European Journal of Soil Biology 79 (2017) 48–56

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Root colonization by host-specific rhizobacteria alters indigenous root endophyte and rhizosphere soil bacterial communities and promotes the growth of mandarin orange

Elizabeth Thokchom [a], Dwipendra Thakuria [b], Mohan Chandra Kalita [c], Chandradev K. Sharma [a], Narayan Chandra Talukdar [a, c, d, *]

[a] Institute of Bioresources and Sustainable Development, Takyelpat Institutional Area, Imphal, 795001, Manipur, India
[b] School of NRM, College of PG Studies, Central Agricultural University, Umiam, 793103, Meghalaya, India
[c] Department of Biotechnology, Gauhati University, Guwahati, 781014, Assam, India
[d] Institute of Advanced Study in Science and Technology, Under Department of Science and Technology, Govt. of India, Paschim Boragaon, Guwahati, 35, Assam, India

ARTICLE INFO

Article history:
Received 27 March 2016
Received in revised form
5 February 2017
Accepted 15 February 2017
Available online 2 March 2017

Handling Editor: Christoph Tebbe

Keywords:
PCR-DGGE fingerprint
Electron microscopy
PGPR
Rhizosphere
Root endophyte

ABSTRACT

We assessed whether colonization of mandarin orange roots by host-specific plant growth promoting rhizobacteria (PGPR) can influence the composition of root endophyte, rhizosphere and non-rhizosphere bacterial communities and plant growth. The ability to colonize root by four host-specific PGPR, namely *Enterobacter hormaechei* RCE1, *Enterobacter asburiae* RCE2, *Enterobacter ludwigii* RCE5 and *Klebsiella pneumoniae* RCE7, was tested in axenically grown mandarin orange seedlings. The combined results of scanning and transmission electron microscopic images showed that PGPR inoculants successfully colonised on surface and within root tissues of axenic mandarin orange seedlings. The effect of root inoculation of 90 days old mandarin orange seedlings grown in natural soil with RCE1, RCE2, RCE5 and RCE7 either individually or as a consortium was evaluated in terms of plant biomass yield at 90, 180 and 360 days of post inoculation (dpi). Inoculation thus enhanced plant biomass yield over the uninoculated control. NMDS and hierarchical clustering of PCR DGGE fingerprints indicated that both the PGPR inoculation and plant age affected bacterial communities in root tissues and rhizosphere soils. Based on sequencing, the majority of the root endophyte and rhizosphere PCR-DGGE bands were from *gammaproteobacteria,* and the other were from *Firmicutes* and *Deinococcus-Thermus.* In conclusion, host-specific PGPR successfully colonized mandarin orange root tissue and rhizosphere soil and enhanced plant growth.

1. Introduction

Beneficial bacteria usually colonize on surface and within tissues of different parts of the host plant, where they live either commensally or execute beneficial functions for the host [7,23]. Depending upon the site of colonization, these beneficial bacteria are broadly categorized into endophytes that live within plant tissues like root, leaf, stem and seeds and into rhizobacteria, tightly- and loosely-adhered rhizosphere soil bacteria [29,47]. Plant seeds usually fall on the soil, a complex microbial habitat, and lay dormant waiting for environmental signals to germinate. As seeds germinate, seed endophytes emerge as important founders of the seedling bacterial community [20]. With the establishment of seed bacterial community, plants also start exerting modifying effects in the rhizosphere through secretion of root exudates for selective recruitment of certain members of the native soil rhizobacteria [15,20,23]. Thus, the interactions between endophytic and rhizobacterial communities of the host plant may play a defining role in the success of bioinoculant technology employed for enhancing crop production. Although there is no clear understanding on overall role of the plant microbiome, there is substantial evidence that these communities are involved in disease control, nutrient acquisition, production of growth hormone and providing benefits to habitat-adaptive fitness of the host plant [27,36–38,40].

* Corresponding author. Institute of Bioresources and Sustainable Development, Takyelpat Institutional Area, Imphal, 795001, Manipur, India.
E-mail address: talukdar_narayan@rediffmail.com (N.C. Talukdar).

http://dx.doi.org/10.1016/j.ejsobi.2017.02.003
1164-5563/

Therefore, the tripartite interactions among root endophyte-host plant-rhizobacteria deserve full attention for understanding the mechanisms of soil-plant-microbe continuum for crop rhizosphere engineering.

Rhizobacteria as bioinoculant may survive and proliferate in the rhizosphere of plant upon inoculation and may even induce shifts in rhizosphere microbial community of its host species [9,14,16,20,34]. Root treatment with rhizobacteria could cause shift in the abundance of native endophytes in different plant parts [3,5,6]. The ability of PGPR strain to colonize host-plant when reintroduced as inoculant is an important criterion for competitive exclusion in the process of rhizosphere interactions. Though recent studies revealed the facts on biological interactions in the context of the soil-plant-microbe continuum, such information are not conclusive enough to answer a few key questions: (1) what is the fate of host-specific rhizobacteria in the root and rhizosphere at different growth stages of the host-plant and (2) does the inoculation of plant roots with host-specific rhizobacteria cause change in the abundance and composition of the native endophyte, rhizosphere and non-rhizosphere bacterial communities? Most of the studies on rhizosphere and endo-rhizosphere communities were conducted in field crops [10] or a few fruit tree species [48]. Although beneficial effect of rhizobacteria inocula on seedling growth of mandarin orange was reported earlier [1,2], information on the effect of inoculation of rhizobacteria on the endophytic bacterial community composition in fruit trees taking into account plant growth stages is not available.

