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Full Length Article

Effects of Rotation of Indian Mustard on Cucumber Seedling Rhizosphere Fungal Community Composition

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Abstract

It is well established now that cropping system has great influence on soil microbial communities, but still advance techniques are required to study more of changes in soil microbiota during cropping systems. Here we used high-throughput sequencing to explore the effects of crop rotation with Indian mustard on composition of cucumber rhizosphere fungal community in pot experiment. In our results, an average of 35,748 quality sequences were obtained and these were classified into more than 450 Operational taxonomic units (OTUs) at 97% sequence similarity. The rotation with Indian mustard changed the rhizosphere fungal community composition of cucumber seedlings, but had no effect on fungal community alpha diversity. Rotation with Indian mustard was dominated by *Ascomycota* phyla, *Leotiomycetes* and *Ascomycota Ineertae sedis* classes, orders of *Sordariales*, *Eurotiales*, *Agaricomycetes Incertae sedis* and unclassified *Sordariomycetes*. In the rotation of Indian mustard, the relative abundance of *Humicola*, *Remersonia*, *Myrothecium*, *Scedosporium* and *Mycothermus* spp. was higher, but the relative abundance of *Pseudallescheria*, *Mortierella*, *Chaetomium*, *Ilyonectria*, *Gibellulopsis* and *Metacordyceps* spp. was lower. Overall, this study has provided great insights of changes in fungal community during crop rotation system. © 2020 Friends Science Publishers

Keywords: Crop rotation; Cucumber; Fungal community; Indian mustard; Rhizosphere

Introduction

Cucumber is one of the most popular greenhouse vegetables throughout the world. However, continuous monocropping is one of the factor causing "soil sickness" which leads to poor plant growth, increase in soil-borne pathogens and finally reduce crop production (Zhou *et al.* 2017). Soil sickness may be related to changes in soil microbial communities because of autotoxicity (Jin *et al.* 2020). Previous studies have shown that cropping systems, such as rotation, intercropping and interplanting systems, could significantly improve soil health for better crop production (Zhou *et al.* 2017). For example, rotation of tomato-celery-cucumber-Chinese cabbage with cucumber could overcome the soil sickness of cucumber (Zhou *et al.* 2017). Previous study found that incorporation of *Brassica juncea* inhibit the growth of pathogenic *Rhizoctonia solani* and *Fusarium oxysporum* (Friberg *et al.* 2009).

Crop rotation is the practice of rotating different crops sequentially between seasons and years in the same field (Wibberley 1996). Previous studies have shown that cucumber rotation with tomato, soybean, wheat and celery was beneficial to maintain the diversity and activity of soil microbes and inhibited the harmful microorganisms that were higher in continuously monocropped cucumber rhizosphere (Wu *et al.* 2011). For example, Jin *et al.* (2019b) reported that rotation with Indian mustard could suppress cucumber Fusarium wilt disease and increase plant-beneficial bacteria in rhizosphere.

It has been shown that *Brassica* spp. crops (*i.e.*, Indian mustard) are commonly grown to reduce soil-borne pathogenic fungi (Larkin and Griffin 2007) because when their tissues are disrupted, the glucosinolate releases isothiocyanate, which is toxic to many soil pathogenic microorganisms (Motisi *et al.* 2009). It was found that Indian mustard and wild rocket green manures increased cucumber rhizosphere bacterial diversity and abundance of potential plant-beneficial species, decreased Fusarium wilt disease and enhanced expression of defense-related genes in cucumber seedling roots (Jin *et al.* 2019c). In this study, we collected Indian mustard- and the fallow-treated soil samples, and further studied the effects of rotation of Indian mustard on diversity and composition of

cucumber fungal rhizosphere using high-throughput sequencing technology.

Materials and Methods

Greenhouse experiment

Cucumber continuous cropping soil was collected from soil upper layer (0–15 cm) of a greenhouse in the experimental station (45°41'N, 126°37'E) of Northeast Agricultural University, Harbin, China, where the cucumber has been cultivating since 2006. The soil type used for pot experiments was sandy loam and the physicochemical properties were determined by method as previously used by Zhang *et al.* (2018), which were as follow: EC (1:2.5, w/v) 0.43 mS cm^{-1}; pH 7.64 (1:2.5, w/v); organic matter 3.51%; inorganic N (NH_4^+-N and NO_3^--N) 146.60 mg kg^{-1}; Olsen P 284.20 mg kg^{-1}; and available K 341.80 mg kg^{-1}.

