Bacterial community variations in paddy soils induced by application of veterinary antibiotics in plant-soil systems

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ABSTRACT

Soil bacterial communities have complex regulatory networks, which are mainly associated with soil fertility and ecological functions, and are likely to be disturbed due to antibiotics applications. The impact of antibiotics, particularly in mixtures form, on bacterial communities in different paddy soils is poorly understood. Using pyrosequencing techniques of 16 S rRNA genes, this study investigated the synergistic effects of veterinary antibiotics (sulfadiazine, sulfamethoxazole, trimethoprim, florfenicol, and clarithromycin) on bacterial communities in a soil-bacteria-plant system. Rice was grown under controlled greenhouse conditions where unplanted and planted treatments were doped with $200\,\mu g\,kg^{-1}$ of combined antibiotics over a period of 3 months. Bacterial richness remained unaltered, while a significant decline was observed in bacterial diversity due to antibiotics in the four paddy soils. *Bacteroidetes* and *Acidobacteria* were increased, while *Actinobacteria* and *Firmicutes* decreased under antibiotics exposure. Despite antibiotics perturbation, compositional variations were mainly attributed to the different paddy soils which harbor distinct bacterial communities. *Haliangium* and *Gaiella* were among the sensitive genera that were negatively correlated to antibiotics perturbation. Additionally, electrical conductivity, total organic carbon, and total nitrogen of soil solution were the key physiochemical indices which significantly influenced the structure of bacterial communities in the paddy soils. These findings expanded our knowledge of effects from synergistic antibiotics application and variations in bacterial communities among different paddy soils.

1. Introduction

Microbes have significant roles in regulating soil fertility, plant growth, and cycling of essential nutrients. Thousands of bacterial, eukaryotic and archaeal taxa can harbor in a single gram of soil which mirrors protein-encoded functions of their taxonomic diversity (Fierer et al., 2012). The soil has a diverse habitat of microbial community around the root zone, which is cooperatively shaped by plant species and soil type (Berg and Smalla, 2009). Microbes in soil converted essential nutrients into stable forms before absorbing by plants. In return, microbes received carbon metabolites from plants in the rhizosphere through root exudation (Schloss et al., 2009), thus makes a close association with each other for mutual benefits. Diversity and richness of bacterial community are closely related to soil systems, which are affected by soil nature, plant, and environmental factors such as temperature, moisture, etc. Despite massive diversity of soil bacterial communities, certain bacterial phylotypes co-occurred dominantly in different soils around the world (Delgado-Baquerizo et al., 2018). The distribution of topsoil microbes is mainly concerned with gradual climatic changes, such as temperature that drive them at continental scales (Garcia-Pichel et al., 2013). Similarly, soil fungal diversity and their community compositions are strongly affected by climatic factors, edaphic variability, and spatial pattern that represent the global key predictors (Tedersoo et al., 2014). Soil pH is also one of the main predictors that influenced bacterial communities. It explained how soil bacterial taxa differ at local, regional, and continental scales (Lauber et al., 2009). Similarly, other factors like nutrients availability and soil moisture also affected the bacterial community structures.

Besides environmental factors mentioned above, the agronomy practices, which greatly influence the soil ecology, needs particular concerns. In recent years, the introduction of veterinary antibiotics (VAs) into the environment is enormously increasing due to intensified livestock industries which facilitate the production, growth, and prevention of contagious diseases in animals. Annually, 100,000 to

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Table 1
Location and physiochemical properties of paddy soils.

Location (Abbrev)	Soil texture	SOM (g kg^{-1})	pH$_a$ [*]	pH$_b$ [**]	EC [**] (dS m^{-1})	TOC [**] (mg L^{-1})	TN [**] (mg L^{-1})
Panjin, Liaoning, Soil A	Loam	18.08	7.30	7.62	3.25	32.88	1.22
Qionghai, Hainan, Soil B	Loam	43.71	5.60	7.41	1.45	108.4	34.1
Hangzhou, Zhejiang, Soil C	Loam	29.89	6.10	7.55	1.69	88.41	18.1
Shanggao, Jiangxi, Soil D	Clay loam	24.36	6.40	7.57	1.13	49.15	8.32

SOM: Soil Organic Matter, EC: Electrical Conductivity, TOC: Total Organic Carbon, TN: Total Nitrogen.

[*] pH$_a$: pH determined from air dried soils.

[**] Parameters determined from soil solutions. pH$_b$: pH determined from soil solution.

