Bacillus firmus strain FSS2C ameliorated oxidative stress in wheat plants induced by azo dye (reactive black-5)

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Abstract

This study was conducted to determine the ability of a bacterial strain FSS2C to ameliorate growth of wheat plants grown under induced stress of reactive black-5 (RB-5). The strain was taxonomically identified as *Bacillus firmus* on the basis of its 16S rRNA gene sequence analysis. The *B. firmus* FSS2C was found physiologically potent in phosphate solubilization, indole-3-acetic acid production and ammonia synthesis in the presence of varying concentrations of azo dye RB-5. Moreover, it decolorized RB-5 in vitro with the maximum decolorization (%) found at pH 7 and 30 °C. Inoculation of wheat plants, growing under stress induced by RB-5 dye, with rifampicin-resistant derivatives of the strain FSS2C substantially reduced the cellular oxidative stress, thereby resulting in higher plant biomass as compared to non-inoculated plants. Similarly, the inoculated plants revealed higher nutrient content in shoots as compared to non-inoculated ones. It was concluded that *B. firmus* strain FSS2C alleviated the oxidative stress impairment caused by reactive black-5 in wheat plants. Therefore, the strain can be used as bio-inoculant in wastewater irrigated soils.

Keywords Abiotic stress · Antioxidants · Azo dyes · *B. firmus* · Oxidative damage · Wheat

Introduction

Effluents from different industries, including the textile industry, are major sources of environmental contamination (Abubacker and Mehala 2012). According to a global

estimate, 10–50% of the dye stuff applied to fabric does not adhere and is discharged as industrial effluents which deteriorate the environment drastically (Singh et al. 2014). Usually, the wastewater produced by the textile industries contain about 20–30% of reactive dyes in addition to huge concentration of salts and metal ions (Mahmood et al. 2017). Conventional means of discharge of dyes enhance the chances of their accumulation in the environment due to their recalcitrant nature against temperature, light, detergents and chemicals, etc. (Couto 2009). In water bodies, the textile effluents modify different physicochemical characteristics such as biochemical oxidation demand (BOD), chemical oxygen demand (COD), pH, color and salinity (Mansour et al. 2012). Similarly, when arable soils are irrigated with such wastewaters, dyes adversely alter the soil properties and microbial communities, thereby leading to reduction in soil fertility and crop yields (Imran et al. 2015; Rehman et al. 2018). In several developing countries, irrigation of arable land with wastewater is a common practice in industrial areas due to the unavailability and/or unsuitability of ground water (Mahmood et al. 2017).

Given the ecological hazards associated with the untreated release of dyes especially azo dyes, their degradation is

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critical to mitigate their toxic effects (Mahmood et al. 2017; Chacko and Subramaniam 2011). Azo dyes are distinguished by the presence of azo group ($-N=N-$) and share a major part (approx. 70%) of synthetic dyes being used in various industries due to their persistence and non-degradable nature (Chacko and Subramaniam 2011). Several physicochemical strategies have been used to eliminate the dyes and their derivatives from the effluents. However, these methods could not gain much popularity due to high costs, less sustainability and damaging nature to ecosystems (Mohan and Pittman 2007). On the contrary, sustainability of microbes owing to their ecological adaptation and cost-effectiveness make them ideal candidates for degradation of hazardous azo dyes, and growth promotion and induction of systemic resistance in plants (Aksu et al. 2007; Gkorezis et al. 2016).

Microorganisms, especially the phytobeneficial bacteria, are very effective in decolorizing azo dyes and tolerating metal and salt stress. These characteristics enable them to promote plant growth by inducing tolerance in plants against abiotic stresses by increasing nutrient uptake and mitigating oxidative stress (Mahmood et al. 2017). Phytobeneficial bacteria utilize aerobic and anaerobic metabolic processes to degrade recalcitrant compounds of xenobiotic nature (Narwal and Gupta 2017). Degradation of azo dyes and oxidative stress alleviation in plants by phytobeneficial bacteria has been extensively studied in wastewater-contaminated soils (Hussain et al. 2013; Nigam et al. 2016; Chen et al. 2016; Chacko and Subramaniam 2011), but there has been a limited research on the plant responses to the decolorized dye compounds in soil. When compared to physical and chemical methods of pollutant degradation, the (micro)biological methods have been found cheaper, more effective, more sustainable, less waste-producing, thus cutting the disposal costs and applicable to a wide range of ecosystems (Pearce et al. 2003).

