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RESEARCH ARTICLE

# Phytoextraction of iron from contaminated soils by inoculation of iron-tolerant plant growth-promoting bacteria in *Brassica juncea* L. Czern

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## Abstract

Iron (Fe) is one of the essential micronutrients for all living organisms. Despite its abundance in most of the contaminated soil, it is usually in unavailable forms. The unavailable form of Fe could be mobilized to plants by the use of microorganisms. This study was carried out to show that the Fe-contaminated field soils could be used to accumulate Fe in the plant parts using bacterial inoculation. For this, from a set of bacterial isolates, four Fe-tolerant bacteria were selected and identified based on 16S rRNA gene sequencing. The Fe-tolerant bacteria belonged to the genus *Bacillus toyonensis* (MG430287), *Rhodococcus hoagii* (MG432495), *Lysinibacillus mangiferihumi* (MG432492), and *Lysinibacillus fusiformis* (MG430290). Screening of plant growth-promoting properties of these isolates revealed that all isolates were able to produce indole acetic acid (50.0–84.0 μg/ml), siderophore, and potassium solubilization (except *R. hoagii*). Pot assay using Fe-contaminated ((8.07–8.35 g $kg^{-1}$) soils River Directorate of India) revealed that Fe-tolerant bacteria enhanced the growth of *Brassica juncea* and its biomass. Besides the improved plant growth, the inoculated plants also showed an overall percentage increase in the uptake of iron in root, stem, and leaf (57.91–128.31%) compared with uninoculated plants. In addition to enhanced plant growth attributes, the isolates also improved the total chlorophyll content and antioxidant properties such as total phenol, proline, and ascorbic acid oxidase. Thus, the results clearly indicated that these isolates could be used as a bioinoculant to improve the sequestration of Fe from the contaminated soils and alleviation of Fe stress in plants.

**Keywords** Iron · *Brassica juncea* · Plant growth promotion · Siderophore · Antioxidants · Biofortification

## Introduction

Soil gets polluted because of unlimited increase in industrialization, urbanization, use of toxic chemical in the higher cop production, disposal of waste water, coal combustion residues, and spillage of petrochemicals (Zhang et al. 2010; Khan et al. 2008). The application of these chemicals is the main source of heavy metals. It pollutes soil with various toxic metals such as cadmium (Cd), chromium (Cr), lead (Pb), iron (Fe), mercury (Hg), copper (Cu), and zinc (Zn). Even though many of the metals are essential micronutrients for plant growth, but increase in concentration may be toxic to plants and other living organisms (Stein et al. 2009; Yang et al. 2013). It has been reported that in India, iron is found in upper permissible limit in many provinces (Bureau of Indian Standards (BIS) 1050 (2012)), which causes iron toxicity to plants because of extreme iron uptake in anaerobic soils (Stein et al. 2009; dos Reis et al. 2014). In aerobic soils, iron is in the form of ferric ($Fe^{3+}$) ions, which makes it limited for uptake by plants (Lemanceau et al. 2009).

The excess amount of iron from soils could be safely sequestered by microbe–plant interaction methods. Researchers from worldwide demonstrated the removal of iron from the soil by plant uptake using phytoextraction method (de Souza et al. 2015; Patel et al. 2018). Further increasing the iron concentration in staple food crops may reduce iron deficiency in plants as well as in human beings (Cakmak 2002). This could be achieved by the inoculation of plant growth-

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promoting (PGP) bacteria. It has been reported that inoculation of PGP bacteria produces a variety of phytohormones, which helps to alleviate abiotic stress in many plants (de Souza et al. 2015). In addition to phytohormones, bacteria producing siderophore further facilitate the uptake of iron from the soil to plants by producing several organic acids (Glick et al. 1999; Jin et al. 2014). Considering the importance of iron in plant and animal metabolism, the unavailable iron present in the soil could be biofortified into the plants by inoculation of PGP bacteria (Kabir et al. 2016; Kamran et al. 2017; Patel et al. 2018). Although Fe is considered an essential micronutrient, its presence beyond threshold limit leads to change in membrane lipid peroxidation, change in metabolism, and higher production and accumulation of reactive oxygen species (Paredes-Páliz et al. 2018). Inductions of many defense enzymes are reported by PGP bacteria in defense reaction against metal stress and its detoxification. The oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO) and other oxidative phenols contribute to the formation of defense barriers for reinforcing the cell structure along with inducing the systemic resistance of the plant (Gill and Tuteja 2010; Hernández et al. 2015). Numerous soil bacteria have been reported to exhibit heavy metal tolerance and can be used for mobilization or immobilization of metals in plants (Mishra et al. 2017; Banerjee et al. 2018). Most of these experiments are carried out under artificially contaminated soils; only a few experiments were conducted in the contaminated field soils.

