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Effects of Tobacco Pathogens and Their Antagonistic Bacteria on Tobacco Root Exudates

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Abstract

The research on relationship between rhizosphere microbes and root exudates has a great significance on discussion of interaction between rhizosphere microbes and plants, as well as control of soil-borne diseases and insect pest. GC-MS was used to analyze changes of tobacco root exudates under the antagonistic action of tobacco bacterial wilt and black shank. It turned out that when pathogens of tobacco bacterial wilt and black shank in tobacco root microorganisms increase, tobacco root exudates augmented rapidly among of which organic acids have the biggest growth, followed by amines. When the pathogens of tobacco bacterial wilt and black shank are inhibited by the active substance of antagonistic antibacterial, 20 - 23 kinds of root exudates are added; besides, the content of 7 substances was reduced to 0. Another interesting finding was that the fluctuations of phthalic acid, isophthalic acid and benzoic acid, which have caused continuous cropping obstacles, were very distinct. The results of this study have provided novel clues for the exploration of continuous tobacco cropping obstacles and soil-borne diseases.

Keywords

Root Exudates, Rhizosphere Microbes, Allelochemical, GC-MS

1. Introduction

Plant-microorganism interaction maintains or dominates the ecological functions of terrestrial ecosystems. Microorganisms in soil environment are regarded

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as the largest reservoir of biological diversity in nature [1]. As many as 1011 microbial cells and 30,000 kinds of prokaryotes can be identified from one gram of rhizosphere soil [2] [3]. Rhizosphere microbes [4], considered as the second genome of plants [5], alter the defensive ability of plants by regulating the host immune system [6] [7] or recruit beneficial plant bacteria [8] [9], or inhibit the growth of plant pathogen by changing plant nutrition supply [10]. Therefore, rhizosphere microbes played a vital role in plant growth, health, yield and other aspects of regulation [11]. However, root exudates are the potential and important intermediates in the interaction between rhizosphere microorganisms and plants [12] [13]. The study of the interaction between rhizosphere microorganisms and root exudates is helpful to the study of the interaction between rhizosphere microorganisms and plants. It is of great significance to evaluate plant growth and soil-borne diseases and insect pests.

Recently, the investigation of the relationship between rhizosphere microbes and root exudates mainly focused on the regulation of root exudates on the formation, metabolism, growth and diversity of rhizosphere microorganisms [14] [15]. In fact, rhizosphere microorganisms can affect plant growth by absorbing a specific component of root exudates to stimulate changes in the abundance or quality of root exudates [16] [17] [18] [19]. For example, inoculation with Bacillus subtilis B26 not only increased the yield of rape (*B. distachyon*), but also increased drought resistance [20], even in the absence of nitrogen source. Both (*Azospirillum*) and (*Herbaspirillum*) of *Vibrio herbaceus* could promote the growth of *Brassica napus* [21]. These studies suggest that rhizosphere microbes play a potential role in plant root exudates. However, because of the complexity of soil microbes in heterogeneous environments, it is more challenging to explore how soil microbes directly affect root exudates.

In present study, the components of tobacco root exudates under the action of different rhizosphere microorganisms were detected and analyzed by gas chromatography-mass spectrometry (GC-MS), and the changes of tobacco root exudates were discussed. It provides effective information for the study of how microbes change cell membrane permeability and signal transduction, and provides a new clue for the prevention and treatment of tobacco soil-borne diseases.

2. Materials and Methods

Tobacco (Yunyan 87) was selected as culture material; Yunyan 87 was selected and bred by Yunnan Tobacco Science Research Institute and China Tobacco Breeding Research (South) Center. It was composed of Yunyan No. 2 as the female parent and K326 as the male parent. Besides, it was approved by the National Variety Approval Committee in December 2000. The main agronomic traits of Yunyan 87 were extremely stable, and its coefficient of variation was smaller than that of the control k326. The growth was uniform and widely adaptable in the field. The blade has the similar thickness, and the layer is yellow and easy to bake. It has an average yield about 2613 kg/hm^2. The comprehensive evaluation index is superior to the main production variety k326. Yunyan 87 has

high quality, stable production and wide adaptability, strong resistance to stress, easy to bake and so on. Tobacco bacterial wilt pathogen (*Ralstonia solanacearum*) and its antagonistic bacteria (*Bacillus amyloliquefacien* YH22); black shank pathogen (*Phytophthora parasitica var. nicotianae*) and its antagonistic bacteria (*Bacillus amyloliquefacien* TU1). All of the above bacteria were preserved in our laboratory.

2.1. Tobacco Cultivation and Root Exudate Extraction

First, Tobacco seedling transplanted to peat soil after the Great Cross (1 kg peat soil was added with bacterial wilt pathogen and black shank pathogen bacteria solution 50 ml, 1.0×10^9 CUF/mL), then sampling after 20 days of cultivation. The culture temperature was 30˚C, watering every 5 days and ensure adequate lighting. The remaining samples were supplemented with antagonistic bacteria against tobacco bacterial wilt and black shank (50 ml, 1.0×10^9 CUF/mL), sampling after 20 days of cultivation too. Collection of tobacco root exudates has adopted the method reported in the literature [22]; repeating 3 times for each experiment and 3 parallels at a time. The collected root exudates were extracted with ethyl acetate: soak each uprooted tobacco with 100 ml distilled water, store in the dark for 48 hours, centrifuge the leachate and then leave the supernatant for suction filtration and adjust pH of the solution to acidity (pH2), alkalinity (pH11), and neutral (pH7) separately, with the same volume of ethyl acetate, extracting the solution. Then concentrated the extract under reduced pressure to 1 mL (45˚C), filtered through a 0.45 μm filter, and finally collected the organics three times. The solution was transferred to a rotary evaporation flask, and evaporated to dryness at 50˚C. The residuals were stored at low temperature (−80˚C).

2.2. Derivatization of Root Exudates and GC-MS Detection Conditions

Add mixture of 40 μL 20 mg/mL methoxyamine salt pyridine and 10 μL 5% phenylacetate to the freeze-dried root exudates; then incubating in a 60˚C incubator for 60 min. Finally, 50 μL of the silylating reagent N,O-Bis(trimethylsiyl) acetamide (BSTFA) was added and incubated at 70˚C for 60 min. Add mixture of 40 μL 20 mg/mL methoxyamine salt pyridine and 10 μL 5% phenylacetate to the freeze-dried root exudates; then incubating in a 60˚C incubator for 60 min. Finally, 50 μL of the silylating reagent N,O-Bis(trimethylsiyl)acetamide (BSTFA) was added and incubated at 70˚C for 60 min, cooled to room temperature naturally. GC-MS was analyzed by Thermo Scientific Gas Chromatography/Mass Spectrometer (DSQII). GC condition: the capillary column is TG-5MS (30 m × 0.25 mm × 0.25 μm), the inlet temperature is 250˚C, and the split ratio is 50:1. The heating program of column temperature: from 50˚C to 250˚C at a rate of 3˚C/min, continue to rise to 250˚C at a rate of 25˚C/min, remaining for 4 min. The carrier gas is He (99.999%), the flow rate is 20 psi, and the injection volume is 1 μL. MS condition: ionization energy is 70 eV, ion source temperature is

300˚C, scanning range is 30 - 600 m/z and transmission line temperature is 280˚C.

