韓藥作誌(Korean J. Medicinal Crop Sci.) 24(5) : 360 – 369 (2016)
ISSN(Print) 1225-9306
ISSN(Online) 2288-0186
http://www.medicinalcrop.org
http://dx.doi.org/10.7783/KJMCS.2016.24.5.360

인삼 추출물에 의한 *Cylindrocarpon destructans*의 주화성 반응 연구

허영화*・시 곤*・장애화*・뢰봉걸*・양 학*・조 암*・이 활*
왕이환*・이 충*・김종석**・이승호***・김영창***†
*중국 길림농업대학교 중약재학원, **우석대학교 재활학과, ***농촌진흥청 국립원예특작과학원 인삼특작부

Chemotactic Response Study of *Cylindrocarpon destructans* towards Ginseng Root Exudates

Yonghua Xu*, Kun Chi*, Aihua Zhang*, Fengjie Lei*, He Yang*, Yan Zhao*, Kuo Li*, Erhuan Wang*, Qiong Li*, Jong Seog Kim, Seung Ho Lee*** and Young Chang Kim***†**

**College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun 130118, China.*
***Department of Rehabilitation, Woosuk University, Wanju 55338, Korea.*
****Department of Herbal Crop Research, NIHHS, RDA, Eumseong 27709, Korea.*

ABSTRACT

Background: *Cylindrocarpon destructans* (Zins) Scholten is an important pathogenic fungus that causes ginseng root rot in many ginseng growing areas in China. Although *C. destructans* have been studied worldwide, research on its chemotaxis towards ginseng (*Panax ginseng* C. A. Meyer) root exudates in the rhizosphere remains limited.
Methods and Results: In this study, we collected ginseng root exudates with three different polarities from three-year-old ginseng roots, and performed chemotaxis and spore germination assays to investigate the ability of these exudates to induce the response in *C. destructans.* The results showed that, compared with other conditions, when *C. destructans* cultivated at 20 ℃ and a pH of 6 exhibited a strong positive chemotactic response toward 2 ㎎/ℓ aqueous phase, 20 ㎎/ℓ butanol phase, and 0.2 ㎎/ℓ petroleum ether from ginseng root exudates, the chemotactic moving indexes were 0.1581, 0.1638 and 0.1441, respectively. In addition, the spore germination rate with optimal chemotactic parameters were 48%, 53%, and 41% in the aqueous phase, butanol phase and petroleum ether groups, respectiviely, which were significantly higher than that in the control group (23%) ($p < 0.05$). The mycelial growth rate with optimal chemotactic parameters increased with culture time, and the maximum growth rates in the aqueous phase, butanol phase and petroleum ether groups were 0.425, 0.406 and 0.364 respectively, on the 4th day. The optimal chemotactic parameters were 39.73 ㎎/50 ㎎/ℓ, 48.93 ㎎/50 ㎎/ℓ, and 31.43 ㎎/50 ㎎/ℓ, in aqueous phase, butanol phase and petroleum ether respectively, from ginseng root exudates, compared with 5.5 ㎎/50 ㎎/ℓ, in the control group.
Conclusions: The present study revealed that certain ginseng root exudates containing chemical attractants act as nutritional sources or signals for *C. destructans* and support its colonization of ginseng roots.

***Key Words*:** *Panax ginseng*, Chemotaxis, *Cylindrocarpon destructans,* Root Exudates

INTRODUCTION

Panax ginseng C. A. Meyer (Ginseng, Araliaceae) in China and Korea is a kind of special pharmaceutical crops with great advantages and features, because the ginseng roots containing useful medicinal ingredients are highly valued, which are used world-widely to treat various diseases by herbal medicine practitioners (Jiao *et al*., 2014). However, a lot of factors such as deteriorated soil conditions (Dou *et al*., 1996; Huang *et al*., 1996), auto-toxicity (He *et al*., 2009), and plant diseases (soil sickness) would cause the continuous cropping obstacle of

†Corresponding author: (Phone) +82-43-871-5506 (E-mail) ycpiano@korea.kr
Received 2016 June 6 / 1st Revised 2016 July 11 / 2nd Revised 2016 August 1 / 3rd Revised 2016 September 21 / Accepted 2016 September 28

ginseng, which restricted the healthy growth of ginseng for long periods, and this also becomes a serious problem that restricts the development of ginseng industry. This study primarily focuses on plant diseases (Zhao, 1995).

Because of the long-period plantation in the same area, soil-borne pathogens including bacteria, fungi, and nematodes get the opportunity to infect the ginseng root, which makes the ginseng plant vulnerable and low quality (Kim *et al*., 2006; Ohh *et al*., 1986). Among them, fungi are the major pathogens to cause ginseng root diseases, for example, ginseng rust rot disease caused by the infection of *Cylindrocarpon destructans* which is one of most popular and well known fungi. *C. destructans* usually affects the surface of ginseng root (Chung, 1975; Lee *et al*., 2015; Yu, 1987). Ginseng rust rot disease is widespread in many regions and countries with an incidence of 25% - 44%, or in even serious area, 80% - 100% to badly influence the production and value of ginseng root.

It has been reported that the indirect activities of ecological effects of plant root exudates and imbalanced proportion of soil microbial population are considered to be the main factors to cause plant diseases. Some populations of microbes could make use of specific components of root exudates to achieve rapid growth by chemotactic response, thus inhibiting the growth of other beneficial microbial to change the composition and quantity of root exudates, which further provides more carbon and energy for the chemotactic and pathogenic microorganisms to create a vicious cycle resulting in stunting plant growth (Dixon *et al*., 1993; Zhou *et al*., 2010). Some report also said that chemotaxis may present a competitive advantage for certain detrimental microorganisms in early establishment on the root of many crop plants leading to reduce root vigor (Ling *et al*., 2011).

