Biological Control 94 (2016) 11–24

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Rhizosphere and endorhiza of oilseed rape (*Brassica napus* L.) plant harbor bacteria with multifaceted beneficial effects

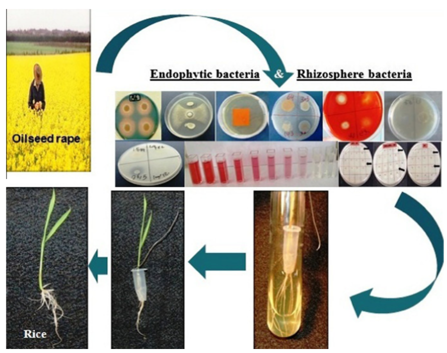
Hassan Etesami *, Hossein Ali Alikhani

Department of Soil Science, University of Tehran, Iran

HIGHLIGHTS

- PGP traits were different between endophytic and rhizosphere isolates.
- Both endophytic isolates and rhizosphere isolates showed good antifungal activity.
- IAA production of isolates was positively correlated with antifungal activity.
- IAA production may be a useful trait to screen antagonistic bacteria.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 June 2015
Revised 30 November 2015
Accepted 5 December 2015
Available online 8 December 2015

Keywords:
Rhizobacteria
Endophytic bacteria
Oilseed rape
Rice
PGPR
Multiple PGP traits

ABSTRACT

In this study, bacteria were isolated from the rhizosphere and inside the roots of oilseed rape (*Brassica napus* L.) plant grown in the field in Iran. A total of 150 isolates were obtained from the rhizosphere (90 isolates) and endorhiza (60 isolates) of *B. napus* plants grown in rotation with rice. All of the isolates were characterized for production of plant growth promoting (PGP) traits and antifungal activity against some rice plant pathogen fungi. The results showed the endophytic and rhizosphere isolates had different PGP traits in terms of both the number and the production amount of these traits. Only one rhizosphere isolate and two endophytic isolates showed highly inhibitory effects against the mycelial growth of all fungal rice pathogens tested in this study. In addition, thirty-seven isolates inhibited only the mycelial growth of some the fungal rice pathogens. A higher percentage of the endophytic isolates were positive for production of IAA, siderophore, and ACC deaminase as compared to rhizosphere isolates respectively. The best bacterial isolates (one endophytic isolate and one rhizosphere isolate), based on multiple PGP traits and inhibitory effects against the mycelial growth of all fungal rice pathogens, were identified. Based on biochemical tests and by comparison of 16S rDNA sequences, the endophytic isolate CEN_3 and the rhizosphere isolate CEN_5 were closely related to *Bacillus subtilis* and *Bacillus cereus* respectively. These bacteria exhibited a broad-spectrum antifungal activity towards all rice phytopathogenic fungi. We also studied the PGP effect of these isolates as single and co-inoculation on rice seedlings. All treatments resulted in significant increases ($p < 0.05$) in root length, root fresh weight, and shoot dry weight, as compared to control. When these isolates were applied as co-inoculation on rice seedlings, the highest increase in rice plant growth indices and population size of these isolates after 20 days was observed as compared to treatments receiving these isolates alone. In conclusion, the *B. subtilis* CEN_3 and *B. cereus*

* Corresponding author at: Soil Science Department, Faculty of Agricultural Engineering and Technology, University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran.

E-mail address: hassanetesami@ut.ac.ir (H. Etesami).

http://dx.doi.org/10.1016/j.biocontrol.2015.12.003

CEN_5 strains analyzed here exert multiple PGP and antagonistic mechanisms, show positive root colonization capability, and represent an excellent option to be used as either potent bio-promoting or bio-control agents in crops such as rice.