2.3. Data Statistics and Analysis

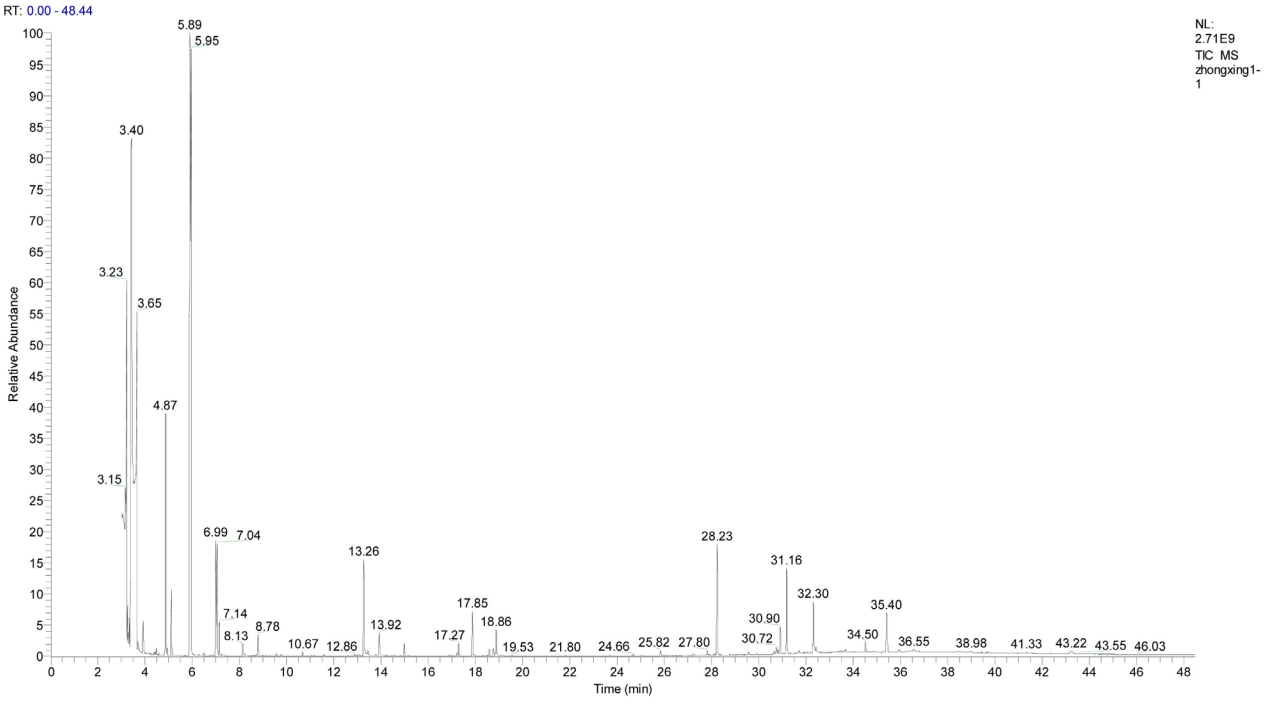
Processing and analyzing data with Origin 8.0 and SPSS 16.0, and GC-MS data was analyzed by Trace Finder 4.0. Statistics was obtained by Trace Finder Data Library. The relative content of root exudates was calculated by peak area normalization method.

3. Results and Discussion

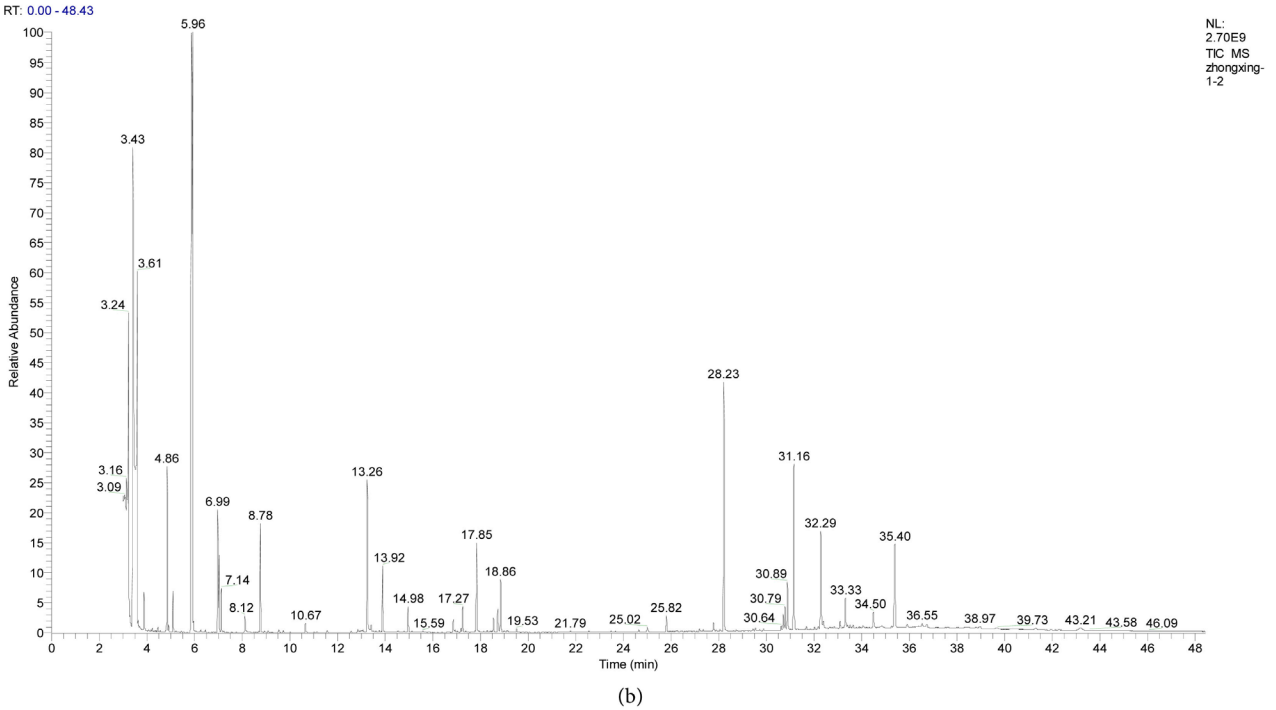
1) Analysis of tobacco root exudates after treating by pathogen of tobacco bacterial wilt and its antagonist.