Chemotaxis of specific microorganisms toward root exudates in soil is related to attraction toward specific components detected in the exudate (Li *et al*., 2014). During the time of ginseng growth, the ginseng root usually secretes many kinds of secondary metabolites, which are regarded as the specific components in the root exudates, and as it happens, the most productive period of secretion of ginseng root exudates are coincidentally the sameas the period of ginseng diseases outbreak. There is no relevant report showing whether exudates affect the outbreak of main soil-borne diseases and chemotactic response of major pathogens of ginseng, or which kinds of pathogens would be induced by the response, or what the relevant factors are, or what the mechanism is, or how to control the happening of plant diseases by exudates.

Therefore, in this paper, three polarities (the aqueous phase, butanol phase and petroleum ether) of ginseng root exudates are used as experimental materials to research the chemotaxis response of *C. destructans* towards ginseng root exudates. This study will help us to understand the real process of energy exchange and information transfer between ginseng root and its surrounding soil environment, and to explore the important theoretical and practical significance to solve the problem of continuous cropping obstacle in ginseng.

MATERIALS AND METHODS

1. Microorganisms and microconidia

The fungal strain *C. destructans* used throughout this study was isolated from the ginseng root in *Panax ginseng* of Ginseng Engineering Research Centre of Jilin, Jilin Agriculture University, Changchun, China. This strain was routinely incubated on potato dextrose agar (PDA) medium (Sigma-Aldrich Co., St. Louis, MO, USA) in petri dishes in the dark at 20℃ for 7 days and maintained at −80℃ in 30% glycerol for long-term storage. The conidial suspension of *C. destructans* was prepared according to Zhang *et al.* (2013). The conidia concentration was 1.0×10^6 CFU/㎖, which was determined by direct observation on the hemocytometer (Ningbo Hinotek Technology Co., Ltd., Ningbo, China).

2. Preparation of experimental samples

The aqueous phase, butanol (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) phase and petroleum ether (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) phase of three-year-old ginseng root exudates with the purity of 95% were obtained from Ginseng Engineering Research Centre of Jilin, Jilin Agriculture University, Changchun, China. The exudates samples of each polarity were rehydrated with sterilized Milli-Q water to 0.2, 2, 20 and 200 ㎎/ℓ respectively, which were filtered by mixed cellulose ester membrane filters (0.22 and 13 ㎜) (Whatman Co., Maidstone, England) to remove

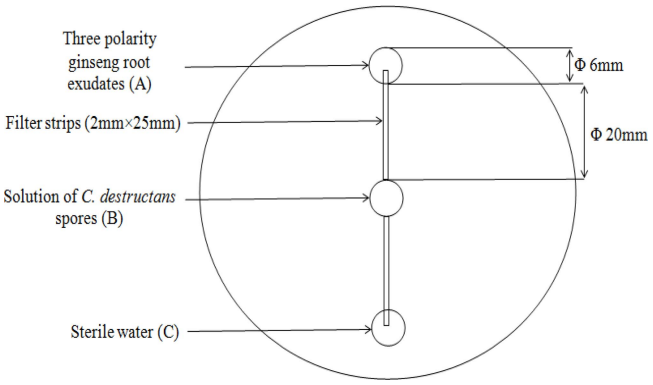

Fig. 1. The test operation map of chemotaxis response of *C. destructans*.

bacteria. The rose bengal medium (containing peptone 5%, KH_2PO_4 1%, $MgSO_4$ 0.5%, glucose 10%, chloromycetin 0.1%, bengal 0.033% and agar 18.5%) (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) was used to cultivate the fungi during the experiments.

3. Experiments of chemotaxis

As shown in Fig. 1, a modified plate assay was used for quantitative chemotactic measurements, based on the materials described above (Morris *et al*., 1992). Briefly, three holes were punched by a hole puncher (Φ = 6 ㎜) in the rose bengal medium from top to down, which was orderly marked as A, B and C (A represents the test group with samples of ginseng root exudates, B represents solution of *C. destructans* spores, C represents the control group with sterile water) containing 30 ㎕ of corresponding solution in each hole. Subsequently, the distance between the three holes was 20 ㎜; A, B and C were connected with filter strips with the size of 25 ㎜ × 2 ㎜. Then, the assay were cultured at 25℃ for 4 days under dark conditions, and five replications were prepared for each treatment.

4. Measurements of indicators

1) Chemotactic migration index (CMI) and mycelial growth rate (MGR)

The calculation standard of moving distance (calipers is used to measure the moving distance) was defined by the longest distance that the most hyphae could move, with *a* representing the moving distance of hyphae from B to A, *b* representing that of B to C; the CMI was *a/b -1*, the MGR was *a - b/d* (*d* represents cultivating days). The measurements of indicators were designed as follows in Table 1.

Table 1. The measurements of indicators.

Value	Implication
CMI > 0	*C. destructans* had positive chemotaxis towards ginseng root exudates
CMI < 0	*C. destructans* had negative chemotaxis towards ginseng root exudates
CMI = 0	*C. destructans* had no chemotaxis towards ginseng root exudates

2) Spore germination rate (SGR)

Spore germination rate (SGR) was determined by the spore germination within one hundred spores observed through the microscope (10x eyepiece × 40x objective lens), and the SGR was calculated by N/100 in percent (N represents the number of spore germination in one hundred spores) (Li *et al*., 2009).

