Biological Control xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biological Control

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

Plant growth promoting and inducible antifungal activities of irrigation well water-bacteria

Nassira Tabli[a], Abdelwahab Rai[a,b,c,d], Leila Bensidhoum[a], Gianna Palmieri[b], Marta Gogliettino[b], Ennio Cocca[b], Carmela Consiglio[b], Fabrizio Cillo[c], Giovanni Bubici[c], Elhafid Nabti[a,*]

[a] Laboratoire de Maitrise des Energies Renouvelables, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria
[b] Institute of Biosciences and Bio Resources (IBBR). National Research Council (CNR), Naples, Italy
[c] Institute for Sustainable Plant Protection (IPSP), National Research Council (CNR), Bari, Italy
[d] Université de Sétif-1, FSNV, Département de microbiologie, Elbez, 19000 Sétif, Algeria

ARTICLE INFO

Keywords:
Pseudomonas
Serratia
Biocontrol
Biological Control Agents
Plant Growth Promoting Bacteria (PGPB)

ABSTRACT

Different bacterial groups in irrigation well water are strongly implicated in soil health and plant development. Herein, 48 bacterial strains were isolated from agricultural well water in northern Algeria. Among them, four strains were selected based on their antifungal potential and their ability to express Plant Growth Promoting traits such as Indole Acetic Acid (IAA), hydrolytic enzymes, siderophores etc. The isolates were identified as *Pseudomonas* sp. (B, D and N strains) and *Serratia* sp. (C strain) by 16S rRNA gene sequencing. Mycelial growth inhibition against *Botrytis cinerea* and *Aspergillus niger* ranged from 60 to 90% for the four strains. Moreover, volatiles compounds emission by the isolates resulted in Plant Growth Inhibition values ranging from 13 to 50%, specifically against *B. cinerea*. Impressively, the strains' antifungal activity showed high inducibility as it was obtained only by the filtered supernatants from bacterial cultures previously in contact with the fungus. Finally, a greenhouse assay, carried out to determine the strains' efficacy in promoting plant growth and protecting seedlings under *Pythium aphanidermatum*-infected soil, revealed that the strain N markedly enhanced pea germination (+250%) and fresh weight (+43%) and tomato fresh weigh (+10%). The results constitute an attempt for better use of the bacterial functional diversity from irrigation wells in sustainable agriculture.

1. Introduction

The worldwide demographic explosion, together with environmental degradation, has the unfortunate consequence that global food production may soon become insufficient to feed the world's population. It is therefore essential to increase agricultural productivity within the next few decades (Glick, 2012). Both biotic and abiotic stresses in agriculture such as drought, salinity, elevated temperature, metal toxicity, nutrient deficiencies and plant diseases caused by fungi, bacteria, viruses, and nematodes are more to more aggressive and constitute serious problems affecting final yields of the most consumed crops (El Khoury and Makkouk, 2010; Borges et al., 2014). Thus, achieving sustainable crop production to protect the environment and ensure enough food requires strategic measures to manage these ecological stresses such as water scarcity and climate change, commonly related to pathogens development and aggressiveness (Itier, 2008; Haggag et al., 2015). However, better understanding of the mechanisms underlying plant resistance/tolerance-related characters is of a great help to develop fruitful new agricultural strategies (Borges et al., 2014).

In the last few decades, the application of chemical fertilizers, pesticides, herbicides and fungicides was the dominant tool to enhance plant growth and to control pathogens propagation. However, such approaches have led to soil degradation and resistance emergence in plant-pathogen populations (Pal and McSpadden Gardener, 2006). In addition, the harmful effects of such chemicals on both human health and the environment obliged researchers to seek for secure and eco-friendly alternatives (Reuveni, 1995; El Khoury and Makkouk, 2010). Certain soil bacteria, commonly described as Plant Growth Promoting Bacteria (PGPB), are able to enhance plant yield and to control phytopathogens, constituting the most widely studied and increasingly used tool in modern agriculture. In future, PGPB are expected to replace chemical fertilizers, pesticides and artificial growth regulators that have numerous side effects on sustainable agriculture (Tsegaye et al., 2017).

