Zinc solubilizing bacteria (*Bacillus megaterium*) with multifarious plant growth promoting activities alleviates growth in *Capsicum annuum* L.

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Abstract

The present study was designed to isolate an array of zinc solubilizing bacteria (ZSB) and to characterize them for plant growth promotion (PGP) attributes with respect to *Capsicum annuum* L. For this purpose, seventy bacteria were procured from cow dung and screened for zinc solubilization (ZnO and ZnCO₃). Where, isolate CDK25 was found to be the most potent owing to its maximum zinc solubilization (ZnO) ability (5.0 cm). For quantitative assay, atomic absorption spectroscopy (AAS) was used, where CDK25 showed markedly higher solubilization of ZnO (20.33 ppm). It was investigated that CDK25 also endowed with multiple PGP attributes viz., Phosphate solubilization, Phytase production, Indole acetic acid (IAA) and Siderophore production. Quantitative study revealed isolate CDK25 to solubilize and produce maximum quantity of phosphate (281.59 µg/ml) and IAA (13.8 µg/ml) respectively. ZSB was applied in different treatments under pot culture assay, where T3 (seeds + CDK25) showed significant impact on plant growth parameters, besides showing maximum zinc content in fruit (0.25 mg/100 g). Hence, isolate CDK25 expresses highest potential throughout the experiments; as zinc solubilizer, PGP strain, and based on 16S rRNA gene sequencing identified as *Bacillus megaterium*. Therefore, meticulous use of this bacterium could aid in providing adequate amount of soluble zinc along with enhanced plants growth, nutrient uptake and yield in sustainable manner.

Keywords Zinc solubilizing bacteria (ZSB) · Cow dung · *Capsicum annuum* L · *Bacillus megaterium* · PGP

Introduction

Zinc, one of the eight micronutrients obligatory by every living organism in small quantity, nevertheless indispensable for their proper growth and development. About 10% of enzymes require zinc as a cofactor viz., in tryptophan synthetase, an enzyme responsible for tryptophan synthesis in indoleacetic acid (IAA) biosynthesis (Hafeez et al. 2013). Plant requires zinc in the range of 5–100 mg/kg, but is critical for: membrane function, photosynthesis, protein synthesis, carbohydrate and auxin metabolism and influences the development of root, grain yield, uptake of water etc. (Tavallali et al. 2010). Plants can uptake zinc as divalent cation (Zn²⁺) in soluble form, but major amount of zinc in soil is present in insoluble fractions (Alloway 2008). Indian soils are deficient in zinc, affecting low crop production

(Sunithakumari et al. 2016). To overcome such problems, researchers suggested the use of efficient biofertilizers in place of chemical fertilizers. (Bakhshandeh et al. 2017). Application of zinc solubilizing microbial inoculants possessing plant growth promoting traits, influencing towards increment of crop yield. To enrich soil profile by enhancing its texture, supplementation of organic residues such as cow dung is being introduced, along with the use of microorganism inoculants also encourages accomplishment of required plant growth and soil health (Kloepper et al. 2004).

Capsicum annuum L. is one of the primary commercial crops, but cash value, consumption rate and yield is substantially low in India, inspite of the large production area (930,000 ha). This can be overcome by the use of efficient bacteria capable to fulfilling the requirements of essential minerals for plants. However, considering the above facts, selection and identification of effective zinc solubilizing bacteria is the first and foremost step in this study along with characterization of bacteria from cow dung possessing plant growth promoting (PGP) attributes, zinc solubilizing capability, and evaluation of growth parameters of *C.*

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annuum L. Thus, identification of an elite isolates capable of transforming insoluble fraction of zinc into soluble form will be an alternative tactic for alleviating zinc deficiency in plants and soil respectively.

