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Elucidating the Potential of Dye-Degrading *Enterobacter cloacae* ZA14 for Cultivation of *Solanum lycopersicum* Plants with Textile Effluents

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Abstract: The presence of textile effluents in water bodies is a matter of concern due to toxicity caused by textile dyes, associated heavy metals and salts. Endophytic bacteria have been reported to reduce the phytotoxicity of textile wastewater (TWW) and improve crop potential. The purpose of this study was to sequester dye-degrading endophytic bacteria with the ability to remediate textile effluents and promote tomato plant growth. Six strains showing the highest dye decolorization were screened from the dye decolorization assay. Selected strains also showed plant growth-promoting traits and improved tolerance to heavy metals and salt. The results revealed that *Enterobacter cloacae* ZA14 showed the highest decolorization (90%) using 200 mg L^{-1} of dye concentration, high minimum inhibitory concentration (MIC) of heavy metals and improved salt tolerance. In a sand culture experiment, the T4 (25% TWW (consisting of 25 mL TWW with 75 mL distilled water) + ZA14) treatment showed an increase in root length (9.3%), shoot length (5.5%), improved chlorophyll contents (7%), and membrane stability index (5%), whereas maximum oxidative stress was indicated by T10 (100% TWW) with an increase of 122% in MDA and 80% in H_2O_2 as compared to T1. An increase of 41% in ascorbate peroxidase (APX), 37% increase in sodium oxide dismutase (SOD), 27% in peroxidase (POD), and 24% in catalase (CAT) by T4 treatment showed the least production of antioxidants as compared to plants receiving 50%, 75% and 100% TWW along with ZA14 application. These results suggested that 25% TWW is beneficial for crop production with the use of an appropriate approach like *Enterobacter cloacae* ZA14 to mitigate textile effluents efficiently and to improve crop production.

Keywords: endophytic bacteria; dye decolorization; bioremediation; textile wastewater; plant growth

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1. Introduction

Synthetic textile dyes are one of the most dangerous pollutants [1], because they are hard to degrade in natural conditions. Release of textile effluents into bodies of water leads to eutrophication [2], pH shift, dissolution of gases like oxygen, along with other appalling

impressions. Heavy metals are related to such a group of metals that are not biodegradable, remain persistent in the environment, carcinogenic, mutagenic and genotoxic. The fatal effects of heavy metals in plants are related to the restricted elementary biological practices such as photosynthesis, mineral nutrition, water relation, as well as by mass production of radicals, lipid peroxides, superoxides, etc. [3].

Textile effluents consist of textile dyes, heavy metals, suspended and dissolved solids, and salts [4]. Effluents coming from textile units also mixed with urban wastewater are widely used by farmers to irrigate agricultural land. These effluents are beneficial when nutrients are present, which promote the growth of plants but also toxic when they contain dyes, heavy metals and salts beyond the optimal range. Due to the extensive application of a large range of dye products, the contamination generated by textile effluents is progressively distressing and has to be managed because of their effect on rising civic unease, and they also cause decreased efficiency of photosynthetic plants under water and hence disrupt the natural life in water [5]. The pollution of heavy metals is a major factor in the declined growth of a crop and is responsible for the growing food insecurity [6].

Dyes and heavy metals are reported to disrupt deoxyribonucleic acid and proteins [7] in plants at higher concentration levels, therefore causing disrupted biomolecules. Plants at cellular levels have the ability to reclaim heavy metals by generation of phytochelatins and metallothioneins [8]. These ligand–metal complexes can be eliminated from certain points or may be sequestered by vacuole in plants [7]. In addition to these mechanisms, plants also have the ability to activate a natural defense system by triggering the production of antioxidants [9].

Various physical approaches like membrane filtration, ultra-filtration, reverse osmosis and chemical approaches like solvent extraction, neutralization, ion exchange and a biological approach are reported for mitigation of textile pollutants. However, among existing approaches for remediation of textile pollutants, the use of bacteria has been proved the most cost-effective approach to remediate harmful effects of textile effluents. Notwithstanding the fact that conventional and chemical agents act fast on pollutants, these are also associated with the cons. These include very high cost and a large production of sludge, as these methods remove the pollutants but they cannot degrade the pollutant. As an alternate strategy, the microbial approach is becoming popular in this era due to its better potential to remove color, it is cost effective and produces degraded products, which become less toxic than the earlier form. It is true to say the potential of microbes to remediate textile effluents has gained attention recently [10].

Endophytic bacteria are those bacteria that reside in plants and are also associated with plant growth promotion [11]. Since there is a lot of usage of textile industrial wastewater for irrigation purposes, the use of dye-degrading amendment along with plant growth promotion is the best strategy to adopt. The dye-degrading endophytic bacteria are capable of growing inside the plant tissues and remediate the contaminants very efficiently [12]. Endophytic bacteria have the ability to adsorb heavy metals on them in the process of biosorption. Endophytic bacteria exhibited improved development and growth in plants by plant microbe association by provision of nutrients like phosphorus, iron, and zinc, nitrogen fixation and production of growth hormones [13]. It is reported that bacterial strains produce antioxidants under stress to promote growth in plants by scavenging reactive oxygen species [14].

Tomato (*Solanum lycopersicum* L.) is a widely used vegetable, having abundant antioxidants and vitamins. Tomato is grown in peri-urban areas of Pakistan and is irrigated with untreated wastewater containing contaminants from industries, which, hence, undermines the safety of public health [15]. It has recently been reported that endophytic bacteria can mitigate stress caused by dyes, heavy metals, and salts of textile effluents. We hypothesized that dye-degrading endophytic bacteria isolated from contaminated sites have the potential to mitigate textile effluents by reducing phytotoxicity for growth promotion in plants. Therefore, we designed a study to isolate indigenous dye-decolorizing endophytic bacteria to mitigate textile effluents like dyes, heavy metals and salts and improve the growth of

plants irrigated with water containing textile effluents, hence ensuring the sustainable use of textile wastewater.

2. Materials and Methods

Two samples from different sources (water and plants) were taken from dye contaminated areas for the isolation of dye-decolorizing endophytic bacteria. In order to obtain endophytic bacteria from the plant sample, the leaves of the orchid tree (*Bauhinia variegata* L.) growing along the drain receiving textile wastewater in Faisalabad were collected. An extract was obtained by grinding the surface-disinfected leaf of *Bauhinia variegata*, and from this extract, dye decolorizing endophytic bacteria were isolated. The isolation of bacteria from the textile wastewater sample was performed by using mineral salt medium (MSM), and for the isolation of bacteria from the plant sample, Luria-Bertani agar media (LB) was used. Both media were spiked with a 200 mg L^{-1} concentration of textile dye, direct blue-71, to isolate bacteria by the dilution plate method followed by the streak method on petri plates. Isolation of bacterial strains was conducted by following the method of Prasad and Rao [16]. Plates with a bacterial strain were incubated for 24 h. The bacterial strains showed growth over media containing textile dye, were capable of degrading dye, and therefore selected for further analysis.

