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Unraveling the characteristics of the microbial community and potential pathogens in the rhizosphere soil of *Rehmannia glutinosa* with root rot disease

Ruifei Wang[a,1], Yan Wang[a,1], Qingxiang Yang[a,*], Chunxiao Kang[a], Mingjun Li[a,b]

[a] College of Life Sciences, Henan Normal University, Xinxiang 453007, China
[b] Engineering Technology Research Center of Nursing and Utilization of Genuine Chinese Crude Drugs in Henan Province, Xinxiang 453007, China

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Abstract: The roots of *R. glutinosa* are a high-demand traditional Chinese medicine with many pharmacological functions and great economic value. However, root rot disease leads to a dramatic reduction in the yield and quality of *R. glutinosa*. Here, we combined high-throughput sequencing technologies and cultural approaches to investigate characteristics of the microbial community in the rhizosphere soil of *R. glutinosa* with root rot disease and identified potential pathogens. The results indicated the following: 1) Root rot of *R. glutinosa* had an intimate relationship with the imbalance of the microbial community. Some genera, such as *Pseudomonas*, Un-s-*Sordariales* and Un-s-*Ascomycota*, were more abundant in rhizosphere soil from diseased *R. glutinosa* than that from healthy *R. glutinosa*, while other genera, such as *Sphingomonas*, *Streptomyces* and *Myrothecium*, displayed the opposite trend. 2) The six isolates (*Pseudomonas hibiscicola*, *Rhizopus stolonifer*, *Fusarium solani*, *Actinomucor elegans*, *Enterobacter aerogenes* and *Aspergillus tubingensis*) from rotten roots were confirmed to be pathogens for root decay of *R. glutinosa* in pot experiments with different pathogenicities of 33.33%–100%, suggesting the potential synergy of several pathogens in causing root rot in *R. glutinosa*. Quantitative PCR and pathogenicity tests indicated that *Pseudomonas hibiscicola* of the genus *Pseudomonas* was probably the main microbe responsible for root rot in *R. glutinosa*.

1. Introduction

Root health is essential for plant development and controls of plant water and nutrient uptake (Peña et al., 2013). Root rot diseases negatively influence root function, cause root decay and finally plant death, leading to a dramatic reduction in plant yield and quality.

It is widely accepted that plant diseases are often caused by one species of microbial pathogens or even by a specific strain. Correspondingly, the current recognition of the plant-pathogen interaction is largely based on the isolation of a single microbial pathogen grown in pure culture (Lamichhane and Venturi, 2015). However, the interactions between plants and pathogens cannot be simplified to just trench warfare between the two parties (Berendsen et al., 2012). Rather, the successful colonization of plant roots and subsequent growth of pathogens could be influenced by many factors, especially the rhizosphere soil microbial community (Chapelle et al., 2016; Lareen et al., 2016). The different pathogens in rhizosphere soil probably display synergistic interactions, which induce more severe disease symptoms than the individual pathogens, although the mechanisms are currently unknown (Glen et al., 2007; Lamprecht et al., 2011; Petkowski et al., 2013). Furthermore, the pathogens must compete with other microbes for resources or for space in the rhizosphere and break through the protective microbial shield to infect plant roots (Weller et al., 2002). Therefore, investigating the changes in the rhizosphere microbial community compositions and abundances with root rot disease would greatly facilitate our understanding of the total population of microbial species involved in plant root rot diseases as well as the underlying mechanisms by which pathogens infect the root.

Culture-independent methods, especially high-throughput sequencing technologies, have recently been widely applied to investigate the

* Corresponding author at: College of Life Sciences, Henan Normal University, Jianshe Road 46, Xinxiang 453007, China.
E-mail address: yangqx@htu.cn (Q. Yang).
[1] Both authors contributed equally to this work.

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relationship between the dynamic changes in the microbial community structure with plant disease (Lamichhane and Venturi, 2015; Dong et al., 2016; She et al., 2017; Karlsson et al., 2017). These methods can provide comprehensive recognition of potential plant pathogens, which will guide the direction for further isolating and confirming potential pathogens by traditional culture-dependent methods. Therefore, combining culture-independent and culture-dependent methods should be a good strategy to analyze the microbes intimately related to plant diseases, including root rot disease; however, few studies have investigated this.

Rehmannia glutinosa has been used in traditional medicine due to pharmacological properties influencing hematologic conditions and tumor growth as cited by Yang et al. (2017). Indeed, more than 15,000 tons of *R. glutinosa* roots are consumed annually, and they have great medical and economic value. Under field conditions, *R. glutinosa* is normally planted in April and harvested in December every year. From July to October of the growth period, roots of *R. glutinosa* rapidly swell by absorbing nutrition and water. Unfortunately, root rot disease frequently occurs because of the hot and muggy weather conditions in this period, which results in a dramatic decrease in *R. glutinosa* yield and quality (Qi et al., 2009). Although members of the genus *Fusarium* are thought to be causal agents for *R. glutinosa* root rot (Lim et al., 2005), agricultural practices have indicated that fungicides against *Fusarium* often have a limited efficacy, suggesting that other rhizosphere microbes are probably also involved in root rot disease of *R. glutinosa*.