A pot experiment was performed during July to September 2016 for cultivation of Indian mustard consisting of two treatments in greenhouse (32°C day/22°C night, with a 16 h light/8 h dark and 60–80% relative humidity. Total of 30 seeds of Indian mustard (cv. Xuelihong) were germinated in each pot (diameter 20 cm, height 17 cm) of total 10 pots in first treatment (R). Same number of pots without Indian mustard seeds were kept as control treatment (M), and treatments were replicated thrice to make 30 pots in total for each treatment. Each pot contained 2.5 ± 0.1 kg of fresh cucumber continuous monocropping soil. After germination, thinning of seedlings was done to minimize the density of seedlings to 10 by removing bad/extra seedlings in each pot. Each treatment was replicated thrice to make a total of 30 pots in each treatment. Pots of both treatments were placed randomly without any order and their place was changed after every third day. Distilled water was added every second day to keep soil moisture at about 65% of its water content and no fertilizer was applied.

After 40 days after sowing, the ground portion of Indian mustard was harvested and the underground portion was left in the soil. Each pot was wrapped in a black polyethylene plastic film, and the soil moisture content was maintained at around 65% and incubated for 30 days. Cucumber seedlings with two cotyledons (cv. Jinyan 4) were then planted in pots, one cucumber seedling per pot. The cultivated conditions of cucumber seedlings were same as described above for Indian mustard.

Soil sampling and DNA extraction

After 30 days of plantation, the cucumber rhizosphere soil was collected according to the method previously used by Zhou *et al*. (2017) and sieved through 2 mm mesh. Sample of 10 plants from each replicate was mixed to prepare a composite soil sample and stored at -80°C for DNA extraction.

Rhizosphere soil DNA was extracted from 0.25 g soil from each sample in triplicate using PowerSoil DNA Isolation Kit (MO BIO Laboratories, C.A., U.S.A.) following the manufacturer's instructions. The extracted DNA (in triplicate) was then combined to make a composite sample and stored at -80°C for further analysis.

Illumina miseq sequencing and data processing

As previously mentioned (Zhou *et al.* 2018a), amplification of the ITS1 region of the fungal rRNA gene was done using the ITS1F/ITS2 primer The forward and reverse primers also had a unique 6 bp barcode for each sample. The three composite sample DNA solutions were separately subjected to PCR amplification, then the PCR product was collected and purified and paired-end sequencing (2×300) was performed on the Illumina Miseq platform of Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

The de-multiplexing, quality filtering and processing of the raw sequence reads were performed by FLASH (Zhou *et al.* 2017). Identification and removal of chimeric sequences was done with USEARCH 6.1 in QIIME (Caporaso *et al.* 2010). Sequences were classified by the agglomerative clustering algorithm in USEARCH (Edgar, 2010) as an Operational taxonomic units (OTUs) with 97% sequence similarity. Each representative OTU sequence was then taxonomically classified by BLAST in the Unite database (Koljalg *et al.* 2013).

Statistical analysis

To avoid possible deviations due to sequencing depth, a random subsample of 30,740 sequences was performed for each sample. The defined OTUs were used to calculate the taxon cumulative curve. The alpha diversity analysis was performed by calculating the Shannon and inverse Simpson indices. The differences in fungal community structures by Beta diversity analysis were assessed using the UPGMA hierarchical clustering analysis based on Bray-Curtis distance. The shared and unique OTUs between treatments were calculated and their distribution was shown in a Venn diagram. Differences in alpha diversity indices and relative abundances of microbial taxa between treatments were analyzed using Student's t test. All of these analyses were done in 'R' (version 3.3.1).