2.1. Dye Decolorization Assay

The dye degradation potential of isolated strains was investigated by a dye decolorization assay. The flasks containing pure isolates in sterilized growth media were placed in the incubator (28 °C) to obtain inoculum of each bacterial strain. The bacterial strains were grown till an optical density (OD) of 0.8 was attained at a wavelength of 600 nm. A solution containing 200 mg L^{-1} direct blue-71 dye was sterilized.

The study of dye decolorization was performed by using 10 mL glass tubes, and bacterial inoculum was applied into the colored medium at a 10% inoculum rate of growth media containing dye. The glass tubes were kept in an incubator at 28 °C for three days under static conditions, whereas the sterilized dye solution without adding bacterial inoculum was also kept under the same conditions as a control. All treatments had three replications. A 2 mL aliquot from each glass tube was taken after 72 h. To obtain supernatant, the sample was rotated by centrifugation at 10,000× *g* for 12 min and analyzed with a spectrophotometer at the wavelength of direct blue 71 dye (587 nm) against un-inoculated (control) medium.

$$\%\text{decolorization} = (\text{A initial} - \text{A final})/\text{A initial} \times 100$$

Here, A initial is the decolorization of un-inoculated media containing dye, and A final is the decolorization of inoculated media (containing dye) after three days. The bacterial isolates displaying prominent decolorization potential were selected for further studies [17].

2.2. Growth and Biochemical Characterization of Selected Strains

Bacterial growth and biochemical characterization tests were performed on six efficient dye degrading bacterial strains (W7, W9, W11, ZA10, ZA12 and ZA14) with three replicates. Isolates were characterized for Gram reaction. This test was conducted by using freshly prepared bacterial isolates. The fully developed colony of a selected strain was collected with the sterilized loop (about 3 mm) and placed on a sterilized glass slide. A few drops of 3% KOH (aqueous) were added on the glass slide and mixed with the KOH solution with the bacterial strain. When the loop was raised from the mixture on the glass slide, a string formation occurs, or the formation of visible gel suspension takes place. The reaction is KOH positive, and the isolate would be Gram-negative and vice versa [18].

The growth medium was spiked with 1% carboxy methyl cellulose (CMC), and the bacterial inoculum was added to the solid agar plates. In control, no inoculum was added. The solid agar plates were kept in an incubator at a temperature of 28 °C for one to two weeks. After 7–14 days, the growth of bacteria indicated the utilization of cellulose [19].

Confirmation of the cellulose-degrading potential of bacteria was also recorded. The formation of gas bubbles indicates catalase enzyme activity by the bacterial strains, and thus the test is positive [20].

The fixation of atmospheric nitrogen can be assessed by culturing bacteria on nitrogen-free media [21]. Various levels of sodium chloride were supplemented to the starch casein agar medium for the determination of sodium tolerance (0, 10, 20 and 30% w/v). The clear bacterial growth indicates the test as positive [19]. The solubilization of the inorganic phosphate was investigated. The developed zones that indicated P solubilization were measured to compute the phosphorous solubilization index (PSI) [22] and phosphorous solubilization efficiency (PSE).

An investigation of endophytic bacteria for their zinc solubilization ability was performed by using the media recommended by Tadashi [23]. The slants were inoculated with bacteria and kept in the incubator for one week at 37 °C [24]. After 7 days, the results of the samples were noted. The endophytic bacteria were characterized for their ability to release hydrogen cyanide (HCN) and were evaluated by the method of Lorck [25]. The bacterial cultures (OD adjusted to 0.1) were spot-inoculated on sterile plates containing starch agar media. After 48 h, the plates are flooded with iodine solution.

A colorless zone surrounding the bacterial colonies indicated production of amylase and starch hydrolysis [26]. The cultures (OD adjusted to 0.1) were spot-inoculated on sterile skim milk agar plates. The bacteria with the ability to degrade protease enzyme showed a cleared zone around the bacterial colony. Each strain with three replications was tested for the production of Indole-3-acetic acid (IAA) [27] and ammonia in peptone water by the method devised by Cappuccino and Sherman [28].

The selected strains with the highest dye decolorizing ability were used in this study to assess their resistance against heavy metals (Pb, Cd, and Cr) by an agar plate dilution procedure [29,30]. The growth media containing agar was spiked with lead (Pb), chromium (Cr) and cadmium (Cd) at concentrations of 100, 200 and 300 mg L^{-1}. The metal level that visibly inhibited the growth of bacterial strains was designated as the minimal inhibitory concentration (MIC).

2.3. Sand Culture Experiment on Tomato Plants

Samples of textile wastewater were collected from local production units of the dying industry, from various locations in Faisalabad, and mixed thoroughly. The sample of textile effluents was stored at −4 °C for further use [25]. A sand culture experiment was conducted to evaluate the phytotoxic effects of various concentrations of textile wastewater (TWW) with a remedial role of endophytic bacteria ZA14 for mitigation of oxidative stress of TWW and to enhance growth, physiology and biochemical responses in tomato plants.

The seeds of the Sahel variety of tomato plant were used in this experiment. For seed priming, the seeds were inoculated with *Enterobacter* sp. ZA14 for two hours. Treatments used in the experiment are presented as: T1 = control (distilled water (DW)); T2 = ZA14 (endophytic bacteria); T3 = 25% TWW; T4 = ZA14 + 25% TWW; T5 = 50% TWW; T6 = ZA14 + 50% TWW; T7 = 75% TWW; T8 = ZA14 + 75% TWW; T9 = 100% TWW; T4 = ZA14 + 100% TWW.

Each treatment has three replicates. In this experiment, 5 healthy seeds of tomato were sowed in 200 g plastic glass containing sterilized sand. Hoagland nutrient solution was applied to maintain the nutrient status in the sand. Application of DW and TWW was maintained as per the need of the plant. Thinning of seedlings was performed after the development of two leaves in each plant. After 45 days, the plants were reaped, and data were recorded as follows.

2.3.1. Estimation of Plant Growth and Physiological Parameters

The impact of TWW and ZA14 bacteria was observed on the length and biomass of the tomato plant after the harvesting. Chlorophyll SPAD value was observed by the portable

chlorophyll meter. Relative water content (RWC%) was also measured by the following method [31].

$$\text{RWC}(\%) = \frac{\text{Fresh weight of leaf} - \text{Dry weight of leaf}}{\text{Turgid weight of leaf} - \text{Dry weight of leaf}} \times 100$$

To assess the membrane stability index (M.S.I) of the plants, a leaf disc from each treatment was taken, and then first electrical conductivity (EC1) and second electrical conductivity (EC2) was recorded. The EC1 designated as C1 was obtained with the heating disc of the leaf at 40 °C for about half an hour, and then its EC was recorded once the sample was cooled. EC2, designated as C2, was obtained by reheating the sample again in a water bath for ten minutes at 100 °C and observing the EC. The value of EC1 and EC2 was used to calculate M.S.I, as given:

$$\text{M.S.I} = 1 - \frac{\text{C1}}{\text{C2}} \times 100$$

2.3.2. Oxidative Stress Markers

The concentration levels of lipid peroxidation was recorded with some minor changes by the method suggested by Yagi [32]. The absorbance was read at 532 nm. A 0.5 g homogenized leaf sample was combined with 0.1% trichloroacetic acid (TCA). After centrifuge, potassium phosphate buffer and potassium iodide solution were mixed with 1mL supernatant [33], then measured against a 390 nm wavelength.