Amongst the phytobeneficial bacteria, plant growth-promoting rhizobacteria (PGPR) are the most widely studied group for their potential to nourish plants with nutrients bound in soils, fixing nitrogen from atmosphere and stimulate plant growth by producing phytohormones (Tariq et al. 2017). Recently, it has also been documented that PGPR strains, especially *Bacillus* spp., have inherent potential to bioremediate soil heavy metals and salts, and to decolorize azo dyes, thereby enabling plants to tolerate these stresses (Shahid et al. 2015, 2018c; Mahmood et al. 2017).

In this context, the objective of current research was to evaluate the PGPR strain FSS2C for simultaneous bioremediation and plant growth-promoting potential under azo dye (reactive black-5) stress.

Materials and methods

Soil sampling and bacterial isolation

The strain FSS2C was isolated from a sorghum rhizosphere grown on a wastewater-contaminated field, adjacent to Paharang Drain, Near Bawa Chak (31.467° North and 73.051° East), Faisalabad, Pakistan. The sample was serially diluted for the isolation (Somasegaran and Hoben 1994). The roots with adhering soil was vigorously shaken to remove the firmly adhered soil. One g of adhered soil was transferred to a test tube containing 9.0 mL of saline solution (0.85% w/v NaCl) and agitated on vortex. The tubes were serially diluted by adding 1.0 mL from each to the next tube containing 9.0 mL saline solution followed by agitation with vortex. The dilutions 10^{-5} and 10^{-7} were poured (100 μ L) on to the nutrient agar plates. Bacterial isolates were purified by repeated streaking and subjected to evaluation of tolerance for different concentrations (0, 50, 100, 250, 500 mg L^{-1}) reactive black-5 (RB-5) dye based on minimum inhibitory concentration (MIC) (Mahmood et al. 2017). The plates were kept in an incubator at $28 \pm 2^\circ C$ for 48 h to allow a prolific growth of colonies. The isolate FSS2C belonging to the dilution 10^{-7} was repeatedly streaked to achieve maximum purity before storage at $-80^\circ C$. Colony morphology, shape, motility and Gram's reaction were studied under light microscope (Olympus) as described by Vincent (1970).

Taxonomic identification and phylogenetic analysis

Genomic DNA extraction and amplification of 16S rRNA gene of the strain FSS2C were carried out as described by Shahid et al. (2018a). The genomic DNA of strain FSS2C was isolated by the CTAB method as reported by Wilson (2001) and DNA was quantified through spectrophotometer using Nano DropTM 2000/2000c (Thermo Fisher Scientific, USA). Amplification of the 16S rRNA gene was carried out with universal primer pair fD1 (5' AGAGTTTGATCCTGGCTCAG 3') and rD1 (5'AAGGAGGTGATCAGCC 3') (Weisburg et al. 1991). The PCR product was purified using QIAquick PCR Purification Kit (Qiagen, Germany). The purified amplicon was sent to Macrogen, Korea for direct sequencing. The retrieved sequence of 16S rRNA gene was converted to final contig sequence by joining both forward and reverse strands using an online tool (Cap3 assembly). The phylogeny was described by BLASTn and RDP database analysis and by constructing the phylogenetic tree with the type strains. The phylogenetic tree was built by utilizing the neighbor-joining (NJ) technique following multiple sequence alignment result of

ClustalW in MEGA 7.0 software package. The clustering constancy of the tree was estimated by bootstrap study of 1000 data sets. The gene sequence was submitted to GenBank database under accession number MG548385.

