JOURNAL OF
EMERGING INVESTIGATORS

Article

# Lactic acid bacteria protect the growth of Solanum lycopersicum from Sodium dodecyl sulfate

**Lauren Lee[1], Jieun Shin[1]**
[1] Henry M. Gunn High School, Palo Alto, California

## SUMMARY

**Sodium dodecyl sulfate (SDS), used in various detergents, deters the growth of many plants when it remains in soil from cleaning. Plants are likely to be exposed to SDS because it could be washed down the drain and end up in the waterways. To protect plants from environmental hazards and to promote plant growth, it is critical to reduce the phytotoxicity of SDS. Lactic acid bacteria (LAB), a special type of probiotic, have been shown to protect plants from various chemical hazards and boost plant growth. In this study, we hypothesized that LAB could play a role in supporting plant growth against SDS-induced stress based on the multiple plant-proliferation capabilities of LAB. We measured tomato growth in Petri dishes and soil in terms of the germination rate, weight, and length of the sprouts. In Petri dishes, SDS significantly reduced the germination rate and growth of tomato sprouts at concentrations of 0.8 mM. Tomato seeds grown in 0.8 mM SDS solution resulted in a 46% and 48% reduction in sprout length and weight, respectively, compared to the non-treated group. Incubation with 5 million CFUs/mL LAB and 0.8 mM SDS reduced the length and weight of the tomato sprouts by just 6% and 20%, respectively, compared to the non-treated group. In soil culture, the addition of 10 million CFUs/mL LAB to 5 mM SDS solution increased the germination rate and plant growth compared to the SDS-only group. This finding suggests that LAB can help plants sustain in environments contaminated by detergents containing SDS.**

## INTRODUCTION

SDS is included in various detergents at home and in the industry. It is consistently used in domestic and industrial cleaning (1). Residual SDS from wastewater treatment may cause harm to plants and soil (2). Several researchers have revealed that SDS inhibits plant germination and growth (2-8). They also found that the total protein contents and the antioxidant enzyme activities measured in both wheat and barley significantly declined after exposure to SDS (3, 4). Additionally, SDS has been found to cause oxidative stress in tomatoes, resulting in reduced anti-oxidative enzyme activities (5). Tomatoes are a useful food resource (6, 7). Therefore, the adverse effects of SDS on tomato growth are worth further investigation. Experimentally, tomato plants can be easily grown in both aquaculture and soil culture. Given the nutritious value, affordability, and availability of tomatoes, we chose tomatoes as the focus plant for the present study.

Lactic acid bacteria (LAB), one probiotic strain, are known to reduce oxidative stress, boost nutrient metabolism, increase the germination rate, and protect against multiple diseases in many plants (8-10). Probiotic strains, such as LAB, all *Lactobacillus* species, all *Bifidobacterium* species, and *Saccharomyces boulardii*, are live microorganisms that can be naturally found in decomposing plants, fermented food, animals, the human body, and many other organisms (10-12). LAB is the most commonly used probiotic and is well-studied (8-12). Multiple studies have revealed the beneficial effects of LAB on plant growth. In one study, LAB promoted plant growth by improving nitrogen fixation and the uptake of important nutrients such as phosphorus and potassium (10). Some LAB strains are able to secrete phytohormones, such as gibberellins and auxins, which play integral roles in plant growth (11). Another study found that LAB detoxified heavy metals, pesticides, and mycotoxins and helped plants tolerate numerous biotic, such as microbial diseases, and abiotic stresses, such as contamination of chemical compounds (7, 13). Several experiments indicated that LAB reduced diseases of bacterial wilt by 61% in tomatoes (6-8). These findings suggest the potential for LAB to lessen the destructive stress of detergent in the agricultural industry, which in turn can help protect plant growth from abiotic challenges and improve plant yield. However, despite a systematic literature search, we found no study on the direct effects of LAB on SDS-induced harm on plants. To fill this research gap, we investigated whether LAB could offset the inhibition of plant growth caused by a surfactant SDS.

In this study, we hypothesized that LAB would protect tomato plant (*Solanum lycopersicum*) growth from SDS stress. In order to test the effect of LAB on plant growth, we examined the length, weight, and germination rates of tomato sprouts treated with both SDS and LAB compared to those treated with SDS-only grown in both Petri dishes and pots with soil. We observed that tomato growth in cultures with both SDS and LAB exceeded growth in cultures with SDS alone. This observation suggests that LAB may ameliorate SDS-stunted growth and can protect plants from environments contaminated by SDS-including detergents, confirming our hypothesis. Our study provided novel insight into the protective effect of LAB on the phytotoxicity of surfactant to plants.

## RESULTS

### Determination of LAB effect on tomato growth

First, we tested the effect of LAB alone on the length and weight of tomato sprouts in Petri dish culture to see if LAB alone increased tomato growth. We grew tomato sprouts for 14 days in Petri dishes under six conditions: CTL (the non-treated group), LB 1.25 (LAB 1.25 million CFUs/mL), LB

https://doi.org/10.59720/23-192

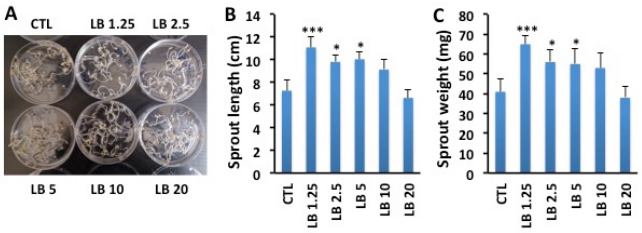


**Figure 1: Effect of LAB on tomato growth in Petri dish culture (n=3). A:** Tomato sprouts in Petri dishes: CTL: Control (non-treated), LB 1.25 (LAB 1.25 million CFUs/mL), LB 2.5 (LAB 2.5 million CFUs/mL), LB 5 (LAB 5 million CFUs/mL), LB 10 (LAB 10 million CFUs/mL), LB 20 (LAB 20 million CFUs/mL). **B, C:** Average length (B) and weight (C) of sprouts grown for two weeks in Petri dishes. Tomato seeds were grown under either control (non-treated) conditions or in an LAB solution of various concentrations for two weeks. Error bars represent standard deviation. *p<0.05,***p<0.001 compared to the control (non-treated), one-way ANOVA.