200,000 t of antibiotics including human and animals have been used worldwide (Min and Tang, 2010). As the largest producer and consumer of the world, China used roughly 162,000 t of antibiotics where 84,240 t (making 52% of total usage) were consumed for livestock production (Zhang et al., 2015). Due to antibiotics residues in livestock products, 29,000–87,000 t of antibiotics residues are annually introduced into agriculture soil via animal feces or urine as manure fertilizer (Zhao et al., 2010). Antibiotics have been detected in the soils at a level of μg kg^{-1} from different regions of the world. For instance, Li et al. (2011) investigated tetracycline, sulfonamide, and quinolone from farmland vegetable soil of China with detection of 44.1, 23.5, and 61.9 μg kg^{-1}, respectively. Similarly, Xie et al. (2012) detected ciprofloxacin, enrofloxacin, and norfloxacin from 0.10 to 288 μg kg^{-1} in the soil of north China. Antibiotics are mainly broad-spectrum drugs which are incompletely absorbed in animals, thus extensively excreted (30–90%) without degradation (Sarmah et al., 2006) and potentially cause selective impacts on different groups of soil microbes. Generally, identification of soil microbial populations has been determined by density gradient gel electrophoresis (DGGE), phospholipid fatty acid analysis (PLFA) (Reichel et al., 2013), and terminal restriction fragment length polymorphism (Islas-Espinoza et al., 2012). Nevertheless, these techniques are quite expensive, time consuming, and the information provided for community structures are limited. High-throughput sequencing of 16 S rRNA genes has been used as a powerful tool for identifying diverse groups of root-associated microbial communities in different plants (Edwards et al., 2015; Peiffer et al., 2013). In recent years, the structure of microbial communities was reported to be changed due to antibiotics exposure using high-throughput sequencing techniques (Lin et al., 2016). Generally, antibiotics are broad-spectrum drugs, which demonstrated selective effects on different microbes that relatively altered their abundance (Ding and He, 2010). Thus, there is a concern that the introduction of antibiotics might change the structure and function of soil microbial communities in the long run.

Rice is the most essential cereal crop and staple agricultural commodity which are widely consumed in Asia (Boonsaner and Hawker, 2012). In paddy soils where rice grows under anaerobic conditions, several reductive processes such as methane production, denitrification, and iron reduction occur in the presence of essential microbes (Chen et al., 2014). In China, antibiotics are detected in paddy soils due to excessive application of manure. Tang et al. (2015) reported the level of antibiotics up to 344 μg kg^{-1} from the long-term consequences of manure application in paddy soils. Although, antibiotics have diverse effects on soil microbial communities and their relations with different soil properties make it more pivotal, classification of soil microbial composition and variation induced by antibiotics have been poorly investigated in rice fields. Moreover, multi-antibiotic effects, often detected in the soil environment, have not received enough consideration so far. Therefore, using 16 S rRNA gene amplicon sequencing, effects of the synergistic antibiotics including sulfadiazine (SDZ), sulfamethoxazole (SMZ), trimethoprim (TRM), florfenicol (FLR), and clarithromycin (CLR) on bacterial communities were assessed in four paddy soils under controlled greenhouse conditions. Correlations between soil physicochemical properties and selected antibiotics with the abundance of bacterial taxa were evaluated. The aim of this study was to provide inclusive information of the response of microbes to antibiotics perturbation in paddy soils collected from four regions of China having different edaphic characteristics.

2. Materials and methods

2.1. Paddy soils collection and experimental design

Soil samples were collected from Panjin, Liaoning (Northeast), Qionghai, Hainan (South), Hangzhou, Zhejiang (East), and Shanggao, Jiangxi (Southeast) paddy fields of China (Fig. S1). Paddy soils were collected from distinct provinces to span a broad range of ecologically diverse paddy soils. The analysis and classification of soils texture are given in Table S1 in Supporting Information (SI). Paddy soils were collected from the plow layer (0–20 cm) of arable lands and were packed immediately after collection in plastic bags. All soil samples were then transported to the greenhouse of Dalian University of Technology. Soils were air-dried for several days at room temperature in shade and sieved through 2 mm screen to remove all debris. After sieving, soils were mixed thoroughly and separated in experimental pots (19 cm × 27 cm each) containing 3 kg of soil per pot. The physiochemical properties of soils are given in Table 1. Soils pH and electrical conductivity (dS m^{-1}) were determined from the soil solutions with WTW Water Quality Analyzer (Multi 4320 SETG, Germany). Similarly, total organic carbon (TOC, mg L^{-1}), and total nitrogen (TN, mg L^{-1}) were also determined from the soil solutions by TOC analyzer (Multi-NlC-2100S), Jena, Germany. Soil organic matter (SOM, mg kg^{-1}) from the soils was determined by $K_2Cr_2O_7$ oxidation and $FeSO_4$ titration method of Walkley and Black, (1934). Aqua regia digestion and ICP-OES (PE Optima 2000 DV) was adopted (Hua et al., 2014) for the determination of Al, Ca, Fe, Mg, Mn, and Na in 4 paddy soils. The detailed methods followed for the analysis of these parameters are available in SI. The rice incubation experiment was conducted in the mid of June 2017 in an artificial greenhouse of Dalian University of Technology, and plants were harvested at the end of September 2017. An average temperature and humidity were kept at 25 ± 2 °C and 50–65% during the entire period. A total of 48 experimental pots were prepared in which each paddy soil was divided into 4 treatments for each soil: unplanted and planted pot (control), unplanted and planted pot (doped with 200 μg kg^{-1} of combined antibiotics). Five VAs, including SDZ, SMZ, TRM, FLR, and CLR were added to antibiotic treatment pots in combined form. An aqueous solution of combined antibiotics was added and mixed evenly with soils as the antibiotics treatments. All pots were incubated for 3 days before rice transplantation. Mature rice seedlings were purchased from plant grower nursery. Each seedling was separated carefully and ten plants were transferred to each pot with an equal distance from each other. Water was given on a daily base to maintain a level of 3–4 cm above the soil surface during the entire period. For the collection of soil solutions, Rhizon samplers (Rhizosphere Research Product, Wageningen, the Netherlands) were installed in each pot (Fig. S2). Approximately 10 mL of soil solution was collected through Rhizon sampler by using a disposable syringe for antibiotics and other analysis.