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Chemotactic response of Ginseng bacterial soft-rot to Ginseng root exudates

Fengjie Lei [a,b], Junfan Fu [a,*], Rujun Zhou [a], Dan Wang [a], Aihua Zhang [b], Wenli Ma [b], Lianxue Zhang [b]

[a] Plant Protection College, Shenyang Agricultural University, Shenyang 110866, China
[b] College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun 130118, China

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ABSTRACT

Our purpose was to evaluate chemotactic response of Ginseng bacterial soft-rot to ginseng root exudates. The exudates of plant roots has a significant influence on the population changes of rhizosphere microorganisms and chemotaxis is an important way in which many pathogens sense the signals of host plants and invade the host plants. In this study, with the capillary method, we tested the chemotactic responses of Ginseng bacterial soft-rot for three ginseng roots exudates under four chemotactic parameters (concentration, temperature, pH and time). The results showed that the chemotatic response of the Ginseng bacterial soft-rot for the ginseng roots exudates at the water layer where pH = 7 and the concentration was 0.0125 mg/L reached its peak value under the circumstance that the exudates was cultivated for 60 min at 25 °C. The chemotatic ratios were respectively 124.89% and 89.44%. For the butanol extract layer and the petroleum ether faction at the concentration of 0.125 mg/L and the pH value at 7, the ginseng roots exudatess reached peak values at 25 °C and 30 °C and 60 min and 75 min respectively, and the chemotatic ratios were respectively 139.64% and101.87%, and 115.29% and 81.36%. The three ginseng roots exudates had positive effects for the chemotaxis of the Ginseng soft-rot bacteria, but the effect declined as the concentration increased.

1. Introduction

There is a chemical communication between plant root exudates and soil microorganism. The influence of plant root exudates on soil microorganism has become a new and hot issue in soil ecology in recently years (Kong and Lou, 2010; Bacilio-Jiménez et al., 2003; Bais et al., 2006). When the nutrient substances in soil are in certain concentration gradients, some bacteria will show Chemotaxis Response based on the instinct of adapting to the environment. As a directional movement of microorganism caused by the instinct of adapting to the environment, chemotaxis can help microorgainsm perceive the change of concentration gardients of chemical substances in surrounding environment, seek food and stay away from toxic environment, which shows competitive advantage from the aspect of survival. More importantly, chemotaxis response is a key apporoch for many pathogenic bacteria to sense the signal of host plant and successfully invade into host (Li and Mu, 2006; Hua et al., 2008). Researches showed that a certain pathogen having the ability of tending to move or grow towards potential host has bigger change to successfully contact host (Sun and Wang, 2009). For example, as a scretion of tobacco or other injured plants, acetosyringone could attract *Agrobacterium tumefaciens* and activate virulent gene of plasmid encodes, which played a role in leading bacterial DNA move towards host (Ashby et al., 1988, 1987). Daidzin and Genistein couldnot only be regarded as chemical attractant for fungal zoospores, but also lead to directional growth of hypha sprouted from rest spore as soybean root can do (Morris et al., 1998; A.H. Zhang et al., 2016; Z.H. Zhang et al., 2016).

Ginseng is an important medical herb in Araliaceae ginseng species. In the production of ginseng, the relatively severe ginseng disease is a bottleneck problem that limits ginseng production and quality. Ginseng bacterial soft-rot has now become one of major bacterial diseases that decrease the yield and quality of ginseng (Bai et al., 2000). Some secondary metabolites secreted from ginseng root are regarded as the nutrition substrates of rhizosphere

* Corresponding author.
E-mail address: fujunfan@163.com (J. Fu).

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microorganisms, which can act as allelopathic factor in adjusting the interaction between plants and bacteria, playing a significant role in affecting plant-environment interaction (Zhang et al., 2009a, 2009b). Some reports showed that phenolic acid secondary compounds in melon root exudates could affect spore germination and mycelial growth of *Fusarium oxysporum* in certain degree (Yang et al., 2014). From data prepared by Wang et al. (2014), that secondary metabolites secreted from roots of different disease-resistant varieties of peppers have inhibiting effect on zoosporangium formation, zoospore release, resting spore germination and mycelial growth of *phytophthora*. Former researches of ginseng secondary metabolites mainly focus on pharmacology and drug efficacy, but negelect its effects on the ecologies and physiologies of the host plants. There have been no reports on whether the ginseng secondary metabolites secreted from ginseng root can cause chemotaxis response of ginseng pathogenic microorganisms, and what the related factors and action mechanism within are. In this experiment, we tested the chemotactic responses of Ginseng bacterial soft-rot upon three componets in ginseng roots exudates using capillary method, in the hope of laying theoretical basis for the in-depth research of ginseng bacterial diseases.

2. Materials and methods

2.1. Materials

2.1.1. Test bacterium

Ginseng Soft-rot Bacteria (*Pseudomonas qessardii*) were collected from Jilin Ginseng Engineering and Technology Research Center, and identified by professor Gao Jie of Jilin Agricultural University.