Decolorization of azo dye

The isolate FSS2C was evaluated for its potential to decolorize the reactive black-5 (RB-5) azo dye in a liquid medium (250 mg L^{-1}). For measuring decolorization potential, Murashige and Skoog (MS) medium was supplemented with varying concentration of NaCl (0%, 2.5%, 5% and 7.5%) at pH 7 and temperature at 30°C . Moreover, decolorization potential was also assessed at different combinations of pH and temperature against the said concentrations of salt. In the second batch, pH of the medium was maintained at 5, 6, 7, 8 or 9, while keeping temperature at 30°C . In the third batch, temperature was varied between 20, 25, 30, 35 and 40°C , while keeping pH at 7. The yeast extract was used as carbon source in the medium. The culture tubes were placed for 72 h at $28 \pm 2^\circ\text{C}$ without shaking. Then, the supernatant was obtained after centrifugation at 24 h, 48 h and 72 h and was subjected to spectrometry for further analysis after every 24 h of incubation. The absorbance was measured at 597 nm by a spectrophotometer (Shimadzu UV/VIS, Kyoto, Japan) and was converted to decolorization % by applying the following formula. A non-inoculated negative control was included in the experiment and % decolorization measured in control was subtracted from the inoculated treatments to ensure the accuracy in results.

$$\text{Percent decolorization} = (a - b)/a \times 100,$$

where 'a' and 'b' represent the absorbance of the negative control and sample inoculated with the strain FSS2C, respectively.

Characterization for plant beneficial traits

Phosphate solubilization

The pure FSS2C culture was tested for phosphate solubilization on Pikovskaya's agar media (Pikovskaya 1948). Phosphate solubilization ability of the isolate was indicated by formation of halozone on the agar plates. The pure culture was then transferred to 500 mL Pikovskaya broth in Erlenmeyer flasks for quantification of phosphate solubilization. The media were supplemented with varying concentration of RB5 ($0, 50 \text{ mg L}^{-1}, 100 \text{ mg L}^{-1}, 250 \text{ mg L}^{-1}$ and 500 mg L^{-1}) followed by shaking in a shaking incubator at $28 \pm 2^\circ\text{C}$ for 240 h. Then, the supernatant was obtained by centrifuging 20 mL of the culture at 13,000g for 10 min. For estimating solubilized phosphate, the phosphomolybdate blue

color method described by Murphy and Riley (1962) was used. For this purpose, the absorbance of the supernatant of the reaction mixture was measured at 882 nm using double-beam scanning spectrophotometer (Shimadzu UV/VIS, Kyoto, Japan). A standard curve was constructed by different concentrations ($0, 1, 2, 3, 4, 5$ and $10 \mu\text{g mL}^{-1}$) of KH_2PO_4 solutions to measure the solubilized phosphate.

IAA synthesis

The IAA synthesis potential of the isolated strain FSS2C was estimated using Gordon and Weber method (1951). To measure the IAA synthesis at varying concentrations of RB-5 (i.e. $0, 50 \text{ mg L}^{-1}, 100 \text{ mg L}^{-1}, 250 \text{ mg L}^{-1}$ and 500 mg L^{-1}), a set of media was amended with tryptophan (100 mg L^{-1}), while the other was left un-amended. After incubation at $28 \pm 2^\circ\text{C}$ for 48 h on an orbital shaker, the supernatant was obtained by centrifuging the cultures at 13,000g. The 2.0 mL Salkowisk's reagent (2% 0.5 FeCl_3 in 35% perchloric acid solution) was mixed in 1.0 mL of the supernatant and the tubes were placed under dark conditions for 30 min to develop pink color. The color quantification was done by subjecting the reaction mixture to spectrometry (Shimadzu UV/VIS, Kyoto, Japan). The absorbance was measured at 540 nm. The standard curve of IAA solutions ($0, 5, 10, 50, 100, 200$, or $500 \mu\text{g mL}^{-1}$) was drawn for comparative measurements.

ACC deaminase activity

The ability of the strain FSS2C to use 1-aminocyclopropane-1-carboxylic acid (ACC) as a sole nitrogen source was measured in 5.0 mL DF salt minimal medium having same concentrations of RB-5 mentioned in above section (Penrose and Glick 2003) as well as 3 L of 0.5 M ACC. Cultures were grown at $30 \pm 2^\circ\text{C}$ for 24 h in a shaker. To determine the ACC deaminase activity, the turbidity of the inoculated cultures was compared to that of non-inoculated control.

Ammonia production

The bacterial strain FSS2C was grown under aerobic conditions in peptone water broth amended with same concentrations of RB-5 as mentioned in above section for 48–72 h at 30°C . The supernatant was harvested and mixed with the Nessler's reagent. A color development of brown to yellow indicated the presence of ammonia. The absorbance was measured at 450 nm using the double-beam scanning spectrophotometer (Shimadzu UV/VIS, Kyoto, Japan) with three replicates. To estimate the amount of ammonia production by the isolate, a standard curve of ammonium sulfate was prepared (Goswami et al. 2014).