Results

Fungal communities alpha and beta diversities

After reading and removing a single OTU by basic quality control filtration, Illumina Miseq. produced an average of 35,748 high quality fungal sequences in each sample with an average read length of 262 bp. A total of 450 OTUs were identified at 97% sequence similarity. The OTU rarefaction curves of all samples tended to be flat (Fig. 1a) and the Good's coverage was larger than 99.5% for

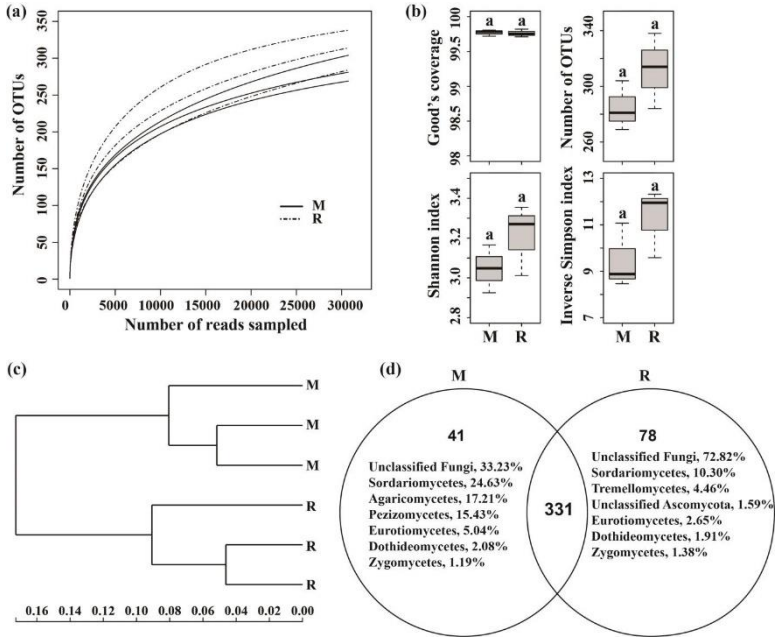

Fig. 1: Rarefaction curves of the number of OTUs (**a**), The Good's coverage, diversity and richness indices of cucumber rhizosphere fungal communities (**b**), Hierarchical clustering tree of Indian mustard (R)- and fallow (M)-treated soil samples at the OTU level (**c**), and Venn diagrams demonstrating the numbers of shared and unique observed fungal OTUs at 97% similarity between Indian mustard (R)- and fallow (M)-treated soil samples (**d**). OTUs were delineated at 97% sequence similarity. Random subsamples of 30,740 16S rRNA gene sequences per sample were used to generate the rarefaction curves and calculate the Good's coverage, diversity and richness indices. Different letters indicate significant difference based on Student's *t* test (P≦0.05). Dendrogram of relatedness of the soil types. Frequencies of OTUs unique to each treatment at the fungal class level were shown

each sample (Fig. 1b). Therefore, the number of sequences was sufficient to assess the diversity of cucumber rhizosphere fungal communities.

Cucumber monocropping and rotation with Indian mustard had similar fungal community richness and diversity indices (Fig. 1b). However, cluster analysis showed that the cucumber rhizosphere fungal community structure differed between R and M treatments (Fig. 1c).

Shared and unique OTUs

For fungal communities, there were 331 OTUs in both treatment samples, accounting for 73.56% of the total OTU observed by the fungi (Fig. 1d). It was found that only a small fraction of OTUs were unique to treatments. The OTUs unique in (M)-treatment samples, fungi were mainly belonging to the classes of *Sordariomycetes*, *Agaricomycetes* and *Pezizomycetes*; while the OTUs unique to (R)-treatment were belonging to *Sordariomycetes*.

Fungal communities composition

A total of 4 phyla were detected in all the samples, among which the *Ascomycota* and *Zygomycota* were dominant, accounting for 84.71 and 12.41% of the total fungi, respectively (Fig. 2a). Compared with monocropped cucumber soil, rotation with Indian mustard had higher *Ascomycota* abundance, but the abundance of *Zygomycota* was relatively low ($P \leqq 0.05$). The top three fungal classes (relative abundance >10%) found were *Sordariomycetes*, *Pezizomycetes* and *Zygomycetes*, accounting for 92.18% of the total fungi (Fig. 2b). Rotation with Indian mustard also increased abundance of *Leotiomycetes*, *Ascomycota Ineertae sedis* and unclassified fungi, and decreased abundance of *Zygomycetes* as compared to monocropped cucumber ($P \leqq 0.05$).