3) Dry weight of mycelial (DWM)

The mycelial growth weight was calculated by the formula; $W_2 = W_0 - W_1$. W_0 represents the weight of mycelia and filter paper after cultivating, W_1 represents the weight of dried filter paper and W_2 represents the mycelial growth weight.

5. Experiments of chemotaxis of *C. destructans* towards ginseng root exudates in different concentrations, temperature and pH

After filtering, three polarity ginseng root exudates with different concentrations (0.2, 2, 20 and 200 ㎎/ℓ) prepared experimental samples were used to conduct the experiments of chemotaxis as described above. On the basis of optimal concentration (2 ㎎/ℓ aqueous phase, 20 ㎎/ℓ butanol phase and 0.2 ㎎/ℓ petroleum ether), different temperatures (10, 15, 20 and 25℃) were investigated; on the basis of concentration and temperature (20℃), the 2 ㎎/ℓ aqueous phase, 20 ㎎/ℓ butanol phase and 0.2 ㎎/ℓ petroleum ether ginseng root exudates were modulated into different pH (5, 6, 7 and 8) to research the chemotaxis response of *C. destructans* under the temperature of 20℃. Five replications were used for each of the above treatment.

6. Response of optimal parameters on the chemotaxis of *C. destructans* towards ginseng root exudates

10 ㎕ spores suspension (1.0×10^6 CFU/㎖) was added

respectively to the aqueous phase (2 ㎎/ℓ), butanol phase (20 ㎎/ℓ) and petroleum ether (0.2 ㎎/ℓ) ginseng root exudates on the microscope slides. Then slides were inverted in a moisturizing petri dish and cultivated in the dark at 25℃ for 12 h in the incubator. After that, the spore germination of one hundred spores were calculated by the microscope (10x eyepiece × 40x objective lens), and five replications were used for each treatment.

7. Determination of the spore germination, mycelial growth rate and mycelial growth weight of *C. destructans* towards ginseng root exudates under the optimal parameters

1) Spore germination

10 ㎕ spores suspension (1.0×10^6 CFU/㎖) was added respectively to the aqueous phase (2 ㎎/ℓ), butanol phase (20 ㎎/ℓ) and petroleum ether (0.2 ㎎/ℓ) ginseng root exudates on the microscope slides. Then slides were inverted in a moisturizing petri dish and cultivated in the dark at 25℃ for 12 h in the incubator. After that, the spore germination of one hundred spores were calculated by the microscope (10x eyepiece × 40x objective lens), and five replications were used for each treatment.

2) Mycelial growth rate

Under the condition of the chemotactic optimal parameters, the hyphae were cultivated for different periods (2, 3 and 4 day) at the end of which, the mycelial growth rate (MGR) was determined and five replications were used for each treatment.

3) Mycelial growth weight

After the cultivation of ginseng root exudates for 7 days under the condition of the chemotactic optimal parameters, the cultivated mycelia were filtered by the quantitative filter paper with known weight (W_1). Then, after the mycelium and filter paper were put into the oven for 2 h (80℃ ± 2℃), they were weighed (W_0) again.

8. Determination of newborn mycelial growth of *C. destructans* towards ginseng root exudates under the optimal parameters

Digital microscope (10x eyepiece × 10x objective lens) was used to observe the newborn mycelial growth of *C. destructans* towards three polarity ginseng root exudates in the optimal chemotactic parameters on the third day of cultivation.

9. Statistical analysis

The differences among the experiments were analyzed via One-way ANOVA, followed by a least significant difference (LSD) test for each assay. All statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). In all statistical tests, *p*-values < 0.05 were considered statistically significance. All data are presented as means ± SD of five sample replicates.

RESULTS

1. Effect of different concentrations of ginseng root exudates on the chemotaxis response of *C. destructans*

In the test, the fungus *C. destructans* showed varying degrees of chemotactic responses toward three polarity ginseng root exudates in different concentrations, but with the increasing concentration, all of them showed an ascending trend at first and then a descending one (Table 2). Among them, *C. destructans* exhibited the strongest positive chemotaxis responses toward ginseng root exudates in 2 ㎎/ℓ aqueous phase, 20 ㎎/ℓ butanol phase and 0.2 ㎎/ℓ petroleum ether, and the CMI was 0.1948, 0.1742 and 0.2024 respectively, which were significantly higher than the other groups ($p < 0.05$). However, when the fungus was cultivated in the ginseng root exudates of 200 ㎎/ℓ petroleum ether, it showed negative chemotaxis response.

2. Effect of temperature on the chemotaxis response of *C. destructans*

Under the cultivation conditions of different temperatures, *C. destructans* showed different chemotactic responses towards ginseng root exudates, and with the increasing temperature, the responses enhanced firstly and then receded (Table 2). Among them, with the optimal concentration, namely, 2 ㎎/ℓ aqueous phase, 20 ㎎/ℓ butanol phase and 0.2 ㎎/ℓ petroleum ether, *C. destructans* displayed the strongest positive chemotactic responses toward ginseng root exudates at the temperature of 20℃, and the CMI was 0.1411, 0.1425 and 0.1334 respectively, which was significantly higher than the other groups ($p < 0.05$). In addition, we found that the chemotactic responses under

Table 2. Chemotactic response of *C. destructans* on the parameters (concentration, temperature and pH) towards ginseng root exudates (aqueous phase, butanol phase and petroleum ether).