The composition of the root exudates of Yunyan 87 was analyzed by GC-MS after irrigating pathogen of bacterial wilt and its antagonist. The main components in the control group were amines, alcohols, acids, lipids, sugars and esters, with the corresponding percentages of 15.78%, 36.84%, 36.84%, 0%, 5.26% and 5.26%. Under the function of pathogen of bacterial wilt, the contents of amines, alcohols, acids, lipids, sugars and esters were 18.42%, 31.57%, 44.73%, 5.26%, 5.26% and 5.26% respectively. While by the antagonistic effect of pathogen of bacterial wilt, the contents of amines, alcohols, acids, lipids, sugars and esters were 11.92%, 23.81%, 38.09%, 9.52%, 11.91% and 4.76% accordingly (**Figure 1**). There were more than 23 types of root exudates of Yunyan 87 compared with the control group, mainly including 3 kinds of amines, 6 kinds of alcohols, 10 kinds of acids, 2 kinds of sugars, 1 kind of esters and cyclopropene respectively (**Table 1**). There are the same components of 5 types with contents decrease and 9 types with contents increase. It was noteworthy that the most remarkable increase was terephthalic acid (96.11%) and benzoic acid (143.75%). After the addition of antagonistic bacteria of bacterial wilt, there were 28 kinds of components increased among of which 9 were newly appeared and 12 of them decreased among of which 8 were completely disappeared, namely thiodiglycol, 1-butanol, and C. acid, isophthalic acid, myristic acid, ethyl benzoate, azelaic acid and propylene.

According to the analysis, we found that the content of propionic acid, isophthalic acid, isophthalic acid, terephthalic acid, myristic acid, ethyl benzoate, benzoic acid and sebacic acid showed the greatest volatility, the content in the control group was 0 (except for terephthalic acid, the content was 0.7792). The treatment group of pathogen of bacterial wilt reached the highest value, while the content decreased to 0 after the addition of antagonistic bacteria (except for terephthalic acid, from 1.5281 to 0.8589, with a decrease of 77.91%). Totally, amines were significantly decreased by loading *B. amyloliquefacien* YH22 (T-test, $p = 0.045$), alcohols content were continuously and significantly decreased by loading *R. solanacearum* and *B. amyloliquefacien* YH22 (T-test, $0.01 < p < 0.05$), the content of acids and lipids were alwalys significantly increased



(a)



(b)

Figure 1. Total ion current of GC-MS with (a) treatment of pathogen of bacterial wilt; (b) treatment of pathogen of bacterial wilt and its antagonistic bacteria.

by loading these two kinds of microbes, the loading of *R. solanacearum* did not change the content of sugars, but significantly increasedby the loading of *B. amyloliquefacien* YH22 (T-test, p = 0.03), the loading of microbes did not obviously change the content of esters in three treatments (**Figure 2**).

Table 1. The content of tobacco root exudates under *Ralstonia solanacearum* and *Bacillus amyloliquefacien* YH22.

Components of tobacco root exudates	Peak area (%)			
	Molecular Formula	Control group	Pathogen of bacterial wilt	Pathogen of bacterial wilt + antagonist
Methylamine	$C_7H_{21}N$	8.9354 ± 0.29	9.1213 ± 0.26	9.5606 ± 0.25
Tyramine	$C_8H_{11}NO$	(-)	2.1331 ± 0.30	1.2131 ± 0.32
Silanamine	$C_7H_{18}N_2$	(-)	5.4464 ± 0.22	6.5480 ± 0.34
Bis(trimethylsilyl)carbodiimide	$C_7H_{18}N_2$	1.2014 ± 0.35	1.6521 ± 0.29	1.2091 ± 0.25
N-Methyltrifluoroacetamide	$C_3H_4F_3NO$	0.1541 ± 0.10	0.2514 ± 0.05	0.2618 ± 0.06
Hexanol	$C_{12}H_{28}O$	4.4790 ± 0.24	3.5823 ± 0.23	3.5856 ± 0.35
Ethylene glycol	$C_8H_{22}O_2$	1.6186 ± 0.22	1.6312 ± 0.32	1.4583 ± 0.23
Diethylene glycol	$C_{10}H_{26}O_3$	1.1490 ± 0.22	(-)	(-)
Phenylethyl alcohol	$C_8H_{10}O$	1.1589 ± 0.15	(-)	(-)
Glycerol	$C_{12}H_{32}O_3$	2.6808 ± 0.40	2.7021 ± 0.19	3.8914 ± 0.39
Thiodiglycol	$C_4H_{10}O_2S$	0.1238 ± 0.06	0.2156 ± 0.06	(-)
D-Pinitol	$C_7H_{14}O_6$	(-)	(-)	0.1423 ± 0.04
D-Mannitol	$C_{24}H_{62}O_6$	(-)	1.1671 ± 0.16	2.1651 ± 0.20
Myo-Inositol	$C_6H_{12}O_6$	(-)	0.5698 ± 0.14	0.5412 ± 0.11
Silanol	$C_{13}H_{14}O$	(-)	0.1296 ± 0.09	0.2315 ± 0.08
1-Butanol	$C_4H_{10}O$	(-)	2.5128 ± 0.39	(-)
2,3-Butanediol	$C_{10}H_{26}O_2$	(-)	0.7250 ± 0.11	0.5283 ± 0.02
1-Monopalmitin	$C_{19}H_{38}O_4$	(-)	1.3210 ± 0.21	1.1593 ± 0.23
Isopropyl alcohol	C_3H_8O	(-)	(-)	1.1452 ± 0.17
Triethylene glycol	$C_8H_{18}O_4$	2.1423 ± 0.17	(-)	(-)
Boric acid	$BC_9H_{27}O_3$	8.4826 ± 0.24	8.5092 ± 0.23	9.5069 ± 0.33
Oxalic acid	C_2H_2O	(-)	4.2156 ± 0.43	3.5623 ± 0.35
Propionic acid	$C_3H_6O_2$	(-)	3.5986 ± 0.38	(-)
Palmitic acid	$C_{19}H_{40}O_2$	(-)	1.1267 ± 0.22	3.1092 ± 0.37
Hydrocinnamic acid	$C_9H_{10}O_2$	(-)	0.0186 ± 0.02	0.8218 ± 0.19
Stearic acid	$C_{21}H_{44}O_2$	5.4667 ± 0.14	4.0571 ± 0.26	7.0821 ± 0.42
Isophthalic acid	$C_8H_6O_4$	(-)	2.5862 ± 0.24	(-)
Terephthalic acid	$C_8H_6O_4$	0.7792 ± 0.25	1.5281 ± 0.25	0.8589 ± 0.18
Myristic acid	$C_{14}H_{28}O_2$	(-)	0.5836 ± 0.15	(-)
Itaconic acid	$C_5H_6O_4$	(-)	0.0782 ± 0.02	0.1290 ± 0.04
Benzimidate hydrochloride	$C_9H_{12}NC_{lO}$	(-)	0.0576 ± 0.02	(-)
Sebacic acid	$C_{10}H_{18}O_4$	(-)	0.3276 ± 0.04	(-)
Lactic acid	$C_9H_{22}O_3$	7.1991 ± 0.29	5.3126 ± 0.24	6.4286 ± 0.29
Benzoic acid	$C_7H_6O_2$	1.5823 ± 0.23	3.8569 ± 0.43	1.2982 ± 0.28
Acetic acid	$C_{10}H_{12}O_2$	2.6790 ± 0.26	3.4087 ± 0.28	4.4589 ± 0.13