In this study, we assumed that two reasons, the balance of healthy microbial community being broken and/or co-existence and synergistic interaction of different potential pathogens in the rhizosphere, are possibly responsible for root rot disease of *R. glutinosa*. By combining high-throughput sequencing with isolation of potential pathogens, we aimed to: 1) determine the characteristics of the microbial community in the rhizosphere of *R. glutinosa*, especially the dynamic relationship between potential pathogens and beneficial microbes, and 2) isolate potential pathogens from the rotten roots of *R. glutinosa*, detect their pathogenicity and discuss the possible mechanism by which these pathogens infect *R. glutinosa* roots. The results of this study will contribute to the biological control of root rot disease in *R. glutinosa* and other medical plants in China.

2. Materials and methods

2.1. Site information and sample collection

Because consecutive monoculture of *R. glutinosa* can result in high occurrence of root rot disease, we selected three fields (each approximately 60 m^2) in which *R. glutinosa* had not been planted for at least 5 years before our experiments. This enabled the incidence of root rot disease under normal planting conditions to be reflected. *R. glutinosa* cultivar “85-5” was cultured in early April 2015 in Wenxian County, Jiaozuo (112°51′E, 113°13′N; a geo-authentic production zone for *R. glutinosa*), Henan Province, China. The main climate conditions of Wenxian County are as follows: annual mean temperature, 14.3 °C; annual average precipitation, 552.4 mm; annual sunshine duration, 2484 h; altitude, 102.3–116.1 m; soil type, loess soil. The rhizosphere soil samples were collected when *R. glutinosa* exhibited serious root rot in July 2015. Four to five sampling points in a portion of the test fields were randomly selected. At each sampling point, plants were carefully dug out and gently shaken off, after which the soil adhering to roots was separated. Following sampling, the soil was taken to the laboratory under sterile conditions. A total of 13 soil samples were divided into two groups: healthy soil (H1–H5, five parallel samples from healthy plants) and diseased soil (D1–D8, eight parallel samples from diseased plants). Before planting and after harvesting, the pH of the experimental soils was 6.94 ± 0.05 and 6.41 ± 0.30, the organic matter content was 8.56 ± 0.54 and 8.04 ± 0.95 g/kg, the total N was 1.00 ± 0.04 and 0.96 ± 0.01 g/kg, and the total P was 0.124 ± 0.010 and 0.115 ± 0.006 g/kg, respectively. All the above mentioned soil characteristics were measured as described by Wu et al. (2013).

2.2. DNA extraction and illumina sequencing

The genomic DNA of the microbes in soil samples was extracted directly using a Soil DNA Kit (Omega Bio-tek, Doraville, USA) according to the manufacturer’s instructions. The concentration and purity of the

Table 1
The top bacterial genera with high contributions to the dissimilarity between the rhizosphere soils of healthy and diseased *R. glutinosa*.

Bacteria	Health Average reads	Disease Average reads	Dissimilarity	Contribution %	Cumulation %
Pseudomonas	336.20	943.13	3.13	8.90	8.90
Sphingomonas	1068.20	871.38	1.90	5.41	14.32
*Ruminococcaceae*_UCG-014	271.80	295.13	1.55	4.41	18.73
Lactobacillus	168.80	342.25	1.21	3.44	22.17
Bacillus	309.40	335.63	0.79	2.24	24.41
Unidentified_*Acidobacteria*	423.00	440.38	0.74	2.10	26.51
Streptomyces	226.20	88.88	0.71	2.03	28.54
*Lachnospiraceae*_NK4A136_group	94.60	221.50	0.63	1.80	30.33
Burkholderia	68.20	92.88	0.63	1.78	32.11
Arthrobacter	242.80	151.00	0.53	1.51	33.62
Stenotrophomonas	127.40	39.38	0.52	1.48	35.10
[Eubacterium]_*coprostanoligenes*_group	86.60	119.88	0.49	1.40	36.50
Rhizobium	245.00	205.13	0.45	1.27	37.77
Haliangium	266.20	258.00	0.37	1.06	38.83
Ohtaekwangia	36.60	107.50	0.37	1.06	39.89
Lysobacter	107.20	102.13	0.35	1.00	40.89
Nocardioides	141.40	74.75	0.34	0.98	41.87
Acidobacter	99.20	113.50	0.32	0.90	42.78
Halomonas	58.80	26.25	0.31	0.89	43.66
unidentified_*Nitrospiraceae*	158.60	123.75	0.30	0.87	44.53
unidentified_GR-WP33-30	188.80	160.13	0.29	0.82	45.35
Opitutus	85.40	53.63	0.29	0.82	46.16
Gaiella	194.20	136.13	0.28	0.80	46.96
Polycyclovorans	118.40	87.00	0.28	0.79	47.75
Gemmatimonas	103.80	95.25	0.28	0.79	48.55
*Psychrobacter*_GR-WP33-30	61.20	17.38	0.28	0.79	49.33
Enterorhabdus	77.60	102.00	0.27	0.78	50.11