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ORIGINAL PAPER

Identification of bacteria associated with underground parts of *Crocus sativus* by 16S rRNA gene targeted metagenomic approach

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Abstract Saffron (*Crocus sativus* L), an autumn-flowering perennial sterile plant, reproduces vegetatively by underground corms. Saffron has biannual corm–root cycle that makes it an interesting candidate to study microbial dynamics in its rhizosphere and cormosphere (area under influence of corm). Culture independent 16S rRNA gene metagenomic study of rhizosphere and cormosphere of Saffron during flowering stage revealed presence of 22 genera but none of the genus was common in all the three samples. Bulk soil bacterial community was represented by 13 genera with *Acidobacteria* being dominant. In rhizosphere, out of eight different genera identified, *Pseudomonas* was the most dominant genus. Cormosphere bacteria comprised of six different genera, dominated by the genus *Pantoea*. This study revealed that the bacterial composition of all the three samples is significantly different ($P < 0.05$) from each other. This is the first report on the identification of bacteria associated with rhizosphere, cormosphere and bulk soil of Saffron, using cultivation independent 16S rRNA gene targeted metagenomic approach.

Keywords Saffron · Rhizosphere · Metagenomics · 16S rRNA gene · Bacterial community

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Introduction

Crocus sativus, commonly known as Saffron, is the world's costliest spice with medicinal value and one kilogram costs around 11,000 US $ (Melnyk et al. 2010 Wani et al. 2011). It is a sterile triploid plant ($3n = 24$) and reproduces vegetatively by underground bulb-like, starch-storing organs known as, corms. The annual life cycle of *Crocus sativus* comprises of three stages, flowering, vegetative and dormant stage. The flowering stage, being investigated in present study, is characterized by absence of leaves, well bloomed flowers on short stalks arising from corm, and fully developed roots.

There is body of literature on plant–microbe associations and interactions (Leveau 2007; Buée et al. 2009; Kumar et al. 2010; Berendsen et al. 2012; Kim et al. 2012 and Ma et al. 2013). Rhizosphere is biologically active zone of the soil, which is very close to the root and contains soil-borne microbes including bacteria and fungi (Hiltner 1904). Rhizosphere bacteria have been reported to influence growth and yield of various plants e.g. rice, tea, cucumber, apple, soyabean and saffron (Johansen and Olsson 2005; Ashrafuzzaman et al. 2009; Joshi and Bhatt 2011; Mahaffee and Kloepper 1997; Mazumdar et al. 2007; Mehta et al. 2010; Wahyudi et al. 2011; Ambardar and Vakhlu 2013). Plants like Banana, Colchicum, Gladiolus and Saffron reproduce asexually by underground corms (Frankova 2006; Singh et al. 2011; Steinitz et al. 1991; Nehvi and Yasmeen 2010). Corms are modified stem in direct contact with soil similar to roots but are different from roots in composition and structure (Esmaeili et al. 2013; Rahmani et al. 2012; Haining et al. 2012; Esmaeili et al. 2011). Part of this study was aimed to elucidate corm bacterial associations, if any, that may exist in the manner of the much studied root-bacterial associations. There are

no reports available on the native microbes/bacteria associated with underground plants like bulb, corm, and rhizome from any plant including Saffron. We have used a term "Cormosphere" for the area under influence of corm (bacteria adhering to the corm sheath) in analogy to rhizosphere and phyllosphere.

Various plant growth promoting bacteria (PGPB) have been isolated by cultivation dependent methods but due to the refraction of most of the bacteria to cultivation under laboratory conditions, complete bacterial diversity of the rhizosphere and cormosphere of a plant cannot be studied using cultivation based approach alone (Amman et al. 1995; Handelsman 2004; Riesenfeld et al. 2004). Cultivation based bacterial diversity studies need to be complemented by cultivation independent technique i.e., metagenomics (Tyson et al. 2004; Venter et al. 2004; Teixeira et al. 2010; Araujo et al. 2012; Thomas et al. 2012). Rhizobacteria possess diverse metabolic capabilities and play a crucial role in plant health, therefore, knowledge of their community structure is imperative for the proper understanding of their individual roles (Buée et al. 2009 and Kumar et al. 2010). The role of metagenomics in the study of bacterial diversity in rhizosphere extends from identifying novel plant growth promoting genes and gene products to characterizing yet-to-be -cultivable microorganisms (Leveau 2007, Inceoglu et al. 2011, Arjun and Kumarapillai 2011). 16S rRNA gene amplicon based metagenomics has been used extensively to study microbial diversity and for prediction of phylogenetic relationships (Kirk et al. 2004, Inceoglu et al. 2011, Peiffer et al. 2013).