Therefore, the present study aimed to study (1) isolation and characterization of iron-tolerant bacteria, (2) influence of iron-tolerant bacteria on iron uptake in *Brassica juncea* plant parts from the contaminated field soils, and (3) influence of iron-tolerant bacteria on the physiological properties of *B. juncea* under contaminated soils.

## Materials and methods

### Soil sample collection and isolation of bacteria

The collection of soil samples was carried out from the nine places along the Tapi River, Surat (21.17° N, 72.83° E), for isolation of bacteria. The physicochemical properties and heavy metal concentration of the soils were analyzed as per the standard procedure (Sparks et al. 1996) using colorimetry, flame photometry, and atomic adsorption spectrophotometry. For the isolation of bacteria, 10-g soil samples were serially diluted and aliquot of 1 ml was spread on nutrient agar (NA) medium. After 24 h, the colonies that appeared on the plates were selected based on color, shape, and size. The isolates were purified by continuous streaking and maintained at 4 °C in 20% glycerol stock. The subsequent experiments were carried out raising fresh culture from the glycerol stock.

### Screening for iron-tolerant bacteria

Screening of iron-tolerant bacteria was carried out in NA amended with different concentrations of $FeSO_4$ (0.1, 0.2, 0.3, and 0.4% (w/v)). On the basis of the screening, the four isolates that showed maximum tolerance to 400 mg/ml were selected for the further experiments.

### Plant growth-promoting properties of iron-tolerant bacteria

Iron-tolerant bacteria were screened for their plant growth promotion ability such as solubilization of phosphate and potassium, and production of siderophore and indole acetic acid (IAA). Briefly, phosphate solubilization capabilities were identified on Pikovskaya medium amended with 0.5 g (100 ml) tricalcium phosphate (Verma et al. 2001). The appearance of a clear zone around the colony indicates solubilization of inorganic phosphate. For checking potassium solubilization capacity, Aleksandrov medium amended with mica powder (1 g/100 ml) was used (Aleksandrov et al. 1967). A clear zone around the bacterial colony represents potassium solubilization. Siderophore production was carried out in chrome azurol S (CAS) agar plates. For this, NA plate was amended with CAS dye (60.5 mg), iron III solution ($FeCl_3.H_2O$ 1 mM), and hexadecyl trimethyl ammonium bromide (72.9 mg). The bacterial cultures were streaked and incubated for 3–5 days. The appearance of a yellow orange zone around the colony indicates production of siderophore (Schwyn and Neilands 1987). IAA production was carried out in the nutrient broth amended with L-tryptophan (200 mg/100 ml). The inoculated broth was kept for 1 week for the analysis of IAA production. After incubation, the supernatant was collected and added of 2 ml Salkowski reagent (50 ml, 35% perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). The mixture was incubated at room temperature for 20 min and the optical density was measured at 535 nm using a spectrophotometer. The concentration of IAA production was calculated using an IAA standard graph and production of IAA by each isolate expressed in μg/ml (Brick et al. 1991).

### Identification of bacteria by 16S rRNA gene sequencing

The bacteria showing iron-tolerant property were selected and identified by 16S rRNA gene sequencing. The bacterial DNA was isolated as per standard procedure (Sambrook et al. 1989). Amplification of partial 16S rRNA gene was carried out in 50 μl PCR reaction mixture with the standard thermocycler condition using the primer pair of 704F (5′-GTA GCG GTG AAA TGC GTA GA-3′) and 907R (5′-CGT CAA TTC MTT TRA GTT T-3′) (Patel et al. 2017; Bhaduri et al. 2018). The PCR products were resolved in 2% agarose gel and purified.