Hence, the present study aimed to investigate cow dung inhabiting bacteria having multifarious benefits. Since, substantial usage of chemical fertilizers in sustainable agricultural systems, results in degradation of soil properties, there by disturbing soil microflora activity. As, livestock manure (cow dung) has numerous properties, because of which it has been in practice since ages. Application of which is indispensable alternatives to chemical fertilizers, which balances the soil properties for a long duration by slowly releasing the nutrients present in manure, thus maintaining the soil microflora (Golabi et al. 2004; Hue and Silva 2000; Fronning et al. 2008; Budaraga 2015; Gudugi 2013). Although, the potential of cow dung in improving soil quality is known to Indian sub-continental farmers for years, but a limited attention is paid on cow dung microorganisms in mediating the nutrients cycling in soil. Cow dung contains high levels of organic materials and various minerals viz., N, P, K, Ca, Na and Fe, P, Mn, Co, Mg and Cl (Devi et al. 2018), besides several microorganisms. The cow dung microorganisms, in particular, bacterial genera is dominated by *Bacillus*, revealed to act as a reservoir of most of the plant growth promoting bacteria, which influences plant biological growth (Swain and Ray 2009). Since, the requirements of biofertilizers are increasing for sustainable agriculture practices. As, due to worldwide large scale use of *C. annuum* L. the potential biofertilizers for this plant is important. So, our ideas facilitate the increased understanding of *C. annuum* L. associated bacteria.

Materials and methods

Enrichment, isolation and screening of zinc solubilizing bacteria (ZSB) from cow dung

Zinc solubilizing bacteria (ZSB) were isolated from cow dung by serial dilution method on petri plates possessing modified Bunt and Rovira media supplemented with 0.1% insoluble zinc sources viz., ZnO and ZnCO₃ were incubated at 30 ± 1 °C for about a week (Bhatt and Maheshwari 2019). Bacterial colonies possessing solubilization zone were screened, purified and enriched in Luria Bertani agar media.

Biochemical and molecular characterization

Gram staining of the unidentified zinc solubilizing cow dung isolates was achieved using the standard procedure described by Dubey and Maheshwari (2012). Biochemical tests were performed as per Bergey's Manual of Bacteriology (Holt

et al. 1994). The most promising isolate was selected and subjected to molecular characterization by 16S rRNA sequence analysis. For which, the genomic DNA was isolated according to Sambrook and Russel (2001) and amplification of 16S rRNA gene sequence was carried out by PCR using primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'. Sequence was aligned by Clustal W using MEGA7 software and identified using nucleotide BLAST search programme of GenBank database (NCBI). Phylogenetic tree was constructed using the neighbour-joining method by means of MEGA version Software.

In vitro assessment of zinc solubilization

All the bacterial isolates exhibiting zone of solubilization were further evaluated for zinc solubilizing ability for two insoluble zinc sources (zinc oxide and zinc carbonate). After 24 h the bacterial colonies were spot inoculated on Bunt and Rovira media plates having zinc sources and kept for incubation at 28 ± 1 °C for about a week (Bhatt and Maheshwari 2019). Bacterial colonies exhibiting larger solubilization zones were selected and the diameters of solubilization zone were recorded. Zinc solubilization index (SI) and solubilization efficiency (SE) was calculated according to Sadiq et al. (2014) and Sharma et al. (2014) respectively. The available zinc concentration of most potent bacterial culture solubilizing zinc was quantitatively assessed, at different time intervals viz. 5, 10 and 15 days using Atomic Absorption Spectroscopy (AAS), expressed in ppm.

SI—(Colony diameter – Halozone diameter)/colony diameter.

SE—Diameter of solubilization halozone/colony diameter.

Beside this, assessment of the acidic and alkaline conditions of the culture filtrates and the control (uninoculated) samples was also recorded at 5, 10 and 15 days of intervals after inoculation. For this, the pH was adjusted using 0.1 M (HCl and NaOH). Zinc solubilizing bacterial isolates was inoculated on the zinc supplemented medium and incubated at 37 ± 1 °C for solubilization.

Plant growth promoting (PGP) ability assays

All the bacterial isolates were evaluated for PGP abilities assays. Phosphate solubilization was assessed qualitatively as well as quantitatively as described by Pikovskaya (1948) and Yu et al. (2011) respectively. The phytase production by bacterial isolates was evaluated as described by Shao et al. (2015). IAA (Indole-3-acetic acid) production by bacterial isolates was determined qualitatively as well as quantitatively using Salkowski reagent as illustrated by Shao et al. (2015) and Palaniappan et al. (2010), respectively.