Present study is the first report on cataloguing of the bacteria associated with rhizosphere, cormosphere and bulk soil of Saffron by cultivation independent 16S rRNA metagenomic approach.

Materials and methods

Sites description and sample collection

Samples from the Saffron bulk soil, rhizosphere and cormosphere were collected during the flowering period (3rd Nov 2010) from Wuyan village (74°58′0″E, 34°1′30″N, 5173ft) in Pulwama district of Kashmir, India. The soil sampling was done as per the protocol standardized by Luster et al. (2009). Composite rhizosphere and cormosphere samples were analysed by collecting the samples from the four corners of three different fields and mixed together. The bulk soil was collected by vigorously shaking the roots and the soil which remains adhere to the roots was taken as rhizosphere soil, whereas the corms sheath was taken to study corm associated bacteria. The samples were collected in triplicate and pooled together. Samples were transported to the laboratory at 4 °C (in ice) and stored at −20 °C till processed further for physicochemical and community DNA extraction.

16S rRNA gene metagenomic library construction

Metagenomic DNA from rhizosphere, cormosphere and bulk soil was extracted following the protocols given by Pang et al. (2008), Wechter et al. (2003), Brady (2007) and Zhou et al. (1996). The isolated metagenomic DNA was further purified by gel elution kit (Macherey–Nagel, Nucleospin Extract II kit), analysed on 1 % agarose gel and stored at −20 °C. Complete 16S rRNA gene corresponding to nucleotide positions 8–1522 was amplified using universal eubacterial primers 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1522R (5′-AAG GAG GTG ATC CAN CCR CA-3′) (Hong et al. 2009). The PCR mixture contained 1–10 ng of DNA extracted from bulk soil, cormosphere and rhizosphere of Saffron, 10 pM of universal primers, 1X PCR buffer (Fermentas), 2.5 mM $MgCl_2$, 2.5U of Taq DNA polymerase (Fermentas), 0.2 mM each deoxynucleoside triphosphate (Fermentas) and sterile filtered MilliQ water was added to make final volume of 50 µl. Negative controls comprised of same assay without the template. PCR amplification was performed in a DNA thermocycler (Eppendorf, India) following the amplification program of, initial denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min 30 s, and a final extension of 10 min at 72 °C. The amplicons of approximately 1,500 bp were analyzed by electrophoresis on 1 % agarose gel and a 1 kb DNA ladder (Fermentas) was taken as the molecular size standard. The amplicon was gel purified using a gel elution kit (Macherey–Nagel, Nucleospin Extract II kit). The 16S rRNA metagenomic gene library was constructed using TA cloning kit (Fermentas). The purified amplicon was further ligated into pTZ57R/T vector with a molar ratio of 2:5 (vector: insert), and Calcium chloride competent *Escherichia coli* Dh5α (prepared as per the protocol of Cohen et al. 1972) were transformed with the ligation mixture. The positive recombinants were screened on AXI plates (Ampicillin-X-gal-IPTG) by blue white selection (~10,000 clones from each library). Each library was constructed in triplicate and random clones were selected for screening. Positive clones were identified by colony PCR using M13 Forward (5́ GTA AAA CGA CGG CCA GT 3́) and reverse primers (5́ CAG GAA ACA GCT ATG AC 3́) of T-vector (pTZ57R/T) using the same program as 16S rRNA gene amplification. ARDRA was performed to remove the redundancy (repetition of same clone) in which the PCR-amplified products of positive recombinants were digested with the restriction enzymes Alu1 (Fermentas).

