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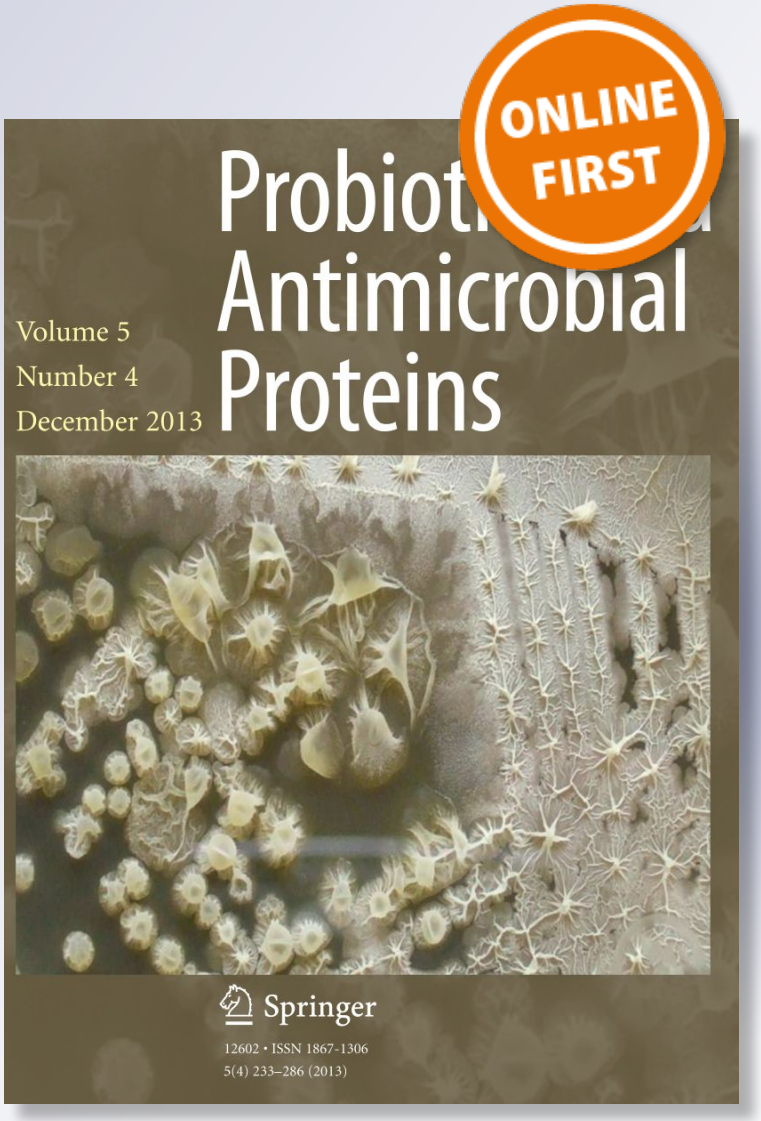
Probiotics and Antimicrobial Proteins

ISSN 1867-1306

Probiotics & Antimicro. Prot.
DOI 10.1007/s12602-018-9417-8



Springer

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https://doi.org/10.1007/s12602-018-9417-8

Plant Growth Enhancement, Disease Resistance, and Elemental Modulatory Effects of Plant Probiotic Endophytic *Bacillus* sp. Fcl1

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Abstract

Endophytic bacteria have already been studied for their beneficial support to plants to manage both biotic and abiotic stress through an array of well-established mechanisms. They have either direct or indirect impact on mobilizing diverse nutrients and elements from soil to plants. However, detailed insight into the fine-tuning of plant elemental composition by associated microorganism is very limited. In this study, endophytic *Bacillus* Fcl1 characterized from the rhizome of *Curcuma longa* was found to have broad range of plant growth-promoting and biocontrol mechanisms. The organism was found to have indole acetic acid and 1-aminocyclopropane-1-carboxylate deaminase production properties along with nitrogen fixation. The *Bacillus* Fcl1 could also inhibit diverse phytopathogens as confirmed by dual culture and well diffusion. By LC-MS/MS analysis, chemical basis of its antifungal activity has been proved to be due to the production of iturin A and a blend of surfactin compounds. Moreover, the organism was found to induce both plant growth and disease resistance in vivo in model plant system. Because of these experimentally demonstrated multiple plant probiotic features, *Bacillus* Fcl1 was selected as a candidate organism to study its role in modulation of plant elemental composition. ICP-MS analysis of *Bacillus* Fcl1-treated plants provided insight into relation of bacterial interaction with elemental composition of plants.

Keywords *Bacillus* sp. · Plant growth promotion · LC-MS · LC-MS/MS · ICP-MS · Lipopeptide antibiotics

Introduction

Plant-microbe interactions have been studied extensively at the rhizobacterial and endophytic level. This has made it possible to design tailor-made microbial formulations for enhancement of plant growth and disease resistance [1]. As agronomic practices are highly dependent upon the bulk use of chemical fertilizers and pesticides, generating natural alternatives have great significance to manage challenges associated with environmental pollution, soil fertility, and human health [2]. Even though microbial formulations have high demand for agricultural application, introduction of new organisms for the same is far beyond the required demand. Hence, exploring the diversity and mechanisms of microbes which are present within plants as endophytes is very significant [3, 4]. Here also, organisms which are present within special parts like seeds and rhizomes can have candidates with consistent plant probiotic features [5, 6]. As these organisms are biologically meant for vertical transmission, highly adapted plant beneficial mechanisms can be expected from them. Among these, microbes with multi-traits to assist biotic and abiotic stress management in plants can have promises for field applications [7]. Among various bacterial genera, *Bacillus* is one of the most common groups with endophytic association in various plants [8, 9].

