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Biodegradation of fungicide Tebuconazole by *Serratia marcescens* strain B1 and its application in bioremediation of contaminated soil

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ABSTRACT

The degradation characteristics of Tebuconazole degrading bacterium *Serratia marcescens* strain B1 in both contaminated soil and culture were investigated in this study. In the liquid mineral salt medium, the optimal temperature, pH value, and inoculation (v/v) for degradation by strain B1 were 30 °C, 7.0, and 4.0%, respectively. When the initial concentration of Tebuconazole was 200 mg L^{-1}, the degradation rate of Tebuconazole was 94.05% in 8 h. As the initial concentrations of Tebuconazole were higher than 300 mg L^{-1}, the biodegradation rates declined as the Tebuconazole concentrations raised. As the concentration was 500 mg L^{-1}, the Tebuconazole was degraded at a rate of only 64.11%. Degradation fit well with the kinetic equation of pesticide degradation as the initial concentrations of Tebuconazole were between 50 and 500 mg L^{-1}. When strain B1 (cultured at 3×10^{14} CFU mL^{-1}) was mixed with the soil contaminated with tebuconzole (200 mg L^{-1} and the concentration of strain B1 of 3×10^{7} CFU g^{-1} dry soil), the Tebuconazole was degraded at a rate of 96.46% in 30 days, while the control soil (with the absence of strain B1) achieved a degradation rate of only 70.42%. These results demonstrate that in contaminated soil, the strain B1 can substantially increase the degradation rate of Tebuconazole. Results of the greenhouse and field experiments indicate that the strain B1 can remove the residues of Tebuconazole in contaminated soil and Chinese cabbage.

1. Introduction

Triazole fungicides are a rapidly growing class of pesticides and are broadly used in the world due to their high efficiency and low toxicity (Angioni et al., 2003; Fenner et al., 2013). These fungicides, such as Tebuconazole, propiconazole, difenoconazole, and flutriafol, could effectively control many plant diseases caused by various basidiomycetes, ascomycetes, and semipermeafilans (Tian et al., 2016). These fungicides also have protective and therapeutic effects that inhibit the synthesis of ergosterol in fungal cell membrane and inhibit the growth of fungi by increasing the permeability of the cell membrane (Song and Nes, 2007; Dong et al., 2016; Zhou et al., 2016).

The most important feature of the triazole fungicides making these pesticides distinguished from other fungicides is that they are capable of significantly regulating plant growth and thus effectively relieving plant stress (Fletcher et al., 2000; Cheruth Abdul Jallel et al., 2008; Kishorekumar et al., 2008; Zhang et al., 2011). The long-term use of these fungicides has caused many problems in many areas. Studies have shown that triazole fungicides interfere the normal functions of the endocrine hormones in both human and other animals. For example, epoxiconazole is very difficult to degrade, thus not only highly toxic to aquatic organisms but also inducing hormone-related cancers in human (Lopes et al., 2010); propiconazole, fluconazole, and Tebuconazole interfere the secretion of male hormones (Kjaerstad et al., 2008; Sancho et al., 2010); myclobutanil, Tebuconazole, difenoconazole, propiconazole, triadimefon, and triadimenol damage the liver in rat and, to some extent, affect the normal development of rats (Goetz et al., 2007, 2009). Both in vivo and in vitro experiments in animals have shown that triazole fungicides have embryonic toxicity and interfere the normal reproductive function of both male and female rats, causing the degeneration in rat placenta (Moreno et al., 2013). A recent study has shown that triazole fungicides inhibit the secretion of progesterone JEG-3 in trophoblast cancer cells in human placenta (Rieke et al., 2014). The residues of the triazole fungicides can stay in the soil for a long term because of their high photochemical stability and low biodegradability (Strickland et al., 2004; EFSA, 2014; Li et al., 2015).

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Diclofenac has a strong affinity with the organic matters in soil, thus degrades slowly in the soil (Schmidt et al., 1993). To date, increasing attention has been paid to the problems caused by the triazole fungicides.

Tezconazole [named as (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol in IUPAC and as (±)-a-[2-(4-chlorophenyl)ethyl]-a-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol in Chemical Abstracts] is a type of triazole with low toxicity, high efficiency, and broad spectrum. This fungicide functions mainly by inhibiting the demethylation of sterols in pathogenic fungi, resulting in the inhibition of the formation of biofilm to kill the fungi. Due to its low dosage, strong absorption, and notable biological activities, Tebuconazole has been used by direct spray and treatment of seeds to treat and prevent many plant diseases such as rust, wheat scab, gray mold, wheat sheath blight, broad bean blight, apple rot disease, and cotton wilt (Chen et al., 2005; D'Angelo et al., 2014; Keinath, 2015; Zhang et al., 2015).

As one of the highly effective triazole fungicides, Tebuconazole has attracted growing attention and has been applied increasingly widely in the world due to its role in the protection of crops. Consequently, humans now have rising opportunities to contact this fungicide. Therefore, it is imperative to not only investigate the protective efficacy of this fungicide but also evaluate the harm to human health caused by Tebuconazole. Tebuconazole is absorbed not only through the gastrointestinal and respiratory tracts but also through intact skin (Goetz et al., 2007), causing pathological changes in organs (e.g., liver and kidney; Pennati et al., 2006). Furthermore, studies on the chronic toxicity of Tebuconazole have shown that Tebuconazole has an accumulative toxicity effect in animals and is more likely to cause potentially chronic poisoning due to its long persistent effect (Yang et al., 2010). Due to the long-term use of this fungicide, the resulting water and soil pollution have severely harmed the ecological equilibrium and endangered human and animal health (Sancho et al., 2010; Montuelle et al., 2010; Papadopoulou et al., 2016). Consequently, it is now urgent to have effective ways to prevent, treat, and control these pollution caused by Tebuconazole.