2.5 (LAB 2.5 million CFUs/mL), LB 5 (LAB 5 million CFUs/mL), LB 10 (LAB 10 million CFUs/mL), and LB 20 (LAB 20 million CFUs/mL) (**Figure 1**). 1.25, 2.5, 5, 10, and 20 million CFUs/mL LAB solution significantly increased the length and weight of tomato sprouts compared to the non-treated group. Sprouts grown in the 1.25, 2.5, 5, and 10 million CFUs/mL LAB solutions had average height increases of 53%, 35%, 38%, and 26%, respectively, compared to the non-treated group. Additionally, the weight of sprouts grown in the 1.25, 2.5, 5, and 10 million CFUs/mL LAB solutions for 14 days increased by 59%, 37%, 34%, and 29%, respectively, compared to the non-treated group. 1.25, 2.5, and 5 million CFUs/mL LAB displayed significantly increasing effects on the length and weight of the tomato plants, while the 10 and 20 million CFUs/mL LAB-treated plants did not have elevated growth (**Figure 1**).

## Determination of SDS effect on germination and tomato growth

To investigate the effects of various SDS concentrations on seed germination and sprout growth, we applied eight SDS concentrations (0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM) to Petri dishes. SDS significantly reduced the growth of tomato sprouts in a dose-dependent manner 14 days after placing the seeds (**Figure 2**). Ninety percent of the tomato seeds germinated in the non-treated group, and 92%, 86%, 77%, and 73% of tomato seeds germinated when treated with 0.025, 0.05, 0.1, and 0.2 mM SDS solution, respectively. These lower concentrations of SDS (0.025, 0.05, 0.1, and 0.2 mM) had no significant (p>0.05) effect on the germination rate. In comparison, 0.4 and 0.8 mM SDS significantly (p<0.001) decreased the germination rate by 25% and 78%, respectively, compared to the non-treated group. Additionally, when treated with 1.6 or 3.2 mM SDS, none of the tomato seeds germinated (**Figure 2** and **Table 1**).

In order to assess the effect of SDS on the growth of tomato seeds, we measured the length and weight of the sprouts grown with various concentrations of SDS in Petri dishes. Both the length and weight of sprouts treated with 0.8 mM SDS were significantly lower than those of the non-treated sprouts (p<0.001). The length and weight of the sprouts significantly decreased as SDS concentration increased. The seeds grown in the Petri dish with 0.8 mM SDS solution resulted in 46% and 48% reductions in the length and weight of sprouts, respectively, compared to the non-treated group (p<0.001) (**Table 1**).

## Determination of LAB effect on tomato growth with SDS

Most importantly, we investigated if LAB helps tomato growth from SDS-induced toxicity with co-application of SDS and LAB. First, 0.8 mM SDS significantly diminished the length (84%) and weight (76%) of tomato sprouts compared to the non-treated group (p<0.001) (**Figure 3B** and **3C**). However, the addition of 2.5 and 5 million CFUs/mL LAB solution reversed the SDS-induced stunted growth. Incubation of the tomato sprouts with 2.5 million CFUs/mL LAB+SDS 0.8 mM solution and 5 million CFUs/mL LAB+SDS 0.8 mM solution for two weeks increased the length of tomato sprouts by factors of 5.6 and 5.7, respectively, and the weight of sprouts by factors of 2.8 and 3.1, respectively, compared to the SDS-only group (**Figures 3B** and **3C**). The growth improvements of tomato sprouts enabled by 2.5 and 5 million CFUs/mL of LAB solutions were statistically significant (p<0.001, one-way ANOVA test) (**Figure 3**).

## Soil culture

Because the Petri dish culture excluded various factors residing in the soil, such as nitrogen, phosphorus, minerals, and soil bacteria, we further tested the effect of SDS and LAB on tomato growth in soil, which is ideal for tomato growth (16, 17). Different concentrations of SDS in the presence of soil may also modify the effects of SDS on tomato growth. Therefore, we performed a preliminary test with three SDS

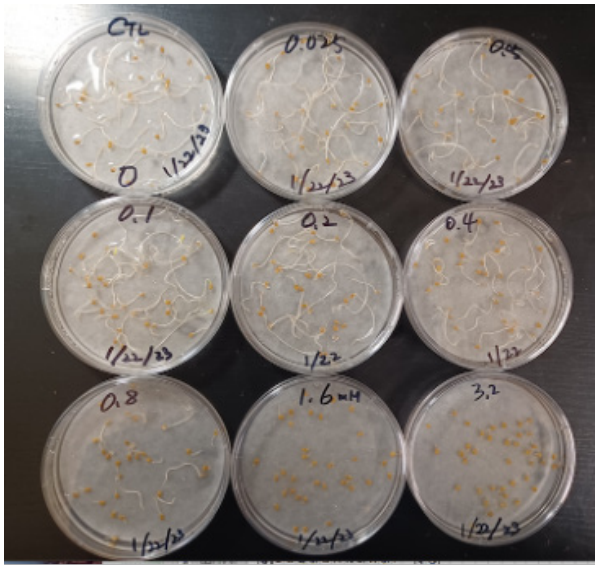


**Figure 2: Effect of different concentrations of SDS on tomato seed germination and sprout growth (n=3).** Tomato sprouts grown in Petri dishes for two weeks. Upper left: Control (ultrapure water), Upper middle: Sodium dodecyl sulfate (SDS) 0.025 mM, Upper right: 0.05 mM, Middle left: 0.1 mM, Middle middle: 0.2 mM, Middle right: 0.4 mM, Lower left: 0.8 mM, Lower middle: 1.6 mM, Lower right: 3.2 mM. The effects of 0.4 mM and 0.8 mM SDS are significant compared to the control (non-treated) tomato culture. Tomato seeds grown in SDS 1.6 mM and 3.2 mM solution did not show germination.