The restricted fragments were analysed by MultiNA, Microchip electrophoretic system (Schimadzu, Japan) and a phylogenetic tree was constructed based on the different banding pattern obtained by ARDRA using the viewer softwares of MultiNA. One clone each was selected from the different clads of phylogenetic tree so that one clone represents about 40–50 clones. 50 clones (having representation in triplicate libraries of each sample) were selected from each metagenomic library and a total of 150 clones were selected from bulk soil, rhizosphere and cormosphere metagenomic libraries. Plasmid isolation was performed using QIAprep spin miniprep kit (QIAGEN) and sent to SciGenom Labs Private Ltd., Cochin, Kerala, INDIA for 16S rRNA gene sequencing.

Sequence analysis

16S rRNA gene sequences obtained from sequencing results was analyzed using bioinformatic tools. The sequences obtained were edited for various quality measures (Q-value = 20, minimum length = 1,500 bp) using CLC sequence viewer, sequence analyser, pairwise alignment and bioedit software (Hall 1999). The resulting nucleotide sequences were assigned bacterial taxonomic affiliations based on the closest match to sequences available at the NCBI database (http://www.ncbi.nlm.nih.gov) using the BLASTn in nucleotide reference database (http://blast.ncbi.nlm.nih.gov). A rarefaction analysis was done to assess the coverage of the bacterial community by the datasets based on the Operational Taxonomy Unit (OTU) clustering results. The sequences from three metagenomic 16S rRNA gene libraries were clustered into OTUs with a cut off value of >97 % sequence similarity. Rarefaction curves were obtained by plotting the sample sizes versus the estimated number of OTUs using the rarefaction tool of Ribosomal Database Project-II Release 9 (http://rdp.cme.msu.edu).

Construction of phylogenetic tree

Approximately 1,477 nucleotides of each ARDRA representative library clones were sequenced using the forward and reverse M13 primers (SciGenom Labs Private Ltd, Cochin, Kerala). The sequences were examined for chimera by the DECIPHER online chimera analysis program (http://decipher.cee.wisc.edu/FindChimeras.html) and assembled with CLC sequence viewer and bioedit software (Hall 1999). The sequences were analysed using BLASTn search version 2.2.3 (Altschul et al. 1997) and classifier tools of Ribosomal Database Project-II Release 9 (http://rdp.cme.msu.edu) to search for the taxonomic hierarchy of the sequences. The 16S rRNA gene sequences along with the reference sequence having close sequence similarity (>97 %) obtained from the National Center for Biotechnology Information (NCBI) Taxonomy Homepage (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/) were aligned using multiple sequence alignment tool ClustalX 2.1 version (Thompson et al. 1997). Phylogenetic and molecular evolutionary analysis was conducted by constructing neighbour-joining tree using algorithm and software package of Phylip 3.69 (Tuimala 2004). The phylogenetic trees were constructed using the neighbour-joining method and 1,000 bootstrap replications were assessed to support internal branches (Hillis and Bull 1993). The phylogenetic trees were viewed using ITOL (http://itol.embl.de/) and edited in MEGA 5.05 software (Tamura et al. 2011).

Bacterial diversity analyses

The bacterial composition was determined by taxonomic assignment performed by RDP Classifier set at 97 % confidence value. The sequences were classified up to genus level using RDP classifier. Relative alpha diversity between bacterial communities was evaluated by calculating the Shannon (Shannon and Weaver 1949) and Simpson's diversity indices. Ribotype richness was calculated according to the abundance based coverage estimate (ACE) and the bias corrected Chao1 values (Chao and Bunge 2002), a non parametric estimate of species richness using EstimatesS software. Intra sample bacterial diversity was analysed using Fast UniFrac program (Hamady et al. 2010) and samples were categorized according to sample source (bulk, cormosphere and rhizosphere). UniFrac tests were performed using 1,000 permutations and calculated with the Fast UniFrac web application (http://bmf2.colorado.edu/fastunifrac/). *P* test significances were used to test whether bacterial communities of each pair of samples were significantly different. Principal coordinate analysis (PCoA) was further performed using the Fast UniFrac metric.

Data availability

The sequences obtained in this study are available at the GenBank under accessions numbers JX260425, JX279932–JX279941, JX289937–JX289942, JX294738–JX294750, JX852636–JX852677, JX945529–JX945568, JX962747–JX962749, KC138682–KC138694, and KC283045–KC283065.