2.3.3. Osmoprotectants

The methodology of Bates et al. [34] was used to determine proline contents by the following equation:

$$\mu\text{mole proline/g FW} = (\mu\text{g}\frac{\text{proline}}{\text{ml}} \times \text{ml of toluene}/115.5)/\text{g of sample}$$

A 0.5 g plant tissue was homogenized, and the sample solution was prepared by mixing filtrate and 2N HCl. From this solution, we took 0.5 mL sample mixture and mixed it with potassium tri-iodide solution [35]. Then distilled water (chilled) and 1,2-dichloromethane were mixed in it, and absorbance at 365 nm of organic layer (lower) was noted.

2.3.4. Secondary Metabolites

A 0.1 g plant (dried) sample was homogenized with 3 mL absolute alcohol. The sample solution was filtered, and in 1 mL of filtrate was added 3 mL of 5% $NaNO_2$, 3 mL of $AlCl_3$ and 4 mL distilled water; the reaction was allowed to occur. After, the addition of sodium hydroxide with the distilled water sample was allowed to stand for 15 min and absorbance at 510 nm was recorded [36].

A 0.25 g dried plant sample was homogenized with 60% ethanol and was heated for 15 min at 65 °C in a water bath. The sample was extracted, and 5 mL final volume was adjusted with 60% ethanol. A 1 mL sample extract was mixed in a glass tube of 5 mL Folin–Ciocalteu (FC) reagent and 4 mL Na_2CO_3. The sample was incubated for 150 min, and later on, absorbance at 765 nm [37] was found. Total anthocyanins were determined by the protocol designed by Mancinelli [38].

2.3.5. Antioxidant Production

The catalase and peroxidase enzymatic activities were recorded by the method devised by Chance and Maehly [39]. The activity of sodium oxide dismutase (SOD) was found by following the protocol by Giannopolitis and Ries [40]. The APX activity was determined by the method of Asada and Takahashi [41].

2.4. Statistical Analysis

The analysis of the data for the decolorization assay was conducted using the computer software R studio (64-bit: R-4.0.4 version) by completely randomized design (CRD). ANOVA and comparison of mean values by least significant difference (LSD) tests were performed by agricolae (installed package) for dye decolorization assay of all isolated strains. The analysis of data was conducted using computer software R studio (64-bit: R-4.0.4 version) by factorial completely randomized design (FCRD) in sand culture experiment. ANOVA and comparison of mean values by least significant difference (LSD) test were performed by doe-bioresearch (package) [42].

3. Results

3.1. Dye Decolorization Assay for Isolation of Bacterial Endophytes

On solid agar medium spiked with direct blue-71 dye, twenty-seven morphologically different colonies with a prominent clearing zone around them were chosen. The isolates from the wastewater and plant samples were designated by codes (W1 to W11, and ZA1 to ZA16), respectively, on their corresponding plates. Isolates were purified five times with the streaking method on solid agar media by choosing colonies of isolates from the designated plate. Purified isolates were preserved in 40% glycerol for further use at −80 °C.

Among all isolated strains, a total of 27 purified strains were selected for the decolorization assay. The decolorization assay of bacterial strains isolated from textile wastewater revealed the best performing strains, W7, W9 and W11, with 79%, 75% and 72% decolorization, respectively (Figure 1A). Similarly, it was found that bacterial strains isolated from the plant sample with the highest decolorization potential were strains ZA10, ZA12 and ZA14, with 82%, 86% and 90% decolorization, respectively (Figure 1B). Among all the isolated strains, six strains (W7, W9, W11, ZA10, ZA12 and ZA14) with the highest decolorization potential were selected for further characterization and analysis of growth promotion in plants.

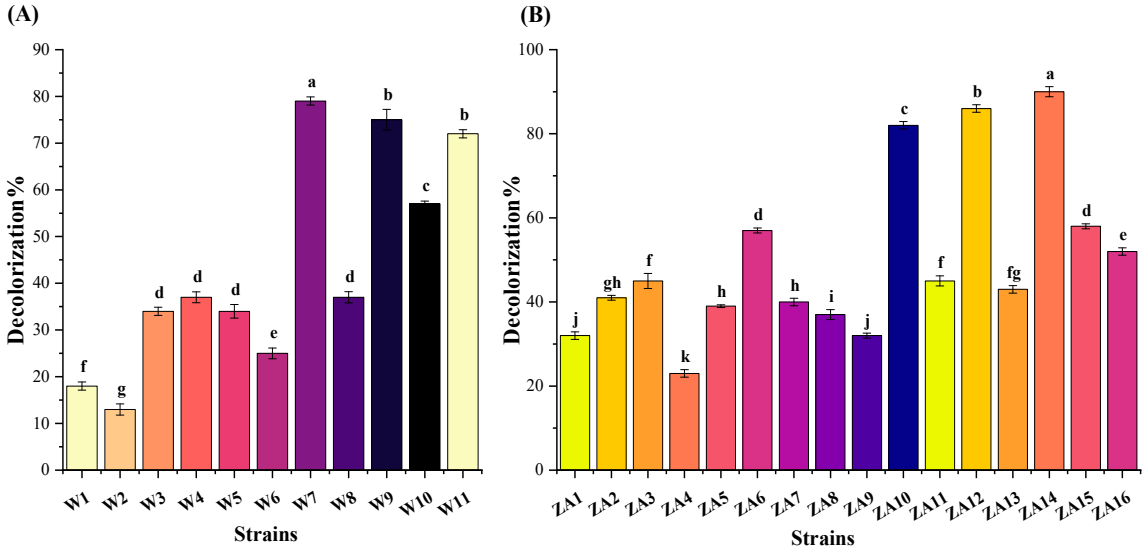

Figure 1. Potential of various endophytic strains to decolorize direct blue (200 mg L^{-1}) azo dye isolated from (**A**) textile wastewater sample and (**B**) plant sample. Data showed means of three replicates at $p < 0.05$.

3.2. Growth and Biochemical Characterization of Bacterial Strains

A significant number of growth and biochemical tests were performed, and the results of six selected bacterial strains are summarized in Table 1. All strains showed positive results in tests for cellulase, catalase, N-fixation, NaCl tolerance, P solubilization and IAA production, while all strains showed negative results in Gram staining, Zn solubilization,

H_2S production and cellulose test. Only the ZA12 strain showed HCN production. Ammonia production was observed in ZA14, while all other strains showed no signs of ammonia production. All the strains were detected as Gram-negative bacteria.

Table 1. Characterization of screened isolated bacterial strains.

Sr. No.	Characteristics	W7	W9	W11	ZA10	ZA12	ZA14
1.	Gram Reaction	−	−	−	−	−	−
2.	Cellulase	+	+	+	+	+	+
3.	Catalase	+	+	+	+	+	+
4.	N-Fixation	+	+	+	+	+	+
5.	NaCl Tolerance	+	+	+	+	+	+
6.	P-Solubilization	+	+	+	+	+	+
7.	Zn-Solubilization	−	−	−	−	−	−
8.	H_2S Production	−	−	−	−	−	−
9.	HCN Production	−	−	−	−	+	−
10.	Amylase	−	+	−	+	+	−
11.	Protease	−	−	−	−	−	−
12.	Ammonia Production	−	+	+	−	−	+
13.	Cellulose	−	−	−	−	−	−
14.	IAA Production	+	+	+	+	+	+

Note: Positive response = +, negative response = −.