https://doi.org/10.59720/23-192

| SDS (mM) | Germination rate (%) | Length (cm) | Weight (mg) |
|---|---|---|---|
| 0 | 90.0±8.8 | 9.006±1.637 | 41.9±10.35 |
| 0.025 | 92.2±6.9 | 8.347±1.504 | 41.8±7.54 |
| 0.05 | 85.6±6.9 | 8.353±2.008 | 42.8±11.56 |
| 0.1 | 76.7±8.8 | 7.550±2.480 | 37.0±11.94 |
| 0.2 | 73.3±6.7 | 7.068±1.735 | 37.9±7.74 |
| 0.4 | 67.8±5.1** | 7.506±1.125 | 37.5±7.52 |
| 0.8 | 20.0±3.3*** | 4.880±3.361*** | 21.8±16.96*** |
| 1.6 | 0 | 0 | 0 |
| 3.2 | 0 | 0 | 0 |

**Table 1: Effect of different concentrations of SDS on tomato seed germination, sprout length, and weight (n=3).** The table shows the means + standard deviation of the length and weight of sprouts grown in Petri dishes for two weeks. p<0.001 and p<0.01 between control (0 mM SDS) and various concentrations of SDS are shown as *** and **, respectively. Tomato seeds grown in SDS 1.6 mM and 3.2 mM solution did not germinate. The one-way ANOVA test was used to analyze significance.

doses (1, 2, and 5 mM) to determine which concentration of SDS significantly decreased the germination of tomato seeds and their early growth in soil. Germination rates of 1, 2, and 5 mM of SDS application decrease the germination by 9%, 12%, and 42%, respectively, 14 days after planting the seeds (**Figure 4**). Based on the results, we selected 5 mM SDS for the soil culture. Then, we compared the tomato plant growth 14 days after planting the seeds in the soil under four different conditions: CTL (non-treated), LB (treated with 10 million CFU/mL LAB), SDS (treated with 5 mM SDS), and SDS+LB (treated with a combination of 10 million CFU/mL LAB and 5 mM SDS) (**Figure 5**). SDS delayed the germination rate by 41% compared to the non-treated group 14 days after planting seeds. The addition of 10 million CFU/mL LAB to the SDS-contaminated restored the germination rate to 76% from 27%, the germination rate of seeds grown in the SDS-only solution 14 days after planting seeds. The seeds grown in the LAB-only solution did not show a significant difference (p>0.05) in germination rate from those in the non-treated group (**Figure 5**). However, seeds treated with both LAB and SDS germinated significantly (p<0.001) faster and grew more than those in the SDS-only group (**Figures 5-6**). Seventeen days after the seeds were planted, the length of sprouts grown with 5 mM SDS solution was 52% shorter than that of the non-treated group, while the length of sprouts grown with 5 mM SDS+10 million CFUs/mL LAB solution was 15% shorter than that of the non-treated group (**Figure 6A** and **6C**). Most interestingly, the sprouts grown with 5 mM SDS+10 million CFUs/mL LAB solution increased in their length by 77% compared to the ones grown with 5 mM SDS-only solution. After 23 days, the length of sprouts grown with 5 mM SDS solution decreased by 36% compared to the non-treated group, while the length of sprouts grown with 5 mM SDS+10 million CFUs/mL LAB solution decreased by 8% compared to the non-treated group. This means SDS+LAB treatment increased the lengths of the plants by 44% when measured 23 days after planting the seeds compared to the plants grown with 5 mM SDS-only solution (**Figure 6B, 6C**). Tomato plants grown with SDS+LAB solution significantly increased their growth compared to those grown with SDS-only solution (p<0.01) 17 and 23 days after planting the seeds (**Figure 6**).

In the natural environment, the pH of the soil is a biogeochemical marker controlled by minerals and acids (14). Investigating the effect of SDS and LAB on soil pH can be critical to finding optimal conditions for natural tomato growth. Therefore, we measured soil pH in each group with a soil pH detector. The pH was measured 15, 24, and 35 days after planting seeds in each pot. The pH in the SDS-only group was 6.22, 6.33, and 5.87 after 15, 24, and 35 days, respectively (**Figure 7**). The SDS-only group was significantly (p<0.001) more acidic than the non-treated group (6.35). The pH in LAB-applied soil (6.20) 15, 24, and 35 days after planting seeds was also lower than the one in the non-treated group (6.35), but the difference was not significant (p>0.05). The SDS+LAB group was significantly (p<0.05) more basic compared to the SDS-only group 35 days after planting seeds but not 24 days after planting seeds (p>0.05). Thirty-five days after seeding, the SDS+LAB solution significantly increased the soil pH of the pots compared to the pots watered with only 5 mM SDS solution, from 5.87 to 6.12 (p<0.05) (**Figure 7**).