Results

Bacterial diversity

Total metagenomic DNA from the bacterial community of all the three composite samples was extracted using various protocols (Zhou et al. 1996, Wechter et al. 2003, Brady

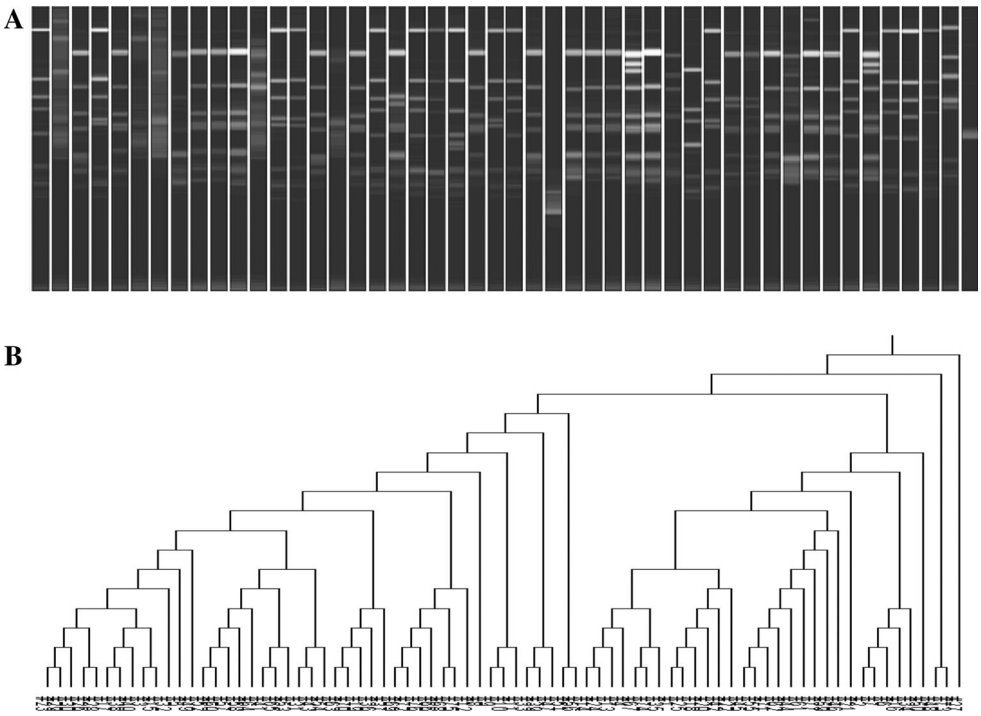

Fig. 1 Amplified ribosomal DNA restriction pattern (ARDRA) of different clones libraries (**a**) and phylogenetic tree (**b**) constructed based on different pattern using MultiNA, Microchip electrophoretic system. Single clone selected from each clad represents 40–50 clones

2007 and Pang et al. 2008) but 16S rRNA gene was successfully amplified from DNA isolated using the protocol developed by Pang et al. (2008). 16S rRNA gene metagenomic library of bulk soil, rhizosphere and cormosphere was constructed by TA cloning kit and ~10,000 clones were picked from, each of the three libraries. Clones were selected randomly for insert confirmation by colony PCR and screened for redundancy by ARDRA. On the basis of ARDRA, a total of 150 clones were selected from the three libraries, with one clone representing 40–50 clones as per phylogenetic tree constructed by viewer software of MultiNA (Fig. 1). The inserts of the selected clones were sequenced by Sanger's method instead of next generation sequencing to get read length up to 1.5 kb, as this read length is long enough for characterization of bacteria up to species level. Pyosequencing though is faster and cheaper but generates 200–500 bp sequence that can be analysed up to phyla level only and error rate is higher than Sanger sequencing (Gottel et al. 2011; Araujo et al. 2012).

