, three strains produced more EPS under the desiccation condition than under the hydrated control condition, a pattern often observed in desiccated soil bacteria (36). Strains MG1655 and S-B31 produced slightly less EPS (although the difference was not statistically significant) under the desiccation condition than the hydrated control condition, which may have been due to EPS production being a generic response to numerous environmental cues (11, 37, 42) and the quality of EPS being as important as its quantity with regard to desiccation resistance (31). The present study did not examine the quality of EPS produced by the different *E. coli* strains.

Trehalose, on the other hand, exhibited a clear role in the desiccation resistance of the *E. coli* strains. Trehalose is the only major intracellular organic solute that exhibited a significant correlation with the *E. coli* die-off rate under the desiccation condition (Table 2). All *E. coli* strains tested responded to the desiccation stress by synthesizing significantly more trehalose (Table 2; Fig. 5). Furthermore, correlation analyses showed that the amounts of trehalose synthesized and accumulated by the different *E. coli* strains corresponded to their respective desiccation resistance capacities. The four soil *E. coli* strains that showed higher desiccation resistance also contained significantly more intracellular trehalose than the reference strain, MG1655.

The clear importance of *de novo* trehalose synthesis in *E. coli* desiccation resistance is in contrast to and is highlighted by the ambiguous effects of exogenous GB uptake, as both trehalose and GB are important osmoprotectants. The different roles played by trehalose and GB were previously demonstrated in *E. coli* strain NCIB 9484, where osmotically induced intracellular trehalose, but not GB, increased cell desiccation resistance (45). The present study provided further evidence for this, as it evaluated the cellular responses of multiple *E. coli* strains under a desiccation condition. The differences between trehalose and GB in *E. coli* desiccation resistance are attributable to their different protection mecha-

nisms, with trehalose being superior to GB in alleviating severe water activity reduction (33). Desiccation often results in greater loss of water than salinity-induced osmotic stresses, as severe desiccation can result in the loss of the last monolayer of water on biomolecules (33). Trehalose was shown to protect cell membranes by lowering the phase transition temperature of the phospholipid membrane and thus help maintain the phospholipid bilayer in the liquid crystalline phase when drying (9), and it can also stabilize desiccated proteins via hydrogen bonding and water replacement, thus keeping dry proteins in their hydrated conformations (27). However, the contribution from GB to *E. coli* desiccation resistance cannot be completely ruled out, as the present study and previous ones all employed rather severe experimental desiccation conditions, and GB may still play a role under relatively mild desiccation conditions.

Both the die-off experiments and the cellular responses under desiccation stress showed that the soil *E. coli* strains were generally more resistant to desiccation stress. The selected soil strains were considered naturalized soil *E. coli* populations, based on their repeated detection in soil samples from the Manoa watershed (Table 1). Given the enormous genotypic diversity of *E. coli* (18), considerable variations in *E. coli* strain responses to any environmental condition are expected. Therefore, when the majority of soil *E. coli* strains exhibit the same trait (i.e., high desiccation resistance), it is reasonable to attribute such a trait to specific habitat adaptation and to consider those strains to be naturalized to the soil environments. This is a more logical explanation than the alternative possibility (i.e., these strains were of recent fecal origin but selected by soil because of their desiccation resistance) because of the isolation patterns of the soil *E. coli* strains (i.e., from multiple locations at multiple times) (Table 1).

The recognition of soils as secondary habitats of *E. coli* not only affects water quality monitoring but may also have public health implications. Traditionally, unlike other enteric bacteria, such as *Salmonella* spp. (46), *E. coli* is not believed to survive extensively once out of its primary habitats. Soils as secondary habitats of *E. coli* imply an *E. coli* life cycle that consists of passage through a host into the environment, adaptation and evolution in the environment, and then passage back into a new host. Such a life cycle would instigate further scientific inquiries about the adaptation and evolution of *E. coli* in natural environments, with particular attention on the emergence of pathogenic *E. coli* strains (43); for example, *E. coli* O157:H7 strains have been found to persist in soil for a long period of time (17, 28), and some Shiga toxin-producing *E. coli* (STEC) strains exhibit higher desiccation resistance than non-pathogenic *E. coli* strains (22), which would also favor their survival in the environment.

In summary, the present study showed that soil *E. coli* strains are resistant to the desiccation stress common to soil environments, exhibiting a trait common among soil-dwelling bacteria. By quantifying cellular responses to desiccation by multiple *E. coli* strains, *de novo* synthesis and accumulation of trehalose was identified to be a common mechanism for enhanced desiccation resistance, a mechanism that is also widely shared by typical soil-dwelling bacteria. Comparison between soil *E. coli* strains and reference strains showed that the former group is generally more desiccation resistant, providing further evidence of *E. coli*'s adaptation and naturalization to soil environments. The recognition of soil as a secondary habitat of *E. coli* will not only have significant implications in water quality monitoring but may also affect how we

approach the adaptation and evolution of *E. coli* in the environment, which is important, given the recent emergence of various pathogenic *E. coli* strains.

