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

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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.

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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

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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.

Different English letters represent there are significant statistical difference between different treatments (P > 0.05).

3. Results

3.1. Influence of three components in ginseng root exudates on chemotaxis of ginseng Ginseng bacterial soft-rot

As showed in Fig. 1, the Ginseng bacterial soft-rot showed chemotactic response upon three components in ginseng root exudates, and the test results were higher than those of control 1 and 2. The chemotaxis of Ginseng bacterial soft-rot decreased as the concentration of water layer ginseng root exudates gradually increased, however when the root water layer root exudates concentration is 0.0125 mg L^{-1}, the chemotaxis reaches the highest level and was significantly higher than results of the same test (P > 0.05), with chemotaxis rate being 102.62% and 68.33%, respectively; the chemotaxis of Ginseng bacterial soft-rot showed a tendency of first increasing and then decreasing as the concentrations of N-butyl alcohol laye and petroleum ether layer root exudates gradually increase, when the concentration reaches 0.125 mg L^{-1}, the chemotaxis is the highest and significantly higher than results of the same test (P > 0.05), with chemotaxis rate being 132.71% and 78.18%, 109.10% and 74.43%, respectively.

3.2. Influence of temperature on chemotaxis of Ginseng bacterial soft-rot

Fig. 2 showed the chemotactic responses of Ginseng bacterial soft-rot to water layer (A), N-butyl alcohol layer (B), and petroleum ether layer (C) under 4 different temperatures. It could be seen that when culturing at 25 °C, Ginseng bacterial soft-rot showed strongest chemotaxis upon water layer and N-butyl alcohol layer, which was significantly higher than results of the same test group (P > 0.05) and decreased with the temperature; however when culturing at 30 °C, Ginseng bacterial soft-rot showed the most sensitive chemotaxis upon petroleum ether layer, which was significantly higher than results of the same test group (P > 0.05) and increased with the temperature. Under such four temperatures, the chemotaxis under 25 °C and 30 °C are higher than those under 15 °C and 20 °C.

3.3. Influence of pH on chemotaxis of Ginseng bacterial soft-rot

As showed in Fig. 3, Ginseng bacterial soft-rot showed chemotactic responses upon water layer (A), N-butyl alcohol layer (B), and petroleum ether layer (C) under different pH values, which showed a tendency of first increasing and then decreasing with the increase of pH values. It could be seen that when pH value was 7 (neutral environment), Ginseng bacterial soft-rot showed strongest chemotaxis upon water layer, N-butyl alcohol layer, and petroleum ether layer, which was significantly higher than results of the same test group (P > 0.05). From an overall perspective, the chemotaxis upon water layer and N-butyl alcohol layer in netural and alkaline environment (pH = 7, pH = 8) were significantly higher than those in acid environment (pH = 5, pH = 6). Under the same pH value (pH = 7 or pH = 8), the intensities of chemotaxis upon different exudatess could be sequenced as N-butyl alcohol layer > water layer > petroleum ether layer.

3.4. Influence of time on chemotaxis of Ginseng bacterial soft-rot

As showed in Fig. 4, Ginseng bacterial soft-rot showed chemotactic responses upon water layer (A), N-butyl alcohol layer (B), and petroleum ether layer (C) at different culture time, which were all significantly higher than that when culture time is 0 min and showed a tendency of first increasing and the decreasing with the extending of culture time. It could be seen that Ginseng bacterial soft-rot reacheed the strongest chemotaxis upon water