Various *Bacillus* spp. have been reported to have highly specilized plant beneficial mechanisms [10, 11]. By IAA production, they have been demonstrated to modulate cell elongation, division, and differentiation in plants. By phosphate solubilization through the production of organic acids, they could release phosphorous in an available form to the plants. ACC deaminase of *Bacillus* origin has been suggested to regulate ethylene biosynthesis by enzymatically cleaving the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12602-018-9417-8) contains supplementary material, which is available to authorized users.

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Published online: 13 April 2018

ethylene precursor ACC to α-ketobutyrate and ammonia [12]. Also, *Bacillus* limits the availability of iron to pathogen through the production of siderophores. The antifungal mechanisms of *Bacillus* spp. are also remarkably significant as it involve production of structurally diverse and functionally unique chemical scaffolds [13]. Because of its plasticity towards plant beneficial features, many *Bacillus* spp. have been isolated from diverse plants as endophytes. Many endophytic *Bacillus* spp. have been reported to produce diverse antimicrobial compounds of lipopeptide family [10]. These lipopeptides generally include surfactin, fengycin, and iturin groups and are synthesized by non-ribosomal peptide synthetase enzymes [14]. Mechanistically, the binding of lipopeptides to the target membrane causes depolarization, membrane translocation, attack to intracellular components, and generation of leaky channels. Its multimode of action makes the resistance development to be difficult for the target organism [15].

Bacillus spp. are one of the commonly recruited endophytic organisms in plants. Even though most of its plant beneficial mechanisms are studied for specific objectives, these can have interrelated and synergistic effects. Hence, the network of events which are globally regulated by endophytic *Bacillus* within plants are highly fascinating. In the study, plant growth and biocontrol properties of endophytic *Bacillus* Fcl1 isolated from *Curcuma longa* have been demonstrated by both in vitro and in vivo methods. Experimental confirmation of broad phytointeractive properties of the isolate in non-host plant indicated its remarkable impact on plants. Hence, the *Bacillus* Fcl1 was taken as a candidate organism to study its influence on elemental modulation in plants by ICP-MS. The plant beneficial mechanisms of *Bacillus* Fcl1 and its in vivo function might have directly or indirectly be formed the basis of observed changes which make the study unique. However, the impact of this can be naturally determined by the synergistic effect of the microbial community.

Materials and Methods

Isolation and Identification of Endophytic Bacteria

Endophytic bacteria were isolated from rhizome of *Curcuma longa* after surface sterilization with 70% ethanol and 1% sodium hypochlorite followed by plating and incubation at room temperature for 24 h along with control. Genomic DNA isolated from purified bacteria was used for PCR amplification of 16S rDNA using the primers 16S F(5′-GAG TTT GAT CCT GGC TCA G-3′) and 16S R(5′-GAT ATT ACC GCG GCG CCT G-3′). The PCR product was further sequenced and the data thus obtained was used for similarity searching using Ezbiocloud. The phylogenetic analysis of the 16S rRNA sequence was conducted by MEGA 7 using neighbor-joining method with 1000 bootstrap replicates [16].

In Vitro Antagonistic Activity

The bacterial isolate Fcl1 was tested for antagonistic activity against *Rhizoctonia solani*, *Phytophthora infestans*, *Pythium myriotylum*, *Sclerotium rolfsii*, *Colletotrichum acutatum*, *Fusarium oxysporum*, and *Corynespora cassiicola* by dual culture [13]. For the large-scale production of antagonistic compound, Fcl1 was made to 3 L nutrient broth culture. The cell-free supernatant of this was acidified to pH 3 and was extracted twice with equal volume of ethyl acetate and the organic phase was subjected to evaporation in a rotary vacuum evaporator at 40 °C. Methanolic extract was prepared by dissolving the dried powder in methanol. *after evaporation*. The extract was further checked for activity against *P. myriotylum*, *C. cassiicola*, *R. solani*, *C. acutatum*, and *F. oxysporum* by well diffusion along with methanol control [17].

LC-MS and LC-MS/MS-Based Analysis for the Identification of Antifungal Compounds

The crude extract was filtered through a 0.22-μm syringe-driven filter (Nylon 66) and was subjected to LC-MS analysis [Acquity H-Class (Waters) ultra performance liquid chromatography with BEH C18 column (50 mm × 2.1 mm × 1.7 μm) and a Xevo G2 (Waters) and mass spectrometer]. Acetonitrile and water (1:9) were used as mobile phase with a flow rate of 0.2 mL/min. The electrospray ionization with a scan range of m/z 50 to 2000 in both positive and negative mode with scan time of 9 min was used. The source voltage, desolvation temperature, and capillary voltage were 135, 350 °C, and 4.50 kV respectively. The molecular ion mass obtained from LC–MS analysis was further subjected to LC–MS/MS analysis [17].

In Vivo Biocontrol Effect of Fcl1 on *Vigna unguiculata*

For in vivo studies, surface-sterilized seeds of *Vigna unquiculata* were allowed to germinate. The germinated seedlings were treated with 24 h grown culture of Fcl1 for 1 h and used for biocontrol studies along with control. In each set of study, 10 seedlings per set were used with triplicates. After 5 days of plant growth, 500 μL of spore suspension of *R. solani* was added at the base of plant and shoot tip and observed periodically for 3–5 days [18].