The purified products were sequenced at the gene sequencing facility available at Gujarat State Biotechnology Mission (GSBTM), Gujarat. The obtained sequences were BLASTn-analyzed using reference sequences available at NCBI, GenBank (Altschul et al. 1997). The analyzed sequences were submitted to GenBank at NCBI (https://www.ncbi.nlm.nih.gov/genbank/), and identified cultures were submitted under Bank a Bug program in GSBTM, Gujarat (https://btm.gujarat.gov.in/gujarat-biodiversity-gene-bank-biogene.htm).

## Growth promotion and uptake of iron from contaminated soil

The effect of iron-tolerant bacteria on plant growth promotion and iron mobilization and induction of antioxidant properties in response to stress were studied in contaminated soils brought from Tapi River, Surat. The soil samples were sterilized by autoclaving at 121 °C for 15 min for 3 consecutive days. The experiment was carried out in pot culture under greenhouse conditions. Before inoculating the seeds into the soil, the Indian mustard seeds (*B. juncea*) were surface-disinfected with 2% sodium hypochlorite for 2 min, subsequently 70% ethanol for 2 min, and washed several times with sterile distilled water. The seeds were blot-dried and soaked in fresh inoculum of iron-tolerant bacteria with concentration of $10^8$ (CFU/ml) for 2 h. After 2 h, the seeds were inoculated in pots filled with 2 kg iron-contaminated soil. The seeds soaked in distilled water served as control. Pots were arranged in the completely randomized factorial design. For each treatment, three replicates were used for the experiment. The pots were maintained in greenhouse conditions at 30–35 °C and 58% relative humidity under a day/night cycle of 13/14 h. The pots were watered on a regular basis to maintain the moisture content. After 1 month of seedlings, *B. juncea* plants were harvested for the measurement of root and shoot length, dry and wet weight of stem, and root. Quantification of iron from mustard plant tissues of root, stem and leaf, and soil samples was carried out using an atomic absorption spectrometer (Freitas et al. 2004).

## Influence of iron-tolerant bacteria on physiological properties of *B. juncea*

### Chlorophyll estimation

Total leaf chlorophyll was estimated as per the method described by Hiscox and Israelstam (1979). Acetone (80%)-extracted compounds from the leaf were analyzed using the spectrophotometer at 645 and 663 nm. Total chlorophyll was calculated using the formula (20:2($A_{645}$) + 8.02 ($A_{663}$) × (*V*/1000 W). Where $A_{645}$ and $A_{663}$ are absorbance of chlorophyll extract at 645 and 663 nm, *V* the total extract volume, and W the fresh weight of the leaves expressed in milligrams per gram.

### Proline estimation

Estimation of proline was carried by homogenizing 0.5 g of fresh leaves in 3% sulfosalicylic acid. The content was centrifuged and an equal amount of glacial acetic acid and ninhydrin was added to the supernatant. The reaction mixture was boiled at 100 °C for 30 min. The reaction mixture was cooled in an ice bath and 4 ml toluene was added with stirring the mixture for a short time. The proline content was measured at 520 nm using toluene as a blank. The proline content was expressed as micromole per gram tissue (Bates et al. 1973).

### Phenol estimation

Total phenolic content was estimated by mixing methanolic extract of leaf (0.5 ml), 10% (v/v) Folin–Ciocalteu reagent (2.5 ml), and 7.5% $Na_2CO_3$ (2 ml). The content was heated for 40 min at 45 °C, and the absorbance was measured at 765 nm. The phenol content was expressed in milligram of gallic acid equivalent to per gram extract using the gallic acid as a standard (Kumazawa et al. 2004).

### Estimation of ascorbic acid oxidase

Ascorbic acid oxidase (AAO) was measured in 500 mg mustard leaf tissues homogenized in 10 ml ice-cold 0.1 M potassium phosphate buffer. The mixture was centrifuged and 3 ml l ascorbic acid was added to the supernatant prior to the measurement of the AAO. The absorbance was measured at 265 nm and expressed in 1 μmol ascorbic acid per minute at 25 °C (Oberbacher and Vines 1963).

## Statistical analysis

The data presented in the study are the mean of three replicates ± S.E. The statistical analysis of the data was carried out using SPSS software (version 16.0). To meet the assumptions of normality, the data were transformed prior to the analysis of the data. Duncan's multiple range test was used to determine the significant variation among the treatments at the 5% level of significance.