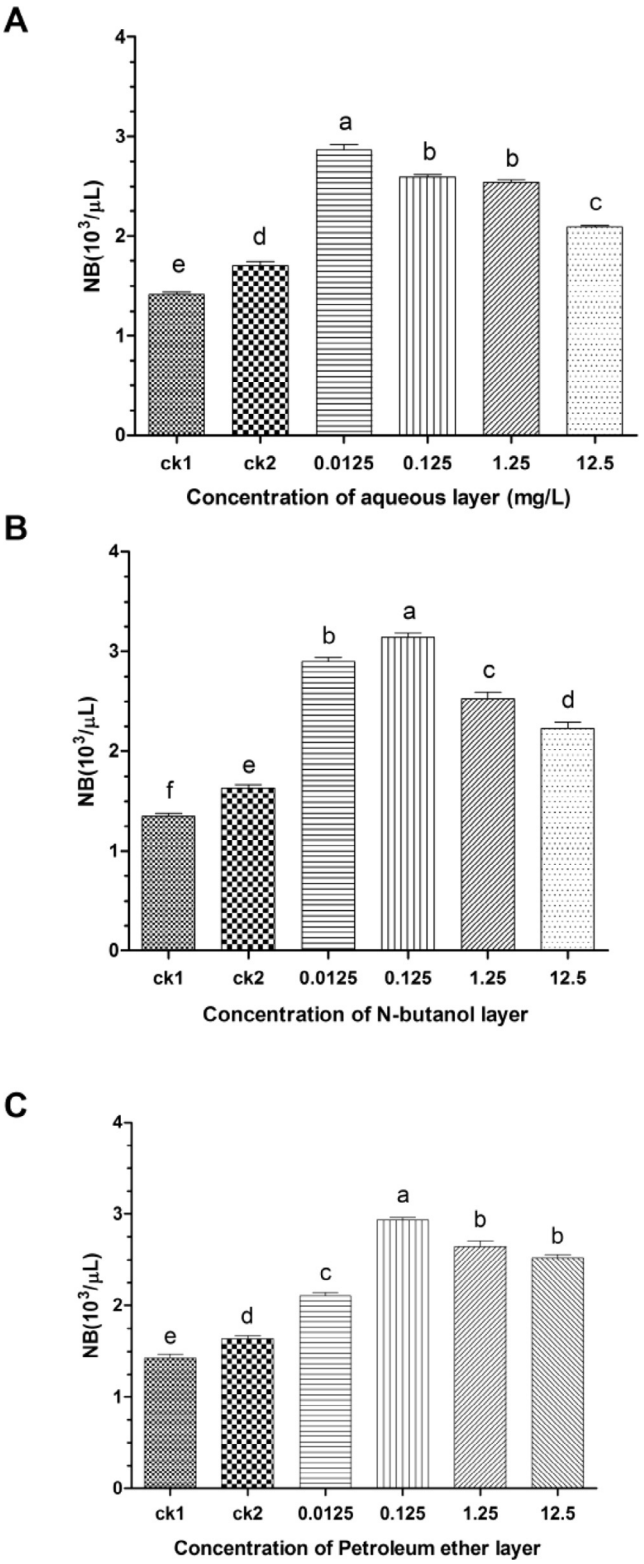

Fig. 1. Chemotactic response of *Erwinia carotovora* on aqueous (A), N-butanol (B) and Petroleum ether layer (C) components of ginseng root exudates (mean ± SEM, *n* = 5).