In Vitro and In Vivo Analysis for Plant Growth Promotion

Fcl1 was screened for IAA production by mixing 1 mL of the culture supernatant with 2 mL of Salkowski reagent (2 mL of 0.5 M $FeCl_3$ and 98 mL 35% $HClO_4$) followed by observing the color formed [19]. Nitrogen fixation property was screened by culturing Fcl1 on Jensen's media (sucrose 20 g/L, K_2HO_4 1 g/L, $MgSO_4$ 0.5 g/L, NaCl 0.5 g/L, $FeSO_4$ 0.1 g/

L, Na_2MoO_4 0.005 g/L, $CaCO_3$ g/L, and agar 15 g/L) [20]. For the phosphate solubilizing property screening, Fcl1 was cultured on Pikovskaya medium (glucose 10 g, $Ca_3(PO_4)_2$ 5 g, $(NH_4)_2SO_4$ 0.5 g, NaCl 0.2 g, $MgSO_4{\cdot}7H_2O$ 0.1 g, KCl 0.2 g, $FeSO_4{\cdot}7H_2O$ 0.002 g, yeast extract 0.5 g, $MnSO_4{\cdot}2H_2O$ 0.002 g, agar 20 g, d.H_2O 1 L, pH 7.4 ± 0.2) containing 2.4 mg/mL bromophenol blue [21]. ACC deaminase production analysis was conducted by culturing Fcl1 in DF salt minimal medium (KH_2P0_4 4 g, Na_2HPO_4 6 g, $MgSO_4{\cdot}7H_2O$ 0.2 g, $FeSO_4.7H_2O$ 0.1 g, H_3BO_3 10 μg, $MnSO_4$ 10 μg, $ZnSO_4$ 70 μg, $CuSO_4$ 50 μg, MoO_3 10 μg, glucose 2 g, gluconic acid 2 g, citric acid 2 g, agar 12 g, d.H_2O 1 L, pH 7.4 ± 0.2) amended with 2 g/L ammonium sulfate [7]. The isolate was cultured in peptone water (Peptic digest 10 g/L, NaCl 5 g/L, dH_2O 1000 mL pH 7.2) and to the culture 2–3 drops of Nessler's reagent (K_2HgI_4 and NaOH or KOH) was added to identify ammonia production [20]. Fcl1 was inoculated onto nutrient agar containing 4.4 g/L of glycine and HCN production was analyzed as per previous report [22]. Fcl1was also checked for protease, chitinase, and catalase production [23]. For in vivo study, surface-sterilized seeds of *V. unguiculata* were used. Germinated seedlings were bioprimed with overnight grown culture of Fcl1 for 1 h along with control in triplicates of 10 seedlings per set. After 7 days, the plants were collected and growth parameters such as shoot length, root length, and root number were measured [24].

ICP-MS-Based Elemental Profiling of Fcl1-Treated Plants

For ICP-MS analysis, the Fcl1-treated plantlets of *V. unguiculata* were dried in an oven at 65 °C for half an hour. Dried samples were powdered and aliquots of 0.1 g were digested with 8 mL HNO_3 (65%, supra pure) in a microwave digester. After that, it was made up to 25 mL with ultra pure water and stored in sterile vials. Samples for ICP-MS (Thermo fisher, iCAP, Q) analysis were then diluted to 10% acid. One milliliter from the digested sample was again diluted to 25 mL. After analysis, the concentrations of each element in ppb were converted to μg/mg using the formula (ICPMS result × 25 × 0.025) ÷ 0.1 [25]. The analysis was conducted in triplicates.

Statistical Analysis

The results were analyzed using statistical programme Origin Pro 7. One-way analysis of variance was used for comparison among the three groups. Post hoc multiple comparison test was used to determine the significant difference among groups. $P < 0.05$ was considered significant.

Results

Isolation and Identification of Bacterial Endophyte

The surface sterilization procedure used in the study was satisfactory as there was no microbial growth observed on control plate. The bacterial isolate selected for the study was designated as Fcl1. This was identified as *Bacillus* sp. (accession number: MH021186) by 16S rRNA sequence analysis (Fig. 1).

In Vitro Antagonistic Activity Assay

Bacillus Fcl1 showed antifungal activity to most of the selected phytopathogens (Fig. 2). The maximum mycelial inhibition was 53.33% towards *F. oxysporum*, followed by 52% to *C. cassiicola*, 49% to *P. myriotylum*, 41.66% to *R. solani*, and 33.33% to *C. acutatum*. The restricted mycelial growth of phytopathogens indicated the potential of endophytic *Bacillus* Fcl1 to produce various antifungal compounds. Further, the methanolic extract of *Bacillus* Fcl1 also showed inhibition of *R. solani*, *P. myriotylum*, *C. acutatum*, *F. oxysporum*, and *C. cassiicola*. This further confirmed the presence of antagonistic compounds in the extract made from *Bacillus* Fcl1 (Fig. S1).

Identification of Bioactive Antagonistic Compounds by LC-MS and LC-MS/MS Analysis

LC-MS analysis revealed the presence of antifungal compounds of iturin A and surfactin group in the extract from *Bacillus* Fcl1. Presence of Iturin A was indicated by m/z 1001.4128 which was further confirmed by the specific fragmentation pattern 230.1033, 382.2538, 506.2712, 643.2451, 811.3624, and 915.3884 (Fig. 3). LC-MS/MS-based analysis of surfactin derivative C_{13} yielded the $M+H^+$ 1008.6602 with fragmentation pattern of 990, 895.5737, 877, 685.4493, and 441.2703. The surfactin C_{14} derivative ($M+H^+$:1036.6932) yielded the fragmentation pattern such as 923.6058, 685.4498, 596.4261, 554.3540, 483.3424, and 441.2700. Also, surfactin C_{16} derivative ($M+H^+$: 1050.7067) yielded the peaks at 937.6205, 824.5306, 685.4490 610.4414, 554.3541, and 441.2699 (Fig. 4). This indicated the capability of *Bacillus* Fcl1 to be explored as a biocontrol agent. As fungi are major phytopathogens, the observed result can be indication of biosynthetic specialization in endophytic *Bacillus* Fcl1 to act against fungal pathogens.

