Applied Soil Ecology 93 (2015) 78–87

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Identification of benzoic acid and 3-phenylpropanoic acid in tobacco root exudates and their role in the growth of rhizosphere microorganisms

Yanxia Liu, Xiang Li *, Kai Cai, Liuti Cai, Ning Lu, Junxiong Shi

Guizhou Academy of Tobacco Science, North Yuntan Road, Guanshanhu District, Guiyang, Guizhou Province 550000, China

ARTICLE INFO

Article history:
Received 21 August 2014
Received in revised form 7 April 2015
Accepted 12 April 2015
Available online xxx

Keywords:
Tobacco root exudates
Pathogen
Antagonist
Rhizosphere microorganism
Soil microbial functional diversity

ABSTRACT

Phenolic compounds are secondary metabolites often implicated in plant-microorganism interactions. However, how these compounds would affect the growth of both pathogen and antagonists is not yet fully understood. In this study, the two main phenolic acids (benzoic acid and 3-phenylpropanoic acid) were screened and identified by ultra-performance liquid chromatography-quadrupole-time of flight-mass spectrometry (UPLC-Q-TOF-MS), and their contents in tobacco root exudates were evaluated. Furthermore, their effects on the biomass and activity of rhizosphere microorganisms, especially the bacterial wilt pathogen *Ralstonia solanacearum* and its antagonist *Brevibacillus brevis* in both liquid culture and soil were investigated. The results showed that the concentrations of benzoic acid and 3-phenylpropanoic acid were 0.25 $\mu g\,g^{-1}$ and 1.15 $\mu g\,g^{-1}$ dry roots, respectively. Both of them could promote the growth of the pathogen and antagonist at low concentration (benzoic acid $\leq 2\ \mu g\,L^{-1}$ and 3-phenylpropanoic acid $\leq 3\ \mu g\,L^{-1}$), while at high concentration, the growth of bacteria was inhibited. The minimum suppressed concentrations of these phenolic acids for *R. solanacearum* were higher than that for the antagonist. The growth of pathogen and antagonist together with the rhizo-microbial functional diversity significantly reduced by adding 4 $\mu g\,kg^{-1}$ benzoic acid or 8 $\mu g\,kg^{-1}$ 3-phenylpropanoic acid in the soil. In conclusion, pathogen adapted better to the accumulation of tobacco root exudates than antagonist, which might be the cause of tobacco bacterial wilt outbreak in mono-cropping system.

1. Introduction

Economic crops growth and development are sensitive to soil-borne diseases causing reduction in crop yield and quality, in a continuous mono-cropping system ever and again (Hao et al., 2010a). Continuous mono-cropping on tobacco production is very prevalent in Guizhou province, southwestern China, which results in serious continuous cropping obstacle of the crop. In the continuous mono-cropping system, tobacco is generally suffered from plant auto-toxicity due to the accumulation of the root exudates (Guo, 2006). The root exudates secreted from the plant root system during the growth age (Chou, 2010) have a significant influence on the soil properties, microbial communities and soil functions (Haichar et al., 2014; Shi et al., 2011). Recently, researches on root exudates mainly focused on rice (Hao et al., 2010a), watermelon (Ling et al., 2011; Wu et al., 2008) and cucumber (Chen et al., 2011; Zhang et al., 2013) root exudates etc. Research on tobacco root exudates usually focused on its effect on tobacco seeds germination, plant growth and enzyme activities (Guo, 2006). However, little information is available on the types and contents of tobacco root exudates, and their influence on rhizosphere microorganisms, especially on pathogen of soil-borne disease and its antagonists.

Phenol and phenolic acids from the root exudates have been identified in tobacco and cucumber plants (Gao et al., 2012; Hu et al., 2007). Previous research showed that many phenolic acids including scopoletin, coumarin, *p*-hydroxybenzoic acid, vanillic acid, 3-hydroxyhydrocinnamic acid, benzoic acid, phenylacetic acid, and hydrocinnamic acid were considered as plant growth inhibitors (Bertin et al., 2003; Inderjit Seastedt et al., 2008; Lannucci et al., 2013). Very recently, effect of phenolic acids on soil microbial biomass, activity, and diversity was studied (Qu and Wang, 2008; Tan et al., 2013; Zhou and Wu, 2012).