Hypocreales, *Mortierellales*, *Sordariales*, *Pezizales* and *Microascales* were the dominant orders (average relative abundance >10%) in all the samples (Fig. 2c). Futhermore, *Agaricales*, *Rhizophlyctidales*, *Thelebolales*, *Sordariomycetes Incertae sedis*, *Agaricomycetes Incertae sedis*, *Eurotiales*, *Xylariales*, *Ascomycota Incertae sedis*, *Onygenales*, *Tremellales*, *Pleosporales*, unclassified *Sordariomycetes*, unclassified fungi and unclassified *Ascomycota* were also detected at relatively higher abundance (average relative abundance > 0.1%) (Fig. 2d). Compared with monocropped cucumber, rotation with Indian mustard had higher relative abundance of *Sordariales*, *Eurotiales*, *Agaricomycetes Incertae sedis*, unclassified *Sordariomycetes* and unclassified Fungi and lower relative abundance of *Mortierellales*, *Microascales*, *Agaricales*, *Thelebolales* ($P \leqq 0.05$).

At the genus level, more than 137 fungal genera or

Table 1: Relative abundances (%) of main fungal genera in cucumber rhizosphere soils

Fungal genera	M	R	Fungal genera	M	R
Pseudallescheria	**25.69 ± 1.64**	**17.46 ± 1.10**	*Aspergillus*	0.07 ± 0.00	0.28 ± 0.08
Mortierella	**15.74 ± 0.25**	**5.46 ± 1.13**	*Thielavia*	0.18 ± 0.01	0.16 ± 0.02
Humicola	**1.39 ± 0.13**	**15.04 ± 1.84**	*Trichoderma*	0.29 ± 0.17	0.02 ± 0.01
Chaetomium	**10.92 ± 0.06**	**5.08 ± 0.16**	***Ilyonectria***	**0.19 ± 0.01**	**0.04 ± 0.01**
Fusarium	6.12 ± 1.05	8.68 ± 1.52	***Gibellulopsis***	**0.13 ± 0.01**	**0.04 ± 0.02**
Pseudaleuria	6.93 ± 1.81	7.09 ± 1.42	*Arachnomyces*	0.09 ± 0.02	0.07 ± 0.03
Kernia	2.28 ± 0.45	1.65 ± 0.10	*Zygopleurage*	0.06 ± 0.01	0.10 ± 0.02
Acremonium	1.54 ± 0.17	2.09 ± 0.25	*Rhizophlyctis*	0.04 ± 0.01	0.12 ± 0.05
Preussia	0.15 ± 0.00	1.39 ± 0.58	*Penicillium*	0.08 ± 0.00	0.07 ± 0.02
Zopfiella	0.45 ± 0.04	0.78 ± 0.17	***Metacordyceps***	**0.10 ± 0.00**	**0.05 ± 0.01**
Cryptococcus	0.60 ± 0.10	0.60 ± 0.08	*Gibberella*	0.05 ± 0.01	0.08 ± 0.01
Remersonia	**0.40 ± 0.02**	**0.73 ± 0.04**	***Mycothermus***	**0.03 ± 0.00**	**0.09 ± 0.02**
Monosporascus	0.59 ± 0.29	0.50 ± 0.25	*Gymnoascus*	0.04 ± 0.01	0.06 ± 0.02
Chrysosporium	0.41 ± 0.08	0.51 ± 0.07	*Phialemonium*	0.04 ± 0.01	0.05 ± 0.01
Microascus	0.35 ± 0.05	0.32 ± 0.11	*Phialosimplex*	0.04 ± 0.01	0.05 ± 0.01
Myrothecium	**0.03 ± 0.01**	**0.57 ± 0.15**	*Scutellinia*	0.04 ± 0.00	0.04 ± 0.01
Scedosporium	**0.19 ± 0.01**	**0.35 ± 0.04**	*Papulaspora*	0.06 ± 0.03	0.01 ± 0.00
Myriococcum	0.01 ± 0.01	0.51 ± 0.31	*Nectria*	0.05 ± 0.02	0.01 ± 0.00
Cephaliophora	0.33 ± 0.10	0.14 ± 0.02	*Arthrographis*	0.03 ± 0.01	0.03 ± 0.01
Wardomyces	0.21 ± 0.05	0.20 ± 0.00	*Coniochaeta*	0.02 ± 0.00	0.03 ± 0.01
Podospora	0.25 ± 0.05	0.12 ± 0.01	*Guehomyces*	0.02 ± 0.00	0.03 ± 0.02