In Vivo Biocontrol Effect of Fcl1 in *Vigna unguiculata*

During in vivo biocontrol studies, the *Bacillus* Fcl1-treated plants of *V. unguiculata* were found to have elicited induced systemic resistance, as evidenced by the resistance to fungal pathogen *R. solani*. In the case of control plants, damping off

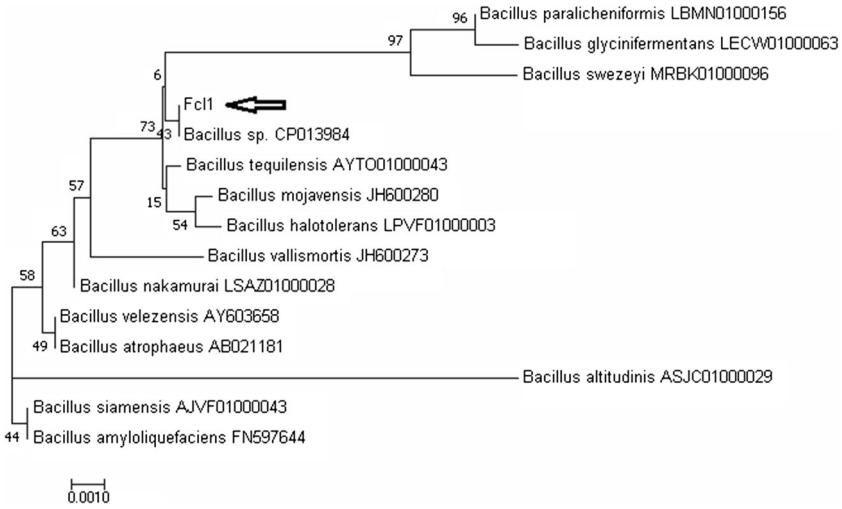

Fig. 1 Phylogenetic analysis of partial 16S rRNA sequence of the bacterial isolates from *Curcuma longa* along with other sequences from NCBI using MEGA 7 neighbor-joining method with 1000 bootstrap replicates

were observed and bioprimed plants were found to have increased shoot length with more number of leaflets in addition to resistance to infection (Fig. 5).

Plant Growth-Promoting Properties

The culture supernatant of *Bacillus* Fcl1 was positive for IAA due to the color change when treated with Salkowski reagent. Growth of *Bacillus* Fcl1 on DF salt minimal medium indicated the production of ACC deaminase. The bacterial isolate was also positive for nitrogen fixation. During assessment of enzymatic activity, *Bacillus* Fcl1 was found to be positive for protease and catalase activity but negative for HCN production. Surface-sterilized seedlings of *V. unguiculata* when treated with *Bacillus* Fcl1 showed significant enhancement in shoot length, root length, and root numbers when compared with the control (Fig. S2). The result obtained was statistically significant and hence proved the ability of *Bacillus* Fcl1 to enhance plant growth (Figs. S3a and S3b). When treated with *V. unguiculata* seedlings, *Bacillus* Fcl1 caused significant enhancement in shoot length (17.42 ± 1.38 cm), root length (8.66 ± 2.65 cm), and root number (12 ± 0.35). The same for distilled water control (shoot length 11.88 ± 1.07 cm, root length 2.35 ± 0.93 cm, root numbers 8 ± 1.50) and nutrient broth control (shoot length 11.97 ± 1.58 cm, root length 2.54 ± 0.62 cm, root numbers 8 ± 1.50) clearly confirmed the plant growth-promoting potential of *Bacillus* Fcl1 (Table 1). The statistical analysis showed result as significant and hence confirmed the plant growth enhancement effect of *Bacillus* Fcl1.

Elemental Profiling of Fcl1 Treated *Vigna unguiculata* by ICPMS

The macro and microelements of *Bacillus* Fcl1 treated plants were profiled by ICP-MS. The result indicated *Bacillus* Fcl1

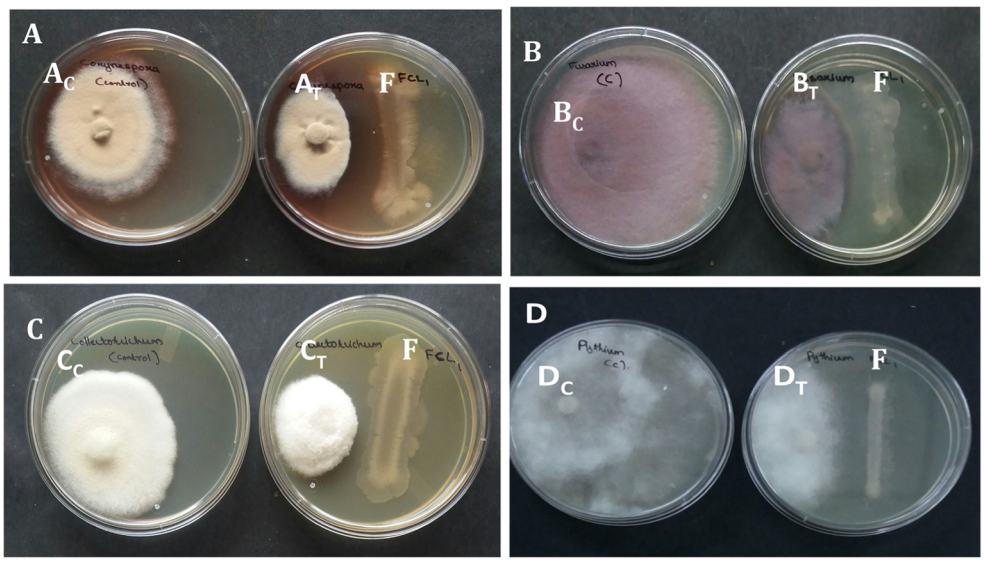

Fig. 2 Antifungal activity of isolated endophytic bacteria *Bacillus* Fcl1 against selected phytopathogens. A_C—*Corynespora cassiicola* control, A_T—*Corynespora cassiicola* test, B_C—*Fusarium oxysporum* control, B_T—*Fusarium oxysporum* test, C_C—*Colletotrichum acutatum* control, C_T—*Colletotrichum acutatum* test, D_C—*Pythium myriotylum* control, D_T—*Pythium myriotylum* test, F—Fcl1