Continued

Octanoic acid	$C_8H_{16}O_2$	1.5820 ± 0.30	1.5692 ± 0.23	1.8263 ± 0.26
3,4-Dimethoxybenzoic acid	$C_9H_{10}O_4$	(-)	(-)	0.0350 ± 0.01
Dehydroabietic acid	$C_{20}H_{28}O_2$	(-)	(-)	1.0464 ± 0.13
Caffeic acid	$C_9H_8O_4$	(-)	(-)	0.0805 ± 0.04
3-Hydroxybutyricacid	$C_4H_8O_3$	(-)	(-)	0.0428 ± 0.01
Sucrose	$C_{12}H_{22}O_{11}$	(-)	(-)	0.0025 ± 0.0009
D-Trehalose	$C_{12}H_{22}O_{11}$	(-)	1.3090 ± 0.17	0.1690 ± 0.13
Galactopyranose	$C_6H_{12}O_6$	(-)	(-)	0.0158 ± 0.006
D-(+)-Mannose	$C_6H_{12}O_6$	(-)	0.0431 ± 0.01	0.0543 ± 0.01
Phenethyl acetate	$C_{10}H_{12}O_2$	5.4376 ± 0.26	5.0627 ± 0.25	5.0289 ± 0.21
Valtrate	$C_{22}H_{30}O_8$	(-)	(-)	0.5030 ± 0.07
Glycerol monostearate	$C_{21}H_{42}O_4$	(-)	(-)	0.3082 ± 0.03
Dodecanoic acid	$C_{24}H_{48}O_2$	(-)	(-)	0.0150 ± 0.03
Propyl heptanoate	$C_{10}H_{20}O_2$	(-)	0.0591 ± 0.008	0.0523 ± 0.007
Cyclopropane	C_3H_6	(-)	0.0112 ± 0.009	(-)
Urea	CH_4N_2O	0.0039 ± 0.001	0.0036 ± 0.0009	0.0153 ± 0.001

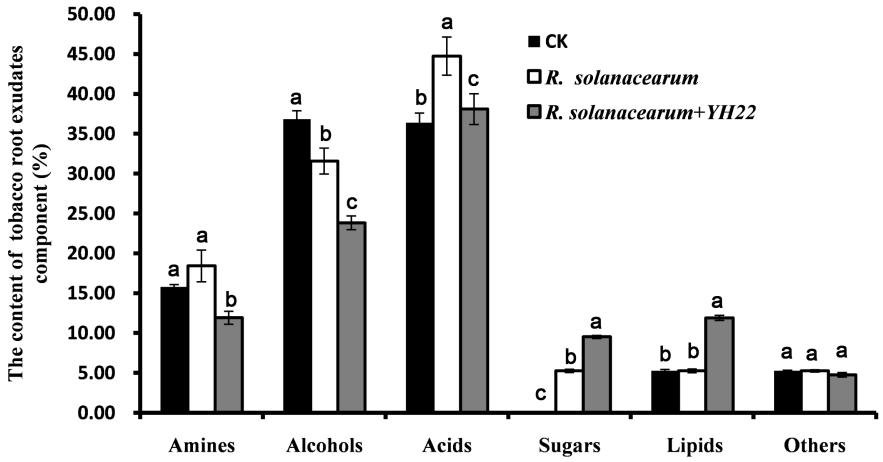
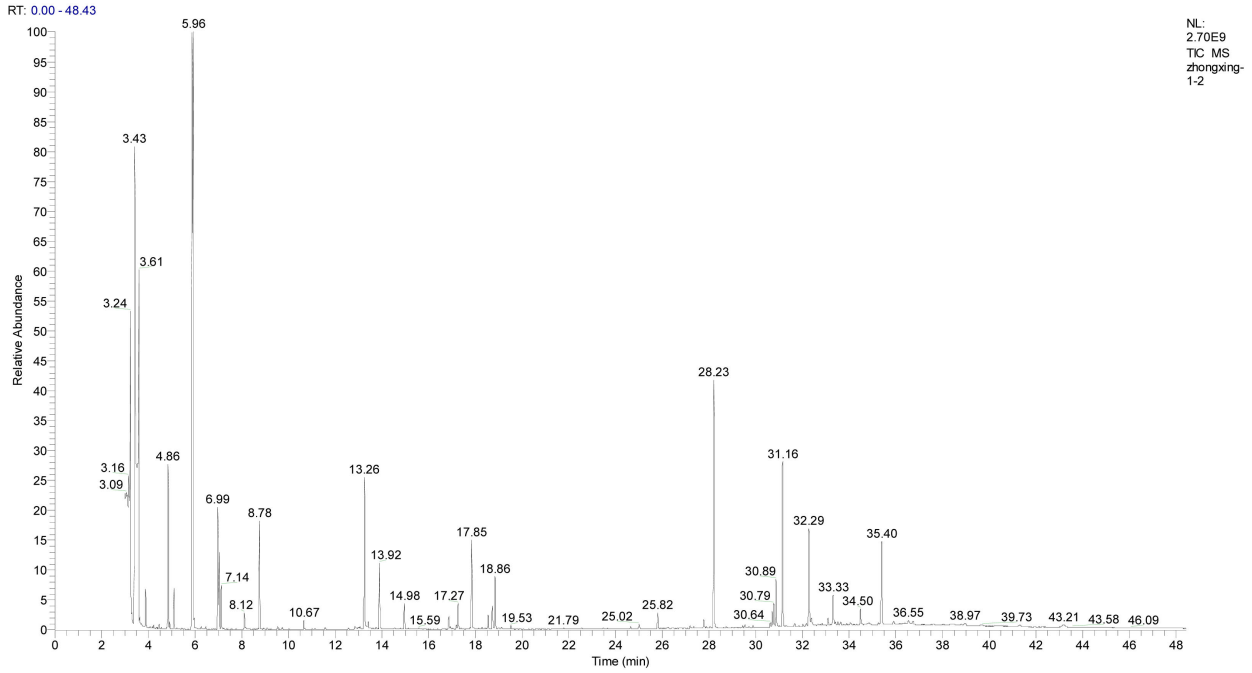