Pot experiment

Comparative growth and inoculum preparation

Rifampicin-resistant derivatives of strain FSS2C (FSS2C_{rif}) were prepared according to Akram et al. (2016) and derivative strain was subjected to fitness analysis by comparing its growth with wild-type strain (FSS2C_w) by plate count method as described by Shahid et al. (2012). The inoculum of strain FSS2C_{rif} was prepared by culturing the strain in nutrient broth to a cell density of 1×10^9 CFU mL⁻¹, collecting the cells by centrifugation followed by washing and re-suspending the pellet in distilled water to adjust the inoculum density up to 1×10^7 CFU mL⁻¹.

Experimental design and material

The clay loam soil with known physicochemical properties (having 9.2 mg kg⁻¹, 0.96 g kg⁻¹, and 104 mg kg⁻¹ of available P, total N and available K respectively, 1.4% organic matter and 7.2 pH) was sampled from University of Agriculture, Faisalabad, Punjab, Pakistan. Out of the total 30 pots filled with 700 g soil per pot, 15 pots were pre-inoculated with the strain FSS2C_{rif}. The pre-inoculation of soil was done by adding inoculum (7.0 mL of inoculum per 100 g of soil) of FSS2C_{rif} (inoculated soil) or by mixing the same amount of distilled water (non-inoculated soil). Wheat seeds were surface sterilized by the method described by Shahid et al. (2012). Then, the seeds were dipped in FSS2C_{rif} inoculum or in sterile ddH₂O separately for 20 min. Both inoculated and non-inoculated seeds were sown in pots (eight seeds per pot). After seedling emergence, plants were thinned to five plants per pot. The plants were irrigated with half strength Hoagland solution (Arnon 1949) without phosphorus source. After seedling emergence, various levels (0, 50 mg L⁻¹, 100 mg L⁻¹, 250 mg L⁻¹ and 500 mg L⁻¹) of azo dye (RB-5) stress was induced along with Hoagland solution.

Measurement of growth parameters and bacterial population density

The plants were uprooted and lengths (cm) of root and shoot were measured with the help of a scale followed by the estimation of fresh weight (g) on electronic balance. The root and shoot samples were dried by placing in an oven at 80 °C for 48 h for the measurement of dry weight. The strain FSS2C_{rif} was recovered from wheat rhizosphere on antibiotic medium plates at different time intervals by plate count method (Somasegaran and Hoben 2012).

Measurement of physiological parameters

Lipid peroxidation and proline content

Lipid peroxidation products of fully grown young leaves were measured by thiobarbituric acid (TBA) reaction (Heath and Packer 1968) and proline content by Bates et al. (1973) method. These methods involve the homogenization of 1 g leaves followed by centrifugation and incubation steps. Lipid peroxidation was estimated at 532 nm and proline content at 520 nm by UV–Vis spectrophotometer (Hitachi U-2910, Tokyo, Japan).

H₂O₂ content and total phenolic content

Plant H₂O₂ content was measured in 1 g of fully grown leaves by the method described by Jana and Choudhuri (1982), while a method described by Julkunen-Tiitto (1985) was used to estimate the total phenolic content. Colorimetric measurements of H₂O₂ and phenolic contents were done by reading the absorbance at 410 nm and 750 nm, respectively, on a UV–Vis spectrophotometer (Hitachi U-2910, Tokyo, Japan).

Catalase and peroxidase activity

The activities of catalase and peroxidase (CAT and POD) were determined by methods described by Aebi (1984) and Chance and Maehly (1955), respectively. One gram of fully grown young leaves was homogenized in 50 mM phosphate buffer (pH 7.8) followed by spinning at 10,000 g for about 10 min. The absorbance was read at 240 nm and 470 nm for CAT and POD, respectively, on a UV–Vis spectrophotometer (Hitachi U-2910, Tokyo, Japan).