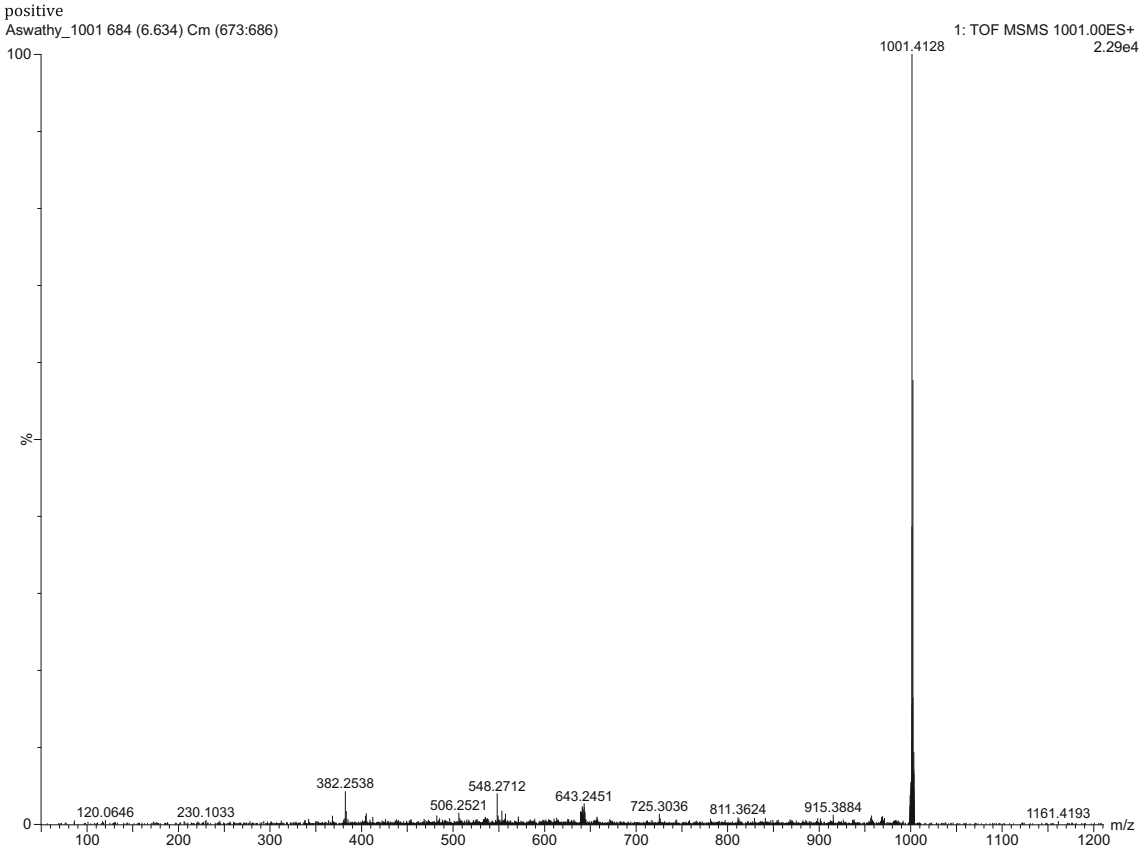

Fig. 3 LC-MS/MS-based fragmentation analysis of iturin A (m/z 1001.4128) from methanolic extract of *Bacillus* Fcl1

to have modulatory effect on elemental composition of *V. unguiculata.* The analysis of macroelements indicated enhancement of phosphorus, calcium, and magnesium content in *Bacillus* Fcl1-treated plants. However, potassium was slightly low when compared to control. In the case of microelements, aluminum content in *Bacillus* Fcl1-treated plants was higher when compared to control. The iron content was also slightly higher whereas the amount of zinc was observed to be lower than control. Manganese content of *Bacillus* Fcl1-treated plants was higher, but copper content was same to that of control. Nickel and sodium contents were comparatively lower. Strontium, chromium, boron, cobalt, and vanadium contents of *Bacillus* Fcl1-treated plants were higher. Rubidium and barium showed almost equal to control. Lead, cerium, and lanthanum contents were significantly lower than that of control. The *Bacillus* Fcl1-treated plants also showed higher amount of lithium, scandium, and cadmium uptake when compared to control, whereas the yttrium, gallium, praseodymium, selenium, gadolinium, samarium, arsenic, and dysprosium contents were comparatively low. Cesium content was higher; however, contents of thorium, erbium, ytterbium, europium, terbium, holmium, and uranium were low. Rhenium content was much higher in *Bacillus* Fcl1-treated plants; also, thallium, beryllium, and indium contents were higher than controls. However, thulium, lutetium, and bismuth contents were lower (Table 2).

Discussion

Due to their ubiquitous distribution, *Bacillus* spp. are one of the common organisms that are associated with plants as endophytes and also used as plant probiotics [9]. Many *Bacillus* spp. with plant growth-promoting effects have previously been isolated as endophyte from diverse plants such as *Capsicum annuum*, *Elettaria cardamomum*, *Curcuma longa*, and *Zingiber officinale* [26] [27]. To identify highly plant beneficial organisms, current study specifically selected rhizome tissue for endophyte isolation where the likely vertical transmission of organism is high. Also, many endophytic bacteria with antiphytopathogenic mechanisms have also been expected from rhizome. In vitro antagonistic analysis of Fcl1 against selected phytopathogens showed its potential to produce antifungal compounds. This was further confirmed by the crude extract-mediated inhibition of phytopathogens. LC-MS analysis of extract showed the presence of iturin A (m/z 1001.4128) and three surfactin derivatives ($M+H^+$:1008.6602, 1036.6932, 1050.7067) as confirmed by LC-MS/MS analysis. The fragmentation pattern obtained was same as per previous report [28]**.** The antifungal activity of iturin lipopeptide is due to its ability to interact with cytoplasmic membrane of target cells which thereby increase K^+ permeability through the formation of ion conducting pore [29]. The LC-MS/MS of surfactin C_{13},