## DISCUSSION

To test whether LAB could rescue tomato plants from the stunted growth effects of SDS, we examined the length and weight of tomato sprouts and their germination rates when both SDS and LAB were present compared to SDS only in both Petri dishes and pots with soil. In the Petri dish culture, LAB (less than 20 million CFUs/mL) significantly (p<0.001) increased tomato growth compared to the non-treated group. However, the highest concentration of LAB (20 million CFUs/mL) led to the lowest tomato growth among various concentrations (1.25, 2.5, 5, 10, and 20 million CFUs/mL) of LAB compared to the non-treated group. This implies that the ability of LAB to improve tomato growth was sensitive to the concentration of LAB in the culture (15). Next, SDS significantly diminished the germination rate and growth of tomato sprouts in a dose-dependent manner. SDS caused phytotoxicity in the Petri dish culture. However, LAB promoted tomato growth despite the presence of SDS. SDS (0.8 mM) significantly (p<0.001) inhibited tomato germination compared to the non-treated group, but adding LAB to the SDS solution led to a significant (p<0.001) increase in germination rate

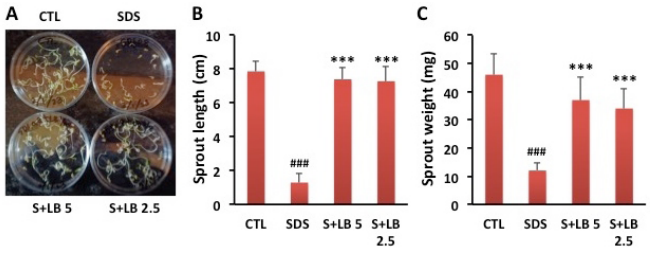


**Figure 3: Effect of LAB on SDS-treated tomatoes grown in Petri dish culture (n=3). A:** Tomato sprouts in Petri dishes: CTL (control, i.e., the non-treated group, ultrapure water only), SDS (0.8 mM SDS), S+LB 5 (0.8 mM SDS plus 5 million CFUs/ Lactic acid bacteria), S+LB 2.5 (0.8 mM SDS plus LB 2.5 million CFUs/mL). **B, C:** Means of the length (B) and weight (C) of sprouts grown in Petri dishes for two weeks. Tomato seeds were grown under either control conditions or in 0.8 mM SDS with various concentrations of LAB solution. Error bars represent standard deviation. ***p<0.001 compared to SDS 0.8, ###p<0.001 compared to CTL, one-way ANOVA.

https://doi.org/10.59720/23-192

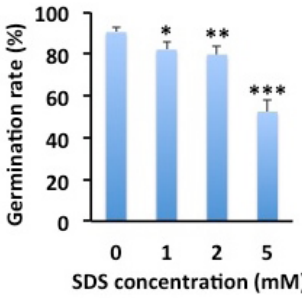


**Figure 4: Effect of SDS on tomato germination in soil culture (n=3).** Tomato seeds (n=28) were grown under four conditions for 21 days after being planted in each soiled pot using ultrapure water and SDS solution (in ultrapure water) of different concentrations. The graph shows the means of germination rate in %. Error bars represent standard deviation. *p<0.05, **p<0.01, and ***p<0.001 between S0 and S1, S0 and S2, and S0 and S5, respectively, one-way ANOVA.

of tomato seeds compared to the one grown with the SDS solution alone.

In soil culture, 5 mM SDS consistently lowered the germination rate, but the addition of LAB to the SDS solution significantly boosted seed germination and plant growth when compared to the SDS-only solution. The pH in soil was significantly lower in the SDS-only group, while the pH in LAB+SDS was comparable to the non-treated group. LAB primarily plays a role in producing lactic acid from carbohydrates, which can reduce pH and release short peptides and free amino acids (18). Consistently, the LAB-only group tended to decrease the pH compared to the non-treated group. However, the significant pH increase in the LAB+SDS group compared to the SDS-only group 35 days after planting seeds suggests that LAB could help the tomato plant sustain despite SDS-induced acidity during persistent exposure to the chemical (19, 20). The detailed mechanisms are unknown and should be investigated in future studies.

LAB could play a role in detoxifying SDS in the growing solution or reverse the reduction in antioxidant enzyme activity caused by SDS. As recent research shows, SDS attenuated the activity of antioxidant enzymes superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase, resulting in excessive $H_2O_2$ contents in wheat and barley (3, 4). In particular, SDS caused protein denaturation, DNA damage, and lipid peroxidation in barley seedlings (4). LAB can either release or activate anti-oxidative enzymes such as catalases and NADH peroxidase, superoxide dismutase, and thioredoxin reductase. Thus, LAB potentially protects the host organism from the toxic effects of reactive oxygen species (12, 18). Therefore, LAB may ameliorate the oxidative state of plants when exposed to harmful chemicals such as SDS (21). Another mechanism of LAB's growth-promoting effect on tomato plants in the presence of SDS could be that probiotics help plants increase nutrient uptake by breaking down the nutrients, thereby improving growth and health (22). *Bacillus* in probiotics can affect micronutrient availability by solubilization, chelation, and oxidation-reduction (23, 24). Furthermore, LAB provides stress tolerance against environmental harm, such as chemical contamination, through anti-oxidative mechanisms (25, 26). The possible implication of the protective effect of LAB against the SDS stress in this study is that LAB could help plants balance SDS-induced nutrient decomposition by producing amines and fatty acids (27). This is supported by another study, which found that high concentrations of SDS (720 mg/L, 2.5 mM) decreased protein content in wheat seedlings, whereas sugar and proline content increased (3). This suggests that LAB, when in SDS culture, can potentially balance the alteration of nutrient