ACKNOWLEDGMENTS

We thank Bunnie Yoneyama for her technical support in the laboratory.

This project was supported by Agriculture and Food Research Initiative competitive grant no. 2009-35102-05212 from the USDA's National Institute of Food and Agriculture (to T.Y.).

REFERENCES

- Bainor A, Chang L, McQuade TJ, Webb B, Gestwicki JE. 2011. Bicinchoninic acid (BCA) assay in low volume. *Anal. Biochem.* 410:310–312.
- Boch J, Kempf B, Bremer E. 1994. Osmoregulation in *Bacillus subtilis*: synthesis of the osmoprotectant glycine betaine from exogenously provided choline. *J. Bacteriol.* 176:5364–5371.
- Boehm AB, et al. 2009. Faecal indicator bacteria enumeration in beach sand: a comparison study of extraction methods in medium to coarse sands. *J. Appl. Microbiol.* 107:1740–1750.
- Boncompagni E, Osteras M, Poggi M-C, Le Rudulier D. 1999. Occurrence of choline and glycine betaine uptake and metabolism in the family Rhizobiaceae and their roles in osmoprotection. *Appl. Environ. Microbiol.* 65:2072–2077.
- Byappanahalli MN, Fujioka RS. 1998. Evidence that tropical soil environment can support the growth of *Escherichia coli*. *Water Sci. Technol.* 38:171–174.
- Byappanahalli MN, Whitman RL, Shively DA, Sadowsky MJ, Ishii S. 2006. Population structure, persistence, and seasonality of autochthonous *Escherichia coli* in temperate, coastal forest soil from a Great Lakes watershed. *Environ. Microbiol.* 8:504–513.
- Cayley S, Lewis BA, Record MT. 1992. Origins of the osmoprotective properties of betaine and proline in *Escherichia coli* K-12. *J. Bacteriol.* 174:1586–1595.
- Chen J, Lee SM, Mao Y. 2004. Protective effect of exopolysaccharide colanic acid of *Escherichia coli* O157:H7 to osmotic and oxidative stress. *Int. J. Food Microbiol.* 93:281–286.
- Crowe JH, et al. 1988. Interactions of sugars with membranes. *Biochim. Biophys. Acta* 947:367–384.
- Cytryn EJ, et al. 2007. Transcriptional and physiological responses of *Bradyrhizobium japonicum* to desiccation-induced stress. *J. Bacteriol.* 189:6751–6762.
- Danese PN, Pratt LA, Kolter R. 2000. Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. *J. Bacteriol.* 182:3593–3596.
- Dinnbier U, Limpinsel E, Schmid R, Bakker EP. 1988. Transient accumulation of potassium glutamate and its replacement by trehalose during adaptation of growing cells of *Escherichia coli* K-12 to elevated sodium chloride concentrations. *Arch. Microbiol.* 150:348–357.
- Feng F, Goto D, Yan T. 2010. Effects of autochthonous microbial community on the die-off of fecal indicators in tropical beach sand. *FEMS Microbiol. Ecol.* 74:214–225.
- Foster RC. 1981. Polysaccharides in soil fabrics. *Science* 214:665–667.
- Freeman BC, Chen C, Beattie GA. 2010. Identification of the trehalose biosynthetic loci of *Pseudomonas syringae* and their contribution to fitness in the phyllosphere. *Environ. Microbiol.* 12:1486–1497.
- Fujioka RS, Tenno K, Kansako S. 1988. Naturally occurring faecal coliforms and faecal streptococci in Hawaii's freshwater streams. *Toxic. Assess.* 3:613–630.
- Gagliardi JV, Karns JS. 2002. Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. *Environ. Microbiol.* 4:89–96.
- Goto DK, Yan T. 2011. Effects of land uses on fecal indicator bacteria in the water and soil of a tropical watershed. *Microbes Environ.* 26:254–260.
- Goto DK, Yan T. 2011. Genotypic diversity of *Escherichia coli* in the water and soil of tropical watersheds in Hawaii. *Appl. Environ. Microbiol.* 77:3988–3997.
- Hardina CM, Fujioka RS. 1991. Soil: the environmental source of *Escherichia coli* and enterococci in Hawaii's streams. *Environ. Toxicol. Water Qual.* 6:185–195.
- Higo A. 2006. The role of a gene cluster for trehalose metabolism in dehydration tolerance of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. *Microbiology* 152:979–987.