Determination of plant nutrient elements

For elemental analysis, plants were washed twice with sterilized ddH₂O, dipped in 20 mM EDTA and washed again. The digestion of the oven-dried samples was performed using wet digestion method in HNO₃: HClO₄ (7:3 V/V). The clear samples were mixed with distilled water up to 50 mL followed by elemental analysis using Atomic Absorption Spectrophotometer (Hitachi U-2910, Tokyo, Japan).

Data analyses

The data were analyzed statistically by two-way Analysis of Variance (ANOVA) (Steel et al. 1997) using Statistix (ver. 8.1) software package. The means of three replicates per treatment were compared using least significance difference (Fisher's LSD) at 95% confidence level.

Results

Morphology and identity of the strain

Out of the 11 morphologically different bacterial isolates purified on the media, only the isolate FSS2C tolerated the RB-5 up to 250 mg L⁻¹ in MIC experiments. Rest of the isolates were either sensitive to the tested dye levels or tolerated up to 50 mg L⁻¹ RB-5, thereby leading their exclusion from further studies. Rod-shaped cells of Gram-negative isolate FSS2C were clearly seen under light microscope. The 16S rRNA gene sequence analysis revealed the taxonomic status of the isolate as *B. firmus* strain FSS2C. BLASTn analysis of 1009 bp sequence showed 99.80% identity with *B. firmus* strain BTNGPSA5 (Acc # MK958537), whereas in RDP database search, the sequence showed similarity with *B. firmus* KAV2P (HQ851083). In phylogenetic tree, the strain FSS2C formed cluster with *B. firmus* SA47 (MK467562) and *B. firmus* BTNGPSA5 (MK958537) (Fig. 1). The 16S rRNA sequence was submitted in GenBank database under the accession number MG548385.

Azo dye decolorization

The strain FSS2C proved an efficient decolorizer of RB-5 and this decolorization potential was inversely proportional to the increasing salt stress (0–7.5%). *B. firmus* strain FSS2C efficiently decolorized the RB-5 even at 5% NaCl concentration. The maximum color removal was found at 0% (99.78%) and lowest (52.97%) at 7.5% NaCl concentration after 72 h of incubation. The highest decolorization at pH 7 viz. 55.29%, 57.62% and 89.91% was found after 24, 48 and 72 h, respectively. Similarly, at 30 °C, the

decolorization percentage (34.06%, 78.60% and 80.74%) was estimated to be the highest after 24, 48 and 72 h, respectively (Fig. 2). An increasing trend of dye decolorization efficiency was measured with time of incubation.

Phytobeneficial potential of the strain FSS2C

Phosphate solubilization

Bacillus firmus strain FSS2C showed high potential to solubilize inorganic phosphate at various RB-5 concentrations. The highest phosphate solubilization (29.61 µg mL⁻¹) was measured when no dye was added in culture medium, but a significant amount of soluble phosphate viz., 22.6, 20.45, 9.14 µg mL⁻¹ was estimated even at 50, 100, and 250 mg L⁻¹ RB-5 in the culture medium, respectively (Table 1). No soluble phosphate was recorded when concentration of RB-5 was increased to 500 mg L⁻¹.

IAA synthesis

The strain FSS2C produced a considerable amount of IAA after 48 h (e.g., 19.74, 11.09 and 10.67 µg mL⁻¹ at 0, 50 and 100 mg mL⁻¹ RB-5 concentration in the presence of tryptophan, respectively) (Table 1). The strain was unable to synthesize IAA in the culture without tryptophan addition.

ACC deaminase activity and ammonia production

The strain FSS2C did not show any ACC deaminase activity under in vitro conditions. Moreover, it produced ammonia viz., 6.94, 5.75, 5.63 and 3.63 µmol mL⁻¹ at 0, 50, 100 and 250 mg L⁻¹ RB-5 concentration, respectively (Table 1).