Measurement indicators		*C. destructans*		
		Aqueous	n-Butanol	Petroleum ether
		Chemotactic migration index (CMI)		
Concentration (㎎/ℓ)	0.2	0.0593 ± 0.0137^{dC}	0.1021 ± 0.0067^{d}	0.2024 ± 0.0065^{aA}
	2	0.1948 ± 0.0175^{aA}	0.1249 ± 0.0035^{c}	0.1149 ± 0.0016^{bB}
	20	0.1258 ± 0.0051^{bB}	0.1742 ± 0.0149^{a}	0.0675 ± 0.0158^{cC}
	200	0.1069 ± 0.0023^{cB}	0.1432 ± 0.0032^{b}	-0.0672 ± 0.0122^{dD}
Temperature (℃)	10	0.0833 ± 0.0132^{dC}	0.0889 ± 0.0131^{dC}	0.0640 ± 0.0075^{dD}
	15	0.1118 ± 0.0015^{cB}	0.1138 ± 0.0066^{cB}	0.1035 ± 0.0028^{cC}
	20	0.1411 ± 0.0097^{aA}	0.1425 ± 0.0075^{aA}	0.1334 ± 0.0029^{aA}
	25	0.1270 ± 0.002^{bA}	0.1287 ± 0.0054^{bAB}	0.1193 ± 0.0020^{bB}
pH	5	0.1264 ± 0.0045^{bB}	0.1562 ± 0.0104^{bA}	0.1232 ± 0.0094^{bB}
	6	0.1734 ± 0.0064^{aA}	0.1667 ± 0.0056^{aA}	0.1517 ± 0.0082^{aA}
	7	0.1110 ± 0.0093^{cB}	0.0929 ± 0.003^{dC}	0.1074 ± 0.0079^{cC}
	8	0.0834 ± 0.0104^{dC}	0.1144 ± 0.0054^{cB}	0.0847 ± 0.0060^{dD}

Different letters indicate that the values are significantly different at the 0.05 level with small letters (a - d) and 0.01 level with capital letters (A - D) by Duncan's Multiple Range Test.

the temperature of 20℃ and 25℃ tests were higher than that of 10℃ and 15℃ tests.

3. Effect of on the chemotaxis response of *C. destructans*

It was found that when *C. destructans* was cultivated with ginseng root exudates of different pH, all the fungus showed positive chemotactic responses, and with the increasing pH, the responses presented strong at first and later became weak (Table 1). Among them, *C. destructans* exhibited the strongest positive chemotactic responses towards weak acid (pH = 6) ginseng root exudates in all three polarity, namely, aqueous phase, butanol phase and petroleum ether, and the CMI was 0.1734, 0.1667 and 0.1517 respectively, which was significantly higher than in the other groups ($p < 0.05$). Furthermore, the chemotactic responses under the condition of acid (pH = 5) and weak acid (pH = 6) were stronger than that of neutral (pH = 7) and alkaline (pH = 8). And the responses from the strongest to the weakest at the same pH (pH = 6) were aqueous phase, butanol phase and petroleum ether.

4. Response of optimal parameters on the chemotaxis of *C. destructans*

As shown in Fig. 2, *C. destructans* showed chemotactic responses towards three polarity ginseng root exudates in the optimal parameters, and the maximum CMI was 0.1581, 0.1638 and 0.1441, which appeared in the weak acid (pH = 6) ginseng root exudates of 2 ㎎/ℓ aqueous phase, 20 ㎎/ℓ butanol phase and 0.2 ㎎/ℓ petroleum ether in 20℃. Meanwhile, it indicated that there might be a link between chemotaxis response of *C. destructans* and the biological characteristics of fungus itself (Fan *et al*., 1995; Wang, 2001).

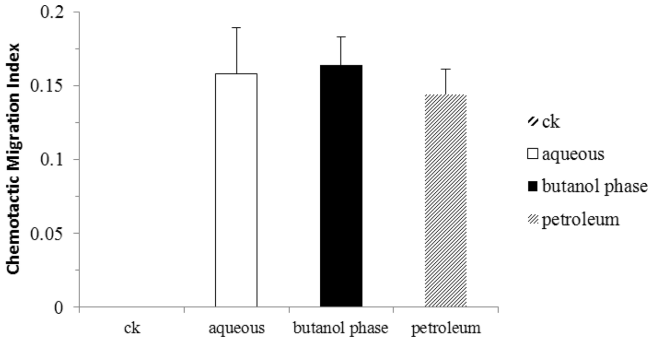

Fig. 2. Response of optimal parameters on the chemotaxis of *C. destructans* towards ginseng root exudates. 2 ㎎/ℓ aqueous phase, 20 ㎎/ℓ butanol phase and 0.2 ㎎/ℓ petroleum ether; both of the was 6 and cultivation temperature was 20℃. Vertical bars represent the standard errors of means from three experiments (n = 15).

5. Effect of optimal parameters of ginseng root exudates on the pathogen spores germination of *C. destructans*

In the experiments, the spores of *C. destructans* germinated normally in the ginseng root exudates with three polarities under the optimal chemotactic parameters

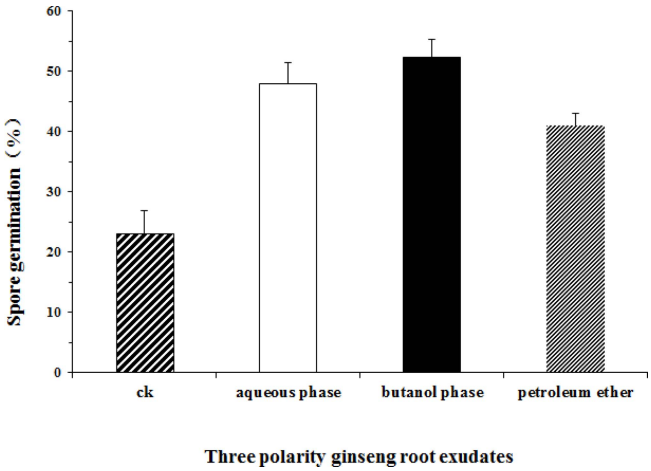

Fig. 3. Effect of optimal parameters of ginseng root exudates on the pathogen spores germination of *C. destructans*. ck; control group, sterile water. Vertical bars represent the standard errors of means from three experiments (n = 15).