# Results and discussion

## Sample collection and isolation of iron-tolerant bacteria

Nine soil samples were collected from the different places of Tapi River, Surat. The physiochemical analysis of the soils

**Table 1** Physico-chemical properties of soil samples collected from Tapi river regions

| Properties | Value |
|---|---|
| pH | 7.50–8.28 |
| Electric conductivity (EC) | 0.42–6.15 |
| Phosphorus ($P_2O_5$) (mg $kg^{-1}$) | 19.0–126.0 |
| Potash ($K_2O$) (mg $kg^{-1}$) | 59.0–1111 |
| Magnesium (Mg) (mg $kg^{-1}$) | 8.4–52.8 |
| Sulfur (S) (mg $kg^{-1}$) | 215.0–1389 |
| Total nitrogen | 75.0–200.0 |
| Zinc (Zn) (mg $kg^{-1}$) | 70.0–87.0 |
| Copper (Cu) (mg $kg^{-1}$) | 103.0–123.0 |
| Iron (Fe) (g $kg^{-1}$) | 8.07–8.35 |
| Cobalt Co (mg $kg^{-1}$) | 38.0–51.0 |
| Nickel (Ni) (mg $kg^{-1}$) | 50.0–95.0 |
| Chromium (Cr) (mg $kg^{-1}$) | 122.0–206.0 |
| Lead (Pb) (mg $kg^{-1}$) | 6.0–9.0 |
| Cd (mg $kg^{-1}$) | < 0.01 |
| As (mg $kg^{-1}$) | < 0.01 |

showed the pH ranged from 7.50 to 8.28. Presence of different micronutrients in high concentration and heavy metals was also observed in the collected soil samples (Table 1). The concentration of Fe was found to be 8.07–8.35 g $kg^{-1}$ in the soil samples. The presence Fe in upper permissible limits (Bureau of Indian Standards) may lead to soil acidification and loss of availability of essential phosphorus and molybdenum. Fe is an essential micronutrient that is involved in many biological processes in plants (Kobayashi and Nishizawa 2012). In higher concentration, iron toxicity leads to oxidative stress in plants (Stein et al. 2009) and alters community structure, microbial diversity, and total biomass of the microorganisms (Khan et al. 2009). PGP bacteria contribute in alleviating iron toxicity in the contaminated soils. A total of 110 bacterial colonies recovered from the soil samples were screened for iron-tolerant capability. The results revealed that more than 85% of the isolates had tolerance of 1 mg/ml Fe concentration. The subsequent increase in Fe concentration resulted in the selection of isolates SBTS3, SBTS2, SKTS9, and SKTS29, which showed 4 mg/ml Fe concentration. Therefore, these isolates could be used to study the interaction between bacteria and plants in mobilizing Fe into the plants. Selection of culturable bacteria associated with plant growth promotion under stress condition is the prerequisite for improving tolerance against metal toxicity (Ambrosini et al. 2012).

## Identification and plant growth promotion properties

The 16S rRNA gene sequencing revealed that the isolates belonged to three genus (Table 2). The isolate SBTS3 was identified as *Bacillus toyonensis* (MG430287), SBS2 as *Rhodococcus hoagii* (MG432495), SKTS9 as *L. mangiferihumi* (MG432492), and SKTS29 as *Lysinibacillus fusiformis* (MG430290). The presence of this genus in the contaminated soils has been reported by many authors (Manchola and Dussan 2014; Kartik et al. 2016; Płociniczak et al. 2017). Screening of PGP properties of these isolates revealed that all isolates were able to produce IAA and siderophore. The isolate *L. mangiferihumi* showed maximum IAA production (84.0 μg/ml) followed by *B. toyonensis* (80.0 μg/ml), *R. hoagii* (58.0 μg/ml), and *L. fusiformis* (50.0 μg/ml) (Table 2). None of the isolates were found to be positive for phosphate solubilization. However, all isolates except *R. hoagii* were found positive for potassium solubilization in plate assay.

Identification of iron-tolerant bacteria with siderophore production may facilitate the iron mobilization from soil to plant of siderophore (Freitas et al. 2015). Further, siderophore promotes the IAA synthesis by chelating $Fe^{3+}$, enabling them to enhance plant growth and protecting the phytohormone from degradation (de Souza et al. 2015). Identifying such bacteria along with metal-tolerant and PGP properties may help in alleviating metal stress.