Fitness study and recovery of strain FSS2C from wheat rhizosphere

Both the strains FSS2C_w and FSS2C_{rif} showed comparatively optimal growth in plate count method (Fig. S2) exhibiting the fitness of the strain to be inoculated in soil. Both the strains showed an optimal cell density (approx. 10 CFU mL⁻¹) at exponential phase in the growth curve. The population density of FSS2C_{rif} before cultivation was estimated to be 7.7 × 10⁷ CFU g⁻¹ of soil. The strain was successfully recovered from the wheat rhizosphere up to 30 days of growth at a population density of approx. 1 × 10⁶ CFU g⁻¹ of rhizosphere soil at 0 mg L⁻¹ RB-5 after 30 days of sowing. The population density of strain FSS2C_{rif} decreased with the increasing concentration of RB-5 and it was calculated as 3.9 × 10³ CFU g⁻¹ of rhizosphere soil at 500 mg L⁻¹ RB-5 after 30 days of sowing. Overall, the strain FSS2C_{rif} maintained an optimal population density in wheat rhizosphere up to a stress level of 250 mg L⁻¹ RB-5.

Fig. 1 Phylogenetic tree of *B. firmus* FSS2C with the type strains and BLASTn-matched strains of genus *Bacillus*. The evolutionary history was inferred using the Neighbor-joining method. The percentages ($\geq 50\%$) of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site

Fig. 2 Decolorization potential of RB-5 by bacterial strain FSS2C at different concentrations of NaCl (a), pH values (b) and temperature (c)

Table 1 Phosphate solubilization and IAA synthesis of strain FSS2C at various RB-5 concentrations

	RB 5 concentration (mg L ⁻¹)				
	0	50	100	250	500
Phosphate solubilization (μg mL ⁻¹)	29.61 ± 2.37	22.6 ± 32.19	20.45.67 ± 3.87	9.14 ± 3.87	–ve
IAA synthesis (μg mL ⁻¹)	19.74 ± 2.37	11.09 ± 3.91	10.67 ± 4.14	–ve	–ve
ACC deaminase	–ve	–ve	–ve	–ve	–ve
Ammonia production (μmol mL ⁻¹)	6.94 ± 2.15	5.75 ± 1.89	5.28 ± 1.19	3.63 ± 1.38	–ve

Each value is the mean of three experimental replications ± standard errors

Effect of inoculation of *B. firmus* strain FSS2C on wheat growth

The inoculated wheat plants showed significantly (Fisher's LSD; $P \leq 0.05$) improved growth characteristics as compared to non-inoculated plants at up to 100 mg L⁻¹ RB-5 stress (Fig. 3). A significantly higher root length

(2.987 cm) was measured in inoculated plants at 100 mg L⁻¹ RB-5 which was 14.75% greater than un-inoculated plants. Similarly, shoot length was also significantly higher in the inoculated plants at up to 250 mg L⁻¹ RB-5 concentration. The inoculation of *B. firmus* strain FSS2C significantly promoted the biomass of wheat plants under the RB-5 stress. Inoculated plants showed 49.46% and 62.37%

Fig. 3 Effect of strain FSS2C inoculation on various growth parameters of wheat under induced RB-5 stress

more root fresh and dry weights as compared to non-inoculated plants even at 250 mg L⁻¹ RB-5. The root and shoot lengths as well as the plant biomass significantly decreased with increasing RB-5 stress (Fig. 3). Moreover, a stress level of 500 mg L⁻¹ of RB-5 was found to have negative affect on bacterial growth and physiological potential and no comparative increase in plant growth was measured at this level.

Effect of inoculation of *B. firmus* strain FSS2C on wheat physiology

With the increasing concentration of RB-5 up to 500 mg L⁻¹, the lipid peroxidation showed an increasing trend (Fig. 4). However, the inoculation of *B. firmus* strain FSS2C reduced the oxidative damage caused by lipid peroxidation. At 100 mg L⁻¹ RB-5, the MDA of inoculated wheat plants was 26.33% lower than non-inoculated ones. The H₂O₂ and

Fig. 4 Oxidative stress-responsive species and antioxidants of wheat plants as affected by inoculation of strain FSS2C under induced RB-5 stress

proline contents also gradually increased with increasing dye concentration. The inoculated wheat plants showed significantly less H_2O_2 and proline contents (39.14% and 4.57%, respectively) at 250 mg L^{-1} RB-5. Conversely, the concentration of antioxidants (CAT and POD) significantly increased with the increasing dye concentration. The CAT and POD contents were significantly higher (20.35% and 18.58%, respectively) in inoculated wheat plants at 100 mg L^{-1} RB-5 (Fig. 4). Similarly, the total phenolic contents of inoculated wheat plants were significantly higher (24.12%) at 100 mg L^{-1} RB-5 contrary to control plants. The total phenolic contents were found to significantly increase with the increasing dye concentration in the soil (Fig. 4).