Siderophore production was estimated on CAS medium (Chrome-azurol S) as described by Schwyn and Neilands (1987).

Pathogenicity of isolates

The isolates possessing zinc solubilization ability was subjected for “hemolytic activity”, to check their pathogenic nature. For this purpose, the isolates were spot inoculated on blood agar plates and were incubated for 48–72 h at 37 ± 1 °C. The plates were checked for appearance of clear (β), greenish-brown (α), and no zones (γ), indicating complete, partial, and no hemolytic activity respectively (Russell et al. 2006).

Development of bacterial consortium

Zinc solubilizing isolates retaining the plant growth promotion abilities were analysed for their compatibility with each other, for formulation of bacterial consortium (Pandey et al. 2018). Isolates were grown in Luria Bertani broth independently, from which 100 μ l of each culture was spread on separate Luria Bertani agar plates. The plates were incubated at room temperature for about 15–20 min, followed by spot inoculation of the other isolates in each of the above plates, and incubated at 28 ± 1 °C for 24–48 h. Isolates with no zone of inhibition were detected to be compatible with each other, and considered as appropriate for the development of consortium.

Plant experiment and analysis of biological growth parameters

Based on initial screening of zinc solubilizing and plant growth promoting abilities, two bacterial isolates CDK15 and CDK25 were selected for evaluation of plant growth promotion of *C. annuum* L. Healthy seeds of *C. annuum* L. were bacterized as described by the method Weller and Cook (1983). Seeds were surface sterilized by 95% ethanol for 1 min followed by 4% NaOCl for 2–3 min, washed with distilled water and air dry in sterile conditions (Hameeda et al. 2006). Bacterial isolates (24 h old) were centrifuged at $10,000 \times g$ at 4 °C for 10 min. Pellets obtained were resuspended in sterile distilled water so as to obtain 10^8 cfu/ml of population density, thereafter mixed with 1% carboxy methyl cellulose (CMC). The slurry so obtained was coated on the surface of the seeds. The experiment was carried out in randomized block design with five seeds per pot (12"dia) having pre-sterilized field soil; each treatment was used in triplicate. Bacterized seeds of *C. annuum* L. were sown in pots with following sets of treatments, T1: Control (*C. annuum* L. seeds + without bacteria), T2: *C. annuum* L. seeds + CDK15, T3: *C. annuum*

L. seeds + CDK25 and T4: *C. annuum* L. seeds + Consortium (CDK15 + CDK25). Analysis of biological (vegetative and reproductive) plant growth promoting parameters viz., plant length, stem girth, number of branches, weight of shoot and root (fresh and dry), were carried out after 60 days of sowing (DAS). Fruit samples harvested after 120 DAS, from each treatment were further analyzed for Zn content. All the samples per treatment were finely crushed and 1.0 g of sample was thoroughly digested in 10 ml diacid mixture ($\text{HNO}_3\text{:HClO}_4$, 4:1 v/v) and diluted to 50 ml using distilled water. Furthermore, the mixture was heated at 70 °C and diluted using 50 ml distilled water. The extract obtained was filtered through a Whatman's filter paper (Jepkoech et al. 2013) and analysis of total zinc content in respective samples was evaluated by atomic absorption spectroscopy. All the data on different parameters were statistically analyzed.

Statistical analysis

The data recorded in the study presented in the means of three replicates with standard error ($\pm$ SEM). Data obtained in the present investigation were subjected to one-way analysis of variance (ANOVA), as described by Bhatt and Maheshwari (2019).

Results

About, seventy bacterial isolates were procured from cow dung. After primary screening, only eight (based on solubilization zone, solubilization index and amount solubilized) were capable of solubilizing zinc sources (ZnO and ZnCO_3), designated as: CDK6, CDK15, CDK25, CDK27, CDK59, CDK61, CDK63 and CDK70.

Biochemical characterization and molecular identification of the selected isolates

Based on morphology, gram staining and biochemical tests, cow dung bacterial isolate were characterized as per Bergey's manual of determinative bacteriology. Based on 16S rRNA sequence analysis, the most potent isolate CDK25 was identified was *Bacillus megaterium*. Nucleotide sequences have been submitted to the GenBank nucleotide sequence database under the accession number (MG774438). A nBLAST search for the 1446 bp 16S rRNA nucleotide sequence of isolate was phylogenetically 97% similar to *B. megaterium* ATCC 14,481 (Accession number NR113670) as shown in Fig. 1.