## Growth promotion and uptake of iron from the contaminated field soils

The inoculation of iron-tolerant PGP bacteria showed enhanced root and shoot length of *B. juncea* in the contaminated

**Table 2** Identification and PGP properties of iron tolerant bacteria

| Identification | | | | PGP growth promoting properties | | | |
|---|---|---|---|---|---|---|---|
| Isolate name | BAB ID* | Genera identified | Accession number | IAA (μg/ml) | Siderophore production | Phosphate solubilization | Potassium solubilization |
| SBTS 3 | BAB 6882 | *Bacillus toyonensis* | MG430287 | 80 | + | − | + |
| SBTS 2 | BAB 6881 | *Rhodococcus hoagie* | MG432495 | 58 | + | − | − |
| SKTS 9 | BAB 6875 | *Lysinibacillus mangiferihumi* | MG432492 | 84 | + | − | + |
| SKTS 29 | BAB 6880 | *Lysinibacillus fusiformis* | MG430290 | 50 | + | − | + |

*Cultures are preserved at Gujarat Biotechnology Mission under Bank a Bug Program; +, positive; −, negative

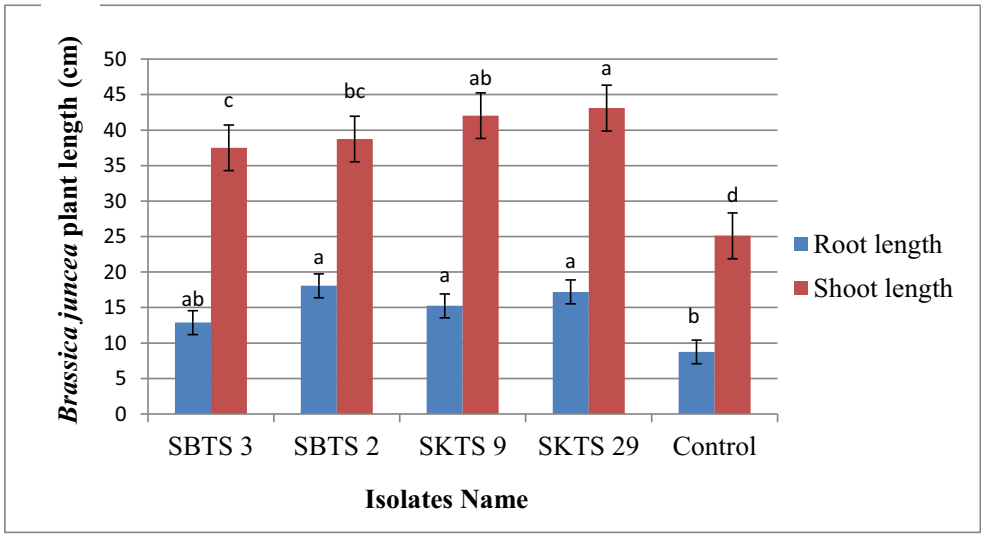


**Fig. 1** Root length and shoot length of *Brassica juncea* under contaminated field soils. Values are means ± S.E. ($n = 3$). Data were analyzed using two-way ANOVA and treatment means were compared

field soils compared with control. The inoculated strains increased the root length from 47.1 to 106.4% and shoot length from 49.40 to 71.71% (Fig. 1). It has been reported that the production of auxins by PGP bacteria can positively influence the regulation of the cell division and the activities of nutrient accumulation and metabolism, which improve plant growth activity (Kong and Glick 2017). All the isolates significantly enhanced *B. juncea* growth compared with the control in natural contaminated soil ($p < 0.05$). The same trend was also observed in the dry biomass of shoot and root of inoculated plants compared with noninoculated plants (Fig. 2). This result is in agreement with previous reports of many researchers that inoculation of metal-tolerant bacteria enhances plant growth parameters under stress condition (Freitas et al. 2015; Kartik et al. 2016). It is well known that iron is an essential micronutrient for the plant growth and several plant metabolisms (Kobayashi and Nishizawa 2012). However, in aerobic condition, the uptake of iron in plants is limited due to the ferric ions ($Fe^{3+}$), which leads to poor iron solubility (Lemanceau et al. 2009). Whereas in anaerobic and the acidic soil condition, formation of more $Fe^{2+}$ from $Fe^{3+}$ leads to iron toxicity due to more uptake (Stein et al. 2009). This condition could be improved by inoculation of PGP bacteria possessing iron-tolerant properties along with the ability to release phytohormones. In this study, all the isolates able to produce IAA may be responsible for improving plant growth under iron stress. Many researchers also reported that enhancement of plant growth might be due to production of several phytohormones under stress condition (Ma et al. 2009; Islam et al. 2015; Shaikh and Saraf 2017).