1. Introduction

In recent years, interest in the use of plant growth promoting rhizobacteria (PGPRs) to promote plant growth due to the social claim for clean agriculture has increased. The use of beneficial bacteria as agricultural inputs for increasing crop production needs the selection of competent rhizobacteria with plant growth promoting (PGP) traits. This application can help to minimize dependence on chemical fertilizers, which in the long term can have adverse effects on environment. PGPRs are rhizosphere bacteria that can enhance plant growth by a wide variety of mechanisms (Bhattacharyya and Jha, 2012). Evidence suggests that plant growth stimulation is the net result of multiple mechanisms that may be activated simultaneously (Martinez-Viveros et al., 2010). Bacteria are common inhabitants of both the surfaces (epiphytes) and the internal tissues (endophytes) of most plants and may have diverse effects on host plant growth. Endophytic bacteria use the same mechanisms to influence plant growth (Lugtenberg and Kamilova, 2009). However, regardless of the mechanism of plant growth promotion, to be more effective in the rhizosphere and endorhiza, PGPRs must maintain a critical population density for a longer period. Successful root colonization and persistence of PGPRs in plant rhizosphere and endorhiza are required in order to exert their beneficial effect on the plant (Elliot and Lynch, 1984). In general, the rhizosphere harbors an extremely complex microbial community including saprophytes, epiphytes, endophytes, pathogens and beneficial microorganisms. In natural systems, these microbial communities tend to live in relative harmony where all populations generally balance each other out in their quest for food and space (Belanger and Avis, 2002). *Brassica napus* (oilseed rape) and *Brassica oleracea* var. *botrytis* (broccoli and cauliflower) are the most economically important species of *Brassica* worldwide. *B. napus* is cultivated in rotation with rice (*Oryza sativa* L.) in Iran. There are many studies showing *Brassica* species harbor microorganisms with beneficial potential in terms of PGP traits and antagonistic activities (Zhang et al., 2014; Card et al., 2015). Naturally, plants select PGPRs that are competitively fit to occupy compatible niches without causing pathological stress on them. However, when screening bacteria for PGP agents, it is better to screen them for the most promising isolates having suitable colonization and PGP traits. The use of endophytic, epiphytic, and rhizosphere bacteria in agricultural production depends on our knowledge of the bacteria–plant interaction and our ability to maintain, manipulate and modify beneficial bacterial populations under field conditions (Hallmann, 1997). In addition, an understanding of the mechanisms enabling these rhizobacteria and endophytes to interact with plants will be worthwhile to completely achieve the biotechnological potential of efficient plant–endophyte and rhizosphere partnerships for a range of applications. The study of the structure of endophytic and rhizosphere microbial populations, their distribution, interaction and functions within and outside of their host is important for understanding their ecological role. There are two approaches to evaluate the diversity of PGP bacteria; one is by isolating cultivable bacteria and other approach is to check the presence of non-cultivable bacteria using random amplified polymorphic DNA (RAPD) and temperature gradient gel electrophoresis (DGGE) (Goswamia et al., 2014). Only cultivable bacteria can be used to develop bio-fertilizers for commercial applications. Hence, the present study was designed to isolate cultivable bacterial rhizosphere and endophytic isolates from the rhizosphere and endorhiza of *B. napus* and characterize them in terms of PGP traits and antagonistic activities so that they can be exploited as a potential bio-inoculant for rice and oilseed rape plants. In addition, the PGP effect of the most promising antagonistic bacterial endophytic and rhizosphere isolates (as a secondary effect) as single and co-inoculation on rice seedlings in the absence of pathogens was studied. We also tried to understand the possible mechanism (s) involved in the promotion of rice plant growth and antifungal activity upon inoculation of antagonistic bacterial isolates.

2. Materials and methods

2.1. Isolation of endorhiza and rhizosphere bacteria

Rhizosphere soil and roots of the oilseed rape (*B. napus* L.) were collected from the Dashte Naz Research Farm (36°37′North, 53°11′East, and about 16 m above sea level) in Iran. Healthy *B. napus* plants, at flowering, were collected randomly from different locations of a field plot where rice had been rotated with *B. napus* and conveyed to the laboratory in coolers. Endorhiza and rhizosphere bacteria were isolated as previously described by Etesami et al. (2014). Serial dilutions (up to 10^{-8}) were made and 0.1 ml aliquots were spread on to nutrient agar (NA) plates. All the plates were incubated at 28 ± 2 °C for 3–5 days at which time the number of colony-forming units (CFU) was counted. Numbers of endophytic and rhizosphere bacterial cells recovered were expressed as CFU g^{-1} fresh tissue weight (wt) and soil respectively. Three replicates per dilution were made. Finally, bacterial isolates identified as individual CFU were selected and subcultured onto NA. Similar bacterial isolates were grouped based on phenotypic characteristics such as shape, motility, color, rate of growth, culture morphology, and Gram-staining reaction, as there has not been a possibility of obtaining repeated strains in the collection, and stored (maintained on the respective slants) in a refrigerator at 4 °C for further studies. For long-term storage, bacterial cultures were maintained at −80 °C in nutrient broth (NB) that contained 20% glycerol. Culture media were supplemented, when required, with cycloheximide (100 mg ml^{-1}) to inhibit the growth of fungi.

2.2. Assay for PGP activities

Production of siderophore, indole-3-acetic acid (IAA) (μg ml^{-1}), and hydrogen cyanide (HCN) were determined as described by Schwyn and Neilands (1987), Patten and Glick (2002), and Lorck (1948) respectively. The presence of orange halos around the colonies on chrome azurol S (CAS) agar media was considered as indicator for the presence of siderophore. Development of colors of light brown (one point), dark brown (two points), and reddish brown (three points) on the Whatman filter paper indicated production of HCN and was considered as an index for evaluating HCN producing isolates. Ability of phosphate-solubilizing of the bacteria was determined on Pikovskaya agar (1948) and proposal suggested by Bashan et al. (2013) was taken into account during the screening of effective phosphate-solubilizing bacteria. Mineral phosphate solubilization and siderophore production index were