Fig. 1 Phylogenetic analysis of CDK25 isolate on the basis of 16S rRNA gene sequencing

In vitro assessment of zinc solubilization

Zinc solubilization potential of the bacterial isolates was evaluated by determining the zone diameter in plate assay. Among all eight isolates, exhibited varying degree of zinc solubilization in both zinc supplemented medium (ZnO and ZnCO_3). Maximum zone solubilization (5.0 cm) was exhibited by CDK25 followed by CDK15 (3.9 cm) in zinc oxide supplemented medium. On the contrary, zinc carbonate supplemented medium, exhibited similar solubilization by CDK25 (3.1 cm) followed by CDK15 (2.1 cm) as shown Table 1. Quantitative assessment of Zn solubilization revealed, maximum zinc solubilization occurred due to CDK25 (20.33 ppm) followed by CDK15 (14.23 ppm), in zinc oxide supplemented medium. Whereas, CDK15 (14.0 ppm) followed by CDK25 (12.0 ppm), in zinc carbonate supplemented medium, after 15 days of incubation. Solubilization of zinc is directly proportional to prolonged incubation of 15 days of time interval (Table 1 and Fig. 2a). The assessment of acidic and alkaline conditions on the Zn

solubilization revealed reduction of pH up to 4.5 to 5.0 from the initial pH – 7.0, after 15 days of incubation (DAI). Cell free culture supernatant of isolate CDK25 showed the lowest pH value of 4.5 followed by CDK15 with the value of 5.5 in case of zinc oxide. On the contrary, 5.0 and 5.5 by the same isolates respectively. The lowering of pH, indicate a higher acidity (Fig. 2b, c).

Plant growth promoting (PGP) ability assays

All the eight zinc solubilizing bacteria were further assessed for PGP ability (phosphate solubilization, phytase production, indole-3-acetic acid production and siderophore production). Among eight zinc solubilizing bacteria, maximum phosphate solubilization was observed by CDK25 (281.59 $\mu\text{g/ml}$) followed by CDK15 (264.04 $\mu\text{g/ml}$) after 8 days of incubation (Table 2; Fig. 3a). Out of eight, only three isolates (CDK6, CDK15 and CDK25) exhibited phytase production ability (Table 2; Fig. 3c).

Table 1 Zinc solubilization zone, solubilization index, solubilization efficiency and amount of zinc solubilized by inoculation of zinc solubilizing cow dung bacterial isolates

Isolates	Zone of solubilization (cm) 7DAI		Solubilization index (cm) 7DAI		Solubilization efficiency (%) 7DAI		Amount of solubilized zinc (ppm) 15DAI	
	ZnO	ZnCO ₃	ZnO	ZnCO ₃	ZnO	ZnCO ₃	ZnO	ZnCO ₃
CDK6	3.7±0.06	2.0±0	3.1±0.04	1.8±0	219.6±3.9	86.9±0	11.9±0.03	10.3±0.12
CDK15	3.9±0.03	2.1±0.03	3.0±0.01	2.1±0.01	207.0±1.7	112.2±1.7	14.2±0.03	14.0±0
CDK25	5.0±0.03	3.1±0.03	4.3±0.03	3.1±0.02	335.5±2.2	208.8±2.2	20.3±0.03	12.0±0.3
CDK27	3.6±0.03	2NAN	2.5±0.01	2.1±0.06	157.9±1.4	117.6±0.02	10.2±0.03	9.2±0.05
CDK59	3.4±0.03	1.9±0	2.7±0.07	2.1±0	165.0±1.5	90.4±0	10.8±0.06	8.9±0.06
CDK61	3.5±0.05	1.9±0	3.1±0.03	2.1±0.02	218.7±3.6	118.7±0	11.5±0.05	8.5±0
CDK63	3.5±0.05	1.7±0	3.3±0.05	2.1±0	231.3±5.6	100±0	9.7±0.1	6.5±0.06
CDK70	3.5±0.03	1.7±0	3.0±0.02	2.1±0	207.8±1.9	100±0	10.5±0.06	9±0