Besides promoting plant growth and biomass, the inoculated bacteria also improved the uptake of iron from the soil (Fig. 3). The uptake of iron from the contaminated soil was correlated with the concentration of iron in the soil (8.07–8.35 g $kg^{-1}$). The accumulation of iron was higher in leaf followed by root (except SBTS2) and stem. The increased amount of iron in leaves may be due to induced transpiration of iron into leaves once absorbed by the root system (Freitas et al. 2015). The overall uptake of iron ranged from 1.22 to 1.77 g in the inoculated plants compared with the control plants (0.77 g). The overall percentage

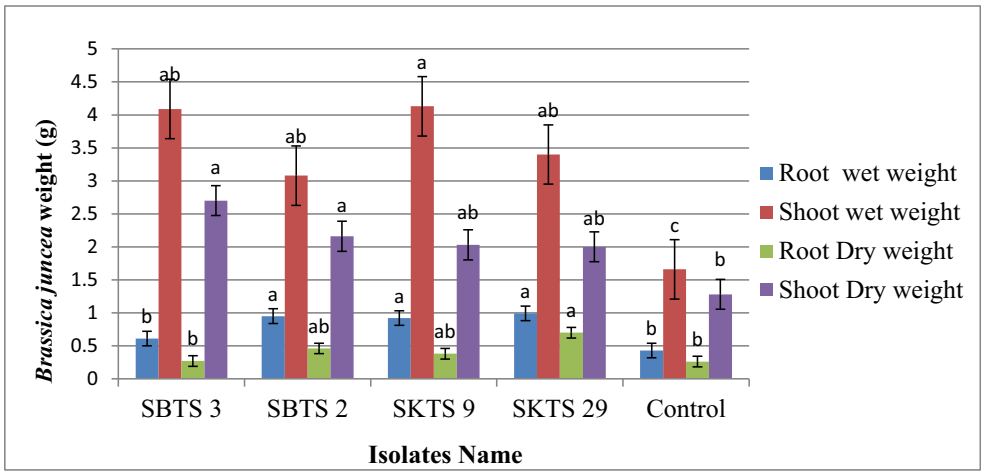


**Fig. 2** Effect of iron stress and PGP bacteria inoculation on *Brassica juncea* root and shoot dry and wet weight. Values are means ± S.E. ($n = 3$). Data were analyzed using two-way ANOVA and treatment means were compared

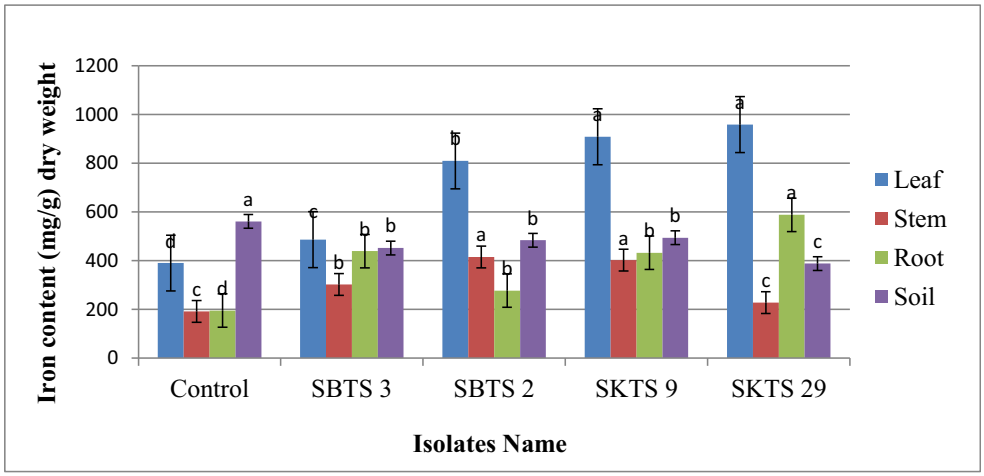


**Fig. 3** Effect of PGP bacteria on iron contaminated field soils on the uptake of iron by *Brassica juncea* root, shoot, and leaf grown for 30 days. Error bars are standard deviation