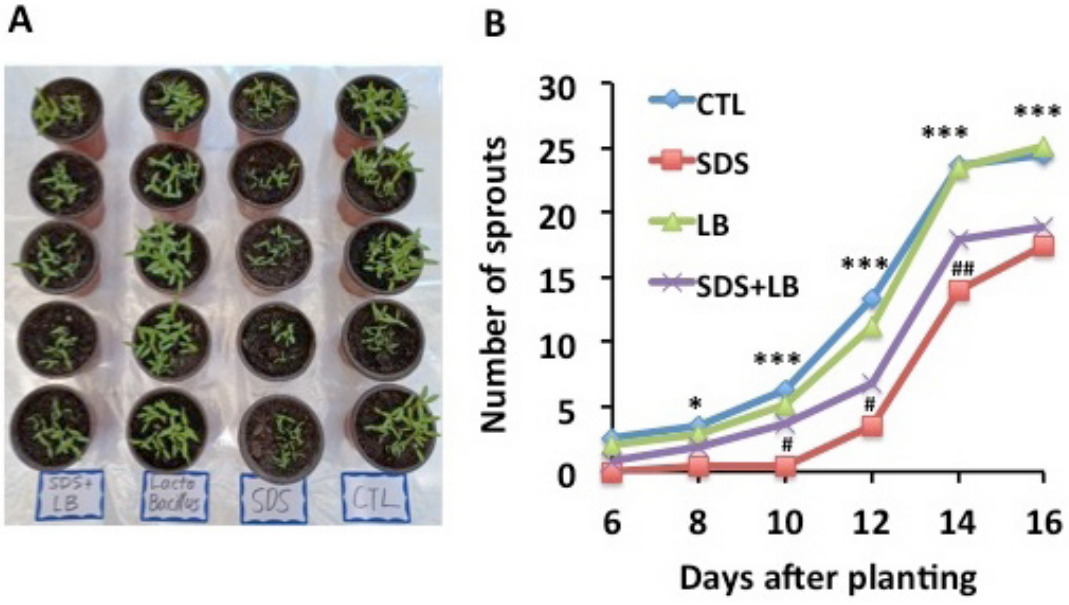


**Figure 5: Effect of LAB and SDS on tomato germination in soil culture (n=3). A:** Tomato sprouts 13 days after planting the seeds. From the left column to the right, SDS+LB: SDS 5 mM and LAB 10 million CFUs/mL LAB, LactoBacillus: 10 million CFUs/mL LAB only, SDS: SDS 5 mM only, CTL: Control (ultrapure water). **B:** Average number of sprouts germinated in 5 individual pots for 6, 8, 10, 12, 14, and 16 days after planting 28 seeds. Tomato seeds were grown under either control conditions or in various dilutions of LAB solution for two weeks. #p<0.05 and ##p<0.01 between SDS and LB+SDS. *p<0.5 and ***p<0.001 between CTL and SDS, one-way ANOVA.

https://doi.org/10.59720/23-192

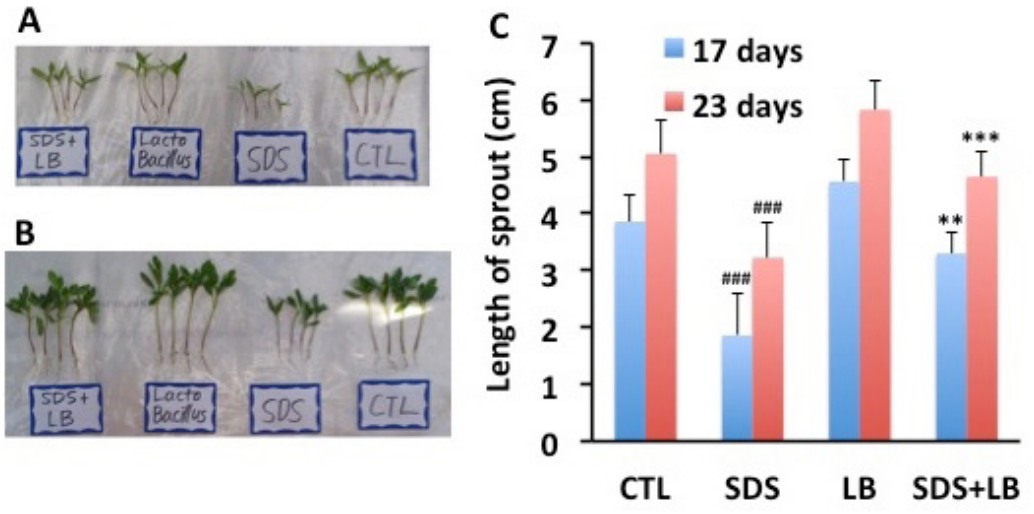


**Figure 6: Effect of LAB and SDS on tomato growth in soil culture (n=3). A:** Tomato sprouts in soil culture 17 days after planting the seeds. CTL: Control (ultrapure water), SDS: 5 mM SDS, LactoBacillus: 10 million CFUs/mL LAB only, SDS+LB: 5 mM SDS and 10 million CFUs/mL LAB only. **B:** Tomato sprouts in soil culture 23 days after the seeds were planted. **C:** Average length of the sprouts grown in pots after 17 and 23 days. Tomato seeds were grown under control conditions, SDS solution in the presence or absence of LAB solution, or only LAB solution for five weeks. Error bars represent standard deviation. ###p<0.001 compared to CTL. **p<0.01 and ***p<0.001 compared to SDS, one-way ANOVA.