2.1.2. Chemical substances

Water layer, N-butyl alcohol layer, and petroleum ether layer in three-year-old ginseng root exudates (purity of 95%). Sodium chloride (AR), Beijing Chemical Works; Beef extract (BR) and peptone (BR), Beijing AOBOX Biotechnological Co., Ltd; Agar powder (H8145), Shanghai Jiafeng Garden Supplies Co., Ltd.

2.1.3. Culture medium

Beef extract-peptone culture medium.
Liquid culture medium (g/L): beef extract 5.0, peptone 10.0, NaCl 5.0, pH 7.2–7.4.
Solid culture medium (g/L): beef extract 5.0, peptone 10.0, NaCl 5.0, agar 20, pH 7.2–7.4.

2.2. Methods

2.2.1. Preparation of bacteria liquid

Inoculate the bacteria stored in −70 °C into beef extract-pepton solid culture medium, and then culture at 25 °C for 24 h. Select the single colony and put it into appropriate amount of diluent (0.90% NaCl, pH 7.2) for fully shaking and mixing, resulting in bacterial suspension (10^8 CFU mL^{-1}, $OD_{625\ nm}$ = 0.1), which was then diluted into 10^7 CFU mL^{-1} bacterial suspension for future use.

2.2.2. Preparation of chemotaxis liquid

Control group: the control group 1 is a negative control group, of which the composition is mainly sterile water; the control group 2 is a positive control group, of which the composition is mainly sterile broth culture solution.

Prepare ginseng root exudates solution with water layer, N-butyl alcohol layer, and petroleum ether layer in concentrations of 0.0125 mg L^{-1}, 0.125 mg L^{-1}, 1.25 mg L^{-1}, 12.5 mg L^{-1}, respectively. Conduct filtration sterilization using 0.22 μm millipore filter for future use.

2.2.3. Chemotactic response test

Improved capillary method was adopted for chemotactic response test (Zou et al., 2009; Toole et al., 1999). One end of glass capillary tube (inner diameter of 0.5 mm) sucked chemotaxis liquid, while the other end was sealed by hot melt glue. Insert the glass capillary tube into 1 mL injector (containing 500 μL of bacterial liquid), and the incubate at 25 °C for 60 min. Wash the outer wall of capillary tube with sterile water to remove attached bacterial liquid, break the capillary tube and then transfer inside content into EP tube, add 40 μL of sterile water for 3 times dilution, and then suck out solution and evenly smear them on solid plate. The whole processes were repeated 5 times. After that, the plate was cultured at 25 °C for 4 h, and then record the average number of single colonies of 5 repeated tests. Therefore the chemotactic response intensity of Ginseng bacterial soft-rot can be measured by the number of bacteria in capillary tube.

2.2.4. Influences of three componets in ginseng root exudates to chemotactic response of ginseng soft-rot bacteria

Prepare ginseng root exudates solution with water layer, N-butyl alcohol layer, and petroleum ether layer in concentrations of 0.0125 mg L^{-1}, 0.125 mg L^{-1}, 1.25 mg L^{-1}, 12.5 mg L^{-1}, respectively. Conduct filtration sterilization using 0.22 μm millipore filter. Conduct chemotactic response test according to the method in Section 2.2.3.

2.2.5. Influence of temperature to chemotactic response of ginseng soft-rot bacteria

Chemotactic response tests were conducted according to method in Section 2.2.3 under temperature of 15 °C, 20 °C, 25 °C, 30 °C, respectively.

2.2.6. Influence of pH value to chemotactic response of ginseng soft-rot bacteria

Prepare ginseng root exudates solutions with water layer, N-butyl alcohol layer, and petroleum ether layer in pH value of 5, 6, 7, 8, respectively, and then conduct chemotactic response test according to method in Section 2.2.3

2.2.7. Influence of time to chemotactic response of ginseng soft-rot bacteria

Based on the method in Section 2.2.3, test Chemotaxis response of bacteria to ginseng root exudates solutions with water layer, N-butyl alcohol layer, and petroleum ether layer under culture time of 0 min, 30 min, 45 min, 60 min, 75 min, respectively.

2.2.8. Chemotactic response of Ginseng bacterial soft-rot to three components in ginseng root exudates under optimal chemotaxis parameters

Screen out the parameters for the most significant chemotaxis phenomenon under four conditions, and then conduct chemotaxis test according to the method in Section 2.2.3.

2.2.9. Data analysis

Chemotaxis rate = $(S - Sck)/Sck \times 100\%$ (wherein, S represents the chemotaxis parameter for Ginseng bacterial soft-rot to ginseng root exudates component, S_{ck} repsrents the chemotaxis parameter for Ginseng bacterial soft-rot to two control groups). Test data were processed using Excel (2007 edition). The significant variance analysis of statistic results were conducted using One-Way ANOVA in SPSS 18.0. Diagrams were charted using GraphPad Prism 5.0.