Root may secrete exudates containing auto-toxic phenol and phenolic acids in response to attacks by pathogens (Ma et al., 2005). Chen et al. (2011) identified that phenolic acids, including 4-hydroxybenzoic acid, vanillic acid, ferulic acid, benzoic acid, 3-phenylpropionic acid and cinnamic acid, were the major

* Corresponding author. Tel.: +86 851 84117106; fax: +86 851 84116909.
E-mail address: iversonlyx@sina.com (X. Li).

http://dx.doi.org/10.1016/j.apsoil.2015.04.009
0929-1393/

components in cucumber root exudates in mono-cropping system. In addition, the soil-borne pathogen *Fusarium oxysporum* f. sp. *cucumerinum* increased during continuous monoculture as a result the *Fusarium* wilt of cucumbers occurred. Similar phenomenon was observed on tobacco, that its root exudates could possibly promote the biomass and activity of *Ralstonia solanacearum* in rhizosphere soil, leading to the burst of tobacco bacterial wilt (Haichar et al., 2014). According to Ke (2009), in the tobacco successive cropping system, root exudates potential contained autotoxins, which might attribute to the increase of pathogenic bacteria. The rhizosphere micro-environment of tobacco was deteriorated, resulting in hindering the metabolic activity of root system and the efficiency of nutrient utilization. Eventually, the growth of tobacco was inhibited. Pathogens increased while beneficial microbes decreased as the root exudates were produced, which led to an imbalanced soil microbial environment and the burst of disease (Han et al., 2006; Ruan et al., 2003).

Soil microbial community is thought to be responsible for biological processes that is necessary for maintaining a healthy soil and suppressing plant diseases (Garbeva et al., 2004; Raaijmakers et al., 2009). It has been shown that a decrease in soil microbial diversity was responsible for the development of soil-borne plant diseases (Mazzola, 2004). Root exudates could not only impact the microbial biodiversity and abundance of rhizosphere soil (Nayyar et al., 2009) but also manipulate biological and physical interactions between roots and soil microorganisms (Bais et al., 2006; Broeckling et al., 2008; Gundel et al., 2014). The indirect disadvantage of the accumulation of the root exudates is attributed to their ability to disrupt the microbial community balance by modifying the community population and structure in rhizosphere and promoting growth and virulence of pathogen (Kong et al., 2008; Nayyar et al., 2009; Wu and Wang, 2006). The mechanisms involved, however, are not totally understood yet and subjects of ongoing research. Therefore, considerable interest was paid on monitoring the changes of soil microbial communities after the application of the phenolic compounds identified in the continuous mono-cropping system.

The purpose of this study was to decipher and quantify the main types of tobacco root exudates by ultra-performance liquid chromatography-quadrupole-time of flight-mass spectrometry (UPLC-Q-TOF-MS), and to monitor variation of rhizosphere microbial community in tobacco continuous cropping soils after tobacco root exudates application through real-time PCR and Biolog EcoPlate System in a pot experiment. The objectives of this research were (1) to evaluate whether the developed application way accord with its ecological requirements, (2) to detect the dynamic change of *R. solanacearum* and its antagonist and (3) to determine the impact of tobacco root exudates on diversity of soil microbial communities in the continuous mono-culture system. We tried to provide novel evidence to explain bacterial wilt occurrence in tobacco mono-cropping system and to propose a new strategy to overcome tobacco bacterial wilt.