composition by exerting protective mechanisms against the SDS challenge. Specifically, a prolidase (PepQ) found in both *Lactococci and Lactobacilli* is essential to the release of free proline (27). Accordingly, LAB provides the required enzyme to release proline, which can serve as an adaptive signaling molecule in expressing essential genes to counter SDS stress (28). In the present study, LAB addition to SDS-treated seeds resulted in more growth than those treated by only SDS, which could be an outcome of the specific proteolytic activity of LAB. Although an increasing number of detergents have replaced SDS with Sodium Laureth Sulfate (SLES), a derivative of SDS that is less harmful to skin and hair in humans, SDS still dominates large-scale detergents at a concentration of 17.4 mM (1). Due to spillage into sewer systems from domestic or industrial activities, surfactants may affect plant development when plants are irrigated with wastewater. Surfactants are also commonly present in pesticides and used as adjuvants in seed coatings of agricultural plants, such as lettuce (*Lactuca sativa L.*) and onion (*Allium cepa L.*) (16). Selecting protective microorganisms for SDS treatment is a big challenge. The current finding that LAB reduced the SDS toxicity in plant growth points to a protective effect of LAB on plant growth. Nonetheless, this research included several experimental limitations. First, when LAB solution was prepared in 500 mL of ultrapure water with one capsule of LAB (10 billion CFUs), each concentration of LAB solution, such as 1.25, 2.5, 5, 10 million CFUs/mL, may not have included the precise number of bacteria. This is because the LAB was not completely soluble in the water, and the insoluble portion easily precipitated in the bottom of the container. We could count the exact number of LAB through a microscope in each diluted solution in the Petri dishes. Second, whether all of the 12 strains of LAB that we used in the experiment were beneficial for tomato growth under SDS treatment is not clear. Future studies should test individual strains or other plant-based probiotics on the tomato seeds. Additionally, we could try to measure nutrient content such as sugar, protein, and proline and examine the function of essential anti-oxidative enzymes, including catalases, NADH peroxidase, superoxide dismutase, and glutathione reductase in the tomato sprouts grown in the presence of SDS and LAB. Notwithstanding the limitations of this study, our finding that LAB can remediate the detrimental effects of SDS on the growth of tomato plants and largely restore the germination rate and plant growth rate has important implications. It suggests that LAB may protect plants from SDS-stunted growth and can help plants sustain in environments contaminated by SDS-containing detergents.

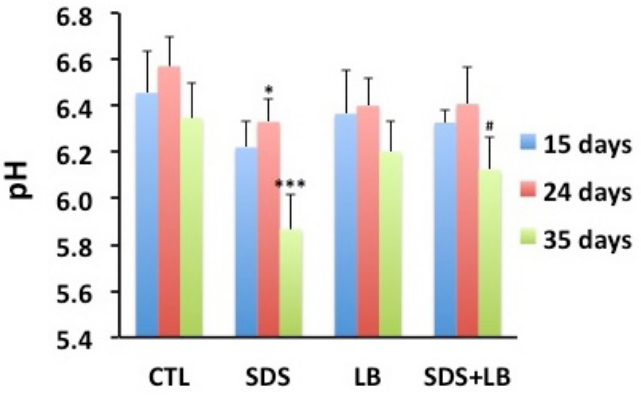


**Figure 7: Effect of LAB and SDS on pH in soil culture (n=3).** pH measured in the soil of pots, including tomato sprouts grown with each treatment (CTL, SDS, LB, and SDS+LB) 15, 24, and 35 days after planting the seeds. CTL: Control (non-treated, ultrapure water only), SDS: Sprouts watered with 5 mM SDS only, LB: 10 million CFUs/mL LAB only, SDS+LB: 5 mM SDS and LAB 10 million CFUs/mL LAB. Error bars represent standard deviation. ***p<0.001 between SDS and LB and between SDS and SDS+LB shown as ***compared to SDS, *p<0.05 and ***p<0.001 compared to CTL. #p<0 compared to SDS. The one-way ANOVA test was used to analyze significance.

https://doi.org/10.59720/23-192

## MATERIALS AND METHODS

### Plant materials and LAB materials

Seeds were isolated from ripe tomatoes (*Solanum lycopersicum*) and placed in sterile plastic Petri dishes. Before placing the tomato seeds in the Petri dish, we sterilized the seeds with ethanol, washed them with sterile distilled water twice, and thoroughly dried them for one day at 20±5°C, following the procedures of another study (29). All the plastic ware was sterile. LAB was purchased at a local pharmacy store. One capsule of LAB probiotics (TruNature) contained 10 billion CFUs of 12 different LAB strains (*Lactobacillus rhamnosus GG*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Bifidobacterium lactis*, *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*).

### Determination of LAB effect on tomato growth

We prepared the LAB solution in 500 mL of ultrapure water with one capsule of LAB probiotics (10 billion CFUs). We diluted the LAB solution to 1/2 (10 million CFUs/mL LAB), 1/4 (5 million CFUs/mL LAB), 1/8 (2.5 million CFUs/mL LAB), and 1/16 (1.25 million CFUs/mL LAB) of the original concentration using ultrapure water. We cultivated each group of 30 seeds in 30 mL of their respective solution in each Petri dish. We measured the length and weight of the 10 tallest sprouts from each Petri dish 14 days after placing the seeds. The length (in cm) from the tip of the sprouts to the end of the seeds was manually measured with a ruler, and the weight (in mg) was examined with a digital analytical scale of 0.001 g (UCLA Scientific).

### Seed germination assay

Sodium dodecyl sulfate (SDS) (C12H25SO4Na, BioUltra >99.0% (GC), molecular weight: 288.38 g/mol) was purchased as the 10% SDS solution from UFC Bio. We carried out the seed germination assays according to the USEPA guidelines (30). The seed germination assays were as follows: We made SDS solutions with concentrations of 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM for seed germination assay from 10% SDS solution. We cultivated each group of 30 seeds in 30 mL of their respective solution in each Petri dish at a concentration specified above or ultrapure water (the non-treated group). The sealed Petri dishes were placed in a dark incubator at 25±5°C with a humidity of 30-40%. When more than 90% of the seeds in the non-treated group had germinated and developed sprouts that were at least 20 mm long, the germination test was ended, the number of germinated seeds were counted, and the lengths (cm) and weights (mg) of the sprouts in each Petri dish were measured.