increase in the uptake of iron in root, stem, and leaf ranged from 57.91 to 128.31% of the inoculated plants compared with the uninoculated plants. It has been reported that Indian mustard (*B. juncea*) is a naturally hyper-metal-accumulating plant (Weerakoon and Somaratne 2009; Rajkumar et al. 2013). However, along with its hyperaccumulating nature, iron accumulation was further enhanced with the application of PGP bacteria. That resulted in agreement with Li and Wong (2012) whose experiments with hyperaccumulating plant *Sedum alfredii* revealed that inoculation with PGP bacteria reduced the Cd and Zn toxicity. Several authors also reported that inoculation of PGP bacteria helps plants to mitigate metal stress by enhancing their nutrient acquisition capacity (Islam et al. 2015; Freitas et al. 2015; Kartik et al. 2016). In this study, all the isolates were able to produce substantial amounts of IAA production that could have improved plant growth and reduced metal stress (de Souza et al. 2015). Further, siderophore production in the root region might enhance metal bioavailability in plants and consequently increase in metal uptake by producing organic acids in the soil (Sessitsch et al. 2013).

## Total chlorophyll production

The assessment of total chlorophyll production under stress conditions revealed that the inoculated plants showed elevated levels of chlorophyll content compared with the control plants (Fig. 4). The inoculation of isolate SBT3 (*B. toyonensis*) showed a significant increase ($p < 0.05$) in chlorophyll content followed by SKTS29 (*L. fusiformis*), SBTS9 (*L. mangiferihumi*), and SBTS2 (*R. hoagii*) (Fig. 4). It was interesting to note that the accumulations of iron content in the leaf of SBTS3-treated plants were lower compared with other treated plants. The overaccumulation of iron in the leaf of other treated plants might be responsible for lower chlorophyll content. Iron is an important nutrient for plants and is involved in several cellular processes, including chlorophyll biosynthesis (Kobayashi and Nishizawa 2012). Irrespective of iron content in the leaf, the inoculated plants had enhanced total chlorophyll. The results are in agreement with several results that inoculation of PGP

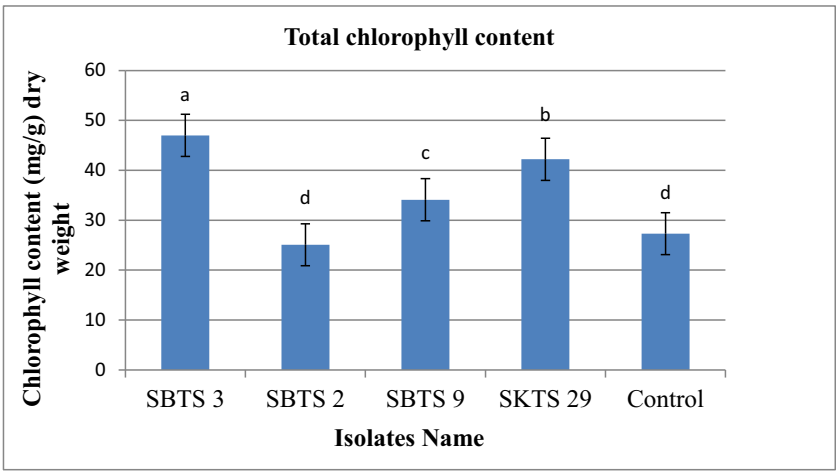


**Fig. 4** Effect of PGP bacteria under iron contaminated field soils on total chlorophyll content. Data are represented as average of three replicates. Data were analyzed using two-way ANOVA and treatment means were compared