All the value is the mean of three replicative observations with ±SE

Fig. 2 **a** Assessment of amount of zinc solubilized (ppm), where ZO zinc oxide, ZC zinc carbonate. **b** Evaluation of solubilization efficiency and pH profiles of zinc solubilizing bacteria in zinc oxide,

after 15 days of incubation. **c** Evaluation of solubilization efficiency and pH profiles of zinc solubilizing bacteria in zinc carbonate, after 15 days of incubation

Table 2 Plant growth promoting attributes of potential zinc solubilizing cow dung bacterial isolates

Isolates	IAA	Sidero- phore production	Phosphate solubilization		
			Organic		Inorganic Pikovskaya's medium
			Phytase produc- tion		
			CP	SP	
CDK6	+	+	++	++	+++
CDK15	++	++	++	++	+++
CDK25	++	+++	+++	+++	+++
CDK27	–	+	–	–	++
CDK59	–	+	–	–	++
CDK61	–	+	–	–	++
CDK63	–	+	–	–	++
CDK70	–	+	–	–	++

IAA indole-3-acetic acid, CP calcium phytate, SP sodium phytate, + positive test/minimum, – negative test, ++ moderate, +++ maximum

In addition, the IAA production assay revealed that three zinc solubilizing isolates (CDK6, CDK15, and CDK25) exhibited the ability of IAA production, on addition of

Salkowski's reagent after 24–72 h incubation (Table 2). The maximum production was observed by CDK25 (13.8 µg/ml) followed by CDK15 (11.6 µg/ml), after 8 days of incubation (Fig. 3a). Out of eight zinc solubilizing bacteria, five of them (CDK6, CDK15, CDK25, CDK63, and CDK70) showed siderophore production, where maximum production was exhibited by CDK25 (Table 2 and Fig. 3b).

Pathogenicity of isolates

The bacterial isolates (CDK15 and CDK25), selected for plant experiment were found to be non-pathogenic as they did not display any haemolytic activity on blood agar medium, ensuring its safe use for further studies.

Plant experiment and analysis of biological growth parameters

Plant experiment results manifested that, among all the treatments, T3 (seeds treated with CDK25), were significantly better in terms of seed germination percentage, plant height, stem girth, number of branches, weight of shoot and root (fresh and dry), along with bio volume and vigor index, after 60 DAS. Furthermore, maximum fruits number was

Fig. 3 Assessment of zinc solubilizing bacteria for plant growth promoting attributes. **a** Effect of protracted incubation on phosphate solubilization. **b** Effect of protracted incubation on IAA production. **c** Venn diagram illustrating the PGP attributes along with zinc solubilization, *PS* phosphate solubilization, *PP* phytase production, *IAA* Indole acetic acid production, *SP* siderophore production. The Venn diagram was drawn using VENNY 2.0 (<https://bioinfo.cnb.csic.es/tools/venny/>)

Fig. 4 **a** Effects of different treatments on fruit yield and assessment of zinc content in fruits of *C. annuum* L., after 120 DAS (days of sowing). Each value is the mean of three replicates with bars representing $\pm$ standard error. Where, T1: Control (*C. annuum* L. seeds + without bacteria), T2: *C. annuum* L. seeds + CDK15, T3: *C. annuum* L. seeds + CDK25 and T4: *C. annuum* L. seeds + Consortium (CDK15 + CDK25)

also recorded in treatment (T3), after 120DAS (Fig. 4). Zinc content of fruits of inoculated plants showed significantly increment as compared to control (T1), after 120 DAS as represented in (Fig. 4). Interestingly, treatment (T3:

seeds + CDK25) showed the highest quantity of zinc with the value of 0.25 mg/100 g. So, the effects on biological (vegetative and reproductive) growth parameters varied depending on the treatments; in the order: T3 > T4 > T2 > T1 (Table 3).