Note: Values (mean ± SE) highlighted in bold are significantly different among treatments of cucumber monocropping (M) and rotations of Indian mustard (R) at the 0.05 probability level (Student's *t* test)

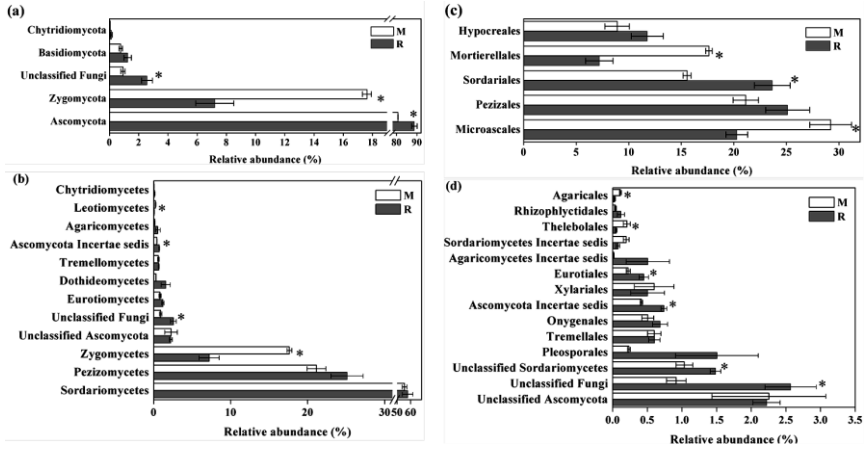

Fig. 2: Relative abundances of main fungal phyla (**a**), classes (**b**), order (**c**, **d**) in cucumber rhizosphere. Fungal phyla and classes with average relative abundances >10% in at least one treatment were shown. Fungal orders with average relative abundances >10% (**c**) and >0.1% (**d**) were shown in at least one treatment. M and R represent treatments of cucumber monocropping and rotations with Indian mustard. Values are expressed as mean±standard error. Asterisks indicate significant difference between treatments based on Student's *t* test ($P \leqq 0.05$)

groups were detected in both treatment soil samples (data not shown). In (R)-treatment, the relative abundance of *Humicola*, *Remersonia*, *Myrothecium*, *Scedosporium* and *Mycothermus* spp. was higher, but that of *Pseudallescheria*, *Mortierella*, *Chaetomium*, *Ilyonectria*, *Gibellulopsis* and *Metacordyceps* spp. was lower (Table 1).

Discussion

Soil fungal community, acting as pathogen, decomposers, and mutalists, play essential roles in many ecosystem processes such as energy flow, nutrient cycling and organic matter turnover (Philippot *et al.* 2013). The composition of fungal could be changed by soil environment (such as soil type, soil pH and soil carbon content) (King and Blesh 2018). Moreover, plants are able to shape their rhizosphere microbiome by releasing exudates containing various compounds (Berendsen *et al.* 2012).

Our results indicated that the structure of Indian mustard rotation and monocropped cucumber fungal community were distinct, which were consistent with the previous findings (Jin *et al.* 2019a), demonstrating that crop rotation changes the rhizosphere environment, thus altered the soil microbial communities composition and structure. Miseq. sequencing showed that the main phyla was *Ascomycota* across all soil samples, and Indian mustard rotation increased the abundance of *Ascomycota*. *Ascomycota*, a group of resident soil fungi, rely on the

