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ORIGINAL ARTICLE

Biochemical Changes and Induced Salinity Stress Conditions in Tomato (*Solanum Lycopersicum* L) Treated With Biosurfactant Produced By A Novel Marine Bacterial Isolate *Bacillus Tequilensis* KM15

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ABSTRACT
Salinity stress occurs when the concentration of salts, particularly sodium chloride (NaCl), in the soil or irrigation water, is high. This condition can negatively affect plant growth and development, including tomatoes. Excess salt levels interfere with water uptake by plant roots and disrupt the osmotic balance within the plant cells, leading to physiological and biochemical changes that affect plant growth and productivity. Biosurfactants, which are surface-active compounds produced by microorganisms, have gained attention for their potential to alleviate the negative effects of salinity stress on plants. They can improve plant growth under saline conditions by several mechanisms: To induce salinity stress conditions on tomatoes using biosurfactants, you would typically expose tomato plants to elevated salt levels in the growing medium or irrigation water. The biosurfactants can be applied to the soil or incorporated into the irrigation water to assess their effects on mitigating the salinity stress. The specific concentration and application method would depend on the type of biosurfactant being used and its compatibility with tomato plants.
***Keywords**: Biochemical; induced salinity; stress conditions; tomato; treated; Biosurfactant; novel marine*

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INTRODUCTION

Biosurfactants are microbial secondary metabolites with surface active properties that pose a wide range of applications in pharmaceutical, biotechnology industries, and agriculture. Due to their lower toxicity, more biodegradability, better compatibility with the environment, and capacity to be synthesized from renewable feedstocks, BS have an advantage over their synthetic counterparts. BS can be produced commercially in several forms for a variety of applications in food, pharma, cosmetics, and petroleum industries and agriculture since they are less hazardous and environmentally benign (1). BS produced by rhizobacteria is well studied as these biomolecules are known to enhance seed germination and also have antagonistic properties against phytopathogens. BS produced by rhizobacteria facilitates biocontrol mechanisms such as induced systemic resistance, competition, hypovirulence, and antibiosis (2). Several biocontrol compounds derived from microbes are thought to be promising for attaining sustainable agriculture since they have antibacterial activity against plant diseases. Members of the Bacillus genus have been shown to produce lipopeptides such as fengycin, surfactin, and iturin which inhibit a variety of fungi and protect crop plants (3).

Abiotic stressors reduce crop yield by 70% wo rldwide each year. Salinity stress among all abiotic stresses is the major cause of reduced productivity as it influences all the plant mechanisms from seed germination to fruit setting. When the salt concentration exceeds 4 dSm^{-1}, 25 electrical conductances (EC), the soil is categorized as saline. One billion hectares of land are damaged by salinization in the world. Under salt stress conditions, various plant systems such as protein biosynthesis, lipid metabolism, and photosynthesis are disrupted ultimately reducing seed germination and yield. A higher concentration of Na^+ inhibits cellular metabolism, and membrane functioning and also induces osmotic stress which causes water deficit conditions in cells by lowering water potential (4). Salt induces oxidative stress by the generation of ROS resulting in membrane deterioration, decreased antioxidant activities, cellular function blockage, and electrolyte leakage. The higher buildup of toxic ions exerts osmotic pressure at the cellular level, plants maintain cellular homeostasis by storing the toxic ions in vacuoles or by producing osmolytes including glycine betaine, sugars and proline to be protected from ionic disturbance. The accumulation of osmolytes is directly linked with salinity tolerance (5). Inorganic solutes such as Na^+, K^+, and Ca^{2+} ions uptake plays an important role in ionic homeostasis that is disturbed by higher levels of NaCl in plants. The buildup of NaCl causes osmotic stress in plants, resulting in ionic imbalance and cell death. Bacterial metabolites help the plants to combat various stressors including salinity stress. Biosurfactant is one such compound reported to lessen salinity effects s in plants and exogenous application is better than the use of synthetic fertilizers to improve the growth of plants. The Biosurfactant present in various plant tissues and salinization works as a signaling factor. Biosurfactant increases the resistance of the tomato plant to dehydration and salinity. Plants treated with Biosurfactant resist membrane malfunctioning which increases under high salts. The Biosurfactant promotes the synthesis of antioxidants and accelerates their activities by lowering ROS production and Na^+ and Cl^- concentration. The Biosurfactant also helps in enhancing photosynthesis, maintaining ionic balance, cellular stability, membrane protection, and redox homeostasis under abiotic stressors (6).