All strains showed IAA production. Bacterial strains showed growth on nitrogen fixation media. H_2S production was not detected in any of the selected strains. Cellulase and catalase activity were observed in W11, ZA12 and ZA14. Amylase activity was only detected in ZA12. HCN production was only observed in ZA12 strain. Phosphorus solubilization was observed in all bacterial strains. However, the highest solubilization index was detected in W11 and ZA14 with values of 2.7 and 4.5, respectively (Table 2), while maximum solubilization efficiency was noticed in W11 and ZA14 with values of 178 and 350, respectively. All strains showed IAA production. Bacterial strains showed growth on nitrogen fixation media. H_2S production was not detected in any of the selected strains. Cellulase and catalase activity were observed in W11, ZA12 and ZA14. Amylase activity was only detected in ZA12. HCN production was only observed in ZA12 strain. Phosphorus solubilization was observed in all bacterial strains. However, the highest solubilization index was detected in W11 and ZA14 with values of 2.7 and 4.5, respectively (Table 2), while maximum solubilization efficiency was noticed in W11 and ZA14 with values of 178 and 350, respectively.

Table 2. P Solubilization by selected dye decolorizing strains.

Strains	Colony Diameter (cm)	Halozone Diameter (cm)	Solubilization Index	Solubilization Efficiency
W7	0.8	1	2.25	125
W9	0.7	1	2.43	143
W11	0.9	1.6	2.78	178
ZA10	0.9	1.5	2.67	167
ZA12	1	1.4	2.4	140
ZA14	0.6	2.1	4.5	350

NaCl tolerance at various levels was also detected in all strains (Table 3). The maximum bacterial growth was observed in ZA14 at 10% NaCl as compared to others, and growth was also noticed in ZA14 at 30% NaCl. The endophytic bacteria showed improved NaCl tolerance, even at higher concentrations (30%).

Table 3. NaCl tolerance by selected strains.

Strains	SL1	SL2	SL3	SL4
W7	+++	++	−	−
W9	+++	++	+	−
W11	+++	++	−	−
ZA10	+++	++	+	−
ZA12	+++	++	+	−
ZA14	+++	+++	++	+

Notes: Here, SL1 = 0% NaCl, SL2 = 10% NaCl, SL3 = 20% NaCl, SL4 = 30% NaCl. Growth level is shown with indication of sign: + = slight growth, ++ = moderate growth, +++ = excellent growth, − = zero growth.

3.3. Minimum Inhibitory Concentration (MIC)

Different concentration levels of 100, 200 and 300 mg L^{-1} of Cd, Cr and Pb were maintained to find MIC by all selected strains W7, W9, W11, ZA10, ZA12 and ZA14 (Table 4).

Table 4. MIC for different heavy metals by selected strains.

Strains	Cd			Cr			Pb		
	ML1	ML2	ML3	ML1	ML2	ML3	ML1	ML2	ML3
W7	+++	+	−	+	−	−	+++	++	+
W9	++	+	−	+	−	−	+	−	−
W11	+++	++	+	+	−	−	++	−	−
ZA10	++	+	−	+	−	−	+	−	−
ZA12	+++	++	+	++	+	−	+++	++	+
ZA14	+++	++	+	+++	++	+	+++	++	+

Notes: Here ML1 = 100 mg L^{-1}, ML2 = 200 mg L^{-1}, and ML3 = 300 mg L^{-1}, (Growth level is shown with indication of sign: + = slight growth, ++ = moderate growth, +++ = excellent growth, − = zero growth).

ZA12 and ZA14 showed better resistance to elevated levels of all three metals. ZA12 and ZA14 showed moderate growth at 200 mg L^{-1} of Cd and Pb. ZA12 showed moderate resistance towards Cr at 100 mg L^{-1}. In contrast, ZA14 showed very resistant behavior towards Cr, and a slight growth was observed even at 300 mg L^{-1} of Cr, where no other strain showed any growth (Table 4).

3.4. Identification of Bacterial Strain

The sequence of amplified 16S rRNA was deposited in GenBank and provided with the accession number OM570257. The 16S rRNA sequence of the bacterial strain exhibited 99.0% homology to *Enterobacter cloacae* ZA14.

3.5. Sand Culture Experiment

3.5.1. Estimation of Plant Growth and Physiological Parameters

The application of endophytic bacteria ZA14 (T2) showed increased root and shoot growth. Among all treatments, tomato plants grown with seeds primed with endophytic bacteria ZA14 displayed the highest increase in root and shoot length in treatment T2 (Table 5). The inoculation of endophytic bacteria made tomato plants suitable to grow with 25% textile effluents (T4), as the data revealed there was increase in root length by 9.3% and shoot length by 5.5%, as compared to T1. Increased fresh and dry biomass was reported with application of ZA14 strain and 25% TWW (T4). In comparison to T1, the results of T4 confirmed increase of 5% in fresh and 7% in dry biomass of tomato plants (Table 5).

Table 5. Parameters presenting growth assessment in tomato.

Treatments	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)	Dry Weight (g)
T1	15.43 ± 0.15 c	20.23 ± 0.18 c	4.17 ± 0.006 c	1.78 ± 0.02 c
T2	18.77 ± 0.35 a	24.03 ± 0.12 a	4.6 ± 0.01 a	1.98 ± 0.01 a
T3	13.27 ± 0.12 d	17.07 ± 0.09 e	3.93 ± 0.02 e	1.69 ± 0.01 d
T4	16.87 ± 0.20 b	21.33 ± 0.23 b	4.38 ± 0.02 b	1.90 ± 0.01 b
T5	12.27 ± 0.09 e	15.30 ± 0.12 f	3.53 ± 0.03 g	1.48 ± 0.02 f
T6	15.73 ± 0.09 c	19.47 ± 0.09 d	4.01 ± 0.04 d	1.70 ± 0.01 d
T7	10.13 ± 0.18 f	13.23 ± 0.09 g	3.29 ± 0.01 h	1.4 ± 0.01 g
T8	13.80 ± 0.17 d	17.36 ± 0.15 e	3.71 ± 0.01 f	1.52 ± 0.01 e
T9	8.80 ± 0.06 g	11.73 ± 0.09 h	2.93 ± 0.02 i	1.24 ± 0.01 i
T10	12.67 ± 0.26 e	15.20 ± 0.06 f	3.32 ± 0.01 h	1.34 ± 0.01 h

Notes: Here T1 = control (DW); T2 = ZA14; T3 = 25% TWW; T4 = ZA14 + 25% TWW; T5 = 50% TWW; T6 = ZA14 + 50% TWW; T7 = 75% TWW; T8 = ZA14 + 75% TWW; T9 = 100 TWW; T10 = ZA14 + 100% TWW; The data is mean of three replicates ± SE and means Sharing similar letter(s) in each column do not differ significantly at $p < 0.05$.