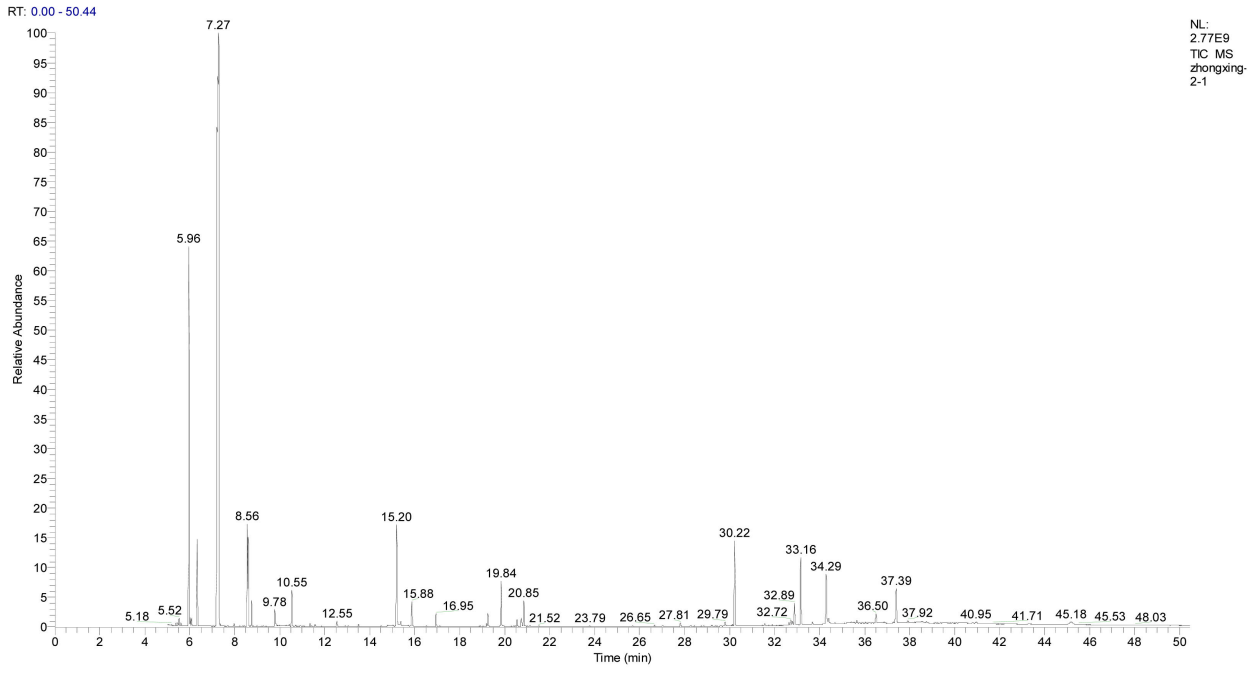
Figure 2. Changes of tobacco root exudates after treatment of bacterial wilt pathogen and its antagonist ((a), (b), (c) represent significant difference).

2) Analysis of tobacco root exudates after treating by pathogen of black shank and its antagonist.

The composition of the root exudates of Yunyan 87 was analyzed by GC-MS after irrigating pathogen of black shank and its antagonist (Figure 3). Under the condition of the pathogen of black shank, the contents of amines, alcohols, acids, lipids, sugars and esters were 14.28%, 28.57%, 48.57%, 2.85%, 2.85% and 2.85% respectively. By the treatment of antagonistic bacteria of black shank, the contents of amines, alcohols, acids, lipids, sugars and esters were 11.36%, 22.72%,



(a)



(b)

Figure 3. Total ion current of GC-MS with (a) treatment of pathogen of black shank; (b) treatment of pathogen of black shank and its antagonistic bacteria.

36.36%, 6.81%, 11.36% and 11.36% separately. There were more than 20 types of root exudates of Yunyan 87 compared with the control group, mainly including 2 kinds of amines, 5 kinds of alcohols, 11 kinds of acids, 1 kind of sugars, 1 kind of esters respectively (**Table 2**). There are the same components of 8 types with contents decreased and 4 types with contents increased. It was noteworthy that

Table 2. The content of tobacco root exudates under *Phytophthora parasitica* and *Bacillus amyloliquefacien* TU1.