22. Hiramatsu R, Matsumoto M, Sakae K, Miyazaki Y. 2005. Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. *Appl. Environ. Microbiol.* 71:6657–6663.
23. Ishii S, Ksoll WB, Hicks RE, Sadowsky MJ. 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* 72:612–621.
24. Kets E, Teunissen P, de Bont J. 1996. Effect of compatible solutes on survival of lactic acid bacteria subjected to drying. *Appl. Environ. Microbiol.* 62:259–261.
25. LeBlanc JC, Goncalves ER, Mohn WW. 2008. Global response to desiccation stress in the soil actinomycete *Rhodococcus jostii* RHA1. *Appl. Environ. Microbiol.* 74:2627–2636.
26. Le Rudulier D, Bouillard L. 1983. Glycine betaine, an osmotic effector in *Klebsiella pneumoniae* and other members of the *Enterobacteriaceae*. *Appl. Environ. Microbiol.* 46:152–159.
27. Leslie SB, Israeli E, Lighthart B, Crowe JH, Crowe LM. 1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Appl. Environ. Microbiol.* 61:3592–3597.
28. Ma J, et al. 2011. Persistence of *Escherichia coli* O157:H7 and its mutants in soils. *PLoS One* 6:e23191. doi:10.1371/journal.pone.0023191.
29. McIntyre HJ, et al. 2007. Trehalose biosynthesis in *Rhizobium leguminosarum* bv. trifolii and its role in desiccation tolerance. *Appl. Environ. Microbiol.* 73:3984–3992.
30. Miller EN, Ingram LO. 2008. Sucrose and overexpression of trehalose biosynthetic genes (otsBA) increase desiccation tolerance of recombinant *Escherichia coli*. *Biotechnol. Lett.* 30:503–508.
31. Mordhorst IL, et al. 2009. *O*-Acetyltransferase gene *neuO* is segregated according to phylogenetic background and contributes to environmental desiccation resistance in *Escherichia coli* K1. *Environ. Microbiol.* 11:3154–3165.
32. Perroud B, Le Rudulier D. 1985. Glycine betaine transport in *Escherichia coli*: osmotic modulation. *J. Bacteriol.* 161:393–401.
33. Potts M. 1994. Desiccation tolerance of prokaryotes. *Microbiol. Rev.* 58:755–805.
34. Purvis JE, Yomano LP, Ingram LO. 2005. Enhanced trehalose production improves growth of *Escherichia coli* under osmotic stress. *Appl. Environ. Microbiol.* 71:3761–3769.
35. Rauch EM, Bar-Yam Y. 2004. Theory predicts the uneven distribution of genetic diversity within species. *Nature* 431:449–452.
36. Roberson EB, Firestone MK. 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Appl. Environ. Microbiol.* 58:1284–1291.
37. Ryu JH, Beuchat LR. 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and curli production on its resistance to chlorine. *Appl. Environ. Microbiol.* 71:247–254.
38. Schnider-Keel U, Lejbolle KB, Baehler E, Haas D, Keel C. 2001. The sigma factor AlgU (AlgT) controls exopolysaccharide production and tolerance towards desiccation and osmotic stress in the biocontrol agent *Pseudomonas fluorescens* CHA0. *Appl. Environ. Microbiol.* 67:5683–5693.
39. Snipen L, Almøy T, Ussery DW. 2009. Microbial comparative pan-genomics using binomial mixture models. *BMC Genomics* 10:385. doi:10.1186/1471-2164-10-385.
40. Streeter JG. 2003. Effect of trehalose on survival of *Bradyrhizobium japonicum* during desiccation. *J. Appl. Microbiol.* 95:484–491.
41. Topp E, et al. 2003. Strain-dependent variability in growth and survival of *Escherichia coli* in agricultural soil. *FEMS Microbiol. Ecol.* 44:303–308.
42. Uhlich GA, Cooke PH, Solomon EB. 2006. Analyses of the red-dry-rough phenotype of an *Escherichia coli* O157:H7 strain and its role in biofilm formation and resistance to antibacterial agents. *Appl. Environ. Microbiol.* 72:2564–2572.
43. van Elsas JD, Semenov AV, Costa R, Trevors JT. 2011. Survival of *Escherichia coli* in the environment: fundamental and public health aspects. *ISME J.* 5:173–183.
44. Vriezen JAC, de Bruijn FJ, Nusslein K. 2007. Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. *Appl. Environ. Microbiol.* 73:3451–3459.
45. Welsh DT, Herbert RA. 1999. Osmotically induced intracellular trehalose, but not glycine betaine accumulation promotes desiccation tolerance in *Escherichia coli*. *FEMS Microbiol. Lett.* 174:57–63.
46. Winfield MD, Groisman EA. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl. Environ. Microbiol.* 69:3687–3694.