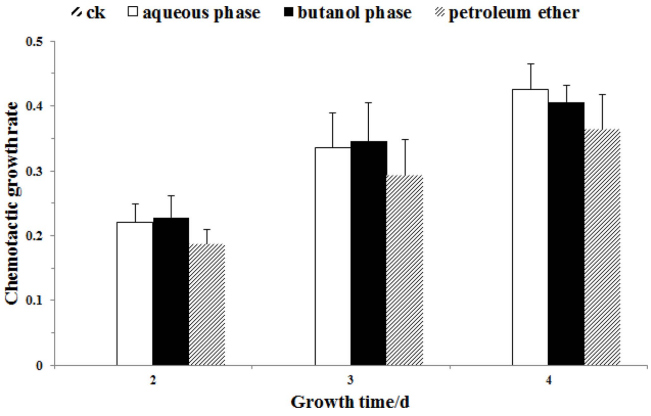

Fig. 4. Effect of optimal parameters of ginseng root exudates on the mycelial growth rate of *C. destructans*. ck; control group, sterile water. Vertical bars represent the standard errors of means from three experiments (n = 15).

shown in Fig. 3. Among them, the spore germination rate was 48, 53 and 41% respectively in aqueous phase, butanol phase and petroleum ether groups, which was significantly higher than that in the control group (23%) ($p < 0.05$). The results implied that different compositions of ginseng root exudates not only induced the germination of pathogen spores, but also promoted the growth.

6. Effect of optimal parameters of ginseng root exudates on the mycelial growth rate of *C. destructans*

The fungi *C. destructans* was cultivated in three polarity ginseng root exudates (aqueous phase, butanol phase and petroleum ether) under the optimal chemotactic parameters. As the culture time went by, the growth rate of the mycelia was increased, and the maximum growth rates appeared on the 4th day when they were 0.425, 0.406 and 0.364 (Fig. 4) respectively.

Interestingly, on the 2nd and 3rd day of cultivation, the speed of mycelial growth rate was that butanol phase group was the fastest, and then aqueous phase group and petroleum ether group, but on the 4th day, aqueous phase group was the fastest. Finally, by conducting the experiments, we found that the chemotactic responses caused by *C. destructans* could offer help to the self-growth and multiplication.

7. Effect of optimal parameters of ginseng root exudates on the mycelial growth weight of *C. destructans*

In spite that the mycelial weight of *C. destructans* was different among the ginseng root exudates of three polarities, they were dramatically still higher than that in control group. Furthermore, the mycelial weight of *C. destructans* in the control group was 5.5 ㎎/50 ㎖, but in the aqueous phase, butanol phase and petroleum ether test groups were 39.73, 48.93 and 31.43 ㎎/50 ㎖ respectively. Meanwhile, after filtering the culture solution, the number of *C. destructans* spores were 2.0, 2.75 and 1.75×10^7 CFU/㎖, respectively, in the aqueous phase, butanol phase and petroleum ether groups, compared with 5.0×10^5 CFU/㎖ in the control group. Another phenomenon was that the of these filtered solutions was increased, because some nitrogenous substances of ginseng

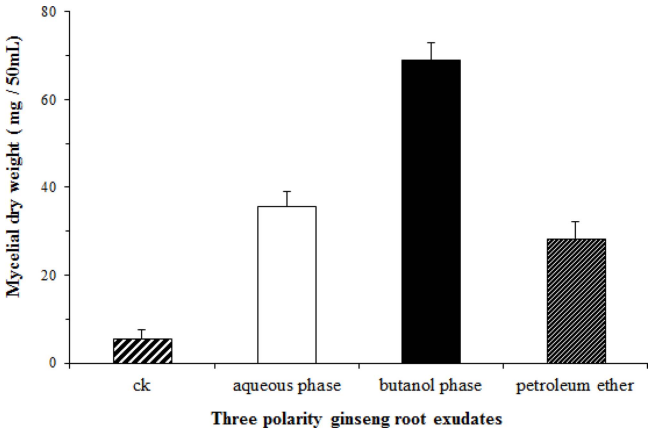

Fig. 5. Effect of optimal parameters of ginseng root exudates on the mycelial growth weight of *C. destructans*. ck; control group, sterile water. Vertical bars represent the standard errors of means from three experiments (n = 15).