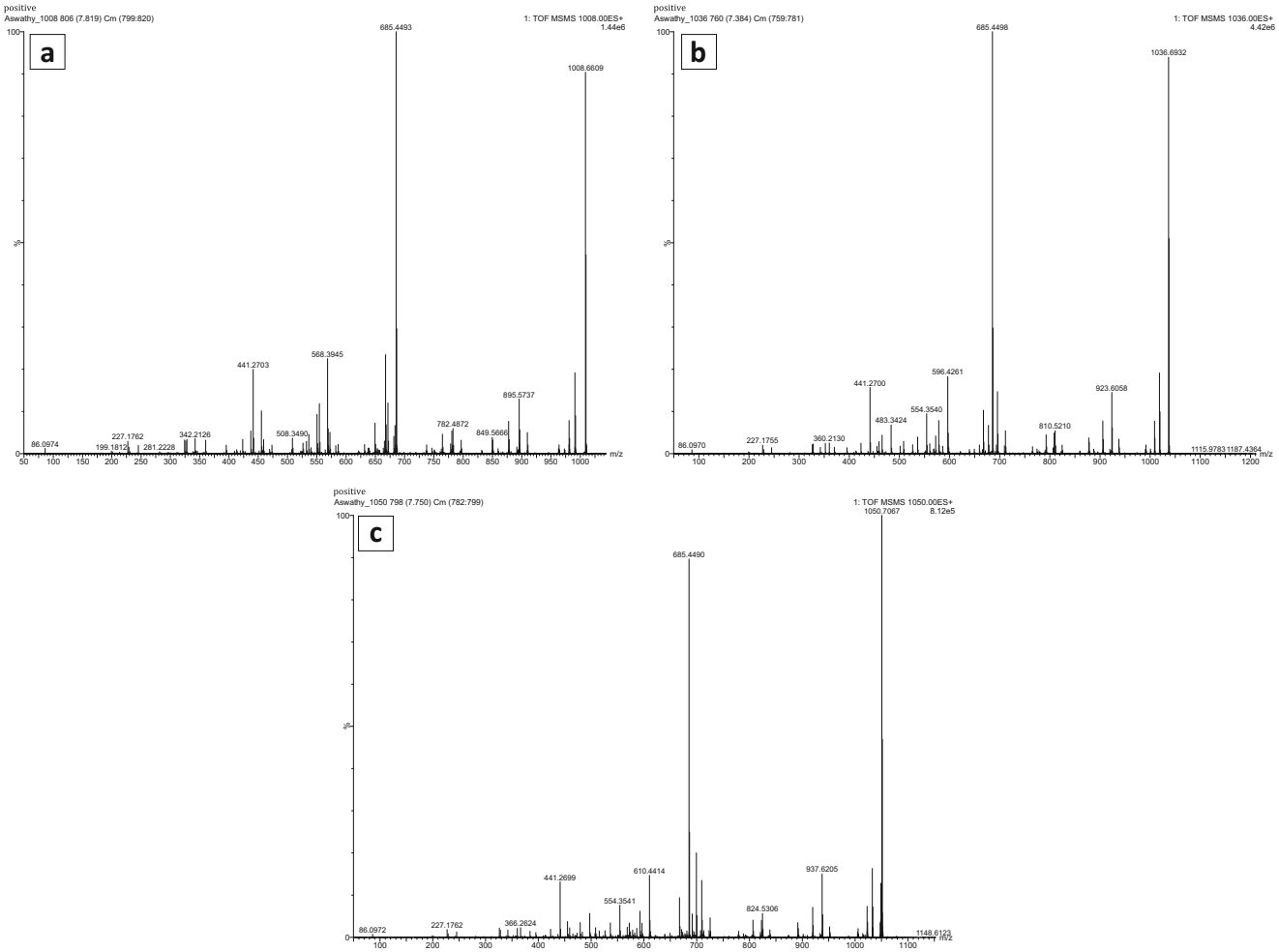

Fig. 4 LC-MS/MS-based fragmentation analysis of surfactin homologs from methanolic extract of *Bacillus* Fcl1. **a** C_{13} M^+H^+: 1008.6602. **b** C_{14} M^+H^+:1036.6932. **c** C_{16} M^+H^+:1050.7067

C_{14} and C_{16} homologs also showed confirmatory fragmentation pattern as per previous reports [30] [31] [28]. Various studies have reported the lipopeptides of *Bacillus* origin to have antagonistic activity against phytopathogens [32]. As the lipopeptides are environmentally acceptable, they have been used as potent weapons to deal with diverse phytopathogens. Surfactins, iturins, and fengycins have been explored for their activity against bacteria, fungi, and oomycetes. However, iturin and fengycin are remarkable for its antifungal activities, while surfactin has potent antibacterial and larvicidal properties [29, 32]. However, the *Bacillus* Fcl1 isolated in the study was found to have production of iturin A and three homologs of surfactin. So the mechanistic basis of its antifungal activity may involve a synergistic action of both iturin and

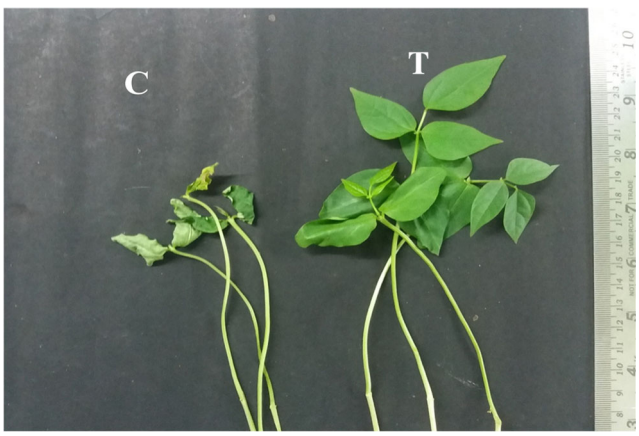

Fig. 5 In vivo biocontrol effect of *Bacillus* Fcl1 in *Vigna unguiculata*. C- control plant after infection with *R. solani*, T- *Bacillus* Fcl1 treated plant after infection with *R. solani*