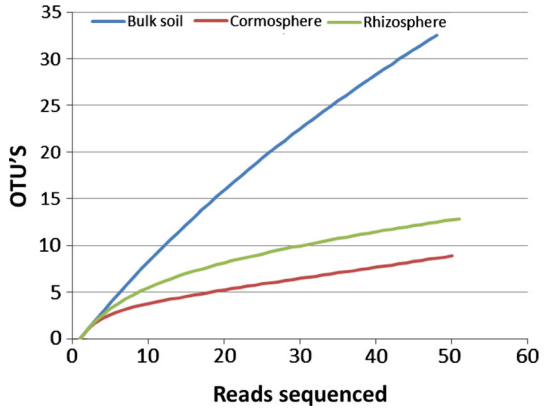

Fig. 2 Rarefaction curves for bacterial OTUs clustering at 97 % rRNA gene sequence similarity. High slope of rarefaction curve indicates more diversity (higher number of OTUs) as compared to low slope curves (Lesser number of OTUs) thereby indicating more bacterial diversity in bulk soil as compared to rhizosphere and cormosphere

Rarefaction curves (97 % identity) in all the three samples did not approach the plateau, indicating less representation of bacterial diversity, which may increase on repetitive sampling and/or use of different DNA isolation protocols (Fig. 2). The bacterial diversity of rhizosphere was represented by 13 OTUs and cormosphere by 8 OTUs, whereas bulk soil was represented by 33 OUT's indicating high genetic (bacterial) divergence in bulk soil as compared to rhizosphere and cormosphere (Fig. 2). Higher bacterial diversity in bulk soil was further complemented by the diversity indices like ACE Mean, ICE Mean, Chao 1 Mean, Chao 2 Mean, Shannon Mean and Simpson Mean (Table 1). Principal coordinate analysis generated by Fast UniFrac further validated the bacterial phylogenetic divergence observed between different samples. UniFrac significance and *P* test significance values for the bacterial communities ($P < 0.05$ for all pairwise comparisons) differed significantly between rhizosphere and cormosphere; rhizosphere and bulk soil; and bulk soil and cormosphere ($P < 0.05$) (Fig. 3). This results were further

Table 1 Diversity indices indicating bacterial diversity and richness in three samples

Diversity indices	Bulk	Cormosphere	Rhizosphere
ACE mean	20.5	12	11
ICE mean	13	6	8
Chao 1 mean	20.5	12	11
Chao 2 mean	13	6	8
Shannon mean	2.21	1.02	1.72
Simpson mean	8.14	2.34	5.05

The bacterial diversity is maximum in bulk soil as compared to rhizosphere and cormosphere indicated by the maximum diversity indice values in bulk soil

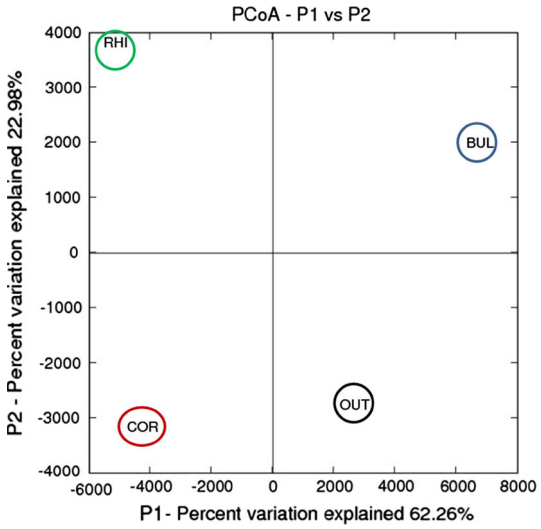

Fig. 3 Principal coordinates (Unifrac) analysis of bacterial community of three samples reflecting significant difference in bacterial diversity as the plots (indicated as *red* for cormosphere, *green* for rhizosphere and *blue* for bulk soil are in different quadrants. COR, RHI, BUL and OUT in figure represents cormosphere, rhizosphere, bulk soil and outgroup respectively. (Color figure online)

complemented by phylogenetic tree constructed using total insert sequences, which cluster the cormosphere, rhizosphere and bulk soil bacterial sequences into separate clads (Fig. 4).

