European Journal of Soil Biology 47 (2011) 374–379

Contents lists available at SciVerse ScienceDirect

European Journal of Soil Biology

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

Original article

Identification and role of organic acids in watermelon root exudates for recruiting *Paenibacillus polymyxa* SQR-21 in the rhizosphere

Ning Ling, Waseem Raza, Jinghua Ma, Qiwei Huang*, Qirong Shen

Provincial Key Lab of Organic Solid Waste Utilization, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

ARTICLE INFO

Article history:
Received 31 March 2011
Received in revised form
20 July 2011
Accepted 18 August 2011
Available online 3 October 2011
Handling editor: Kristina Lindström

Keywords:
Chemotaxis
Paenibacillus polymyxa
Root exudates
Swarming
Watermelon

ABSTRACT

Paenibacillus polymyxa can serve as a biocontrol agent with a broad host range, but knowledge of the possible contribution of root exudates to its colonization of the rhizosphere remains limited. In this experiment, we identified several organic acids in the root exudates of watermelon. Chemotaxis and swarming assays were performed to investigate the ability of these organic acids to induce the motility of *P. polymyxa* SQR-21. Oxalic acid, malic acid and citric acid were present in the root exudates but only the intermediate products in tricarboxylic acid cycle, i.e., malic acid and citric acid, could significantly induce motility in *P. polymyxa* SQR-21. The maximal inducing ability was obtained with malic acid. Values for malic acid were 3.9 and 1.5 times higher than the control in the chemotaxis assay and the swarming assay, respectively. An *in vitro* experiment further confirmed that these intermediate products in tricarboxylic acid cycle could promote recruitment to *P. polymyxa* SQR-21, thereby increasing the population in the rhizosphere. In conclusion, some of the organic acids secreted by roots could play an important role in root colonization of SQR-21. This finding contributes to our understanding of the interactions of bacteria and plants under natural conditions.

1. Introduction

In the past two decades, significant progress has been made in understanding the mechanisms of plant growth promotion and disease suppression by introduced bacteria, but the bacterial traits involved in the process of rhizosphere competence remain poorly understood [1,2]. Root-secreted chemicals mediate multipartite interactions in the rhizosphere, where plant roots continually respond to and alter their immediate environment. These chemicals are mostly from the rhizodeposition processes that serve as a major source of soil organic carbon released by plant roots [3,4]. Some of these chemicals may play an essential role in root colonization of bacterial biocontrol agents [5,6]. To achieve successful and reproducible biocontrol efficacy, knowledge of the ecological interactions taking place between root-secreted chemicals and biocontrol agents in the rhizosphere is required to predict the conditions under which robust biofilms can be formed.

Certain *Paenibacillus polymyxa* strains that associate with many plant species have been effectively used in the control of plant pathogenic fungi and bacteria [7–9]. *P. polymyxa* SQR-21 (SQR-21) isolated by the Provincial Key Lab of Organic Solid Waste Utilization (Jiangsu, China), was found to be highly effective for controlling *Fusarium*-wilt disease [10–12] on watermelon by the production of antibiotics and hydrolytic enzymes [13], systemic alteration of root exudates [14], and biofilm formation. However, information is still needed about the relationship between watermelon root exudates and SQR-21 motility and root-surface colonization.

Motility can have a profound impact on the colonization of surfaces [15]. Most motile bacteria can sense and respond to low concentrations of organic compounds in their environment through the process of chemotaxis [16,17]. We hypothesized that the organic acids secreted by the watermelon roots could activate the chemotaxis and swarming of SQR-21 and could therefore increase its population in the rhizosphere and contribute to biofilm formation on the root surface. To determine the effect of organic acids present in watermelon root exudates on recruitment of SQR-21 in the rhizosphere, we identified some of the organic acids secreted by watermelon roots, evaluated the abilities of these organic acids to activate the chemotaxis and swarming of SQR-21, and further *in vitro* assessed the recruitment to plants of SQR-21 induced by organic acids.

2. Materials and methods

2.1. Bacterial strain

The *P. polymyxa* SQR-21 (CGMCC accession no: 1544; China General Microbiology Culture Collection Center) was isolated and

* Corresponding author. Tel.: +86 25 84396212.
E-mail address: qwhuang@njau.edu.cn (Q. Huang).

1164-5563/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejsobi.2011.08.009

identified by the Provincial Key Lab of Organic Solid Waste Utilization, Jiangsu, China and found to be highly efficient against *Fusarium oxysporum*-caused cucumber and watermelon wilt diseases. The SQR-21 was incubated in Luria-Bertani liquid culture on a shaker at 30 °C and 170 rpm. Bacterial cells were harvested, and the optical density was determined.

2.2. Plant material and growth conditions

Seeds of watermelon (*Citrullus lanatus* L. cv. Jingxin No.1) obtained from the Nanjing Vegetable Research Institute, Jiangsu, China, were surface-sterilized with household bleach (2% NaOCl) for five min, rinsed four times in sterile double-distilled water and then sown in pots containing steam-sterilized perlite. Fifteen days after seeding (first true leaf unfolded), the plantlets were gently transplanted to a plastic pot (volume 650 ml) containing a moist, sterilized mixture of perlite and vermiculite (V:V = 1:1). Each plastic pot contained two plantlets. Perlite and vermiculite were chosen because they could easily be washed from the roots. The transplanted plants were cultivated for an additional 30 days and watered with Hoagland nutrient solution every three days throughout the experiments. All the experiments were performed in a greenhouse at 32 ± 2 °C with a photoperiod of 16 h light/8 h dark. After 30 days of growth, the plants were sampled to harvest the root exudates.