MATERIAL AND METHODS

Greenhouse experiment: Effect of salinity-induced toxicity in Tomato seeds

The tomato plant, known scientifically as *Solanum lycopersicum* L., is the subject of ongoing research. After steeping in distilled water for 5 days, tomato seeds were treated with 0.1% (w/v) hydrogen chloride for 10 minutes to sterilize their surface. To produce seedlings with consistent germination, a controlled environment greenhouse was utilized. The tomato plants were initially placed in 6-liter black plastic pots, which were then filled with a 1:1:1 mixture of peat moss, vermiculite, and perlite, before being enveloped in plastic and moved to a 6-by-40-meter greenhouse. Throughout the growing season, 315 kilograms of nitrogen, 225 kilograms of phosphorus, and 450 kilograms of potassium were applied to the tomato plants. Each container contains added essential nutrients. Approximately 120 mM of soil salt (NaCl) was maintained at a constant level. To prevent osmotic stress, aliquots containing 120 mM NaCl are utilized. The average temperature during the day was 26 degrees Celsius, while the average temperature at night was 19 degrees Celsius. The relative humidity ranged between 65 and 75%.

For the duration of their development, each container contained four separate plants. Following 11 days of initial growth, we began the saline treatment on the plants. Each container contains added essential nutrients. After 11 days of acclimatization to the environment, the three-leafed seedlings are separated into four batches designated T1, T2, T3, and T4.

Lot-1 (T1): Plants in soil treated with 120 mM NaCl and Bacillus tequilensis KM15 (foliar sprayed)

Lot-2 (T2): Plants in soil treated with 120 mM NaCl (Control)

Lot-3 (T3): Plants in soil treated with 120 mM NaCl and foliar sprayed with the extracted biosurfactant.

Lot-4 (T4): Plants in soil untreated with 120 mM NaCl

Each of the 12 distinct experimental conditions was replicated twice, and the entire experiment was organized using a randomized block design. After applying salt for three weeks, the foliage of the plants was sprayed with the Bacillus tequilensis KM15 and extracted Biosurfactant treatments, as well as the water-based control treatment. The concentration of the extracted biosurfactant was one hundred grams per liter, and the discharge volume was one milliliter of Bacillus tequilensis KM15 diluted in one liter of distilled water. Following the collection of the plants, their unprocessed weights were determined using a scale. After a week of exposure to the sun, they were desiccated in an oven heated to 65 degrees Celsius. Using a tape measure calibrated in inches, the length of each plant was determined. By observing and measuring each plant, its growth and physiological properties were determined. Eight weeks after Bacillus tequilensis KM15 and the isolated Biosurfactant for Salinity Induced Toxicity were applied, samples were collected.

Plant Pigments Content

The research conducted by (7) was used to determine the amounts of chlorophyll a and b, as well as carotenoids. The yield from chlorophyll extraction using acetone was approximately 80%. The absorbance was measured at wavelengths 640, 663, and 480 nm using a spectrophotometer. The optical density of the anthocyanin pigment at 535 nm was measured as part of the method described (8), which was used to determine the pigment's concentration.

Activities of Antioxidants (CAT, POD, SOD, and Proline)

The quantification of CAT and POD activity was conducted using the method devised (9). We utilized the method devised (10) to determine the level of SOD activity. Using the method specified (11), a spectrophotometer was utilized to determine the total free proline content of a sample of fresh leaves.

Oxidative Stress Markers

The quantity of malondialdehyde, also known as MDA, was measured with a spectrophotometer, and the amount of hydrogen peroxide, also known as H2O2, was calculated by combining the approaches of Heath and Packer (11).

RESULTS

Plant growth experiments of Salinity Induced Toxicity on Tomato.

Tomato seeds (of the variety Solanum lycopersicum Mill. HTM, which originated from unicorn seeds) were steeped in distilled water. After the treatment, the seeds were allowed to germinate for a total of five days. To produce seedlings with consistent germination, a controlled environment greenhouse was utilized. After an additional three weeks, the plants were eventually prepared for transplanting into the Fields. Approximately 120 mM of soil salt (NaCl) was maintained at a constant level. To prevent osmotic stress, aliquots containing 120 mM NaCl are utilized. Sprays containing Bacillus tequilensis KM15 extracted Biosurfactant, and a control consisting of water were utilized three weeks after the application of salt. The concentration of the extracted biosurfactant was one hundred grams per liter, and the discharge volume was one milliliter of Bacillus tequilensis KM15 diluted in one liter of distilled water. The soil used in this experiment was physically and chemically analyzed, and the results of both analyses were computed. Following the collection of the plants, their unprocessed weights were determined using a scale. After a week of exposure to the sun, they were desiccated in an oven heated to 65 degrees Celsius. Using a tape measure calibrated in inches, the length of each plant was determined. By observing and measuring each plant, its growth and physiological properties were determined. Three weeks after administering Bacillus tequilensis KM15 and the isolated biosurfactant for salinity-induced toxicity, we collected samples.