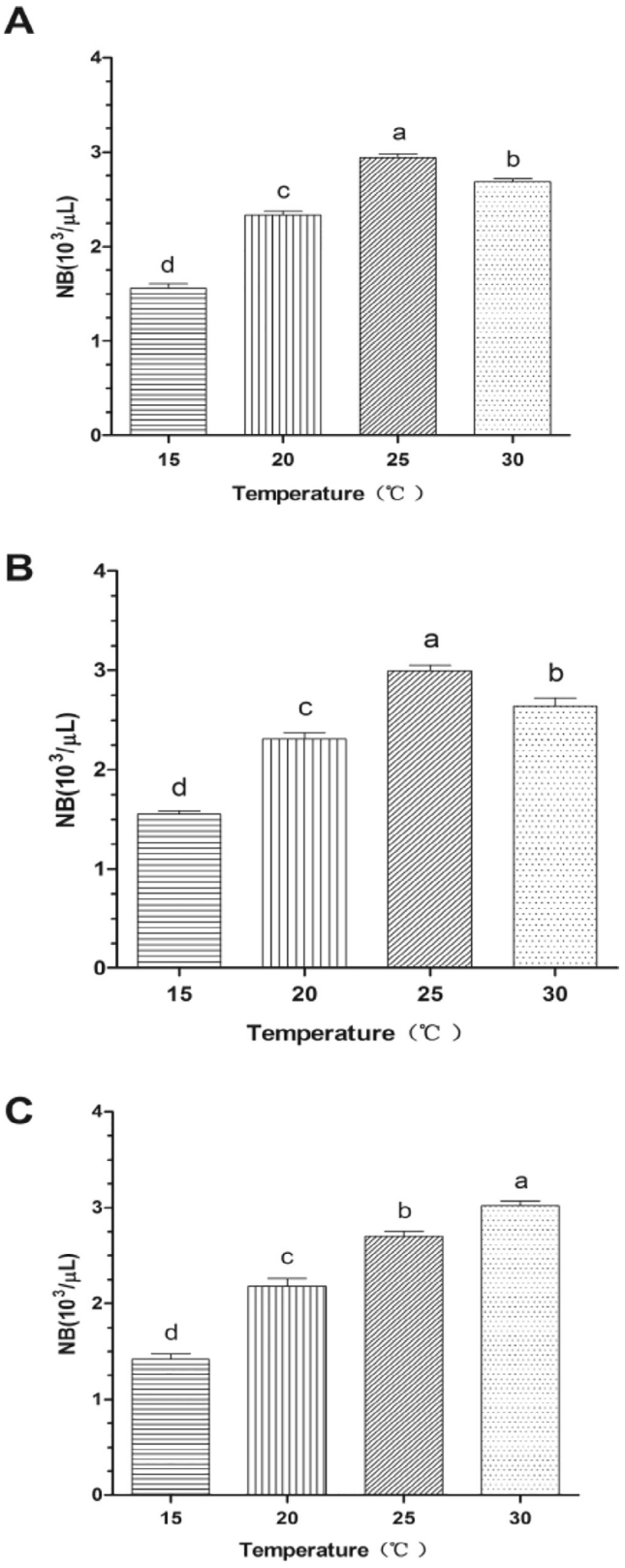

Fig. 2. Effect of temperature on the chemotaxis of *Erwinia carotovora* (mean ± SEM, n = 5).

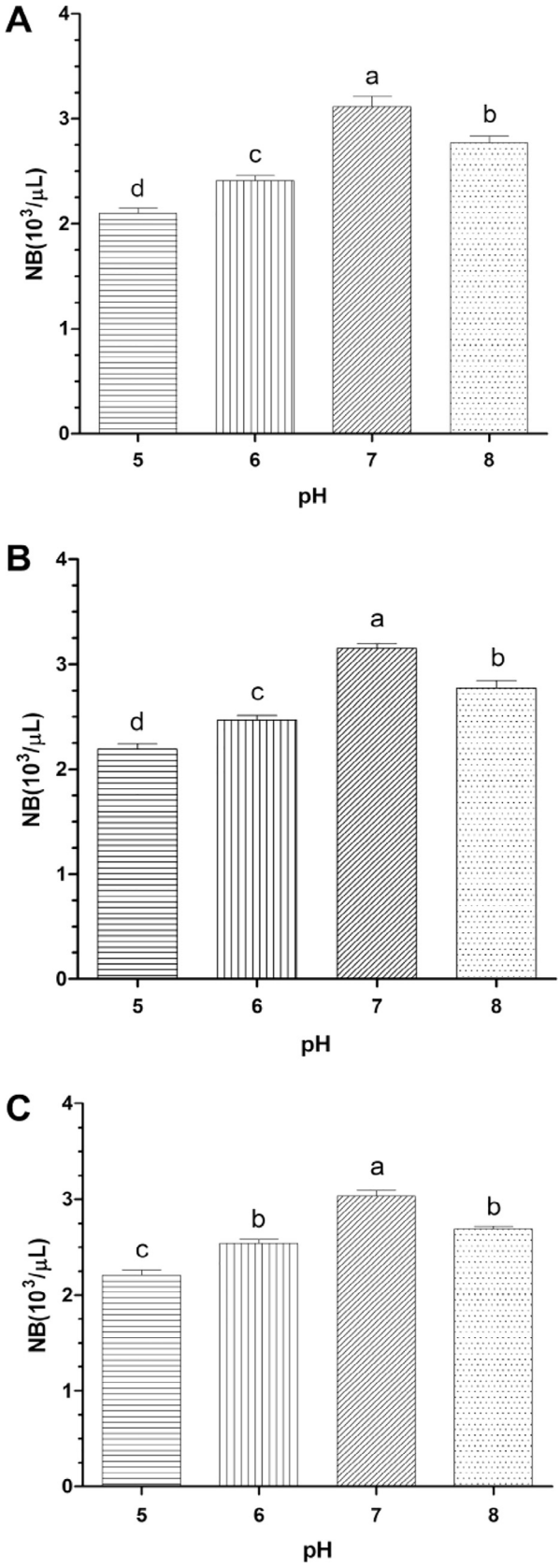

Fig. 3. Effect of pH on the chemotaxis of *Erwinia carotovora* (mean ± SEM, n = 5).

layer, N-butyl alcohol layer, and petroleum ether layer when culture time was respectively 60 min and 75 min, which was significantly higher than results of the same test group ($P > 0.05$). In addition, the chemotaxis upon N-butyl alcohol layer was stronger than those upon water layer and petroleum ether layer at all culture times.