2.3. Collection of root exudates

For collection of root exudates, plants were removed by gently washing the perlite and vermiculite from the roots with tap water. One batch, consisting of four plants, was placed in a beaker containing 200 ml sterile double-distilled water so that the roots were completely submerged. Five beakers (amount to 20 plants) were used in the experiment. All the plants were placed in a plant growth chamber for 24 h (16 h light/8 h dark) at 28–32 °C and subsequently removed from the beaker.

A volume of 1000 ml of the water was then passed through a column containing 10 ml XAD-4 resin (Sigma, USA). The compounds were eluted with 200 ml of spectral-grade methanol (Sigma, American) and evaporated in a rotary evaporator (Yarong Model RE-52A, Shanghai, China) at 40 °C to a volume of 1 ml. The compounds were stored at −80 °C for use in the bioassay and analysis by HPLC.

2.4. Identification of organic acid in root exudates

The standard organic acid compounds used for HPLC were oxalic acid, malic acid, citric acid, succinic acid and fumaric acid. The compounds were identified using an HPLC system (Agilent 1200, USA). The column was XDB-C18 (4.6 × 250 mm, Agilent, USA) and the mobile phase consisted of 5 mmol/L H_2SO_4 (A) and methanol (B) with a gradient elution. The compositions of the gradients were as follows: 0 min, 95% A plus 5% B at the flow rate of 0.4 ml/min → 10 min, 90% A plus 10% B at the rate of 0.4 ml/min → 15 min, 90% A plus 10% B at the rate of 0.4 ml/min → 16 min, 90% A plus 10% B at the rate of 0.5 ml/min → 20 min, 90% A plus 10% B at the rate of 0.5 ml/min → stop. The UV detector wavelength was set at 210 nm. Standard compounds were chromatographed alone and in mixture. Retention times for the standard compounds and the major peaks in the extracts were recorded. Organic acid compounds from each fraction were identified by comparing their retention times and the areas of their peaks with those of the standards [18].

2.5. Chemotaxis assay

A capillary assay was performed according to the method of Rudrappa et al. [19] with some modifications. Briefly, the assay setup consisted of a 200-ml pipette tip as a chamber for holding 100 ml of bacterial suspension (0.1 OD600 prepared from culture grown overnight) in Luria–Bertani medium. A 4-cm 25-gauge needle (Becton-Dickinson) was used as the chemotaxis capillary and was attached to a 1-ml tuberculin syringe (Becton–Dickinson) containing 200 ml of the compound (organic acid compounds with different concentrations were introduced to the syringe separately in Luria–Bertani liquid medium). After 2 h of incubation at room temperature, the needle syringe was removed from the bacterial suspension and the contents were diluted and plated in Luria–Bertani medium. Accumulation of bacteria in the capillaries was calculated as the average from the CFUs obtained in triplicate plates, and the results were expressed as the mean of three separate capillary assays for each determination. The relative chemotaxis index (RCI) was calculated as the ratio of the accumulation value for the bacteria that entered the test capillary to the corresponding value for the control capillary. An RCI of 2 or greater is considered significant for this method [20].

2.6. Swarming assay

The swarming assay was performed as described by Park et al. [2] with modifications. Swarm agar medium (1.0% agar) plates were prepared and air-dried for 30 min in a laminar flow hood. A sterile paper disk (8 mm diameter) was then put in the center of each plate. Inoculum of fresh *P. polymyxa* SQR-21 from colonies was obtained by incubation in Luria–Bertani liquid culture on a shaker at 170 rpm and 30 °C. Cells in the logarithmic growth phase (OD600 nm of 0.4) were resuspended in 2 mL phosphate buffer (50 mM) after gentle spinning at 600 × g and 4 °C for three min. Five microliters of the bacterial suspension loaded on the paper disks was drop-inoculated at the centre of the swarm agar medium (1.0%, w/v, agar) plate containing 30 μM of different organic acids. The bacteria were incubated at 28 °C for 4 d. Colony diameters were measured in three directions on each plate with three replications after incubation for 4 d. The results were expressed as the mean of three separate assays for each determination.

2.7. In vitro assay

An *in vitro* assay was performed in tissue culture bottles containing 50 ml of solid nutrient medium. The solid nutrient medium was prepared as described in our previous report [10]. The germinated seeds of watermelon were sown in the nutrient medium in the

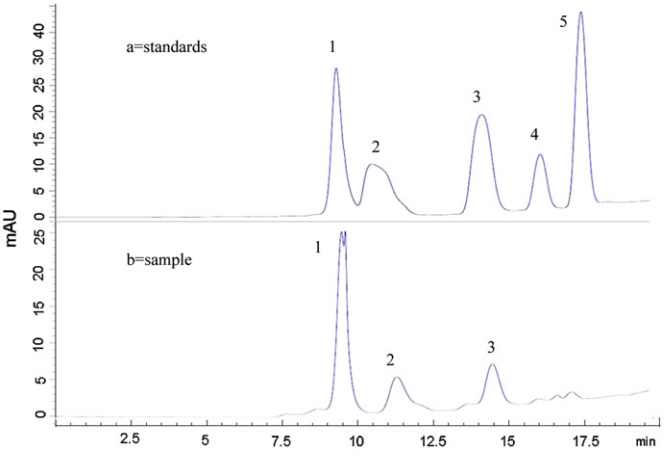

Fig. 1. The chromatogram of organic acids detected with HPLC for chemical standards (a) and for watermelon root exudates (b). The peaks from left to right in the figure represent the different compounds: 1, oxalic acid; 2, malic acid; 3, citric acid; 4, succinic acid; 5, fumaric acid.