Table 1: Effect of *B. tequilensis* KM15 and its extracted biosurfactant on plant growth in induced salinity stress conditions

Treatments	Root length (cm)	Shoot length (cm)	Total weight (g)
T1	15.6±(0.08)a	20.6±(0.02)a	40.2±(0.11)a
T2	6.8±(0.02)d	9.6±(0.04)d	16.4±(0.02)d
T3	13.7±(0.04)b	17.8±(0.02)b	37.2±(0.13)b
T4	8.6±(0.04)c	11.2±(0.14)c	21.7±(0.14)c

Based on the results of Fischer's test for the least significant difference ($p<0.05$), the values are ranked from most significant (a-g) to least significant (a-g).

Plant Pigments Content

The research conducted (12) was used to determine the amounts of chlorophyll a and b, as well as carotenoids. The yield from chlorophyll extraction using acetone was approximately 80%. Utilizing a spectrophotometer, the absorbance at 640, 663, and 480 nm was determined (Hitachi-u, 2001). The optical density was measured using spectrophotometry at 535 nm, and the methodology devised (13) was applied to quantify the amount of anthocyanin pigment present. The results of an analysis of variance and mean square revealed that the growth and coloration of tomatoes in controlled versus salinized environments were substantially different.

Table 2: Effect of *B. tequilensis* KM15 and its extracted biosurfactant on plant pigments in induced salinity stress conditions

Treatments	Chlorophyll a (mg-1/ wt)	Chlorophyll b (mg-1/ wt)	Carotenoids (mg-1/ wt)
T1	6.74±(0.2)a	6.28±(0.2)a	4.44±(0.1)a
T2	3.33±(0.8)d	3.55±(0.1)d	1.31±(0.2)d
T3	6.00±(0.4)b	5.82±(0.2)b	4.29±(0.1)b
T4	5.30±(0.4)c	5.25±(0.4)c	1.65±(0.1)c

Based on the results of Fischer's test for the least significant difference ($p<0.05$), the values are ranked from most significant (a-g) to least significant (a-g).

Activities of Antioxidants (CAT, POD, SOD, and Proline)

Using the method specified (14) a spectrophotometer was utilized to determine the total free proline content of a sample of fresh leaves.

Table 3: Effect of *B. tequilensis* KM15 and its extracted biosurfactant on plant antioxidant defense systems in induced salinity stress conditions

Treatments	Catalase (Units mg-1/ protein)	Peroxidase (Units mg-1/ protein)	Superoxide dismutase (Units mg-1/ protein)	Proline (mg-1/ wt)
T1	0.37±(0.1)a	2.90±(0.2)a	3.52±(0.2)a	3.45±(0.1)a
T2	0.06±(0.2)d	1.12±(0.4)d	1.83±(0.1)d	1.16±(0.2)d
T3	0.29±(0.1)b	2.52±(0.1)b	3.09±(0.2)b	2.81±(0.2)b
T4	0.12±(0.1)c	1.76±(0.1)c	2.41±(0.1)c	1.76±(0.2)c

Based on the results of Fischer's test for the least significant difference ($p<0.05$), the values are ranked from most significant (a-g) to least significant (a-g).

Oxidative Stress Markers

The quantity of malondialdehyde, also known as MDA, was measured with a spectrophotometer, and the amount of hydrogen peroxide, also known as H2O2, was calculated by combining the approaches (15).

Table 4: Effect of *B. tequilensis* KM15 and its extracted biosurfactant on plant oxidant stress markers in induced salinity stress conditions

Treatments	H2O2 (µmol g-1/ wt)	Malondialdehyde (nmol g-1/ wt)
T1	3.03±(0.2)a	3.48±(0.1)a
T2	1.0±(0.1)d	1.54±(0.1)d
T3	2.24±(0.2)b	2.82±(0.2)b
T4	1.82±(0.1)c	2.16±(0.2)c

Based on the results of Fischer's test for the least significant difference ($p<0.05$), the values are ranked from most significant (a-g) to least significant (a-g).

DISCUSSION

Enhanced water availability: Biosurfactants can enhance water penetration and availability in the root zone by reducing surface tension and improving water infiltration into the soil. This can help alleviate the water stress caused by salinity. Ion exchange and leaching: Some biosurfactants can bind to salt ions and facilitate their movement through the soil, reducing their concentration around the plant roots (16). This helps to prevent excessive salt accumulation and supports better nutrient uptake. Cell membrane protection:

Biosurfactants can help stabilize the cell membranes of plant cells, which are often damaged by salt stress. By maintaining the integrity of cell membranes, biosurfactants can mitigate the harmful effects of salt stress on plant cells. Plant growth promotion: Certain biosurfactants have been reported to have direct growth-promoting effects on plants. They can enhance root development, nutrient uptake, and overall plant vigor, thereby enabling plants to tolerate salinity stress better (17).