Effect of inoculation of *B. firmus* strain FSS2C on plant nutrient uptake

Inoculation of wheat plants with *B. firmus* strain FSS2C significantly increased the NPK content. The root N contents were found significantly higher (23.03%) in inoculated wheat plants at 250 mg L^{-1} RB-5 stress, while N contents in shoot were 10.80% higher in contrast to non-inoculated control plants at 100 mg L^{-1} RB-5. Similar trend was observed in plant P contents, where root P contents of inoculated wheat plants were 62.71% greater at 250 mg L^{-1} RB-5 stress, while shoot P contents were found 71.23% higher at 250 mg L^{-1} RB-5. The root and shoot K uptake was also significantly higher (16.86% and 77.08%, respectively) in wheat plants with *B. firmus* strain FSS2C inoculation at 100 mg L^{-1} RB-5, while shoot K contents of inoculated wheat plants was also significantly higher at 250 mg L^{-1} (Fig. 5).

Discussion

The taxonomic identification of strain was confirmed by analyzing its 16S rRNA gene sequence. The sequence similarity analysis with NCBI and RDP databases revealed the bacterial identity as *B. firmus*, whereas phylogenetic tree validated this result (Fig. 1). During the recent years, few bacterial strains of genus *Bacillus* have already been reported as efficient PGPR along with concurrent potential of remediation of pollutants including the textile dyes (Radhakrishnan et al. 2017; Mahmood et al. 2017).

The strain FSS2C showed a good potential to tolerate the presence of NaCl salt during decolorization of RB5 in the medium. This activity is valuable, because the presence of salts negatively affects the microbial growth and activity (Hussain et al. 2013; Mahmood et al. 2017). The strain considerably decolorized RB5 even in the presence of 7.5% of NaCl in the medium. This property of the strain is comparable with few other bacterial strains recently reported for azo dyes decolorization under induced salt

stress (Maqbool et al. 2016; Mahmood et al. 2017; Shahid et al. 2018c). However, relatively lower decolorization of RB5 in the presence of higher concentrations of NaCl might be due to the effect of NaCl either on growth of the strain FSS2C or on the functional enzymes involved in decolorization process (Khalid et al. 2012; Hussain et al. 2013; Abbas et al. 2016). Moreover, the strain FSS2C showed an optimal decolorization of RB5 at pH 7.0. Several previous studies have reported neutral to slightly alkaline pH values for optimum decolorization process (Hussain et al. 2013; Maqbool et al. 2016; Shahid et al. 2018c; Hafeez et al. 2018). The effect of pH on decolorization of RB5 might be because of the fact that growth, survival, and enzymatic activities (including dye decolorizing enzymes) of the organisms are pH sensitive (Johansson et al. 2011; Imran et al. 2015; Maqbool et al. 2016; Shahid et al. 2018c). Similarly, temperature is also one of the key factors for effective dye decolorizing ability of bacterial strains (Mate and Pathade 2012). In the present study, the strain FSS2C carried out an optimal decolorization of RB5 at 30°C (Fig. 2). This range of temperature is in accordance with a number of previous studies reporting an optimal decolorization of RB5 by the bacterial strains at temperature ranging from 25 to 35°C (Hussain et al. 2013; Mate and Pathade 2012; Chan et al. 2011). It should be noted that the site from where strain was isolated and is intended for has an average temperature of 25 – 30°C for most part of the year. Thus, the PGPR strain FSS2C can be considered as a promising biological agent for decolorization of azo dyes even in the presence of different abiotic stresses. However, there is a dire need to investigate the intermediate molecules of azo dye degradation and their environmental impacts.