Components of tobacco root exudates		Peak area (%)		
	Formula	Control group	Pathogen of black shank	Pathogen of black shank + antagonist
Methylamine	$C_7H_{21}N$	8.9354 ± 0.58	8.6354 ± 0.43	8.1213 ± 0.46
Tyramine	$C_8H_{11}NO$	(-)	2.0031 ± 0.36	1.6131 ± 0.30
Silanamine	$C_7H_{18}N_2$	(-)	4.3898 ± 0.27	5.3218 ± 0.33
Bis(trimethylsilyl)carbodiimide	$C_7H_{18}N_2$	1.2014 ± 0.22	1.1304 ± 0.34	1.9807 ± 0.42
N-Methyltrifluoroacetamide	$C_3H_4F_3NO$	0.1541 ± 0.16	1.3543 ± 0.31	0.3318 ± 0.19
Hexanol	$C_{12}H_{28}O$	4.4790 ± 0.42	3.5823 ± 0.52	4.5998 ± 0.35
Ethylene glycol	$C_8H_{22}O_2$	1.6186 ± 0.31	1.1563 ± 0.25	1.2380 ± 0.32
Diethylene glycol	$C_{10}H_{26}O_3$	1.1490 ± 0.43	(-)	(-)
Phenylethyl alcohol	$C_8H_{10}O$	1.1589 ± 0.44	(-)	(-)
Glycerol	$C_{12}H_{32}O_3$	2.6808 ± 0.68	2.0565 ± 0.41	1.9967 ± 0.11
Thiodiglycol	$C_4H_{10}O_2S$	0.1238 ± 0.08	0.1615 ± 0.11	(-)
D-Pinitol	$C_7H_{14}O_6$	(-)	(-)	0.1120 ± 0.04
D-Mannitol	$C_{24}H_{62}O_6$	(-)	1.6160 ± 0.28	2.0615 ± 0.33
Myo-Inositol	$C_6H_{12}O_6$	(-)	0.2298 ± 0.17	0.0982 ± 0.06
Silanol	$C_{13}H_{14}O$	(-)	0.2629 ± 0.14	0.3234 ± 0.08
1-Butanol	$C_4H_{10}O$	(-)	2.5342 ± 0.38	(-)
2,3-Butanediol	$C_{10}H_{26}O_2$	(-)	0.0830 ± 0.05	0.5429 ± 0.19
1-Monopalmitin	$C_{19}H_{38}O_4$	(-)	1.0321 ± 0.29	1.1421 ± 0.29
Isopropyl alcohol	C_3H_8O	(-)	(-)	1.0852 ± 0.27
Triethylene glycol	$C_8H_{18}O_4$	2.1423 ± 0.34	(-)	(-)
Boric acid	$BC_9H_{27}O_3$	8.4826 ± 0.31	8.5014 ± 0.37	9.5421 ± 0.25
Oxalic acid	C_2H_2O	(-)	4.6245 ± 0.53	3.5460 ± 0.54
Propionic acid	$C_3H_6O_2$	(-)	1.5436 ± 0.28	(-)
Oleic acid	$C_{21}H_{42}O_2$	(-)	0.0743 ± 0.04	0.0628 ± 0.05
Palmitic acid	$C_{19}H_{40}O_2$	(-)	1.2187 ± 0.23	3.0752 ± 0.29
Hydrocinnamic acid	$C_9H_{10}O_2$	(-)	0.0567 ± 0.04	0.1822 ± 0.14
Stearic acid	$C_{21}H_{44}O_2$	5.4667 ± 0.36	3.9579 ± 0.29	5.0162 ± 0.37
Isophthalic acid	$C_8H_6O_4$	(-)	2.5536 ± 0.30	(-)
Terephthalic acid	$C_8H_6O_4$	0.7792 ± 0.36	1.2501 ± 0.27	0.8705 ± 0.35
Myristic acid	$C_{14}H_{28}O_2$	(-)	1.5432 ± 0.29	(-)
ITACONIC ACID	$C_5H_6O_4$	(-)	0.1482 ± 0.10	1.1209 ± 0.30
Benzimidate hydrochloride	$C_9H_{12}NC_{lO}$	(-)	0.0942 ± 0.05	(-)
Sebacic Acid	$C_{10}H_{18}O_4$	(-)	0.8764 ± 0.24	(-)
Lactic Acid	$C_9H_{22}O_3$	7.1991 ± 0.42	3.2412 ± 0.26	2.4098 ± 0.26
Benzoic acid	$C_7H_6O_2$	1.5823 ± 0.34	2.2651 ± 0.34	1.0092 ± 0.15

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Acetic acid	$C_{10}H_{12}O_2$	2.6790 ± 0.28	3.8057 ± 0.35	4.4490 ± 0.27
Octanoic acid	$C_8H_{16}O_2$	1.5820 ± 0.29	1.9920 ± 0.74	1.8836 ± 0.34
3,4-Dimethoxybenzoic acid	$C_9H_{10}O_4$	(-)	(-)	0.6530 ± 0.31
Dehydroabietic acid	$C_{20}H_{28}O_2$	(-)	(-)	1.0472 ± 0.09
Caffeic acid	$C_9H_8O_4$	(-)	(-)	0.1054 ± 0.03
3-Hydroxybutyricacid	$C_4H_8O_3$	(-)	(-)	0.0855 ± 0.07
Sucrose	$C_{12}H_{22}O_{11}$	(-)	(-)	0.0355 ± 0.003
D-Trehalose	$C_{12}H_{22}O_{11}$	(-)	(-)	(-)
Galactopyranose	$C_6H_{12}O_6$	(-)	(-)	0.0238 ± 0.002
D-(+)-Mannose	$C_6H_{12}O_6$	(-)	0.1233 ± 0.08	0.0945 ± 0.30
Phenethyl acetate	$C_{10}H_{12}O_2$	5.4376 ± 0.68	5.3637 ± 0.32	5.3490 ± 0.29
Valtrate	$C_{22}H_{30}O_8$	(-)	(-)	0.5789 ± 0.26
Glycerol monostearate	$C_{21}H_{42}O_4$	(-)	(-)	0.8582 ± 0.25
Dodecanoic acid	$C_{24}H_{48}O_2$	(-)	(-)	0.0952 ± 0.03
Propyl heptanoate	$C_{10}H_{20}O_2$	(-)	0.0589 ± 0.04	0.1509 ± 0.03
Urea	CH_4N_2O	0.0039 ± 0.001	0.0136 ± 0.002	0.0134 ± 0.003

the most remarkable increase was terephthalic acid (60.43%) and benzoic acid (43.15%). After the addition of antagonistic bacteria of black shank, there were 24 kinds of components increased among of which 2 were newly appeared and 13 of them decreased among of which 7 were completely disappeared, namely thioethylene glycol, 1-butanol, propionic acid, isophthalic acid, myristic acid, ethyl benzoate and azelaic acid (**Figure 4**).

According to the analysis, we found that the content of propionic acid, isophthalic acid, myristic acid, ethyl benzoate and azelaic acid showed the greatest volatility, the content in the control group was 0. The treatment group of pathogen of black shank reached the highest value, while the content decreased to 0 after the addition of antagonistic bacteria, followed by terephthalic acid and benzoic acid, which increased from 0.78 to 1.53 and 2.27 respectively, and decreased to 0.86 and 1.01 after adding antagonistic bacteria. Totally, amines were significantly decreased by loading *B. amyloliquefacien* TU1 (T-test, $p = 0.048$), alcohols content were continuously and significantly decreased by loading *P. parasitica var. nicotianae* and its antagonistic bacteria *Bacillus amyloliquefacien* TU1 (T-test, $p = 0.03$), the content of acids and lipids were alwalys significantly increased by loading these two kinds of microbes, the loading of *P. parasitica var. nicotianae* did not change the content of sugars, but significantly increasedby the loading of *B. amyloliquefacien* TU1 (T-test, t = 0.02), the loading of microbes did not obviously change the content of esters in three treatments (**Figure 4**).