Table 1 Statistical analysis of variation in shoot length, root length, and root number in *V. unguiculata* seedlings under the influence of *Bacillus* Fcl1

	Shoot length (cm)	Root length (cm)	Root number
DW	11.88095 ± 1.07	2.35714 ± 0.93	8 ± 1.50
NB	11.97619 ± 1.58	2.54762 ± 0.62	8 ± 1.50
Bacillus Fcl1	17.11905 ± 1.74	7.42857 ± 1.39	12.38095 ± 3.09

Data represents mean ± SD, $n = 10$

Table 2 Elemental composition variation in *Bacillus* Fcl1-treated seedlings of *Vigna unguiculata* when compared to control (DW, distilled water and NB, nutrient broth) obtained from statistical analysis of equationally calculated ICP-MS data

Elements	Concentration of elements in μg/mg (DW)	Concentration of elements in μg/mg (NB)	Concentration of elements in μg/mg (*Bacillus* Fcl1 treated)
P	17,204.85 ± 0.04326	18,642.39 ± 0.000289	23,262.36 ± 0.00577
K	5480.208 ± 0.0058	5067.222 ± 0.01732	3056.288 ± 0.00577
Ca	1042.916 ± 0.013978	865.6594 ± 0.0577	1008.112 ± 0.00577
Mg	2332.551 ± 0.044037	2531.073 ± 0.0404	3072.762 ± 0.00577
Al	2773.086 ± 0.004839	2362.629 ± 0.023	5518.251 ± 0.00577
Fe	1248.675 ± 0.57735	1529.133 ± 0.0173	1966.232 ± 0.173205
Zn	518.4937 ± 0.57	1093.37 ± 0.0057	1010.586 ± 0.00577
Na	598.197 ± 0.577	731.22 ± 0.028	630.5497 ± 0.0057735
Mn	615.6643 ± 0.57735	569.3644 ± 0.0115	667.1072 ± 0.057735
Cu	187.652 ± 0.57735	371.3465 ± 0.011547	382.6262 ± 0.057735
Ni	95.41116 ± 0.57735	231.5451 ± 0.01154	178.5957 ± 0.057735
Rb	73.41317 ± 0.57735	65.667 ± 0.005774	72.24814 ± 0.057735
Ba	43.51681 ± 1.154701	50.64808 ± 0.005774	50.59823 ± 0.057735
Pb	18.968 ± 0.57735	40.2741 ± 0.011547	18.58723 ± 0.288675
Ce	17.14381 ± 0.57735	46.94329 ± 0.005774	23.792 ± 0.057735
Sr	33.88729 ± 0.5775	34.03089 ± 0.011547	35.71732 ± 0.46188
La	9.569304 ± 0.5773	22.18043 ± 0.005774	11.7856 ± 0.173205
Nd	7.181556 ± 0.5735	20.55475 ± 0.005774	9.356357 ± 0.000577
Cr	12.03843 ± 0.57735	16.38177 ± 0.005774	17.8189 ± 0.173205
B	9.510833 ± 0.5773	8.235051 ± 0.005774	13.65752 ± 0.005774
Co	12.37857 ± 0.57735	10.21102 ± 0.000577	15.41275 ± 0.005774
V	7.625573 ± 0.5735	7.103661 ± 0.001155	13.03811 ± 0.017321
Y	4.365975 ± 0.57735	11.24482 ± 0.005774	5.617573 ± 0.023094
Ga	4.737815 ± 0.5775	9.072364 ± 0.00063	6.656724 ± 0.005774
Pr	2.182281 ± 0.57735	5.382621 ± 0.005774	2.450031 ± 0.011547
Se	1.724484 ± 0.5773	4.34281 ± 0.00063	2.047903 ± 0.005774
Gd	1.271439 ± 0.154994	4.072109 ± 0.028868	2.151476 ± 0.005774
Sm	1.230168 ± 0.025244	3.881787 ± 0.000173	1.634382 ± 0.002309
Li	2.208819 ± 0.000902	2.45985 ± 0.011576	3.710903 ± 0.005774
As	1.692374 ± 0.002972	3.315715 ± 0.005779	2.592224 ± 0.000231
Se	0.935837 ± 0.027982	2.54004 ± 0.017323	1.337387 ± 0.005777
Dy	0.669462 ± 0.00577	1.961493 ± 0.003464	0.881436 ± 0.011547
Sc	0.847444 ± 0.005774	0.776539 ± 0.000572	1.828174 ± 0.028868
Cd	1.517583 ± 0.005774	0.990791 ± 0.000577	1.648132 ± 0.005774
Th	0.412498 ± 0.002887	0.865954 ± 0.003464	0.577751 ± 0.00547
Er	0.29151 ± 0.005774	0.813317 ± 0.000577	0.366821 ± 0.003464
Cs	0.48896 ± 0.001735	0.441386 ± 0.011547	0.736362 ± 0.011269
Yb	0.184376 ± 0.005774	0.472301 ± 0.034641	0.237221 ± 0.002309
Eu	0.187691 ± 0.002887	0.471954 ± 0.001732	0.262212 ± 0.005774
Tb	0.136285 ± 0.002887	0.426192 ± 0.002309	0.216405 ± 0.023094
Ho	0.123105 ± 0.011547	0.334213 ± 0.001097	0.159595 ± 0.005774
U	0.095682 ± 0.005774	0.2319 ± 0.005758	0.136484 ± 0.005774
Tl	0.095831 ± 0.00577	0.102734 ± 0.002846	0.115224 ± 0.005774
Tm	0.035716 ± 0.002829	0.095468 ± 0.002646	0.044309 ± 0.000577
Be	0.047424 ± 0.002887	0.046614 ± 0.001704	0.070361 ± 0.000577
In	0.054387 ± 0.001155	0.073106 ± 0.001155	0.08424 ± 0.000577
Lu	0.027294 ± 0.000577	0.070174 ± 0.005803	0.044826 ± 0.011547
Re	0.0003 ± 0.00	0.001797 ± 0.000173	0.024364 ± 0.000173
Bi	0.052326 ± 0.000115	0.056857 ± 0.002082	0.05161 ± 0.000566

Data represents mean ± SD

surfactin. This is in accordance with enhanced fungicidal activity by the application of iturin and surfactin in a synergistic manner [29]. Also, lipopeptide production is considered as one of the prominent mechanisms underlying the biological activity of *Bacillus* spp. against various phytopathogens [27].