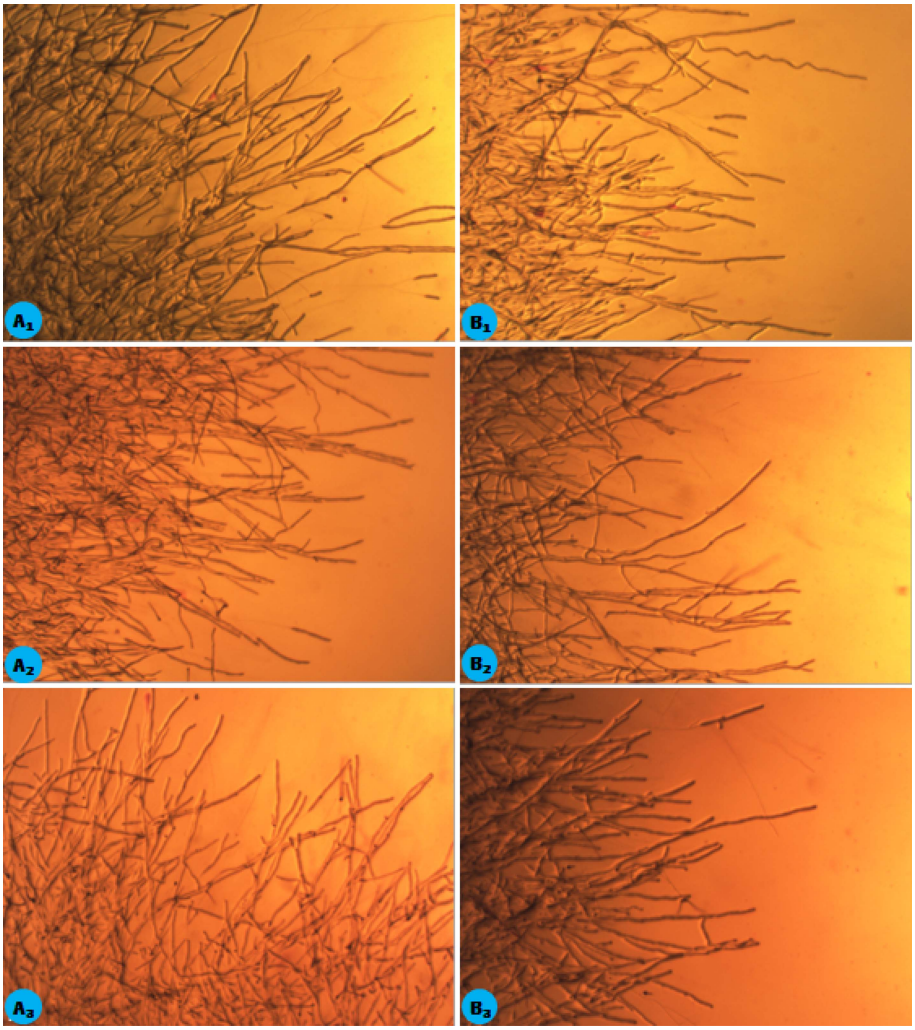

Fig. 6. The microscopic density growth map of mycelium colony edge of *C. destructans* which was affected by ginseng root exudates. Test group; A_1; aqueous phase, A_2; butanol phase, A_3; petroleum ether. Control group; B_1, B_2 and B_3. Magnification; 10x eyepiece × 10x objective lens, photograph of symmetry points.

root exudates may be utilized by *C. destructans* as energy sources.

8. Effect of optimal parameters on the newborn mycelial growth *of C. destructans* towards ginseng root exudates

As shown in Fig. 6, the three groups of micrograph (A_1 and B_1, A_2 and B_2, A_3 and B_3) represented the edge density of the colony of new born mycelial growth of *C. destructans* towards ginseng root exudates, respectively. From the micrograph, we could see that when the newborn mycelia grown from center to both ends (Fig. 1), the mycelia grown in the test group was more intensive and massive with more branches, and the density was also higher than that in the control group due to the ginseng root exudates attraction. The results suggested that some compositions of ginseng root exudates may modulate the activity of new born mycelia and strengthen their capacity to transmit nutrients or moisture, and thereby induced the mycelia to accumulate nutrients for self-growth or multiplication.

DISCUSSION

The previous study has reported that organics produced by photosynthesis could be released into the rhizosphere as root exudates, some of which were all oleochemical and

semio chemicals to provide carbon, energy and nutrition for microbial growth and multiplication. Meanwhile, some soil microorganisms with the help of chemotactic responses to the rhizosphere and root surface for colonization and reproduction. Different root exudates have certain influence in microbe species, microbial flora and microbial physiological characters, and the quantity of microbe is positively correlated with the root exudates accumulation (Badri and Vivanco, 2009; Bais *et al*., 2006). In the soil, pathogenic fungi are frequently affected by the root exudates which may attract or repel the nomadic pathogens, and may stimulate or inhibit the germination of resting vegetative forms. For example, some essential amino acids (arginine, lysine and histidine) secreted by mulberry sapling roots and flavonoids released by macadamias roots could promote conidia germination, mycelia growth and zoospores accumulation of some funguses (Liu *et al*., 2005; Zhenli and Zhiyi, 2005). The root exudates of *leguminous* plants contain some materials as signals to induce rhizobium to produce nodulation factors, which could, thereby, cause identification, infection, colonization and nodulation of rhizobium towards *leguminous* plants (Zhang and Zhang, 2005). However, few studies have reported the effects of ginseng root exudates on the chemotaxis of *C. destructans*, and ginseng rust rot disease is an urgent problem needing to be solved by ginseng-planting regions or countries (Lee *et al.*, 2016).

In China, the growth and development stage of ginseng lies in the period from late May to late June when root exudates gradually increase, reaching the peak period of ginseng rust rot disease (Wang, 2001). Though there are lots of factors that affect the occurrence of *C. destructans,* the exudates of roots definitely play a significant role in it. Our study accurately sought to analyze that the fungus exhibited stronger positive chemotactic responses towards the low and middle concentration of ginseng root exudates in aqueous phase, butanol phase and petroleum ether, and under the optimal chemotactic parameters, the spores germination, mycelial growth rate and dried weights of *C. destructans* significantly increased, whereas all of them decreased as the increase of root exudates concentration. Li *et al.* (2009) reported some of the ginseng root exudates inhibited the colony growths of *C. destructrans* at a high concentration and accelerated the growths at a medium concentration.