Phylogenetic composition of all the three samples was significantly ($P < 0.05$) different, 13 genera were catalogued from the bulk soil, 6 from the cormosphere and 8 from the rhizosphere (Fig. 5). Cormosphere was dominated by *Pantoea* whereas rhizosphere by *Pseudomonas* and bulk soil by representatives of uncultivable *Acidobacteria, GP4* (Fig. 5). Despite the small distance between the bulk soil, rhizosphere and cormosphere, none of the genus was common in all the three soil types.However, there were some genera which were common in two soil types at a time, like *Pseudomonas (P. frederiksbergensis) and Acidobacteria GP6* common in bulk soil and rhizosphere; *Staphylococcus (S.epidermidis)* in bulk soil and cormosphere and *Pantoea (Pa. vagans, Pa. agglomerans* and *Pa. eucrina)* and *Enterobacter* in cormosphere and rhizosphere (Fig. 5). The relative abundance of *Pseudomonas* was significantly higher in the rhizosphere (34 %) than in the corresponding bulk soil (10 %) but were absent in cormosphere (Fig. 5). *Acidobacteria* GP6 was more abundant in bulk soil (12 %) whereas in rhizosphere their number is comparatively less (2 %) and were totally absent from cormosphere (Fig. 5). *Pantoea* showed an increase in comparative abundance in cormosphere (52 %) than rhizosphere (18 %) whereas *Enterobacter* was more in rhizosphere (10 %) than cormosphere (2 %) (Fig. 5). *Staphylococcus* was equally represented in bulk soil and cormosphere (2 %).

Discussion

Saffron rhizosphere and cormosphere is a naïve niche which has not been explored, despite Saffron's prized economic and medicinal value (Sharaf-Eldin et al. 2008; Melnyk et al. 2010; Kamalipour and Akhondzadeh 2011; Chryssanthi et al. 2011). Bacterial diversity of rhizosphere of various plants like rice, tea, cucumber, apple, potato, soya bean and recently Saffron has been studied extensively (Mahaffee and Kloepper 1997; Johansen and Olsson 2005; Ashrafuzzaman et al. 2009; Joshi and Bhatt 2011; Mazumdar et al. 2007; Mehta et al. 2010; Wahyudi et al. 2011; Inceoglu et al. 2011, Ambardar and Vakhlu 2013) using cultivation dependent and independent techniques but cormosphere microbiota has not been studied in any corm bearing plant. The biochemical composition and physiology of root and corm are different as they are two different plant organs (Esmaeili et al. 2013; Rahmani et al. 2012; Haining et al. 2012; Berendsen et al. 2012; Esmaeili et al. 2011; Buée et al. 2009). In the present study, rarefaction analysis (Fig. 2), principle coordinate analysis (Fig. 3), and phylogenetic analysis (Fig. 4) suggests that bacteria inhabiting cormosphere, rhizosphere and bulk soil of Saffron are significantly different, similar to the results of comparative study of bacterial diversity in potato rhizosphere and bulk soil (Inceoglu et al. 2011).

The dominance of *Pseudomonads* in rhizosphere of Saffron is in accordance with reports in literature, as they are chemically attracted to the root exudates and are selected over other microbes due to their PGP properties (Saharan and Nehra 2011). We have reported similar findings earlier, using cultivation dependent approach (Ambardar and Vakhlu 2013) but the number of *Pseudomonads* characterized in present study was more. Seven different species of *Pseudomonads* were found in rhizosphere whereas only three species were identified from bulk soil