2. Materials and methods

2.1. Screening and identification of *R. solanacearum* and its potential antagonistic strain

R. solanacearum was isolated on SMSA medium (Englerbrecht, 1994), which consists of 10 g bacto peptone (Difco), 5 mL glycerol, 1 g casamino acid (Difco), 15 g bacto agar (Difco) and 1 L distilled water and was supplemented with 100 $\mu g\,mL^{-1}$ polymyxin B sulphate (Sigma), 5 $\mu g\,mL^{-1}$ crystal violet, 50 $\mu g\,mL^{-1}$ tetrazolium salt (Sigma), 25 $\mu g\,mL^{-1}$ bacitracin (Sigma), 0.5 $\mu g\,mL^{-1}$ penicillin (Sigma), 5 $\mu g\,mL^{-1}$ chloramphenicol (Sigma) and 100 $\mu g\,mL^{-1}$ cycloheximide (Sigma). The purified isolations were identified by 16S rDNA identification and re-inoculated to tobacco plants to assess the pathogenicity of *R. solanacearum* based on Koch's postulates (Koch, 1893). Roots and rhizosphere soils of healthy tobacco plants from a severely wilt-diseased field were collected. There were totally five locations with one soil sample and one plant sample per location in the same filed using five-point sampling method (Groves et al., 2010). Some properties of the soil in the filed were: pH 5.53, 28.2 $g\,kg^{-1}$ of organic carbon, 2.3 $g\,kg^{-1}$ of total nitrogen, 48.9 $mg\,kg^{-1}$ of available phosphorus, 543 $mg\,kg^{-1}$ available potassium. Soil was applied with 750 kg of chemical fertilizer per hectare with $N/P_2O_5/K_2O$ ratio of 10:10:25. Tobacco plants were uprooted delicately from the trays and shaken gently to remove all but the most tightly adhering rhizosphere soil. The roots were placed into 10 mL of sterile water and sonicated for 15 min to obtain rhizosphere soil (Carlsen et al., 2012). The isolation and purification of antagonists was according to the *in vitro* assay described by Liu et al. (2013). The most potential antagonistic strain was selected for their strong and consistent inhibitory effect of *R. solanacearum* (Fig. 1). The inhibitory effect of the selected strains against *R. solanacearum* was checked by spot-inoculating single and representative colonies on agar media at four locations 2 cm from the center (Opelt and Gerg, 2004). Twelve hours later, cell suspensions of the pathogenic organisms were sprayed evenly over the plates. This procedure was repeated for five generations. The stain with constant suppression to *R. solanacearum* was chosen and stored for the following

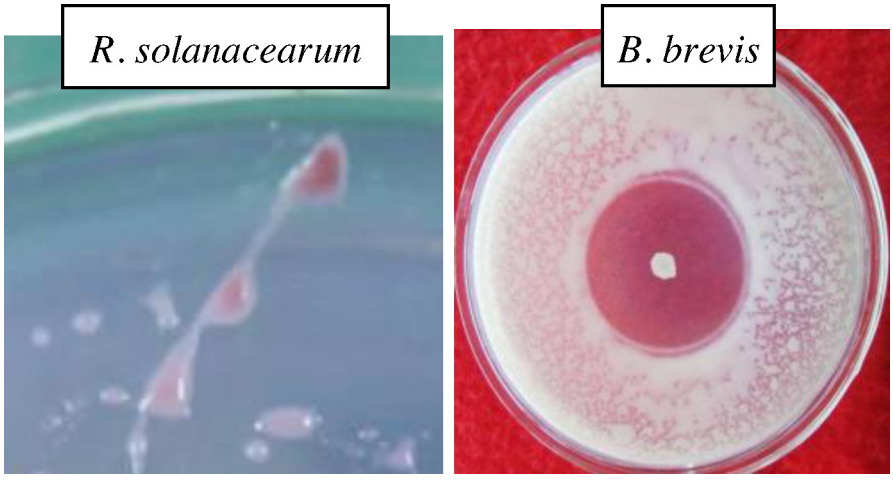

Fig. 1. The colony morphology of *R. solanacearum* (left) and antagonistic activity tested by co-culturing *R. solanacearum* and antagonistic strain *B. brevis* (right).