Discussion

Zinc ameliorates crop productivity, besides providing nutritional security. Intervention like the practicing of soil microorganisms solubilizing unavailable zinc fractions, increases zinc uptake in plant but, exploitation of cow dung bacteria for mediating insoluble zinc fractions is least known. Application of zinc solubilizing bacteria can overcome the unavailability of zinc fractions thereby accomplishing the zinc solubilization. Although, several research has been reported, where a range of microorganisms have been procured from different habitat capable of solubilizing different zinc sources (Di Simine et al. 1998; Fasim et al. 2002; Gandhi et al. 2014; Vidyashree 2016; Dinesha et al. 2018). But little is known about the of cow dung microorganism with respect to zinc solubilization. On the contrary, a number of plant growth promoting microorganisms have been reported, capable of zinc solubilization (Ramesh et al. 2014; Fasim

Table 3 Effect of different treatments on the vegetative parameters of *C. annuum* L. (60DAS)

Labels	Treatments	Plant length (cm)	Stem girth (mm/plant)	No. of branches	Shoot weight/plant (g)		Root weight/plant (g)		Bio volume index (BI)	Vigor index (%)
					Fresh	Dry	Fresh	Dry		
T1	Untreated control	20.33 ± 0.23	12.12 ± 0.01	10.60 ± 0.22	12.65 ± 0.23	1.53 ± 0.03	1.7 ± 0.26	0.3 ± 0.03	246.39	948.59
T2	<i>C. annuum</i> L. seeds treated with CDK15	22.12 ± 0.87	12.21 ± 0.01	11.73 ± 0.41	20.52 ± 0.60	1.93 ± 0.08	2.1 ± 0.21	0.2 ± 0.03	270.08	1179.65
T3	<i>C. annuum</i> L. seeds treated with CDK25	27.84 ± 1.29	15.32 ± 0.01	14.43 ± 0.02	28.51 ± 0.3	2.6 ± 0.05	2.2 ± 0.15	0.4 ± 0.03	426.50	1855.81
T4	<i>C. annuum</i> L. seeds treated with Con-sortium (CDK15 + CDK25)	23.13 ± 0.27	13.30 ± 0.01	11.78 ± 0.45	19.65 ± 0.28	1.9 ± 0.20	1.0 ± 0.12	0.3 ± 0.05	334.22	1387.8

Values shown in table are mean of three replicates

et al. 2002; Abaid-Ullah et al. 2015). But this is the first report demonstrating the cow dung microorganisms as an excellent source of zinc solubilizer, plant growth enhancer and thereby increasing the zinc content of plant (fruit). In the present investigation, among eight bacterial isolates, CDK25 showed a remarkable solubilization potential for zinc oxide by producing clear halo zones, belonging to *Bacillus* genera. Solubilization of zinc is indispensable in nutrient cycling for better growth and development of plant. It can be accomplished by various mechanisms like production of organic acids, proton extrusion or production of chelating agents (Nahas 1996; Sayer and Gadd 1997). Results of the present study clearly indicated that the decrement in pH range from 7 to 4.5 and acidification of the media by CDK25 directly influenced the zinc solubilization mechanism. Similar to this was reported by Desai et al. (2012), where maximum zinc availability was directly related to low pH of the medium. As per our findings, isolate CDK25 exhibited the highest zinc solubilizing potential after 15 days of inoculation with a value of 20 ppm, in case of zinc oxide, indicating prolonged incubation leads to maximum solubilization. Maximum solubilization was observed in the case of zinc oxide; these results agree with earlier reports, where maximum solubilization was achieved in the zinc oxide supplemented medium (Pawar et al. 2015; Mishra et al. 2017).