One of the major PGPR traits of bacterial species is solubilization of soil-bound phosphate. The known mechanism of phosphate solubilization is rhizosphere acidosis by the release of organic acids (such as gluconic acid, citric acid, maleic acid and succinic acid etc.). They reduce the pH of rhizosphere micro-environment and release the cations (Ca^{+2} , Al^{+2} and Fe^{+2}) bound to soil phosphates (Trivedi and Sa 2008; Shen et al. 2011). The strain FSS2C solubilized a substantial amount of phosphate under in vitro conditions, which is comparable to some other studies (Akram et al. 2016; Alibrandi et al. 2018). In addition to phosphate solubilization, phytohormones and ammonia production is the key mechanism of direct plant-growth promotion (Goswami et al. 2014). The IAA is an important signaling molecule involved in root cell proliferation and elongation even in very minute concentrations (Glick 2014; Liu et al. 2018). The strain FSS2C synthesized substantial amount of IAA by tryptophan-dependent and tryptophan-independent pathways (Table 1). Interestingly, the strain is able to considerably solubilize phosphate and produce ammonia even at 250 mg

Fig. 5 Effect of strain FSS2C inoculation on wheat nutrient uptake under induced RB-5 stress

L^{-1} of RB5, while IAA synthesis was found up to 100 mg L^{-1} of RB5 concentration. Previously, Maqbool et al. (2016) reported a physiologically potent strain *Pseudomonas aeruginosa* strain ZM130 for phosphate solubilization and IAA synthesis in the presence of an azo dye (reactive yellow-2). The decrease in phosphate solubilization and IAA synthesis by the strain FSS2C at higher concentration might be due to the toxic effects of RB5 at higher concentrations as already reported in case of the strain ZM130 (Maqbool et al. 2016).

In this study, *B. firmus* FSS2C significantly improved wheat growth under RB-5-induced stress conditions as is evident from the root and shoot length and plant biomass (Fig. 3). A limited amount of the literature is available on the effect of any PGPR strain on plant growth under azo dye stress conditions. Khaliq et al. (2013) reported the increased biomass of tomato plants after *Pseudomonas fluorescens* (Q14) inoculation in the presence of azo dye containing wastewater. Similarly, Mahmood et al. (2017) reported the enhanced seed germination percentage and

plant length of mung bean in the presence of microbially decolorized congo red dye. The growth promotion might be due to better adaptation of the strain FSS2C under induced azo dye stress followed by nutrient mobilization and IAA synthesis. The intense colonization of the strain FSS2C might have resulted in the synthesis of phytohormone IAA, which enabled the plant to have better access to nutrients and water (Couillerot et al. 2011; Shahid et al. 2015).

The cellular increase of reactive oxygen species (ROS) with the increasing concentration of RB-5 up to 500 mg L⁻¹ was the indication of oxidative injury in plant cells (Nasraoui-Hajaji et al. 2012), which reduced the plant growth and biomass. The elevated lipid peroxidation in plant cells negatively affects the water and nutrient uptake from soil and many metabolic processes. The increased cellular antioxidant levels in inoculated plants might be attributed to some unknown signaling mechanisms that are activated as a result of bacterial inoculation (Fig. 4). It has already been established that PGPR induce stress tolerance in plants by triggering the cellular antioxidant levels (Shahid et al. 2018b; Younesi and Moradi 2014). Proline is a very important osmolyte involved in osmoregulation and stabilization of many other macromolecules (Curá et al. 2017). Decreased proline contents in inoculated wheat plants might be attributed to stress alleviation ability of inoculated strain FSS2C. The oxidative stress in plants is characterized by the increased production of superoxide (O₂⁻)/hydroxyl (OH⁻) radicals and hydrogen peroxide (H₂O₂) (Waszczak et al. 2018). In response, plant cells produce antioxidant (enzymatic and non-enzymatic) molecules to ensure the defense against ROS (Sun et al. 2018).

The decreased levels of NPK in wheat roots and shoots with increasing RB-5 concentration may be the consequence of increased Na⁺ and Cl⁻ ion uptake (Fig. 5). The inoculation of *B. firmus* FSS2C mobilized the nutrient from soil to roots and shoots as suggested by the increased NPK content of inoculated wheat plants. The increased nutrient contents of plants after bacterial inoculation has been reported earlier (Forni et al. 2017).

Conclusion

The *B. firmus* FSS2C, being the potent phytobeneficial strain, can be used as bio-inoculant in soils receiving textile effluents loaded with RB-5 to combat abiotic stress and promote plant growth. The strain showed prominent decolorization of RB-5 azo dye under salt stress conditions as well as helped wheat plants to resist against abiotic stress by boosting the oxidative repair mechanisms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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