The result of treatment T4 showed a maximum of 7% increase in chlorophyll content (SPAD value) as compared to control. Similarly, ZA14 inoculated plants also showed improved chlorophyll contents at other level of TWW in contrast to plants without inoculation, as illustrated in Figure 2a. A maximum increase of 3.95% in R.W.C% was recorded in plants with treatment T2. However, with inoculation of ZA14, plants receiving 25% TWW also showed an increase of 1.26% in R.W.C as compared to control, as presented in Figure 2b. The highest M.S.I was observed by plants treated with ZA14 (T2), exhibiting 10.4% elevation as compared to control (Figure 2c). However, the best performing treatment with TWW was Treatment T4, which showed 5% increase in M.S.I by overcoming the drastic effects of textile effluents.

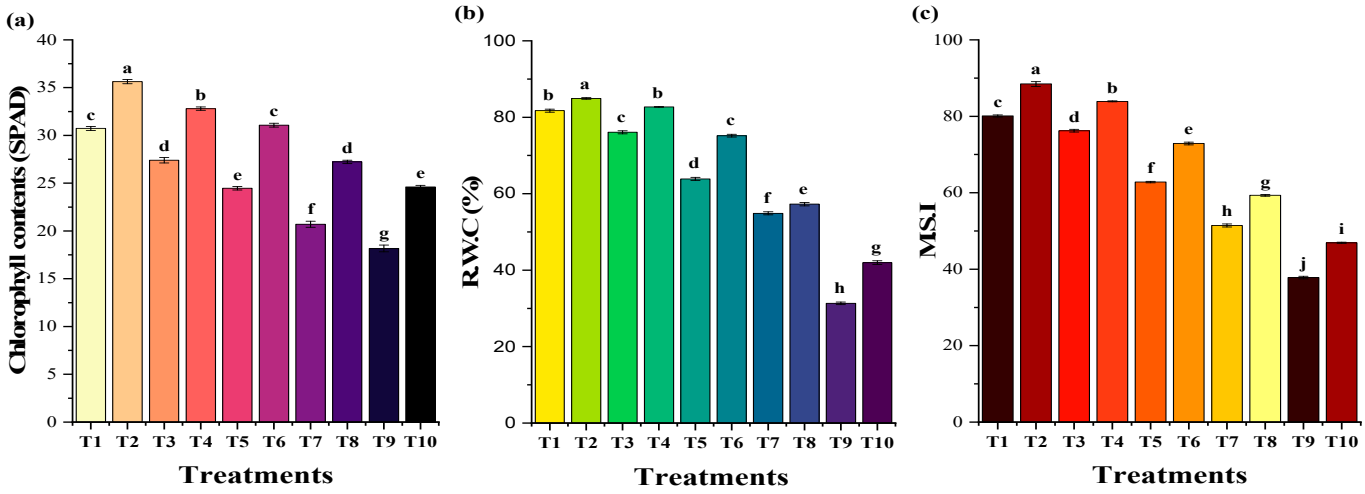

Figure 2. The combined effect of endophytic strains and textile wastewater on (**a**) chlorophyll content (SPAD value), (**b**) relative water content and (**c**) membrane stability index of tomato plants. In each figure, the data is mean of three replicates ± SE and the bars sharing similar letters do not differ significantly at $p < 0.05$; T1 = control (DW); T2 = ZA14; T3 = 25% TWW; T4 = ZA14 + 25% TWW; T5 = 50% TWW; T6 = ZA14 + 50% TWW; T7 = 75% TWW; T8 = ZA14 + 75% TWW; T9 = 100 TWW; T10 = ZA14 + 100% TWW.

3.5.2. Oxidative Stress Markers

The lowest production (13.8 nmol g^{-1} f·wt) of MDA contents was noted with use of ZA14 in plants irrigated with D.W. In the plants, the contents of MDA increased with increased toxicity of TWW (Figure 3a). Whereas, in treatment T9 where 100% TWW was used without bacteria, the plants showed 122% increase in MDA contents as compared to

control. Similarly, H_2O_2 contents were 147% increase in plants treated with 100% TWW with application of bacteria, whereas maximum H_2O_2 contents (180%) were indicated by plants treated with 100% TWW (Figure 3b).

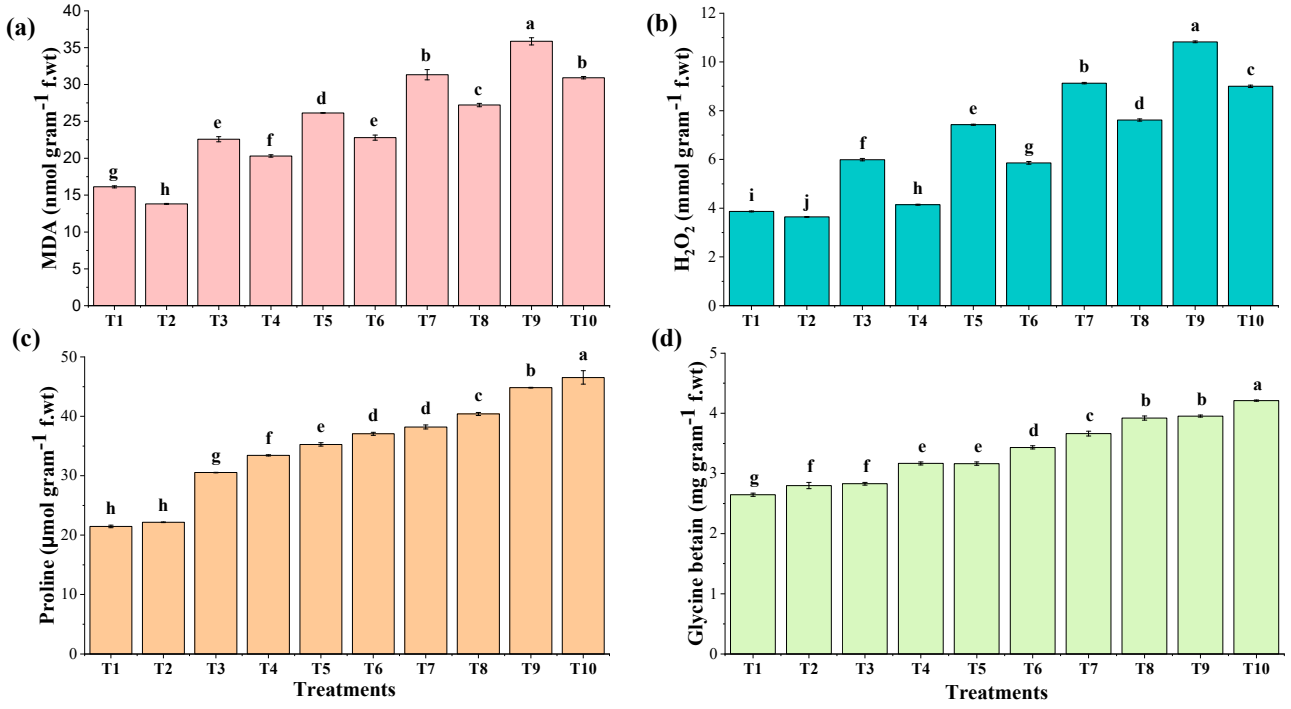

Figure 3. The combined effect of endophytic strains and textile wastewater on the production of oxidative stress markers; (**a**) MDA, (**b**) H_2O_2, (**c**) proline and (**d**) and glycine betaine in tomato plants. In each figure, the data is mean of three replicates ± SE and the bars sharing similar letters do not differ significantly at $p < 0.05$; T1 = control (DW); T2 = ZA14; T3 = 25% TWW; T4 = ZA14 + 25% TWW; T5 = 50% TWW; T6 = ZA14 + 50% TWW; T7 = 75% TWW; T8 = ZA14 + 75% TWW; T9 = 100 TWW; T10 = ZA14 + 100% TWW.