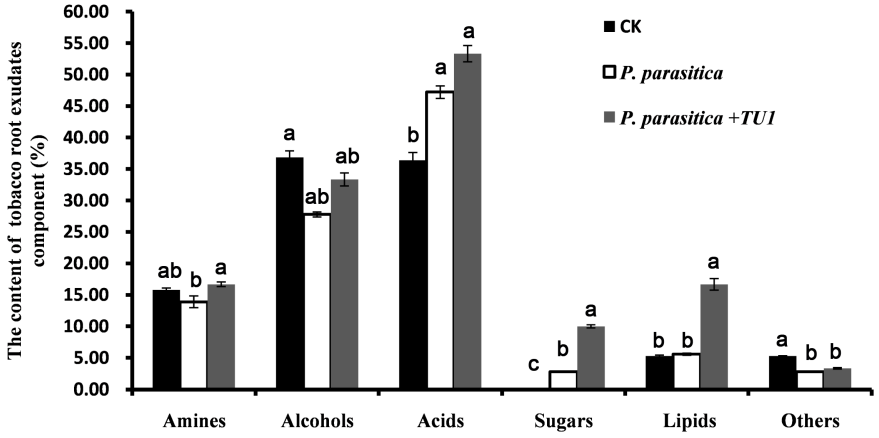

Figure 4. Changes of tobacco root exudates after treatment of black shank pathogen and its antagonist ((a), (b), (c) represent significant difference).

Plant-root exudates-rhizosphere microbes form a cyclical interaction [23], and rhizosphere microorganisms convert organic nutrients of root exudates into inorganic nutrients to facilitate plant utilization, thus affecting comprehensive indicators of plants [24] [25] [26] [27]. By analyzing the changes of root exudates of tobacco under the effect of different microbes, it was found that when the pathogenic microorganisms in the rhizosphere microorganisms increased, the root exudates of tobacco augmented evidently. When the abundance of pathogens of bacterial wilt in the rhizosphere increased, the organic acids, amines and lipids increased by 32.78%, 13.35% and 15.76% respectively among which organic acids displayed the greatest rise, increased by 10.11%; followed by amine, increased by 6.09%. When the pathogen of bacterial wilt was inhibited by the active substances of its antagonistic bacteria, the root exudates changed significantly, and there were newly 19 kinds of elements added, but 1-butanol, propionic acid, isophthalic acid, myristic acid, phenethyl imine acid, sebacic acid and cyclopropene disappeared. Although the mechanism of effect of propionic acid, myristic acid, ethyl benzoate and sebacic acid was unclear, studies have confirmed that terephthalic acid, isophthalic acid and benzoic acid are allelopathic substances to many crops. They are crucial substance that cause continuous cropping obstacles [28] [29] [30] [31] [32]. When the abundance of pathogen of bacterial wilt and its antagonist changed in the rhizosphere, the changes of root exudates were similar to the treatment of bacterial wilt and its antagonist.

There are some differences in the number of tobacco root exudates under different treatments. The proportion of the amount is shown in **Figure 2** and **Figure 4**, but the types of secretions are mainly amines, alcohols, organic acids and sugars. In the control group of tobacco root exudates, there was no carbohydrate, but it was detected in the treatment group of pathogen of bacterial wilt. After adding antagonistic bacteria, the types of carbohydrates increased and the change of organic acids was the most obvious: the control group was 36.84%, and the treatment group of bacterial wilt pathogen was 44.74%. Under the in-

fluence of pathogenic bacteria and antagonist, the types of organic acid increased to 51.61%, with a rise of 21.44% and 15.35% respectively. The change rule of root exudates of black shank pathogen and its antagonist was similar to that of bacterial wilt pathogen and its antagonist. However, the types of organic acid increased to 47.22% and 53.33%, with a rose of 28.18% and 12.93%. Data has shown that through the treatment of bacterial wilt, black shank pathogen and their antagonistic bacteria, the content of propionic acid, isophthalic acid, myristic acid, ethyl benzoate and azelaic acid fluctuated abundantly, while the control group was 0. The treatment group of bacterial wilt pathogen reached the highest its peak, and the content decreased to 0 after the addition of antagonistic bacteria, followed by terephthalic acid and benzoic acid, which increased from 0.78 to 1.53 and 2.27 respectively, yet decreased to 0.86 and 1.01 separately after adding antagonistic bacteria. The results showed that propionic acid, isophthalic acid, myristic acid, ethyl benzoate and azelaic acid were direct inducers of bacterial wilt and black shank pathogens, and their antagonistic bacteria could alleviate harmful bacteria that induced tobacco to produce allelochemicals (terephthalic acid, isophthalic acid and benzoic acid). The dramatical increase of the abundance of pathogen in soil was a vital factor which led to continuous cropping obstacles. It was speculated that the possible mechanism was due to the increase of the abundance of pathogen [33], which induced crops to produce allelopathic substances, thus allelochemicals provide nutrients for the colonization of harmful bacteria. It formed a vicious circle that eventually resulted in continuous cropping obstacles. Although this conjecture needs further confirmation, the study provides new clues for exploring tobacco continuous cropping disorders and soil-borne diseases.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., *et al.* (2012) The Rhizosphere Microbiome and Plant Health. *Trends in Plant Science*, **17**, 478-486. https://doi.org/10.1016/j.tplants.2012.04.001

[2] Egamberdieva, D., Kamilova, F., Validov, S., *et al.* (2008) High Incidence of Plant Growth-Stimulating Bacteria Associated with the Rhizosphere of Wheat Grown on Salinated Soil in Uzbekistan. *Environmental Microbiology*, **10**, 1-9.

[3] Mendes, R., Kruijt, M., de Bruijn, I., *et al.* (2011) Deciphering the Rhizosphere Mi-

crobiome for Disease-Suppressive Bacteria. *Science*, **332**, 1097-1100. https://doi.org/10.1126/science.1203980

[4] Pradeep, R.M. (2015) The Promise of the Plant's Second Genome. *Journal of Investigative Genomics*, 2, 83.

[5] Zamioudis, C. and Pieterse, C.M. (2012) Modulation of Host Immunity by Beneficial Microbes. *Molecular Plant-Microbe Interactions*, **25**, 139-150. https://doi.org/10.1094/MPMI-06-11-0179

[6] Zhang, Y., Du, B.H., Jin, Z.G., *et al.* (2011) Analysis of Bacterial Communities in Rhizosphere Soil of Healthy and Diseased Cotton (*Gossypium* sp.) at Different Plant Growth Stages. *Plant Soil*, **339**, 447-455. https://doi.org/10.1007/s11104-010-0600-2

[7] Lanoue, A., Burlat, V., Henkes, G.J., *et al.* (2010) *De Novo* Biosynthesis of Defense Root Exudates in Response to *Fusarium* Attack in Barley. *New Phytologist*, **185**, 577-588. https://doi.org/10.1111/j.1469-8137.2009.03066.x

[8] Yang, J.W., Yi, H.S., Kim, H., *et al.* (2011) Whitefly Infestation of Pepper Plants Elicits Defence Responses against Bacterial Pathogens in Leaves and Roots and Changes the Below-Ground Microflora. *Journal of Ecology*, **99**, 46-56. https://doi.org/10.1111/j.1365-2745.2010.01756.x

[9] Lee, B., Lee, S. and Ryu, C.M. (2012) Foliar Aphid Feeding Recruits Rhizosphere Bacteria and Primes Plant Immunity against Pathogenic and Non-Pathogenic Bacteria in Pepper. *Annals of Botany*, **110**, 281-290. https://doi.org/10.1093/aob/mcs055

[10] Zhang, L., Xu, H.M. and Zhu, B.L. (2016) Soil Microbiome with the Occurrence and Development of Replant Disease—A Review. *Acta Microbiologica Sinica*, **56**, 1234-1241.