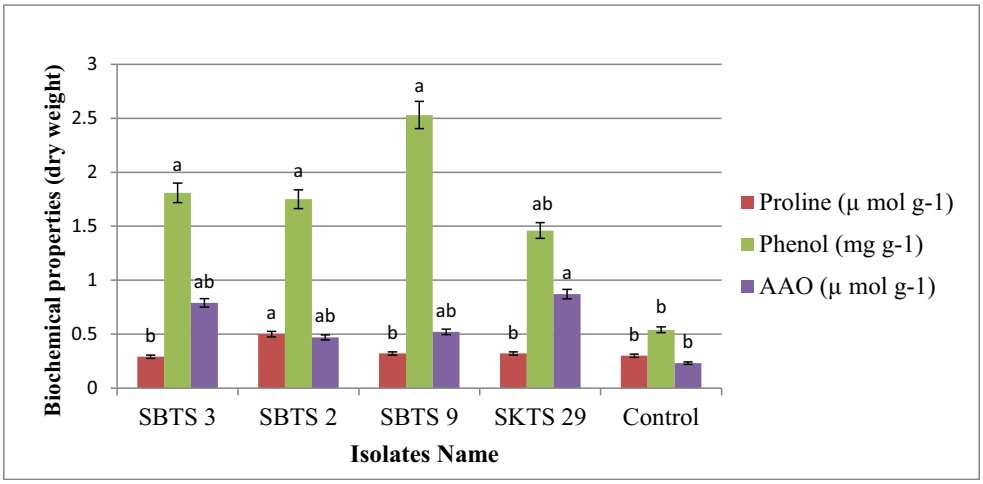


**Fig. 5** Effect of PGP bacteria on biochemical properties (total phenol, proline, and ascorbic acid oxidase) in *Brassica juncea* under iron contaminated soils. Values are means ± S.E. ($n = 3$). Data were analyzed using two-way ANOVA and treatment means were compared

bacteria improves chlorophyll synthesis under metal stress condition (Islam et al. 2015; Kartik et al. 2016; Shaikh and Saraf 2017).

## Influence of iron-tolerant bacteria on physiological properties

Inoculation of iron-tolerant PGP bacteria not only enhanced growth and phytoextraction of iron from the contaminated soils but also improved total phenol, proline, and AAO in *B. juncea* (Fig. 5). Accumulation of proline in the leaf samples of *B. juncea* was not significantly different from that of the inoculated (except SBTS2) and noninoculated plants. However, the production of proline indicates the plant's stress tolerance to the contaminated soil. It has been reported that proline has a major function as a metal chelator and an antioxidant molecule during stress (Singh et al. 2016; Dar et al. 2016).

In the treated plants, inoculation of iron-tolerant bacteria significantly ($p < 0.05$) improved biosynthesis of phenolic compounds from 170.37 to 368.51% compared with the control plants (Fig. 5). Numerous functions of phenolic compounds have been reported in plants. For example, the antioxidant nature of phenolic compounds inhibits lipid peroxidation and chelate metals (Michalak 2006). The increase in phenolic compounds may inactivate iron ions by suppressing reactive oxygen species (Rice-Evans et al. 1996; Arora et al. 2000). Similar induction of phenolic biosynthesis was reported in plants exposed to nickel, cadmium, and copper (Irtelli and Navari-Izzo 2006; Pawlak-Sprada et al. 2011; Ibrahim et al. 2017).

The results of AAO biosynthesis revealed similar enhancement in the inoculated plants compared with the control plants. The enhancement of AAO ranged from 104.34 to 243.47% in the treated plants (Fig. 5). The AAO is an important antioxidant molecule involved in enhancing the photosynthetic pigments, transpiration, and oxidative defense potential (Akram et al. 2017). Further, AAO triggers a number of functions in plants exposed to stress conditions (Akram et al. 2017). The enhancement of antioxidant molecules in inoculated plants might have played a trigger role in minimizing the iron stress, thereby increasing the plant growth and uptake of more iron. Additionally, induction of the synthesis pathway of polyphenols has been reported in plants cultivated on metal polluted soils. Moreover, inoculations with appropriate PGP bacteria induce the expression of phenylalanine ammonia lyase, the first enzyme in the polyphenols synthesis pathway (Paredes-Páliz et al. 2018).

## Conclusions

This study concluded that iron-tolerant bacteria alleviate iron stress and uptake of iron from the contaminated field soils. The data revealed that iron-tolerant bacteria not only promote the plant growth but also increase the uptake of iron into various parts of plants. Further, the bacteria also enhanced the biosynthesis of antioxidant molecules in protecting the plants against the stress. Considering the effect of iron tolerant bacteria in mitigating iron stress and enhancing iron content in the plant, these strains could be formulated as bioinoculants. Further research is aimed at the application of these bacteria in natural ecosystem for iron stress alleviation.

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## Compliance with ethical standards

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

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