3.5.3. Osmoprotectants

The maximum increase of 108.8% in proline was indicated with the use of 100% TWW (Figure 3c). The production of glycine betaine was increased in all treatments under stress of TWW. The production of GB was found to be significantly high (49.62%) in plants irrigated with 100% TWW without application of bacteria. Whereas, in the plants, the application of ZA14 with 100% TWW (T10) caused the highest (50.35%) production of GB of about 4.21 mg g^{-1} fresh weight as compared to T2 having 2.8 mg g^{-1} fresh weight (Figure 3d).

3.5.4. Secondary Metabolites

Textile phytotoxicity in *Solanum lycopersicum* (tomato) plants induced a higher level of production of total flavonoids (Figure 4a), total phenols (Figure 4b) and total anthocyanins (Figure 4c) by 70.66%, 88% and 92.9% in plants treated with 100% TWW alone. However, the application of ZA14 and 100% TWW influenced the production of total flavonoids (71.6%), total phenols (89%) and total anthocyanins (94%) as compared to control having ZA14 only.

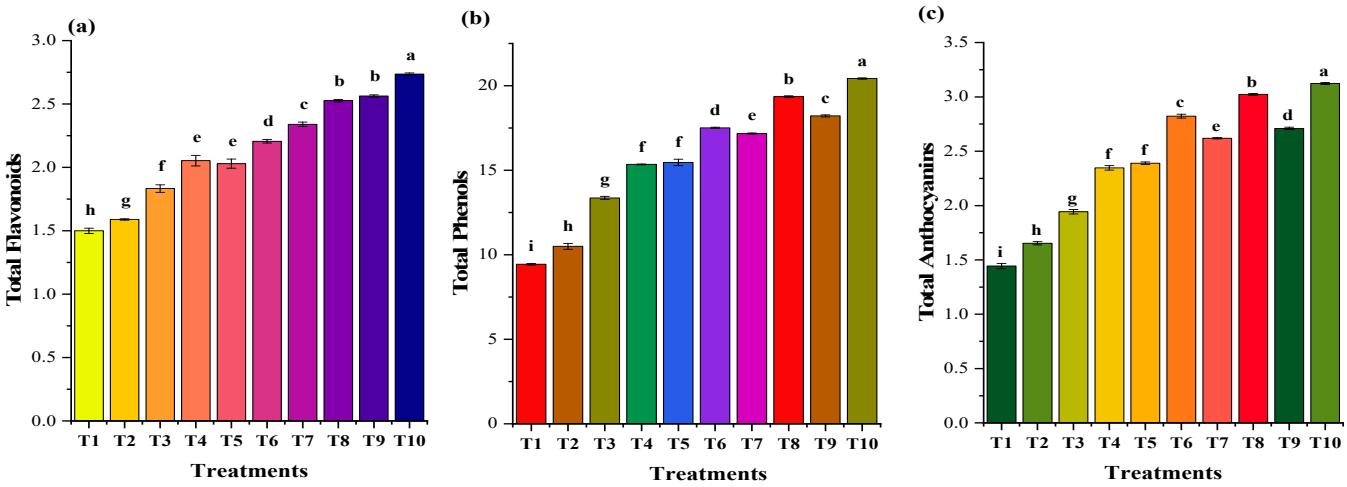

Figure 4. Combined effect of endophytic strains and textile wastewater on production of secondary metabolites; (**a**) total flavonoids, (**b**) total phenols and (**c**) total anthocyanins in tomato plants. In each figure, the data is mean of three replicates ± SE and the bars sharing similar letters do not differ significantly at $p < 0.05$; T1 = control (DW); T2 = ZA14; T3 = 25% TWW; T4 = ZA14 + 25% TWW; T5 = 50% TWW; T6 = ZA14 + 50% TWW; T7 = 75% TWW; T8 = ZA14 + 75% TWW; T9 = 100 TWW; T10 = ZA14 + 100% TWW.

3.5.5. Antioxidant Production

The data showed that a significant increase in production of enzymatic antioxidants were found in plants treated with ZA14 strain along with different levels of TWW as compared to plants without bacterial inoculation. The production of enzymatic antioxidants were found to be minimal at low levels of textile wastewater effluents. It was found that with the increased level of TWW in plants, increased production of antioxidants followed. The data showed maximum production of APX (Figure 5a), SOD (Figure 5b), POD (Figure 5c) and CAT (Figure 5d) by 80%, 85%, 83% and 77% in treatment T10 receiving 100% TWW with supplementation of ZA14 as compared to control.

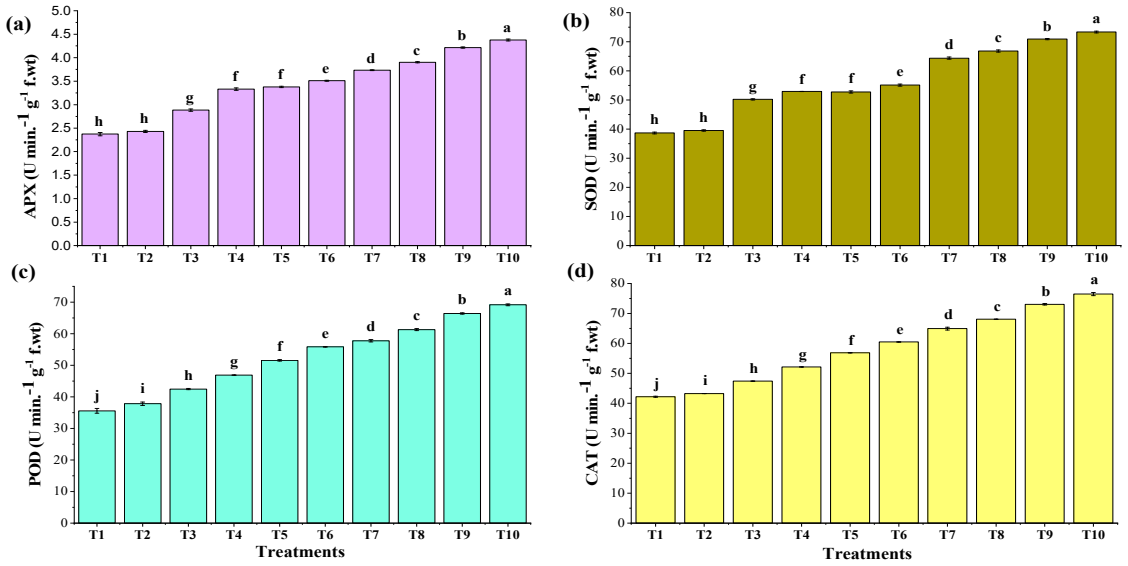

Figure 5. The combined effect of endophytic strains and textile wastewater on production of antioxidants; (**a**) APX, (**b**) SOD, (**c**) POD and (**d**) and CAT in tomato plants. In each figure, the data is mean of three replicates ± SE and the bars sharing similar letters do not differ significantly at $p < 0.05$; T1 = control (DW); T2 = ZA14; T3 = 25% TWW; T4 = ZA14 + 25% TWW; T5 = 50% TWW; T6 = ZA14 + 50% TWW; T7 = 75% TWW; T8 = ZA14 + 75% TWW; T9 = 100 TWW; T10 = ZA14 + 100% TWW.