decomposition of soil organic matter or plant root exudates, play an important role in maintaining soil microbial ecological balance (Wang *et al*. 2016). In this study, the dominant orders includes *Hypocreales*, *Mortierellales*, *Pezizales*, *Microascales* and *Sordariales* (average relative abundance >10%) and *Thelebolales*, *Sordariomycetes Incertae sedis*, *Eurotiales*, *Xylariales*, *Ascomycota Incertae sedis*, *Pleosporales* and unclassified *Ascomycota* (average relative abundance > 0.1%) of the phylum *Ascomycota*, which are considered to be primary straw residue decomposers (Hannula *et al*. 2012; Ma *et al*. 2013; Wang *et al*. 2017; Hu *et al*. 2018). Therefore, from these findings we speculate that crop rotation changed the composition and content of root exudates, which might be the reason of higher abundances of decomposer fungi.

It has been reported that after continuous monocropping of cucumber, phenolic compounds (such as *p*-coumaric acid) could accumulate in the soil, thus promoted the growth of pathogenic fungi (Zhou *et al.* 2018b). The reduction of pathogenic fungi in the soil and the increase in beneficial fungi may be associated with the degradation of phenolic acids. Venter *et al*. (2016) have shown that the increase in soil microbial diversity and abundance can be attributed to increase in crop diversity. These findings suggest that crop rotation can increase the abundance of root residues, resulting in the higher diversity of decomposers in rhizosphere. This phenomenon is caused by the selectivity of rotation for soil fungi. Therefore, we inferred that plant residues and root exudates could provide carbon source for soil microbes and thus changed the composition of soil microbial communities.

It is well understood that not all the fungi are plant pathogenic but some of them can promote plant growth by decomposing plant residues to provide nutrients to the plants (Ahmad *et al.* 2018). Compared with monocropped cucumber, rotation with Indian mustard increased abundance of *Humicola*, *Remersonia* and *Myrothecium* spp., but decreased abundance of *Mortierella*, *Chaetomium* and *Gibellulopsis* spp. ($P \leqq 0.05$). Previously,it has been reported that *Humicola*, *Remersonia* and *Myrothecium* are plant beneficial fungi, which can promote biogeochemical cycles and the absorption of nutrients and inhibit disease development. *Humicola*, a biocontrol fungi, reduced the disease incidence of pepper blight caused by *Phytophthora capsici* and black spot on leaf of cabbage caused by *Alternaria brassicicola* (Ko *et al.* 2011; Yang *et al.* 2014). Krishnan *et al.* (2017) reported that due to the ability to synthesize ligninolytic and cellulolytic enzymes, *Remersonia* can stimulate plant growth. Similarly, *Myrothecium* could produce some secondary metabolites (such as trichothecene macrolides) to inhibit plant pathogen (Liu *et al.* 2016).

Root exudates have been found that root exudates play important role in plant defense against soil-borne pathogens (Park *et al.* 2004). In our study, the relative abundance of *Ilyonectria*, *Gibellulopsis* and *Metacordyceps* spp. ($P = 0.05$) were decreased by rotation of Indian mustard which contains plant pathogens. The genera of *Ilyonectria* has capable of causing black foot rot of *Proteaceae* (Aiello *et al.* 2014). Kawaradani *et al.* (2013) reported that some species of *Gibellulopsis* could cause the seedling rot on chrysanthemum and lettuce. Meanwhile, some species in *Metacordyceps* are predominant genera in pesticide-contaminated agricultural soils (Merlin *et al.* 2014). Cucumber itself could selectively recruit microorganisms in the rhizosphere for its own benefit (Jia *et al*. 2019; Zhou *et al.* 2019). Therefore, we assume that rotation of Indian mustard inhibited the root colonization of soil-borne pathogen as compared to monoculture.

Conclusion

In this study we used Indian mustard as rotation crop with cucumber as main crop to study effects of crop rotation system on soil fungal community. Our results indicated that crop rotation affected the fungal composition and altered the dominant genera, increased the abundance of fungi with potential antifungal ability and decreased the harmful fungi. These results show that rotation with Indian mustard could be beneficial growth and development of cucumber which is an important crop in many parts of the world. Overall, our findings suggest that adopting crop rotation system with suitable crops could cure soil health by altering its microbial communities and alleviate soil sickness.

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