Allelopathic effect on stress response can be dynamic and transformation, such as certain concentration can enhance allelopathic effect, but high concentration decreased allelopathy. It is because that the microorganisms converse between growth and defense by adjusting the external allelopathic material adaptability. Due to the presence of specific chemical receptor on the surface of the microorganisms membranes, it allows the microorganisms to detect changes in the concentration of chemical substances in the extracellular environment, and by intracellular delivery system will feel the transformation of chemical signals into intracellular signals and then effects the microorganisms taxis movement behavior.

Nicol *et al*. (2003) found that ginsenosides secreted by American ginseng root had positive influence in the species composition and growth of the soil fungal (*Phytophthora cactorum* and *C. destructans*) community in the effective concentrations, while it inhibited the growth of *harzianum*. When *Phytophthora megasperma* infects soybean, its zoospores would be strongly attracted by the daidzein and genistein which are secreted in the exudates, and the chemotactic effect happens even in the low concentration, but when *Phytophthora megasperma* infects alfalfa and douglas fir, it does not show this kind of chemotactic response (Nicol *et al*., 2003). Bagga and Straney (2000) reported that apigenin could effectively stimulate the germination of *Nectria haematococca* spores, and such stimulation could take place in lower concentrations, but for other flavonoids, such as luteolin and hesperetin, this impact could be triggered in higher concentrations, and the effect of flavonoids as stimulation factors of spores germination was related with inhibition activity of cAMP phosphatase, thus increasing the ability of cAMP.

Above all, we suggested that ginseng root exudates could be perceived, metabolized and transformed by the rhizo-fungus in the rhizosphere, and in addition, there might be an association between root exudates concentration effect and chemotactic response, colonization, reproduction, community building of soil microbial.

Previous research has shown that the morbidity of *C. destructans* isolated in *P. ginseng* is higher in the roots before and after growth stage than that during the growth stage, while during three periods, there is no obvious difference in the external factors, and thus the rhizosphere

environment plays an important role in ginseng root exudates. Zhang *et al.* (2014) has reported that root exudates contain ginsenoside, organic acid, phenolic acid, and other secondary metabolites, some of which would cause the acid-base imbalance of soil and lead to the chemotaxis attraction of some soil microorganism swimming or colonizing on the surface of plant root. Larsen *et al.* (2004) has reported that chemotaxis of some soil microorganism are sensitive to temperature, increased by the rising of temperature, which is related to methylation and phosphorylation of receptor proteins on the intracellular membrane of soil microorganism because the kinetics of enzymes that take part in the process of methylation and phosphorylation is influenced by the temperature.

So we investigated the effect of and temperature of ginseng root exudates, which seems to be the important factors for chemotaxis of *C. destructans*. In this study, our findings suggested that when *C. destructans* was cultured in different and temperatures, the chemotaxis towards ginseng root exudates was different from that in rose bengal medium, i.e. it was higher in weak acid environment than that in the neutral and basic environment, meanwhile, chemotactic response was the highest at 20℃ compared with that at 10, 15 and 25℃.

The study was not only indirectly verified that root exudates in the rhizosphere environment acted as specific chemoattractant for pathogenic fungus (*C. destructans*) growth and multiplication, but it also indicated that the chemotaxis of *C. destructans* towards ginseng root exudates may supply a positive advantage indetermining the outcome of its colonization.

In this study, although three kinds of inducers couldn't make *C. destructans* grew faster, they could release some volatile substances to forma gradient in the soil which could be detected by *C. destructans*, which led to the regulation of the mycelia activities, and the results were as follows; 1) Activities of mycelia on the side that receives ginseng root exudates was enhanced to promote the transmission capability of nutrition or water, thus the nutrition accumulating faster to reach a degree of saturation condition where more branches of mycelia grow to make the whole fungal colony look fluffier and firmer due to the repulsive interaction and larger angles of mycelia, 2) Another phenomenon was that ginseng root exudates may stimulate and resolve some intracellular molecules of *C. destructans* cells, and objectively improve the concentration of intracellular substances, which made the osmotic pressure of mycelia increase to absorb more water to grow fluffier and firmer. If there were any supportive materials such as membrane or medium around the mycelia, the mycelia easily touched upon the materials to grow along then, and however, if there were no such supportive materials, the growth of branches would be restricted, and as a result, the margin of bacterial colony would be on the decline. Thereby, from the macro view, the results were shown in Fig. 6 that mycelia grew toward ginseng root exudates, which formed the chemotactic response of *C. destructans* that we discovered. As for the formation mechanism, further studies are needed.

ACKNOWLEDGMENTS

This work was funded by the National Natural Science Foundation of China (project No. 31100239, No. 31200224, No. 31470420), the funded projects for Science and Technology Development Plan of Jinlin (project No. 20110926, No. 2011-Z25, No. 20130206030YY, No. 2014 0520159JH, No. 201410297) and the project supported by the Ministry of Science and Technology of China (project No. 2011BAI03B01). And, this work was carried out with the support of cooperative research program for Agriculture Science and Technology Development (PJ01018701), Rural Development Administration, Republic of Korea.

REFERENCES

Badri DV and Vivanco JM. (2009). Regulation and function of root exudates. Plant Cell and Environment. 32:666-681.

Bagga S and Straney D. (2000). Modulation of cAMP and phosphodiesterase activity by flavonoids which induce spore germination of *Nectria haematococca* MP VI(*Fusarium solani*). Physiological and Molecular Plant Pathology. 56:51-61.