PGPB can directly affect plant metabolism through nitrogen fixation, mineral solubilization, plant hormones modulation and plant tolerance improvement to abiotic stresses (drought, salinity, nutrient

* Corresponding author.
E-mail address: elhnabti1977@yahoo.fr (E. Nabti).

http://dx.doi.org/10.1016/j.biocontrol.2017.10.010
Received 6 August 2017; Received in revised form 5 October 2017; Accepted 20 October 2017

deficiency etc.). PGPB also participate indirectly in preventing phytopathogens development either by competition for space and nutrient occupation or by producing antibiotic metabolites, inducing systemic resistance in the whole plant (Niranjan Raj et al., 2006; Ahmadzadeh and Tehrani, 2009; Suresh et al., 2010; Tri Wahyudi et al., 2011; Beneduzi et al., 2012). Bacteria belonging to genera *Acetobacter, Achromobacter, Arthrobacter, Azoarcus, Azospirillum, Pseudomonas, Serratia, Anabaena, Acetobacter, Clostridium, Enterobacter, Flavobacterium, Frankia, Rhizobium* and others are the most used as PGPB for several vegetal species (wheat, rice, pea, chickpea, tomato etc.). In addition, the use of PGPB in the biocontrol of phytopathogenic fungi (*Botrytis*, *Aspergillus*, *Mucor*, *Phytophthora* etc.) is not less adopted (Elad et al., 1996; Limura and Hosono, 1998; Loqman et al., 2009; Glick, 2012; Talibi et al., 2014; Tabli et al., 2014). Unfortunately, as the results obtained in laboratory are not necessarily ensured *in vivo*, the unpredictable character of the most *in vitro*-selected PGPB oblige both scientists and farmers to seek for more competent isolates that are able to ensure better results in soil, a more complex environment than *in vitro* substrates (Przemieniecki et al., 2015).

As water is the most limiting factor for agricultural production, water scarcity could potentially lead to lands abandonment due to economic losses (EEA, 2009). From a microbiological point of view, some bacteria in irrigation well water may constitute potential source of significant metabolic richness with high ability to interact with plants, leading to yields improvement, soil restoration and pathogens biocontrol (Bensidhoum et al., 2016). In addition, PGPB are isolated from different natural sources including soil, plants and mineral waters (Ramadan et al., 2015; Bensidhoum et al., 2016). Herein, we selected four multi PGP- and antagonistic-treats bacteria from an irrigation well water in Northern Algeria. The bacteria were screened for their ability to produce metabolites such as hydrogen cyanide (HCN), ammonia, indole acetic acid (IAA), hydrolytic enzymes, siderophores etc. After identification, the multi-PGP isolates were tested for their *in vitro* ability to inhibit fungal growth and sporulation. The PGP ability of the selected strains was then verified on tomato and pea plants and biocontrol properties against pathogens was confirmed on pea plants growing on *Pythium aphanidermatum*-infected soil.

2. Materials and methods

2.1. Water sampling

Bacteria were isolated from water samples of an irrigation well located in the region of Djebira in Bejaia, northern Algeria (36°41′59.2″N; 5°04′28.8″E). The samples were transported to the laboratory at 4 °C and bacterial isolation was immediately performed on Plate Count Agar medium (PCA). Thus, the samples were serially diluted (up to 10^{-6}) and each dilution served as inoculum for three PCA plates (1 ml/Petri dish). After incubation (72 h at 30 °C), the phenotypically different colonies were purified on the same medium and conserved for further studies.