[11] Sasse, J., Martinoia, E. and Northen, T. (2018) Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science*, **23**, 25-41. https://doi.org/10.1016/j.tplants.2017.09.003

[12] Bais, H.P., Weir, T.L., Perry, L.G., *et al.* (2006) The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. *Annual Review of Plant Biology*, **57**, 233-266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

[13] Weisskopf, L., Le, B.R., Kohler, F., *et al.* (2008) Spatio-Temporal Dynamics of Bacterial Communities Associated with Two Plant Species Differing in Organic Acid Secretion: A One-Year Microcosm Study on Lupin and Wheat. *Soil Biology and Biochemistry*, **40**, 1772-1780. https://doi.org/10.1016/j.soilbio.2008.02.018

[14] He, H., Wang, Z.W., Hu, D., *et al.* (2011) Progress on Interactions between Root Exudates and Rhizosphere Microorganisms. *Journal of Hebei Agricultural Sciences*, **15**, 69-73.

[15] Kawasaki, A., Suzanne, D., Peter, R., *et al.* (2016) Microbiome and Exudates of the Root and Rhizosphere of *Brachypodium distachyon*, a Modelfor Wheat. *PLoS ONE*, **11**, e0164533. https://doi.org/10.1371/journal.pone.0164533

[16] Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., *et al.* (2009) The Rhizosphere: A Playground and Battlefield for Soilborne Pathogens and Beneficial Microorganisms. *Plant Soil*, **321**, 341-361. https://doi.org/10.1007/s11104-008-9568-6

[17] Hoitink, H. and Boehm, M. (1999) Biocontrol within the Context of Soil Microbial Communities: A Substrate-Dependent Phenomenon. *Annual Review of Phytopathology*, **37**, 427-446. https://doi.org/10.1146/annurev.phyto.37.1.427

[18] Garbeva, P., Veen, J.A., Elsas, J.D., *et al.* (2004) Microbial Diversity in Soil: Selection

of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness. *Annual Review of Phytopathology*, **42**, 243-270. https://doi.org/10.1146/annurev.phyto.42.012604.135455

[19] Weller, D.M., Raaijmakers, J.M., Gardener, B.B., *et al*. (2002) Microbial Populations Responsible for Specific Soil Suppressiveness to Plant Pathogens. *Annual Review of Phytopathology*, **40**, 309-348. https://doi.org/10.1146/annurev.phyto.40.030402.110010

[20] Gagne, B.F., Mayer, B.F., Charron, J.B., *et al*. (2015) Accelerated Growth Rate and Increased Drought Stress Resilience of the Model Grass Brachypodium Distachyon Colonized by *Bacillus subtilis* B26. *PLoS ONE*, **10**, 11-16.

[21] Amaral, F.P., Pankievicz, V.C., Arisi, A.C., *et al*. (2016) Differential Growth Responses of *Brachypodium distachyon* Genotypes to Inoculation with Plant Growth Promoting Rhizobacteria. *Plant Molecular Biology*, **90**, 689-697. https://doi.org/10.1007/s11103-016-0449-8

[22] Yu, H., Shen, G. and Gao, X. (2013) Determination of Tobacco Root Exudates by GC-MS. *Acta Tabacaria Sinica*, **19**, 64-71.

[23] Shi, S.J., Richardson, A.E., O'Callaghan, M., *et al*. (2011) Elects of Selected Root Exudate Components on Soil Bacterial Communities. *FEMS Microbiology Ecology*, **77**, 600-610. https://doi.org/10.1111/j.1574-6941.2011.01150.x

[24] Zhang, A., Sun, K., Da, W., *et al*. (2008) Study on the Microorganism of Two Habitats of *Hippophae rhamnoides* Rhizosphere Soil. *Journal of Northwest Normal University* (*Natural Science*), **44**, 69-73.

[25] Lu, Y. and Zhang, S. (2006) Progress on Rhizosphere Microorganisms. *Soil*, **38**, 113-121.

[26] Chen, L., Li, P., Si, L., *et al*. (2002) Ecological Research of Root Exudates. *Journal of Ecology*, **21**, 57-62.

[27] Graham, R.D. (1988) Genotypic Differences in Tolerance to Manganese Deficiency. In: *Zinic in Soil and Plants*, Kluwer Academic Publishers, Dordrecht, 78-85.

[28] Liu, H., Song, Q. and Li, F. (2002) The Roles of Root Exudation on Rhizosphere Nutrient and Rhizosphere Microorganisms. *Acta Botanica Boreali-Occidentalia Sinica*, **22**, 693-702.

[29] Wang, F. (2017) Effect of 1,2-Benzenedicarboxylic Acid on Root Growth of Adzuki Bean. *Qinghai Agriculture and Forestry Science and Technology*, **4**, 12-16.

[30] Liu, Y., Li, X., Cai, L., *et al*. (2016) Identification of Phenolic Acids in Tobacco Root Exudates and Their Role in the Growth of Rhizosphere Microorganisms. *Journal of Plant Nutrition and Fertilizer*, **22**, 418-428.

[31] He, Z., Wang, R., Wang, X., *et al*. (2014) Preliminary Study of Screening of Peanut Autotoxicity Degrading Microorganism and the Degradation Effect. *Chinese Agricultural Science Bulletin*, **30**, 224-227.

[32] Geng, G., Zhang, S. and Cheng, Z. (2008) Allelopathy of 1,2-Benzenedicarboxylic Acid and Its Mechanism. *Journal of Hunan Agricultural University* (*Natural Sciences*), **34**, 656-659.

[33] Gu, Y., Qiu, Q., Wang, Z., *et al*. (2012) Effects of Soybean Continuous Cropping on Microbial and Soil Enzymes in Soybean Rhizosphere. *Scientia Agricultura Sinica*, **45**, 3955-3964.