Multitudinous researchers have illustrated that for the enhanced plant growth and development increments in certain factors, termed as plant growth promotion (PGP) attributes are inevitable. Those are indole acetic acid production, phosphate solubilization, siderophore production, nitrate production and HCN production (Ma et al. 2009; Dell'Amico et al. 2008; Barzanti et al. 2007; Idris et al. 2004). Present study exemplify that seed treated with PGP isolate CDK25 significantly increases the plant vegetative and reproductive growth parameters of *Capsicum annuum* L., later identified as *Bacillus megaterium*. Since, *B. megaterium* isolate was also observed to exhibit the maximum IAA production after 8 days of incubation with a value of 13.8 µg/ml, demonstrating enhancement in indole acetic acid production with prolonged incubation. IAA production by PGP bacteria, besides increasing cell elongation also encourages the growth of root hair and lateral roots in plants, resulting in availability of ample nutrients and water to plant (Davies 2010). Rajkumar and Freitas (2008) also observed that *P. jessenii* promoted *R. communis* plant growth by IAA. As an effective plant growth hormone, higher production of IAA is corresponding with enhanced seed germination and seedling growth parameters. IAA in certain quantity is adequate to produce physio-morphological changes in the young seedlings (Masciarelli et al. 2013; Talboys et al. 2014).

Our results also revealed that the isolate *B. megaterium* (CDK25) was also capable of solubilizing inorganic as well as organic phosphate. Maximum phosphate

solubilization was observed after 8 days of incubation with a value of 281.59 µg/ml, with an interesting fact that increased in the phosphate solubilization corresponding to prolonged incubation till 8 days. Ability to solubilize phosphate by plant growth bacteria could ameliorate uptake of phosphate by the host plant, resulting in increase in the vegetative and biological parameters. In addition to this, our study goes well with that of Dubey et al. (2014), where *Bacillus subtilis* BSK17 solubilizes inorganic phosphate and enhances the yield of *Cicer arietinum*. Moreover, the isolate was capable of producing siderophore which positively influences the plant growth parameter, by ameliorating availability of soluble iron to the *C. annuum* L., resulting in enhancement of its vegetative growth. Since, iron is one of requisite micronutrients required by number of plants for their proper functioning. Our study goes well with the study of Pandey et al. (2018), where *B. pumilus* and *B. subtilis* enhance the growth of amaranth by production of siderophore. Similar to this, Yu et al. (2011) also reported siderophore producing *Bacillus subtilis* CAS15 which enhances the growth of pepper. This increase in the nutrient by the cow dung microorganisms may help in balancing the nutrients in the zinc deficient soils with low crop productivity.

The plant experiment results also exemplify that, the seed bacterized with *B. megaterium* (CDK25), designated as treatment T-3 was found to be superior among all other treatments resulting in increased in various biological plant parameters viz. seed germination, plant height, stem girth, number of branches, fresh and dry weight of root and shoot, and fruit zinc content, as represented in Table 3 and Fig. 4. Similar findings were reported by Pandey et al. (2018), who reported enhancement in amaranth growth parameters by plant growth promoting bacteria *B. pumilus* and *B. subtilis*. Various PGP bacteria have been reported which enhances the planting value parameters in crops (Dubey et al. 2014; Chauhan et al. 2016). The enhancement in plant growth parameters and zinc content can mainly be associated with enhancement in PGP attributes viz. IAA production, siderophore production, phosphate solubilization (Ahmad et al. 2008; Ma et al. 2009). Moreover, several studies related to *B. megaterium* as a phosphate solubilizer, enhancing amino acid content and growth of mustard (Kang et al. 2014) from different habitat have already been reported. But to our best knowledge, this is the first report illustrating the role of cow dung bacteria (*B. megaterium*) as a zinc solubilizer, plant growth promoter and nutrient enhancer, suggesting it as an alternative to chemical fertilizers and also to fulfill the nutrient deficiencies in host plant (*C. annuum* L.), by transforming insoluble/unavailable zinc to the soluble/available one.

Conclusions

To our knowledge, this is the first report of zinc solubilizing plant growth promoting (PGP) cow dung bacteria. This work indicated the potential of zinc solubilizing cow dung bacteria for growth promotion of *C. annuum* L. Besides this, the increase in the nutrient by the cow dung bacteria may help in balancing the nutrients in the zinc deficient soils while enhancing crop productivity. The efficient cow dung isolate reported in this study could be formulated to alleviate zinc deficiency of *C. annuum* L. So, owing to zinc solubilization and plant growth promoting (PGP) ability of bacterial isolate, its application can fulfill the deficiency of zinc along with enhancing plants growth, leading to promising key for sustainable crop cultivation system.

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Author contributions KB assisted during the experiments and prepared the manuscript. DKM corrected and finalized the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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