2.2. Fungal strains and growth conditions

The plant pathogenic fungi used in this work were *Botrytis cinerea* BC1, kindly provided by the laboratory of Mycology (University of Bejaia-Algeria), and *Aspergillus niger* ATCC 9642, available at the Institute of Bioscience and Bio-Resources (IBBR)-CNR, Italy. The two fungal strains were revivified and purified on Potato Dextrose Agar (PDA) medium before use.

2.3. Selection procedure and antifungal activity

Initially, 48 bacterial isolates, obtained from water samples were tested for their antifungal potential against the aforementioned fungal strains, using agar diffusion method as described by Sagahón et al. (2011). Thus, 5 mm diameter PDA plugs, containing young mycelium of either *B. cinerea* or *A. niger*, were taken from actively growing colonies (4 days old) and then deposited in the plate center of the same medium. 10-µL overnight cultures of each bacterial strain, growing on Luria Bertani broth, were spotted at 1.5 cm from the plate edge (three spots/plate). The plates were sealed with parafilm and incubated for 7 days at 21 ± 2 °C (*B. cinerea*) and 25 ± 2 °C (*A. niger*). Controls without bacteria were used and the experiment was done in duplicates. At the end, the percentage of mycelial growth inhibition (PGI) was calculated according to the following formula: PGI% = KR-R1/KR × 100 (KR is the distance in mm covered by the fungi in the control plate; R1is the distance in mm between the fungi and the bacterial colony) (Soylu et al., 2005).

Four isolates, selected following the previous test, were phenotypically characterized (cell/colony shape, Gram, catalase, oxidase, mobility) (Guiraud and Galzy, 1980). They were then phylogenetically identified based on their 16S rRNA sequences and used for further studies.

2.4. Spore's germination inhibition

The selected strains' ability to inhibit fungal spore's germination was screened by mixing 20 µL of the spore suspension (10^6 spores/mL) with equal volumes of 24 h-old bacterial culture grown in LB medium (10^8 CFU/mL). The mixtures were kept in Eppendorf tubes containing 1 mL of 5% glucose solution, prepared in sterile distilled water. The tubes were incubated at 21 ± 2 °C (*B. cinerea*) and 25 ± 2 °C (*A. niger*) for 24 h. Control tubes with only fungal spores were prepared and the experiment was repeated in triplicate. The spores' germination success was hemocytometrically measured by determining the percentage of spores germination inhibition (SGI%) (Sadfi-Zouaoui et al., 2008).

2.5. Antifungal volatiles production

The production of volatile compounds implicated in the strains' antagonistic activities was assayed using the protocol described by Dennis and Webster (1971). A bottom portion of PDA-containing plates was inoculated with 5 mm disc of the pathogen fungi (*B. cinerea* or *A. niger*). A similar bottom portion was streaked with the antagonist. Both bottom plates were placed face to face and sealed with parafilm. Plates without bacteria were used as control. The fungal radial growth was recorded, 7 days later, as compared to the control.

2.6. Metabolic and functional characterization

2.6.1. Hydrolytic enzymes secretion

Hydrolytic enzymes production by the selected strains were tested using agar disk methods. The bacterial strains were initially grown on PCA plates for 48 h at 30 °C. Five mm agar discs, containing the obtained cultures, were used to determine cellulolytic (Carder, 1986), esterasic (Carrim et al., 2006), lipolytic (Carrim et al., 2006), chitinolytic (Kopečný et al., 1996), proteolytic (Carrim et al., 2006), amylolytic (Vinoth et al., 2009) and ureasic (Christensen, 1946) activities.

2.6.2. Phosphate solubilization

The ability of the four strains to solubilize tricalcium phosphate was tested on Pikovskaya's agar medium, as described by Peix et al. (2001). After 3 days of incubation (30 °C), phosphate-solubilizing bacteria generate transparent halos around colonies.

2.6.3. Siderophores production

The bacterial isolates were assayed for siderophores production on the Chrome Azurol-S agar medium, as described by Schwyn and Neilands (1987). After incubation (30 °C/48–72 h), the development of yellow to orange halo around the colony indicated siderophores