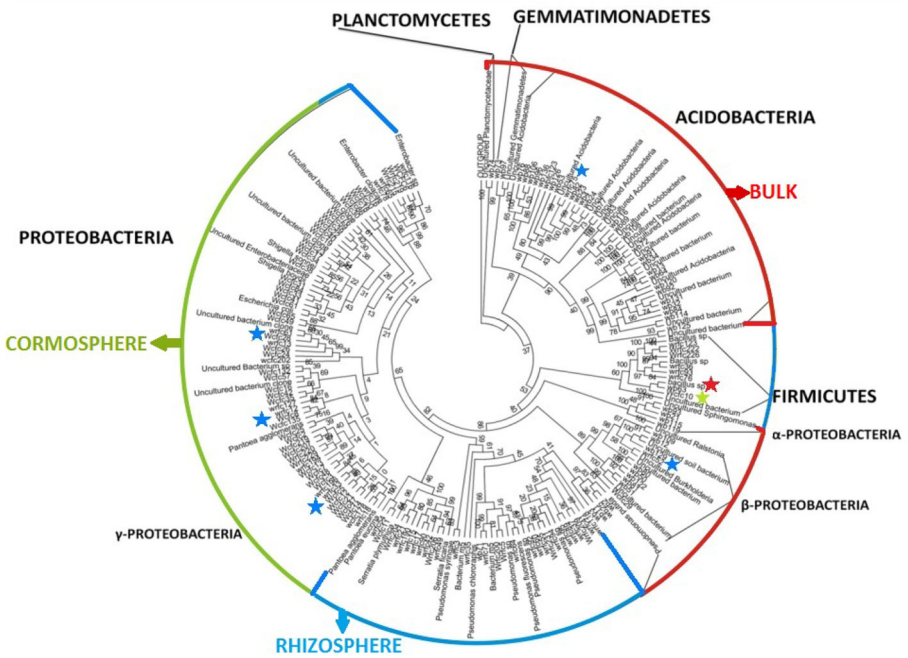

Fig. 4 Phylogenetic tree representing all the sequences of bulk soil (*red*), cormosphere (*green*) and rhizosphere (*blue*) which are clustered in separate clads represented by *different colours*. *Star* in the clad depicts the common bacteria between the two samples i.e. *blue stars* in *green* clad depicts the bacteria common in rhizosphere and cormosphere whereas *red* and *green stars* in *blue* clad represent the cormosphere and rhizosphere bacteria common to bulk soil. (Color figure online)

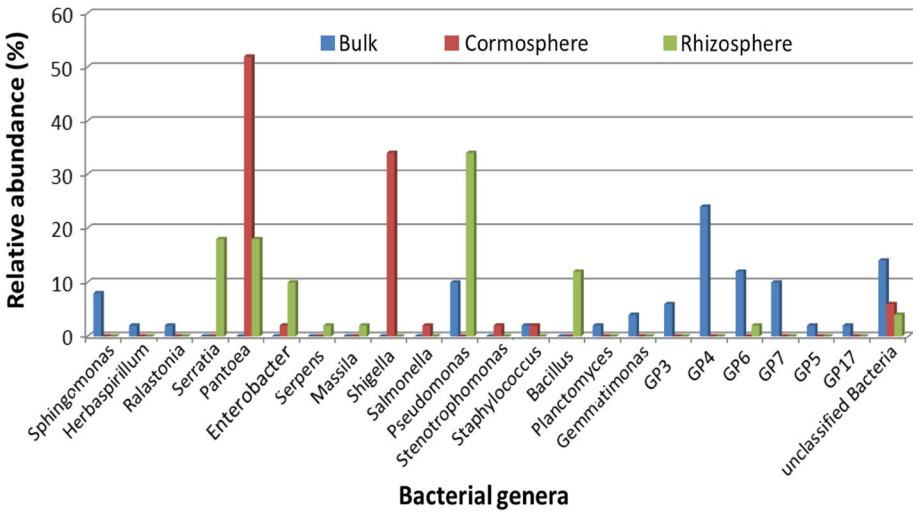

Fig. 5 Relative abundance of bacterial genera in rhizosphere, cormosphere and bulk soil of saffron indicating dominance of *Pseudomonas* in rhizosphere, *Pantoea* in cormosphere and *Acidobacteria GP4* in reference bulk soil

with *Pseudomonas frederiksbergensis* common to both bulk soil and rhizosphere. *Pseudomonas frederiksbergensis* has not been reported from any other plant rhizosphere but from the soil at coal gasification site in Frederiksberg, Denmark (Andersen et al. 2000). *Acidobacteria* GP6 was the other common bacterial group between bulk soil and rhizosphere, with more abundance in bulk soil. The C (Carbon) value of bulk soil was 1.36 % thus allowing the growth of *Acidobacteria* as they are known to be more abundant in environments with low carbon availability and seem to prefer bulk soil to nutrient-rich rhizosphere (George et al. 2011).