In vivo biocontrol studies using non-host plant *V. unguiculata* confirmed induction of resistance, as evidenced by the resistance to fungal pathogen *R. solani.* Here, the control plants were totally damped off and treated plants showed both resistance and an increase in length of shoot and number of leaflets. Various microbial components and products have been reported to induce resistance in plants. Elicitation of induced systemic resistance was found to be mediated by lipopolysaccharides, siderophores, flagella, iron-regulated metabolites, biosurfactants, 2,4-diacetylphloroglucinol, pyocyanin, and volatile organic compounds [27, 33]. The elicitation of ISR as observed in study is confirmatory to the resistance induced by lipopeptide producing *Bacillus* Fcl1. This induced resistance may also be due to the production of IAA by *Bacillus* Fcl1, which could influence plant growth and provide resistance to plant from bacterial as well as

fungal infections. This occurs via the ability of bacterial PAMP or fungal elicitors to elicit miRNAs which target auxin receptors such as TIR, AFB2, and ABF3 with the upregulation of plant defense genes such as pathogenesis-related and hypersensitive response genes [34]. Thus, bacterial endophytes with IAA production can enhance plant growth as well as equip the plant to manage phytopathogenic attack. This is indicative of great promises of *Bacillus* Fcl1 isolated in this study for field application.

Microbial IAA production can contribute to plant growth in various ways. Because of its remarkable influence in plant diverse, pathways like indole-3-pyruvic acid pathway, indole-3-acetamide pathway, indole-3-acetonitrile pathway tryptamine pathway, and tryptophan side chain oxidase pathway, it have been demonstrated for IAA synthesis. The ability of IAA production by various *Bacillus* spp. has been reported previously [35]. Microbially produced IAA can trigger plant's shoot and root elongation which can promote maximal nutrient absorption by plants [36]. ACC deaminase production by *Bacillus* Fcl1 also is an indication of its role on ACC conversion and hence supportive to minimize ethylene-mediated inhibition in plants. *Bacillus* sp. mediated ACC deaminase production has already been reported [35]. These capabilities of *Bacillus* Fcl1 are also indicative of its likely original role in rhizome of *Curcuma longa* from where it has been isolated. Endophytes have an advantage over and rhizobacteria, as it can reside in plant tissues, and hence property of *Bacillus* Fcl1 to fix nitrogen can also be supportive to plant growth [37]. Most remarkably, the isolate *Bacillus* Fcl1 was found to provide both plant growth and disease resistance to non-host plant which is indicative of its likely potential to colonize economically important plants.

Due to the plant growth and biocontrol efficiency of *Bacillus* Fcl1, its interaction with plants is expected to influence significantly the nutritional mobilization and a balanced maintenance of elemental composition. The elemental profiling of *Bacillus* Fcl1 treated *V. unguiculata* plants by ICP-MS showed remarkable modulation of elemental composition when compared with the control. ICP-MS has previously been used to analyze changes in plants as per diverse soil conditions. As most of the plant functions are influenced by the associated microbial community, organisms with highly specialized plant beneficial features like *Bacillus* Fcl1 as identified in the study can expect to have key role in determining soil nutrient mobilization to plants. Majority of the analyzed macroelements were increased except potassium in Fcl1 treated plants. Microelements contents such as V, Li, Mg, Sc, Al, Cd, Fe, Cs, Mn, Tl, Cu, Lu, Rb, Re, Sr, G, B, and Co of Fcl1-treated plants have also increased. Meanwhile Gd, In, Zn, S, Bi, Na, Dy, Ni, Th, Pb, Er, La, Yb, Nd, Eu, Ba, Tb, Y, Ho, Ga, U, Pr, Tm, Se, and Be were decreased with respect to control (Table 2). Current result is the first report on detailed elemental analysis of plants under association with endophytic bacteria. Even though the current study provides insight into key changes in plant under the influence of a single organism, naturally the outcome of these changes can be due to synergistic effects of diverse microbial community. Hence, the current results provide gateway information to investigate impact of endophyte on global nutritional chemistry of plants. The *Bacillus* Fcl1 interaction might have directly or indirectly formed the basis of observed changes in plants. However, more studies are required to identify elemental profile changes in plant as per microbial interaction. Exploring such features is essential to generate tailor-made plant probiotics to specifically engineer nutritional composition of plants as per specific requirement.

In conclusion, the study has resulted in the isolation of endophytic bacteria *Bacillus* Fcl1 having plant growth as well as biocontrol properties along with plant elemental modulatory effect. The organism was positive for IAA, ACC deaminase, nitrogen fixation, and it inhibited pathogens through iturin and surfactin production. The occurrence of both plant growth promotion as well as antifungal properties in the organism with its proven ability to interact with non-host plant can be highly interesting as it could be a good candidate for the development of plant probiotics for plant growth and disease suppression.

Acknowledgments The authors acknowledge Prof. C. T. Aravindakumar, Hon. Director, and Mr. Dineep D., Scientific Assistant of the Inter-University Instrumentation Centre, Mahatma Gandhi University, Kottayam, for LC-MS and LC-MS/ MS analysis.

Compliance with Ethical Standards

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

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