### Determination of LAB effect on tomato growth with SDS

We tested 30 tomato seeds in 30 mL of their respective solution in each Petri dish under four conditions for 14 days; CTL (the non-treated group, ultrapure water), SDS (0.8 mM SDS in ultrapure water), SDS+LB5 (5 million CFUs/mL LAB and 0.8 mM SDS in ultrapure water), and SDS+LB2.5 (2.5 million CFUs/mL LAB and 0.8 mM SDS in ultrapure water). The lengths (cm) and weights (mg) of the sprouts in each Petri dish were measured at the end of the cultivation.

### Soil Culture

For the experiment of cultivation in 500 grams of soil, we planted seeds from tomatoes in 20 plastic pots with a diameter of 10 cm, with five pots per experimental condition. Twenty-eight seeds were planted in each pot with a soil mix for potted vegetables (Miracle Grow Potting Mix). The plants were grown outside under the influence of solar lighting but without considerable natural watering, such as rain. Outside conditions were as follows: temperature 18-26°C, humidity 30-40%, 14 h under sunlight during the day, and 10 h in the dark at night. The conditions differed in the solution used for watering: ultrapure water for the non-treated group, 5 mM SDS for the SDS group, 10 million CFUs/mL LAB for the LAB group, and 10 million CFUs/mL LAB plus 5 mM SDS for SDS+LAB group. We watered the plants once per day with 60 mL of the appropriate solution (explained above) per pot for each group. Every two days, from the 6th to the 16th day, we counted the number of germinated. We measured the length (cm) from the tip of the tallest leaf to the end of the root of the five tallest sprouts from each pot 17 and 23 days after planting the seeds. The measurement was manually performed with a ruler. We examined the pH in each soil pot on the 15th, 24th, and 35th day using a soil pH meter (PCE-PH 18).

### Statistical analysis

We performed both the Petri dish culture and soil culture experiments three times under the same conditions for accuracy and reliability. The data represent the average of each measurement (n=3). The one-way ANOVA (Analysis of Variance) was used to analyze the significance of the differences between multiple groups. Post Hoc Tukey HSD (Honestly Significant Difference) test followed to facilitate pairwise comparison within our ANOVA data. Statistical significance was determined at the level of $p<0.05$. The statistical analysis was conducted in Socscistatistics.com.

## ACKNOWLEDGMENTS

We would like to thank Ms. Karen Saxena of Gunn High School who continued to support my research. Also, we truly appreciate Alan Lee, who provided countless suggestions when compiling and presenting results.

**Received:** August 08, 2023
**Accepted:** January 03, 2024
**Published:** November 15, 2024

## REFERENCES

1. Wolkoff, Pederet, *et al*. “Risk in cleaning: chemical and physical exposure.” *Science of the Total Environment.* vol. 215, no. 1–2, Apr. 1998, pp. 135–156. http://doi.org/10.1016/S0048-9697(98)00110-7.
2. Yilmaz, Fadime, and Bulent Icgen. “Characterization of SDS-degrading Delftia acidovorans and in situ monitoring of its temporal succession in SDS-contaminated surface waters.” *Environmental Science and Pollution Research*, vol. 21, Jun. 2014, pp. 7413-7424. https://doi.org/10.1007/s11356-014-2653-x.
3. Chang, Guohua, *et al*. “Effects of sodium dodecyl sulfate on wheat (*Triticum Aestivum* L.) seedlings.” *American Institute of Chemical Engineers Environment Progress*, vol. 34, no. 4, Feb. 2015, pp. 1142–1147. http://doi.org/10.1002/ep.12107.