Though borne by many plants as a organ for storage and vegetative reproduction, there are no prior studies investigating the microbes/bacteria associated with and underground modified stem like corm, tuber, bulb etc. that could be used as a basis of comparison of the present study. However corm is reported to be rich in monosaccharide like lyxose, xylose, ribose, glucose, mannose, galactose, rhamnose, cellobiose, maltose, lactose, fructose (Haining et al. 2012) which can serve as a source of food for the microbial community and provide incentives to the microbial community for colonizing corms vicinity. In addition, phenolic compounds (Esmaeili et al. 2011), peroxidases (Rahmani et al. 2012) and some metals (Esmaeili et al. 2013) are also reported from the corm. Saffron cormosphere was dominated by genus *Pantoea*, in contrast to

Pseudomonas that was dominant in Saffron rhizosphere. *Pantoea* (*Pantoea vagans, Pantoea agglomerans* and *Pantoea eucrina)* and *Enterobacter* (*E.ludwigi*) were the bacterial genera common to saffron rhizosphere and cormosphere with *Pantoea* more abundant in cormosphere and *Enterobacter* in rhizosphere (Fig. 4). Both *Pantoea* and *Enterobacter,* members of *Enterobacteriaceae* are reported to be PGPR and *Pantoea agglomerans, Pantoea vagans* and *E.ludwigii,* catalogued in the present study have also been reported from the rhizosphere of maize, chickpea, phyllosphere of eucalyptus leaves and *Lolium perenne* rhizosphere respectively (Mishra et al. 2011; Brady et al. 2009; Shoebitz et al. 2009). *Pantoea agglomerans* is reported to produce IAA and solubilise tri-calcium phosphate; *Pantoea vagans* and *E.ludwigii* acts as biocontrol agent in potato and *Lolium perenne* respectively (Mishra et al. 2011; Brady et al. 2009; Shoebitz et al. 2009; Sturz and Nowak 2000), suggesting thereby that these bacteria may be performing similar functions in the cormosphere of Saffron. However, to our knowledge, *Pantoea eucrina* has not been reported from any plant.

The bacteria present in cormosphere were different from the bulk soil, *Staphylococcus* being an only exception. *Staphylococcus epidermidis* was found to be a common bacterial species in cormosphere and bulk soil and to our knowledge has not been reported from any root, corm or underground tuber. The difference in the bacteria isolated from cormosphere and bulk soil suggests that bulk soil is not the reservoir for the cormosphere bacteria. It can be hypothesized that in Saffron cormosphere, bacteria are transferred from mother corm to daughter corm during vegetative propagation during nursing of the daughter corms by mother corms and not from the bulk soil.

The bacterial diversity of Saffron rhizosphere was different from bulk soil and cormosphere; but the pattern of bacterial diversity of rhizosphere was similar to other known plant rhizospheres. Some of the Saffron rhizobacteria catalogued in present study have been reported from other plants by cultivation dependent method e.g. *P. thivervalensis* from wheat (Sachdeva et al. 2010), *P. brassicacearum subsp., Neoaurantiaca* from *Brassica napus* (Elena et al. 2009*), Pantoea agglomerans* from Maize and chickpea (Mishra et al. 2011), *Pa. vagans* from Eucalyptus leaves (Brady et al. 2009), *S.ficaria* from the Angelica trees (Okamoto et al. 2000), *S.plymuthica* from Grass roots (Alstrom and Gerhardson 1987) and *B.drentensis* from Cactus (Garrido et al. 2012). Eleven Saffron rhizobacterial species being reported for the first time from any plant rhizosphere are *P. koreensis, P. frederiksbergensis, P. baetica, P. mohnii and P. reinekei, Pa. eucrina, Pa. conspicua, E. asburiae, E. kobei, B. niacin* and *B. soli.* Out of the various species of *Pseudomonas* catalogued from Saffron rhizosphere by metagenomic analysis, only *P. koreensis* has been isolated using cultivation dependent approach in our previous study (Ambardar and Vakhlu 2013). *P. koreensis* has not been reported from any plant rhizosphere but is reported to produce bio surfactant effective against *Pythium ultimum* and *Phytophthora infestans* (Hultberg et al. 2010).

Conclusion

Corm, the underground organ for storage and vegetative propagation, is as important as root, if not more. Microbial associations with roots are well studied but corm-associated microbes are unexplored and need to be explored and analysed. In Saffron, cormosphere was found to harbour specific bacteria that are different from rhizosphere and the bulk soil. As expected Saffron rhizosphere is rich in *Pseudomonads* but surprisingly no *Bacillus* was identified. Cormosphere on the other hand harbours *Pantoea* but how they interact with corm needs further investigation. What effect these bacterial interactions have on plant growth and development is matter of further investigation.

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