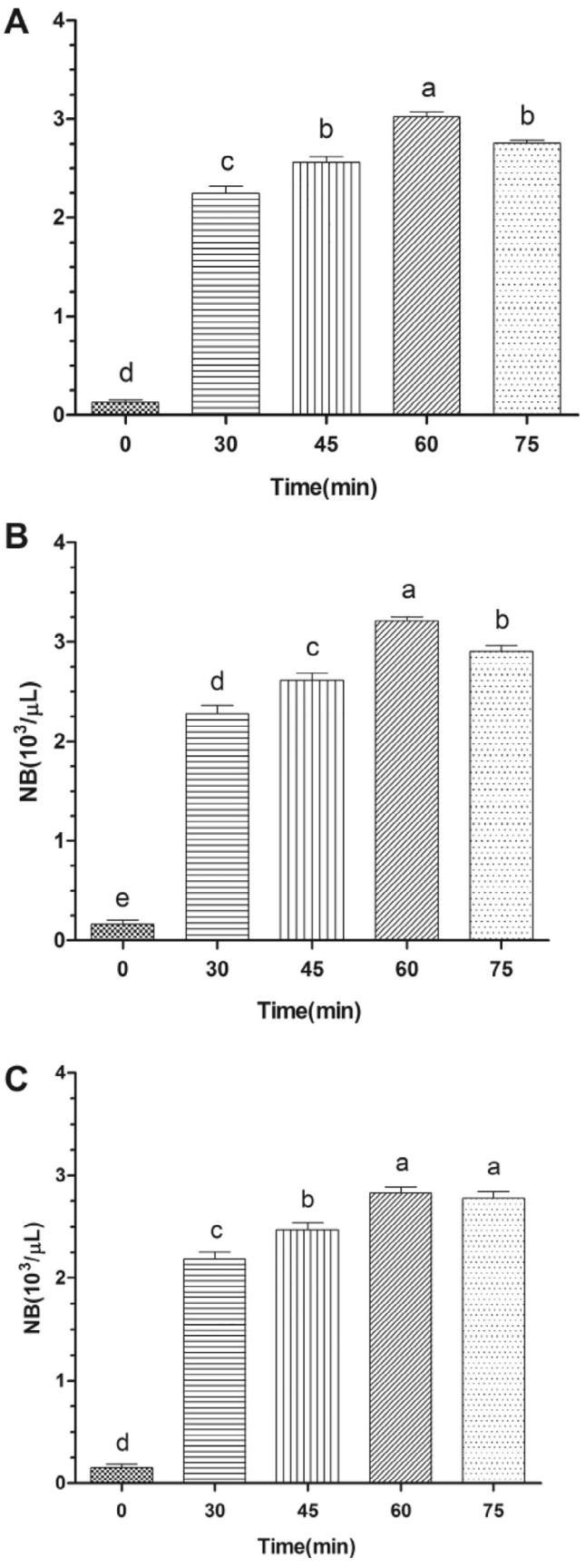

Fig. 4. Effect of time on the chemotaxis of *Erwinia carotovora* (mean ± SEM, n = 5).

3.5. Chemotactic responses of Ginseng bacterial soft-rot upon three components in ginseng root exudates under optimal chemotaxis parameters

As showed in Fig. 5, Ginseng bacterial soft-rot showed chemotactic responses upon water layer, N-butyl alcohol layer, and petroleum ether layer under optimal chemotaxis parameters, which were all significantly higher than those of two control tests. It could be seen that Ginseng bacterial soft-rot reached the strongest chemotaxis upon 0.0125 mg L^{-1} water layer at pH = 7, temperature of 25 °C, and culture time of 60 min, reaching chemotaxis rate at 124.89% and 89.44%; Ginseng bacterial soft-rot reached the strongest chemotaxis upon 0.125 mg L^{-1} N-butyl alcohol layer at pH = 7, temperature of 25 °C, and culture time of 60 min, reaching chemotaxis rate at 139.64% and 101.87%; Ginseng bacterial soft-rot reached the strongest chemotaxis upon 0.125 mg L^{-1} petroleum ether layer at pH = 7, temperature of 30 °C, and culture time of 75 min, reaching chemotaxis rate at 115.29% and 81.36%.

4. Discussion

Different plant root exudates could affect the type, microflora, and physiological properties of soil microorganism. For many soil microorganisms, the amount of soil microorganisms was positively related with the accumulation amount of root exudates (Shi, 2004; Darrah, 1991). The interaction and related mechanism between root exudates and phytopathogen had attracted numerous scholars' attention. May microorganisms showed chemotactic reponse upon root secretion. In soil environment, pathogenic bacteria are often affected by host plant root secretions. Pathogenic bacteria in swimming stage may be attracted or rejected by such compounds, while the germination of non-swiming propagule may be stimulated or inhibited by such compounds.