Bais HP, Weir TL, Perry LG, Gilroy S and Vivanco JM. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology. 57:233-266.

Chung HS. (1975). Studies on *Cylindrocarpon destructans*(Zins.) Scholten causing root rot of ginseng. Reports of the Tottori Mycological Institute. 12:127-138.

Dixon RK, Winjum JK and Schroeder PE. (1993). Conservation and sequestration of carbon: The potential of forest and agroforest management practices. Global Environment Change. 3:159-173.

Dou S, Zhang J, Jiang Y and Song J. (1996). Effect of ginseng

cultivation on chemical properties of soil. Journal of Jilin Agricultural University. 18:67-73.

Fan MZ, Dong JA, Yang JG, Shen SX, Yang WX and Liu GS. (1995). Factors affecting hyphae growth and toxin production of *Alternaria brassicae.* Journal of Agricultural University of Hebei. 18:21-27.

He CN, Gao WW, Yang JX, Bi W, Zhang XS and Zhao YJ. (2009). Identification of autotoxic compounds from fibrous roots of *Panax quinquefolium* L. Plant and Soil. 318:63-72.

Huang CH, Li FY and Guo J. (1996). Ginseng continuous cropping soil chemical element analysis. Special Wild Economic Animal and Plant Research. 1:50-51.

Jiao L, Li B, Wang M, Liu Z, Zhang X and Liu S. (2014). Antioxidant activities of the oligosaccharides from the roots, flowers and leaves of *Panax ginseng* C. A. Meyer. Carbohydrate Polymers. 106:293-298.

Kim JH, Jeon YH, Park H, Lee BD, Cho DH, Park BY, Khan Z and Kim YH. (2006). The root lesion nematode, *Pratylenchussub penetrans*, on ginseng(*Panax ginseng*) in Korea. Nematology. 8:637-639.

Larsen MH, Blackburn N, Larsen JL and Olsen JE. (2004). Influences of temperature, salinity and starvation on the motility and chemotactic response of *Vibrio anguillarum*. Microbiology. 150:1283-1290.

Lee SW, Lee SH, Jin ML, Park KH, Jang IB and Kim KH. (2016). Control of soil-borne pathogens in ginseng cultivation through the use of cultured green manure crop and solarization in greenhouse facilities. Korean Journal of Medicinal Crop Science. 24:136-142.

Lee SW, Lee SH, Park KH, Jin ML, Jang IB and Kim KH. (2015). Inhibition effect on root rot disease of *Panax ginseng* by crop cultivation in soil occurring replant failure. Korean Journal of Medicinal Crop Science. 23:223-230.

Li XG, Ding CF, Hua K, Zhang TL, Zhang YN, Zhao L, Yang YR, Liu JG and Wang XX. (2014). Soil sickness of peanuts is attributable to modifications in soil microbes induced by peanut root exudates rather than to direct allelopathy. Soil Biology and Biochemistry. 78:149-159.

Li Y, Liu SL, Huang XF and Ding WL. (2009). Allelopathy of ginseng root exudates on pathogens of ginseng. Acta Ecologica Sinica. 1:161-168.

Ling N, Raza W, Ma J, Huang Q and Shen Q. (2011). Identification and role of organic acids in watermelon root exudates for recruiting *Paenibacillus polymyxa* SQR-21 in the rhizosphere. European Journal of Soil Biology. 47:374-379.

Liu JF, Yang DM, Ouyang MA, Wang LN, Zhang Y and Zeng M. (2005). Influence of methanol eluates fractionated from *Macadamia integrifolia* roots on spore germination and hyphal growth of AM fungi. Chinese Journal of Plant Ecology. 6:172-176.

Morris PF and Ward EWB. (1992). Chemoattraction of zoospores of the soybean pathogen, *Phytophthora sojae*, by isoflavones. Physiological and Molecular Plant Pathology. 40:17-22.

Nicol RW, Yousef L, Traquair JA and Bernards MA. (2003). Ginsenosides stimulate the growth of soilborne pathogens of American ginseng. Phytochemistry. 64:257-264.

Ohh SH, Yu YH, Cho DH, Lee JH and Kim YH. (1986). Effect of chemical treatments on population changes of *Ditylenchus destructor* and responses of *Panax ginseng*. Korean Journal of Applied Entomology. 25:169-173.

Wang TS. (2001). China ginseng. Liaoning Science and Technology Publishing House. Liaoning, China. p.377.

Yu YH. (1987). Root rot diseases of *Panax ginseng* and their control in Korea. The Plant Pathology Journal. 3:318-319.

Zhang AH, Peng H, Lei FJ and Zhang LX. (2014). Extraction and identification of ginseng root exudates. Journal of Northwest Agriculture and Forestry University. 7:191-196.

Zhang F, Zhu Z, Yang X, Ran W and Shen Q. (2013). *Trichoderma harzianum* T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere. Applied Soil Ecology. 72:41-48.

Zhang Q and Zhang L. (2005). Perception and signal transduction of rhizobium nod factor in leguminous plants. Chinese Agricultural Science Bulletin. 7:233-238.

Zhao YF. (1995). Jilin province *Panax ginseng* and *Panax quinquefolius* L. disease occurring and protection problem and work advice. Special Wild Economic Animal and Plant Research. 4:45-46.

Zhenli YE and Zhiyi TU. (2005). Study on the secretion and the rhizospheric microorganisms of mulberry roots. Science of Sericulture. 1:18-21.

Zhou RJ, Fu JF and Shi HY. (2010). Screening and identification of antagonistic strains against *Cylindrocarpon destructans*. Journal of Shenyang Agricultural University. 4:422-426.