https://doi.org/10.59720/23-192

4. Genisel, Mucip, *et al*. "Evaluation of physiological and biochemical aberration linked to effect of sodium dodecyl sulphate on barley seedlings." *SN Applied Sciences*, vol. 2, Mar. 2020, pp. 514. http://doi.org/10.1007/s42452-020-2289-z.
5. Haidri, Irfan, *et al*. "Enhancing the Antioxidant Enzyme Activities and Soil Microbial Biomass of tomato plants against the stress of Sodium Dodecyl Sulfate by the application of bamboo biochar." *Remittances Review*, vol. 9, no. 2, Apr. 2024, pp. 1609-1633. https://doi.org/10.33282/rr.vx9i2.90.
6. Hamed, Hoda A., *et al*. "In vivo efficacy of lactic acid bacteria in biological control against Fusarium oxysporum for protection of tomato plant." *Life Science Journal*, vol. 8. No. 4, Oct. 2011, pp. 462-468. https://doi.org/10.1016/j.scienta.2016.05.029
7. Markiewicz, Bartosz, *et al*. "Hydroponic cultivation of tomato." *Alternative crops and cropping systems,* edited by Petr Konvalina, IntechOpen, Apr. 2016, pp. 105-129. http://doi.org/10.5772/62263.
8. Konappa, Narasimha Murthy, *et al*. "Lactic acid bacteria mediated induction of defense enzymes to enhance the resistance in tomato against Ralstonia solanacearum causing bacterial wilt." *Scientia Horticulturae*, vol. 207, Oct. 2016, pp. 183-192. https://doi.org/10.1016/j.scienta.2016.05.029.
9. Raman, Jagadeesh, *et al*. "Application of Lactic Acid Bacteria (LAB) in Sustainable Agriculture: Advantages and Limitations." *International Journal of Molecular Science*. vol. 23, no. 14, Jul. 2022, p. 7784. http://doi.org/10.3390/ijms23147784.
10. Jaffar, Nur Sulastri, *et al*. "The potential of lactic acid bacteria in mediating the control of plant diseases and plant growth stimulation in crop production-A mini review." *Frontiers in Plant Science,* vol.13, Jan. 2023, p. 1047945. http://doi.org/10.3389/fpls.2022.1047945.
11. Lamont, John R., *et al*. "From yogurt to yield: Potential applications of lactic acid bacteria in plant production." *Soil Biology and Biochemistry,* vol. 111, Aug. 2017, pp. 1–9. http://doi:10.1016/j.soilbio.2017.03.015.
12. Konstantinos, Papadimitriou, *et al.* "Stress Physiology of Lactic Acid Bacteria." *Microbiology and Molecular Biology Reviews,* vol. 80, no. 3, Jul. 2016, pp. 837–890. http://doi.org/10.1128/mmbr.00076-15.
13. Yu, Annabelle O., *et al*. "Abundance, diversity and plant-specific adaptations of plant-associated lactic acid bacteria." *Environmental Microbiology Reports,* vol. 12. no. 1, Feb. 2020, pp.16-29. https://doi.org/10.1111/1758-2229.12794.
14. Neina, Dora. "The role of soil pH in plant nutrition and soil remediation." *Applied and Environmental Soil Science,* vol. 2019, Nov. 2019, pp. 1-9. http://doi.org/10.1155/2019/5794869.
15. Askarian Fatemeh, *et al*. "The effect of lactic acid bacteria administration on growth, digestive enzyme activity and gut microbiota in Persian sturgeon (*Acipenser persicus*) and beluga (*Huso huso*) fry." *Aquaculture Nutrition*, vol. 17, no. 5, Feb. 2011, pp. 488-497. http://doi.org/10.1111/j.1365-2095.2010.00826.x.
16. Gálvez, Antonia, *et al*. "Effect of different surfactants on germination and root elongation of two horticultural crops: implications for seed coating." *New Zealand Journal of Crop and Horticultural Science,* vol. 47, no. 2, Oct. 2019, pp. 83-98. http://doi.org/10.1080/01140671.2018.1538051.
17. Joseph, Shayne J, *et al.* "Laboratory cultivation of widespread and previously uncultured soil bacteria." *Applied and Environmental Microbiology*. Vol. 69, no. 12, Dec. 2003, pp. 7210-7215. http://doi.org/10.1128/AEM.69.12.7210-7215.2003.
18. Bryukhanov, AL., *et al*. "Antioxidant properties of lactic acid bacteria." *Microbiology,* vol. 91, no. 5, Sep. 2022, pp. 463-478. http://doi.org/10.1134/S0026261722601439.
19. Talwalkar, Akshat, *et al*. "Metabolic and biochemical responses of probiotic bacteria to oxygen." *Journal of Dairy Science,* vol. 86, no. 8, Aug. 2003, pp. 2537-2546. https://doi.org/10.3168/jds.S0022-0302(03)73848-X.
20. Talwalkar, Akshat, *et al*. "A review of oxygen toxicity in probiotic yogurts: influence on the survival of probiotic bacteria and protective techniques." *Comprehensive Reviews in Food Science and Food Safety*, vol. 3, no. 3, Nov. 2004, pp. 117-124. http://doi.org/10.1111/j.1541-4337.2004.tb00061.xh.
21. Konappa, Narasimha Murthy, *et al*. "Lactic acid bacteria mediated induction of defense enzymes to enhance the resistance in tomato against *Ralstonia solanacearum* causing bacterial wilt." *Scientia Horticulturae*, vol. 207, Aug. 2016, pp. 183-192. http://doi.org/10.1016/j.scienta.2016.05.029.
22. De Angelis Maria, *et al*. "Environmental stress responses in Lactobacillus: a review." *Proteomics,* vol. 4, no. 1, Jan. 2004, pp. 106–122. https://doi.org/10.1002/pmic.200300497.
23. Souza, Rocheli de, *et al*. "Plant growth-promoting bacteria as inoculants in agricultural soils." *Genetics and Molecular Biology,* vol. 38, no. 4, Dec. 2015, pp. 401-419. http://doi.org/10.1590/S1415-475738420150053.
24. Hassen, Ahmed Idris, *et al*. "Microbial inoculants as agents of growth promotion and abiotic stress tolerance in plants." *Microbial Inoculants in Sustainable Agricultural Productivity*, vol. 1, Feb. 2016, pp. 23-36. http://doi.org/10.1007/978-81-322-2647-5_2.
25. Abhilash, PC, *et al*. "Plant growth-promoting microorganisms for environmental sustainability." *Trends in Biotechnology,* vol. 34, no. 11, Nov. 2016, pp. 847-850. http://doi.org/10.1016/j.tibtech.2016.05.005.
26. Van Oosten, MJ., *et al*. "The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants." *Chemical and Biological Technologies in Agriculture,* vol. 4, no. 5, Jan. 2017, pp 1-12. http://doi.org/10.1186/s40538-017-0089-5.
27. Kunji, Edmund RS., *et al*. "Reconstruction of the proteolytic pathway for use of beta-casein by Lactococcus lactis." *Molecular Microbiology*, vol. 27, no. 6, Mar. 1998, pp. 1107-1118. http://doi: 10.1046/j.1365-295.
28. Szabados, László, *et al*. "Proline: a multifunctional amino acid." *Trends in Plant Science,* vol. 15, no. 2, Dec. 2010, pp. 89-97. http://doi.org/10.1016/j.tplants.2009.11.009.
29. Hajji-Hedfi, Lobna, *et al*. "Understanding the Influence of Applying Two Culture Filtrates to Control Gray Mold Disease (*Botrytis cinerea*) in Tomato." *Agronomy*, vol. 13, Jun. 2023, pp. 1774. http://doi.org/10.3390/agronomy13071774.
30. Environmental Protection Agency (1996). "Ecological

https://doi.org/10.59720/23-192

effects test guidelines (OPPTS 850.4200): Seed germination/root elongation toxicity test." EPA (712-C-96-154. Washington, DC. https://nepis.epa.gov/ Accessed 29 August 2024.