In dry or semiarid regions, the quantity of salinity in the soil can pose a significant barrier to agricultural production. It has a devastating effect on the plant's capacity to develop and produce food as a result of the reduction in available water. In addition, it causes metabolic abnormalities, which in turn result in a decrease in meristematic activity and cellular elongation in plants, which contributes to a high respiration rate and growth suppression. As a result of a plant's exposure to salinity stress, reactive oxygen species (ROS) are produced (18). These reactive oxygen species consist of superoxide (O2•) and hydrogen peroxide (H2O2). ROS are responsible for oxidative stains in plants and the disruption of chlorophyll, DNA, proteins, and membranes in their normal functions, according to (19). In this study, the presence of salinity (120 mM NaCl) significantly inhibited tomato plant growth, leading to a lower yield. Increased concentrations of Na+ ions and oxidative stress in the form of H2O2 and malonaldehyde were shown to be responsible for the decreased growth and productivity of plants. All of these aspects of plant health, including photosynthetic efficiency, gas exchange, ion balance, disordered membranes, and hydration status, were adversely affected by these variables, which contributed to their demise (20). High concentrations of NaCl salt can result in osmotic pressure, metabolic dysfunction, decreased energy needs, and disruption of cell division. If given the chance, plants would either develop or acquire an antioxidant system to reduce the damage caused by reactive oxygen species (ROS) induced by salinity. This system comprises a large number of enzymatic and non-enzymatic antioxidants with low molecular weight. These antioxidants include proline, carotenoids, ascorbate, glutathione, and -tocopherol, among others. In this specific research endeavor, three distinct rhizobacteria were chosen to function as PGPRs (21). When these rhizobacteria were administered to wheat plants under salt stress, the results were significantly superior to those of untreated plants. The increased growth characteristics of wheat plants following soil treatment with PGPRs may be the result of the production of a variety of phytohormones in the plant rhizosphere and the effective regulation of endogenous levels of these phytohormones (22).

These phytohormones keep the body's cellular development and division mechanisms operating at peak performance so the body can function optimally. Another mechanism that is exploited by PGPRs to promote root cell growth and division is the production of indole-3-acetic acid, also known as IAA (23). According to the findings of this research, the correct growth and nutrient uptake by the roots of wheat plants, particularly the hairy roots, could be linked to any of the three bacterial strains that were tested and recognized as PGPRs. This was found to be particularly true for the hairy roots. Numerous microbial metabolites were synthesized, which improved the root structure and physiology of the plant by allowing it to take in more water and nutrients (24). This was accomplished by the plant being able to take in more water. These metabolites include things like auxins, gibberellins, and cytokinins, to name just a few examples. (25-27). The quick proliferation of wheat can be attributed to several factors, including the IAA and NH4 outputs of the strains that were studied as well as the amount of soluble phosphorus that they contained. However, these are only some instances of the many other things that could be the cause. Because of this, we were able to establish a connection between the features of halo-tolerant PGPRs that stimulate plant development and the improved agronomic parameters and wheat biomass. This demonstrates that not only do they protect against, but also actively boost growth characteristics while the plant is under the influence of salinity stress (28). When working together, plants and microorganisms have the potential to increase rates of re-vegetation and crop yields in agricultural soils (such as those that have salinity as a result of irrigation or those that are located in dry or semi-arid locations). In soils that have been severely degraded by salt (for example, coastal soil), a beneficial consortium between microorganisms and plants known as plant-microbe interaction can occur (29).

CONCLUSION

It is essential to observe that biosurfactants do not always reduce the detrimental effects of salinity stress on tomatoes. This is because the efficacy of biosurfactants depends on a variety of factors, including the type of biosurfactant used, its concentration, the genotype of the tomato plant, the environment in which it is cultivated, and the level of salinity stress. To gain an understanding of how biosurfactants affect the growth and yield of tomatoes when they are exposed to salinity, it would be necessary to undertake controlled experiments and monitor plant responses. In response to salinity stress, tomato plants slowed down several physiological processes, resulting in stunted plant growth. Increasing antioxidants, osmoregulation, ionic homeostasis, and photosynthetic pigments, the Biosurfactant application as foliar

spray under salinity was able to ameliorate these deficiencies. Under conditions of high salt concentration, biosurfactants' antagonistic effect on antioxidants such as H2O2 and MDA is yet another characteristic that distinguishes it from other similar substances. It was discovered that Biosurfactant had a positive effect regardless of the presence or absence of sodium. The results of these studies indicate that biosurfactant has the potential to be utilized successfully in agricultural contexts to protect crops from the damaging effects of salinity.

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