Thokchom et al. [47] isolated and screened several rhizobacteria from the rhizosphere of mandarin orange and identified four rhizobacterial strains as superior inocula based on their multifaceted plant growth promoting (PGP) traits and ability to promote growth of mandarin orange both under *in-vitro* and *in-vivo* conditions. We hypothesized that the colonization of roots of mandarin orange by these four host-specific plant growth promoting rhizobacteria (PGPR) affects the composition of root endophyte, rhizosphere and non-rhizosphere bacterial communities and plant growth. In the present study, these four rhizobacteria were used as test inoculants for root inoculation of mandarin orange seedlings and the aims of the investigation were (1) to test the colonization ability of test rhizobacteria in the roots of mandarin orange seedlings grown in axenic culture and soil, (2) to determine the growth enhancement of plant upon root inoculation of 90 days old seedlings with the test rhizobacteria either individually or as a consortium, and (3) to analyse the changes in the composition of root endophyte, rhizosphere and non-rhizosphere soil bacterial communities at different timepoints during one year of plant growth period.

2. Materials and methods

2.1. Root colonization study

2.1.1. Rhizobacterial strains and treatment combinations

Enterobacter hormaechei (RCE1), *Enterobacter asburiae* (RCE2), *Enterobacter ludwigii* (RCE5) and *Klebsiella pneumoniae* (RCE7) are mandarin orange rhizosphere bacteria. Their full length sequences of 16S rRNA genes are available in Genbank under Accession numbers JN673773, JN673774, JN673777 and JN673779, respectively [47]. *Bacillus megaterium* (MTCC 4126) strain P5, a PGPR of rice rhizosphere origin [46], was used as a positive control. Mandarin orange was inoculated with either individual strains, an RCE1, RCE2, RCE5 and RCE7 consortium (CT), or left uninoculated as a negative control. Inocula were prepared by culturing in Luria Bertani (LB) medium at 30 °C and 120 rpm till 10^8 CFU mL^{-1} of LB broth.

Healthy seeds (uniform size and vigor) from ripe fruits of mandarin orange were surface sterilized in 2% sodium hypochlorite solution for 7 min, 70% ethanol for 2 min and finally five rinses with sterile water. The effectiveness of the sterilization was assessed by incubating the surface sterilized seeds on nutrient agar plates for 2 days. The surface sterilized seeds were immersed in the rhizobacterial suspension (LB broth) according to treatment combinations, shaken at 120 rpm for 30 min and dried under laminar air flow. For the negative control, surface sterilized seeds were immersed in sterile LB broth.

2.1.2. In vitro experiment - endophytic and root surface colonization

The root colonisation assay was conducted in triplicate using 200 mL test tubes containing Murashige and Skoog medium [32]. The inoculated seeds were pre-germinated in Petri-dishes, transferred to the tubes aseptically and grown in a plant growth chamber (LGC–3301R, Daihan Lab. Tech. Co. Ltd.) at 16 h photoperiod, 50–70% humidity and 22–25 °C for 2 months.

2.1.3. Scanning and transmission electron microscopy (SEM and TEM)

Fresh fine root samples (obtained from the assay described in section 2.1.2.) were fixed in 2.5–3.0% glutaraldehyde (Sigma, St. Louis, MO, USA) for 4 h and washed in 0.1 M sodium cacodylate buffer (3 changes) for 1 h. Then, the roots were dehydrated by dipping in increasing concentrations of acetone in water, and finally washed in 100% acetone. The samples were dried using the tetramethylsilane (TMS) method in which the specimens were immersed in TMS for 5–10 min for two changes at 4 °C followed by drying at room temperature (25–26 °C). Finally, root specimens were mounted on brass or aluminium stubs and coated using silver or gold (~35 nm thick) before being examined and digitised under SEM (JSM-6360, JEOL). Likewise, for TEM observation, fresh fine root samples were washed, fixed, sectioned, post-fixed and dehydrated as described above for SEM. The root sections were embedded in Spurr-resin and polymerised at 40–50 °C for hardening the resin. The ultrathin sections (60–90 nm) were deposited onto copper grids coated with formvar and contrasted with 2.5% uranyl acetate and lead citrate. The sections were examined under electron microscope (JM-2100), operating at 200 kV and digitised photographs were obtained.

2.2. Pot experiment

2.2.1. Rhizobacterial inocula preparation

Individual rhizobacterium (RCE1, RCE2, RCE5, RCE7 and P5) and a RCE1, RCE2, RCE5 and RCE7 consortium (CT) were cultured in LB broth and incubated for 48 h at 30 °C and 120 rpm. Finely ground compost (passed through 500 μm sieve) was autoclaved (121 °C at 15 psi for 20 min) 3 times. 200 mL culture broth was mixed with 360 g sterile compost to form slurry with 10^8–10^9 CFU g^{-1}compost.

2.2.2. Pot soil

The pot soil was air-dried, pulverized, and passed through a 3.2 mm sieve and not sterilized (natural soil). Pots were filled with 4 kg soil and 40 g of sterile compost. The bulk density of the pot soil was made at 1.50 g cc^{-1}. Soil filled pots were kept in a greenhouse covered with shade net (75%). The chemical properties of the soil were: 1.10% total organic carbon, 1792 kg ha^{-1} total nitrogen, 224 kg ha^{-1} available N, 672 kg ha^{-1} total phosphorus, 35.4 kg ha^{-1} available P_2O_5, 1210 kg ha^{-1} total K, 379 kg ha^{-1} available K and pH 6.5.