Studies show that microbes generally play a significantly essential role in the degradation of many categories of pesticides (Cao et al., 2013; Cai et al., 2014; Cheng et al., 2017; Rajamanickam et al., 2017). Microbes have abundant biological, physiological, and genetic diversities and can degrade pesticides by using various types of enzymes (Zhang et al., 2010, 2012; Pino et al., 2011; Li et al., 2013; Ramu and Seetharaman, 2014). The microbial degradation method does not cause secondary pollution because the microorganisms used to degrade the pesticides are derived directly from contaminated soil. This technology has been applied in many regions because the efficiency of microbial degradation is significantly better than the degradation of pesticides under natural environmental conditions and the operation is simple and convenient. To date, many microbes capable of degrading or converting pesticides have been isolated and have been recognized as one of the prominent methods of controlling environmental pollution and is showing great prospects (Yong and Zhong, 2010; Maya et al., 2011; Mori et al., 2011l; Thangadurai and Suresh, 2014; Zhang et al., 2014, 2016; Khodaei et al., 2017).

Studies have demonstrated that many species of bacteria, fungi, actinomycetes, and algae have the ability to degrade and transform triazole fungicides (Zheng et al., 2009; Chen et al., 2010; Sarkar et al., 2010; Pinto et al., 2012). These microbes degrade mainly propiconazole and other fungicides, while studies on the degradation and bioremediation of Tebuconazole are sparse. With the goal of effectively controlling the contamination caused by Tebuconazole, we isolated a strain of Tebuconazole degrading bacterium *Serratia marcescens* B1 by enrichment from the soil contaminated by Tebuconazole. The strain B1 is evaluated as non-toxic and kept at the China Center for Type Culture Collection (CCTCC, Wuhan, China). The strain B1 is further studied to characterize its optimal temperature, pH, and initial inoculation concentrations for its degradation. We also evaluate the bioremediation effect of strain B1 on contaminated soil to provide the theoretical foundation for the bioremediation of the contaminated soil by using *Serratia marcescens* strain B1 in practice. The long-term scientific question that we ask is how we can eliminate the residues of Tebuconazole in the polluted environments in order to obtain the bioremediation of contaminated ecosystems.

2. Materials and methods

2.1. Medium and chemical reagents

Tebuconazole had a purity of 95% (the National Reference Material Center, China). All other chemicals including acetonitrile (with a chromatographic purity), methanol, petroleum ether, dichloromethane, and ethyl acetate, were of analytical grade. Luriae-Bertani (LB) liquid medium was generated by dissolving 5 g yeast extract, 10 g NaCl, and 10 g peptone in 1000 mL distilled water, and adjusted to pH 7.0. Mineral salt medium (MSM) contained 0.3 g $MgSO_4{\cdot}7H_2O$, 1.5 g K_2HPO_4, 1 g NH_4NO_3, 1 g KH_2PO_4, 25 mg $CaCl_2{\cdot}2H_2O$, and 8 mg $Fe_2(SO4)_3$, resolved in the final of 1000 mL distilled water (pH 7.0).

2.2. Collection and preparation of soil samples

The soil samples were gathered from the location where the Tebuconazole was never applied (Agricultural Experimental Station, Jilin Agricultural University). The features of the soil samples (identified as meadow chernozemic soil) were as previously reported (g kg^{-1} dry weight; Zhang et al., 2016)): organic matter 28, total phosphorus 1.03, total nitrogen 1.57, and pH 6.4. Top layer of the soil samples (up to 15 cm) was dried at room temperature, sieved (2 mm), and kept in dark (~ 4 °C) in the sealed polyethylene bags (Zhang et al., 2016).

2.3. Preparation of bacterial inoculum

The Tebuconazole degrading bacterium strain B1 was screened and isolated from Tebuconazole contaminated soil (Zhang et al., 2016) and kept at the Jilin Agricultural University. Basic Local Alignment Search Tool (BLAST) at National Center for Biotechnology Information (NCBI) was used to carry out the homology analysis of 16S rRNA sequence of strain B1 (Genbank accession number KX827591).

The bacterial suspension of strain B1 was cultured in 100 mL LB liquid medium in a flask (250-mL) with Tebuconazole (200 mg L^{-1}) and stored and shaked in dark (30 °C and 150 rpm). The bacterial culture was then incubated at 4 °C and centrifuged for 5 min (12,000 rpm) when the bacterial growth reached its logarithmic phase. Bacteria were washed twice using phosphate buffer (50 mM and pH 7.0) and a spectrophotometer (Shimadzu UV-2410) was used to measure the optical density (OD_{600}) of the bacterial suspension for the following experiments. The values of OD600 were used to estimate the concentration of bacterial population. Simply, bacterial growth is positively correlated with the values of OD600, the higher concentrations the bacterial population are, the higher the values of CD600 are. All experiments in our study were repeated thrice and the experimental controls did not contain the strain B1.

2.4. Measurement of degradation rate of Tebuconazole and the growth rate of strain B1

Diluted bacteria (1:25) were planted into a MSM (100 mL) with Tebuconazole (200 mg L^{-1}) and then incubated and shaked at 30 °C and 150 rpm. The degradation rates of Tebuconazole and growth of bacteria were indicated by the concentrations of Tebuconazole and OD_{600} after culturing for 1, 2, 3, 4, 5, 6, 7, and 8 days, respectively.