4. Discussion

The presence of textile dyes and heavy metals can degrade environmental quality and, hence, negatively influence human life [7,43]. Isolation of endophytic bacteria from two different sources was performed. The idea behind this research was to isolate the native bacteria thriving in the textile dye-contaminated environment to explore their dye decolorizing potential. This strategy was followed by researchers and reported in the literature [44].

A total of twenty-seven strains, of them 11 strains from wastewater and 16 strains from the plant sample, were isolated by the dilution and plate method and showed the degradation of textile dye at 200 mg L^{-1}. After repeated purification, the decolorization assay of 27 isolated strains was analyzed to screen the most efficient dye-degrading bacteria. The same method was followed by other researchers [30,45] for screening of dye-degrading bacteria. Among all the isolated strains, six strains (W7, W9, W11, ZA10, ZA12 and ZA14) with the highest decolorization potential were selected for further analysis. Bacteria uses textile dye as a source of energy by degrading the organic dye molecules and further degradation of metabolites. This results in the loss of characteristic dye molecules and eventually loss of color. In a previous finding, bacterial strains showed dye removal potential (98%) for Congo red dye [46]. Bacterial dye decolorization is performed by presence of azo-reductase enzyme of bacteria, which break the strong N-bond of azo dye, thereby reducing azo dye into aromatic amines. These aromatic amines are also subjected to degradation by bacterial enzymes such as laccase. A complete remediation of textile dye crystal violet was found with the use of *Enterobacter* spp. [47]. Our finding about efficient removal of dye effluents by a biological approach as a best strategy was also supported by previous research [48].

The use of endophytic bacteria has been associated with enhanced plant growth without causing any harm to plant tissues [12]. Endophytic bacteria are reported for their growth promotion potential in plants under normal and stress conditions like heavy metals [49]. To promote plant growth and development, several bacterial processes such as production of IAA, N-fixation and P-solubilization have proved beneficial. It is reported that the growth-promoting bacteria in soil convert unavailable nutrients like phosphorous and zinc into the available form for growth [50]. Plant growth-promoting bacteria utilized these unavailable nutrients for growth and development of plants by making them capable of surviving various harsh conditions.

All six selected strains displayed a positive response in characterization tests for cellulose, catalase, N-fixation, NaCl tolerance, P solubilization and IAA production, while all strains showed negative results in Gram staining, Zn solubilization and H_2S production. In potatoes, the IAA synthesis was released by bacterial endophytes, *Burkholderia phytofirmans* PsJN [51]. Therefore, the growth of plants is concomitant with the presence of a plant–microbe relationship, which improves the overall health, development and yield of plants. An endophytic bacterium, *Azoarcus* sp. BH72, was found in nitrogen fixation in a rice crop [52]. It is reported that in rice, maize and wheat, the ability to utilize atmospheric nitrogen and phytohormone secretions was detected in the presence of the bacterial endophyte *Azospirillum lipoferum* 4B [53].

Phosphorus solubilization by all bacterial strains was indicated, and ZA14 strain showed highest PSI and PSE. Phosphorous is the second most vital macronutrient for plant growth, after nitrogen. The availability of phosphorous in soil for the uptake of plants is the main problem. The bacteria solubilize phosphorous by many mechanisms like producing gluconic acid, ion exchange, acidification and chelation, hence increasing P availability to plants, which results in increased growth and yield [54]. Among all the selected strains, the presence of plant growth promotion traits is best displayed by strain ZA14, which indicates its ability to enhance plant growth efficiently.

All strains showed tolerance to NaCl at varying concentration levels. The ZA14 isolate showed the highest salt tolerance level (30%). Endophytic bacteria showed no reduction in the composition of their community diversity [55] under salt stress, making them salt-

tolerant species. The proteobacteria had abundant growth in salt-affected soil [56]. All this research exposed the fact that endophytic bacteria have the ability to survive at high levels of salt. Endophytic salt-tolerant species of *Acinetobacter* (ACMS2) and *Bacillus* sp. PVMX4 strains had the characteristics for promotion of plant growth by reducing the influence of salts [57].

Bacterial strains can manage heavy metal stress by adopting various survival mechanisms, among which exopolysaccharides, metal-phosphates and siderophore production are notable features in the immobilization of heavy metals, hence reducing mobility of heavy metals into plant tissues. Decreased heavy metal concentrations in soil improves plant growth, as reported by a researcher [58]. The minimum inhibitory concentration (MIC) for heavy metals like Cd, Cr and Pb was performed. All selected strains showed growth in metal supplemented media at low concentration of heavy metals. Our results are supported by the fact that various mechanisms like biosorption, bioaccumulation, immobilization, chelation and biotransformation are involved, showing the effective management of heavy metal stress by bacteria [58]. *Enterobacter* spp. K2 strain also showed resistance towards cadmium heavy metal [59]. Bacterial strain AR16 has the ability to degrade Cr metal [60]. This confirms our findings. The selected strains W7, ZA12 and ZA14 exhibited an improved level of tolerance for increased concentrations of the metals Cd, Cr and Pb. However, no strain showed growth at 300 mg L^{-1} Cr. The reduced or absence of growth at high concentration shows reduced resistance of strains at increased concentration of heavy metals. This could be due to reduced bacterial growth and physiological activity, as reported previously [30].

A remarkable reduction in plant growth was observed with application of TWW in plants as compared to plants inoculated with ZA14 bacterial strain. The progressive decline in plant growth under increased levels of textile effluents was observed in all growth responses, indicating the lower level (25%TWW) is more suitable for the growth of plants. Our finding is also supported by the study [61], which stated that effluents with 25% concentration showed improved growth in *Vigna mungo* plants. At lower concentrations of chromium, copper, nickel and zinc, heavy metals increased the germination and growth of alfalfa seedlings [62]; however, with increased concentration levels of these heavy metals, a reduction in germination and plant growth was caused. The high level of TWW for irrigation indicated textile phytotoxicity in the form of stunted growth. The augmented levels of textile dyes, heavy metals and salts are the main contributors to the decline in growth. The use of 80% textile effluents caused maximum reduction in root and shoot length of tomato plants [63], and a decline in dry biomass was also reported at the 80% textile effluents usage. Enterobacteria have up-regulated the growth of the root and shoot of tomato plants. Inoculation of endophytic bacteria helped in degradation of textile effluents like dyes, heavy metals and salts. Dye-decolorizing bacteria are involved in the breakdown of organic textile dye molecules with the help of oxidoreductive enzymes, by converting the large stable dye molecule into small metabolites [64], which are further degraded by microbes. Moreover, use of dye-degrading endophytic bacterial strains has shown improved growth of tomato plants when TWW was used for irrigation. Bacteria have the ability to provide nutrients, phytohormones for plant growth and various enzymes to mitigate stress caused by phytotoxicity.