Some plant secondary metabolites, as allelopathic substances, could exert chemotactic effect to soil microbial populations, which was likely to cause disproportion between beneficial bacterium and harmful pathogens and the frequent occurrence of plant diseases and insect pests (Ju et al., 2002). A.H. Zhang et al. (2016) and Z.H. Zhang et al. (2016) explored chemotactic reponses of ginseng *rhizoctonia solani* and *sclerotinia sclerotiorum* upon ginseng total saponins, found that the total saponins as chemotactic factor

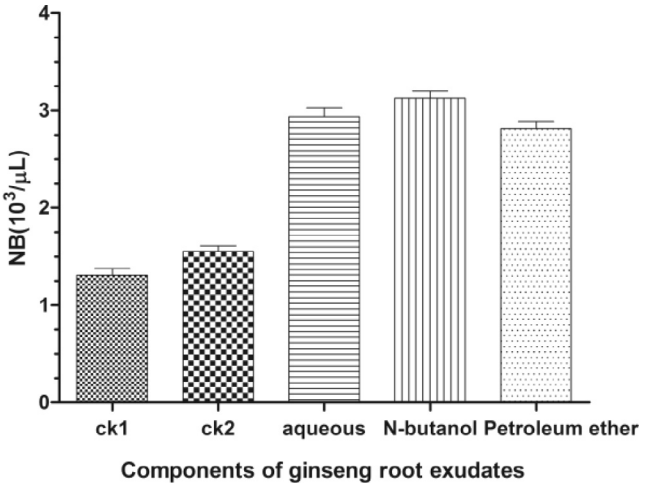

Fig. 5. Response of optimal parameters on the chemotaxis of *Erwinia carotovora* on three-kind components of ginseng root exudates (mean ± SEM, n = 5).

could induce ginseng *rhizoctonia solani* and *sclerotinia sclerotiorum* to develop the chemotactic reponses. Nicol et al. (2003) believed that ginseng total saponins, as a allelopathic factor, promoted the growth of major American ginseng soil-borne pathogenic fungi such as *phytophthora* and *cylindrocarpon destructans*, but inhibited the growth of *trichoderma harzianum* which had exerted antagonism effect. Zhang et al. (2009a, 2009b) also reported that foreign ginseng saponin can exert allelopathic effect to the growths of ginseng soil-borne pathogens, *rhizoctonia solani*, *cylindrocarpon destructans*, *alternaria panax* in different degree.

Under natural condition, the concentration effect of secondary metabolite was of important significance to the illustration of chemotaxis, only in specific concentration could strong chemotaxis be shown. This test showed that ginseng soft-rot bacteria showed relatively strong chemotaxis upon three components in ginseng root exudates in lower concentrations, but the chemotaxis decreased with concentration. This may be due to that the high concentration of root exudates inhibted receptor protein on cytoplasmic membrane to sense extracellular stimulation signal, or due to that the high concentration of root exudates decreased the activity of CheY protein, which slowed the phosphorylation and acetylization process, making it not well combined with other proteins and inibiting the rotation of bacterial flagellum. Researches had indcated that the optimal chemotaxis time for escherichia coli is 60 min, optimal pH is 7.79, however under 15 °C, it would not show chemotaxis activity (Adler, 1973; Larsen et al., 2004; Liu, 2013; Li et al., 2007).

This paper presents the research of chemotactic responses of Ginseng bacterial soft-rot upon three components of different polarities (water layer, N-butyl alcohol layer, petroleum ether layer) in root exudates. Although it could confirm that Ginseng bacterial soft-rot showed chemotaxis upon all the three components, the separation, analysis, and idenfication of certain substance or substances with specific chemotaxis effect in each component were still remained to be further researched.

5. Conclusions

In this test, Ginseng bacterial soft-rot showed strong chemotactic reponse upon water layer, N-butyl alcohol layer, and petroleum ether layer of root exudates respectively at culture time of 60 min and 75 min, at temperature of 25 °C and 30 °C, and pH = 7. This verified that temperature, culture time, and pH value were influencing factors to the chemotactic reponse of bacterium. The exudates of ginseng roots had a significant influence on the Ginseng bacterial soft-rot and chemotaxis was an important way in which Ginseng bacterial soft-rot sense the signals of ginseng plants and invade the ginseng plants.

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