Textile effluents in irrigation water showed a prominent influence on the physiological traits of plants as indicated in our results. In tomato plants at the pre-flowering stage, reduction in chlorophyll content, nitrogen and protein contents by using 80% effluents was noted [65]; however, better chlorophyll content, nitrogen and protein contents were observed at the 20% effluent level. The presence of salts [66] and heavy metals in irrigation water at aggravated levels are reported as a cause of decreased physiological activities in plants and are the ultimate cause of decreased growth [67]. There was a distinguishable increase in chlorophyll content (SPAD value), R.W.C% and M.S.I of tomato plants under application of ZA14 strain in both normal and textile wastewater conditions. Endophytic bacteria are associated with a number of mechanisms for remediation of dyes [45,68] and

heavy metals [69,70], therefore causing degradation of dyes, immobilization of heavy metals, increased nutrient uptake from soil, hence promoting growth, and physiological practices in plants [71].

Textile effluents in irrigation water are the most prominent factors affecting the normal functioning of plants. These abiotic factors cause damage to plants at the cellular level and result in disruption in homeostasis, osmotic balance and dehydration. In order to maintain osmoregulation in plant cells, there is a need for osmolytes in the plant cell. Apart from the proper regulation of water and solute contents in plant cells, the osmolytes play main roles in the management of stress as low-weight chaperones, signaling molecules also called scavengers of reactive oxygen species (ROS) [72,73]. As oxidative stress markers, MDA and H_2O_2 showed strong interaction with increasing levels of stress provided to plants, and as presented in the results, with treatment T9, the maximum production of MDA and H_2O_2 was produced in comparison to plants treated with ZA14 strain. Increased production of MDA and H_2O_2 showed increased levels of stress present in plants. Proline is the most important osmolyte in scavenging of ROS [74]. Besides playing the role of osmoprotectants, proline helps in acquiring carbon as well as nitrogen, hence leading to development of the plant. In conditions of stress, overproduction of proline resulted in helping the maintenance of water and solutes across the plant cell, redox balance and adjustment of osmotic balance for alleviation of oxidative stress and proper cell structure [75]. The increased production of proline contents in plants treated with different levels of TWW showed increased production of proline as compared to plants treated with DW. Whereas, in plants treated with ZA14 strain and different levels of TWW, an increased production of proline contents was observed as compared to the treatment with only ZA14 strain. This present study finds support from the previous work [73] in which contents of glycine betaine also increased in response to stress conditions.

Elevated level of TWW in plants declined the production of secondary metabolites (total flavonoids, total phenol and total anthocyanins) and enzymatic antioxidants (SOD, POD, CAT, APX); plants without bacterial application showed a level of phytotoxicity caused by textile effluents in tomato plants. However, increased production of secondary metabolites and enzymatic antioxidants were recorded with application of ZA14 as compared to uninoculated plants. Phenolics are abundantly found in plants, produced to cope with stress induced by dyes and heavy metals [76]. It is reported that phenols act as a strong responsive agent against heavy metals like cadmium, copper and iron. The abundant generation of flavonoids, phenols and anthocyanins is the indication of strong defense mechanism produced in tomato plants in response to textile phytotoxicity. Whereas, contrary to our findings, it is reported [77] that a decline in the generation of antioxidants like SOD and CAT with inoculation of endophytic bacteria was observed under stress.

The presence of textile pollutants in irrigation water had reduced the soil quality and showed compromised growth and physiological activities in tomato plants. Release of textile pollutants on arable land resulted in reduced plant growth, degraded soil properties, along with bioaccumulation of heavy metals reported by Khan and Malik [78]. The increased mobility of heavy metals from soil to plant tissues could lead to nutrient deficiency leading to stunted growth, disturbed physiological activities and, hence, declined plant yield [79]. However, application of endophytic bacterial strain ZA14 on one side reduced the toxicity of textile dyes and heavy metal with the help of bacterial enzymes and, on the other hand, improved plant growth. This could be possible due to the ability of *Enterobacter* spp. to degrade textile dye and heavy metals [80]. Moreover, *Enterobacter cloacae* ZA14 has exhibited various mechanisms like phosphorus solubilization, nitrogen fixation and production of IAA, which are helpful in plant growth under both normal and stress conditions. Indole-3-acetic acid (IAA) is responsible for increased biomass production and development [81], which trigger the formation of roots, provide plants with nutrients from soil solution and also release organic compounds like root exudates, which are beneficial for plants [82]. Naveed et al. [50] reported that bacteriological interactions with plants have many positive outcomes including provision of nutrients (iron, zinc), reduced heavy metal

mobility to plant tissues and improved physiological processes [83,84]. All these traits of microbes explain well how endophytic strain ZA14 can promote growth of tomato when textile wastewater was applied for irrigation.

5. Conclusions

In this study, we first isolated 27 different strains that have the potential to degrade textile dye. In the dye decolorization assay, all the isolated strains showed decolorization potential when 200 mg L^{-1} of textile dye was added to nutrient media. Among all the isolates, the six best performing isolates, W7, W9, W11, ZA10, ZA12 and ZA14, were selected for further characterization. Heavy metals (Cd, Cr and Pb) tolerance was found to be highest in strain ZA14. The highest salt tolerance (30%) was also exhibited by the ZA14 strain. The sand culture experiment showed that application of endophytic bacteria has the highest growth and physiological potential. However, application of TWW showed a correlation of decreased growth with increased level of textile effluent application in tomato. Farmers use TWW, which is mixed with urban wastewater due to limited availability of fresh water. Use of TWW interrupts the plant growth due to heavy metals and textile dyes. Textile dye is carcinogenic and negatively influences plant growth. Heavy metals are toxic at high concentrations, cause nutrient imbalance in soil, and therefore cause compromised growth of plants, whereas application of ZA14 strain showed improved growth, physiological responses and production of biochemical responses to mitigate textile phytotoxicity. The ZA14 strain can reduce the azo bond of textile dyes, tolerate high concentrations of heavy metals and supply plants with essential nutrients, phytohormones and enzymes to enhance tomato plant growth under textile wastewater toxicity. This study revealed that among all treatment levels of TWW, 25% TWW has the better impact on growth of plants in non-inoculated plants, but the most prominent response was observed in the inoculated plants under application of 25% TWW. Therefore, following a proper mitigation approach like using endophytic Enterobacter sp. ZA14 could help plants to overcome phytotoxicity of textile effluents with improved plant growth. However, further experiments are needed to exploit the potential of strain ZA14 for enhanced plant growth and associated health risk assessment caused by heavy metals present in certain atmospheric and soil conditions.

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