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Changes in tetracycline partitioning and bacteria/phage-comediated ARGs in microplastic-contaminated greenhouse soil facilitated by sophorolipid

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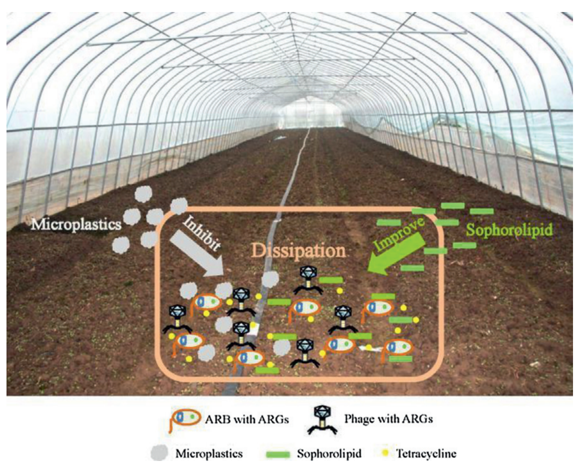
HIGHLIGHTS

- Both bacteria and bacteriophages are important source of ARGs in soil.
- Existence of microplastic inhibited the dissipation of soil antibiotics and ARGs.
- Sophorolipid clearly prompted the dissipation of soil antibiotics and ARGs.
- Sophorolipid outweighed microplastic in impacting soil antibiotic/ARGs attenuation.
- Water soluble TC best positively correlated to ARGs level in bacteria and phages.

GRAPHICAL ABSTRACT



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ABSTRACT

The emerging mixed contamination of antibiotics and microplastics in greenhouse soil has made the control of antibiotic resistant gene (ARG) transmission a novel challenge. In this work, surfactant sophorolipid was applied to enhance the dissipation of tetracycline (TC) and *tet* genes in the presence of microplastics in greenhouse soil. During 49 days of incubation, soil bacteria and phages were both found to be the crucial reservoirs of ARGs. Meanwhile, microplastic's presence significantly inhibited the dissipation of TC and ARGs in the soil. However, sophorolipid application was proved to outweigh the negative impact caused by microplastic existence, and lead to the highest dissipation of soil TC and ARGs. Significant positive correlation was detected between the dissipation rate of water-soluble and exchangeable TC content and bacteria/phage co-mediated ARG levels. This also held true between the two fractions of soil TC and the ratio of ARG level in the bacteria to that in the phages (B_{ARGs}/P_{ARGs}). The opposite impacts of microplastic presence and sophorolipid amendment on the TC/ARG

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dissipation found in this work provides new information for understanding ARG transmission between bacteria and phages in the mixed contaminated greenhouse soil.

1. Introduction

To increase vegetable production and farming profit, an ever-increasing amount of closed greenhouse facilities have been erected [1–3], and various measures such as the organic fertilizers and plastic film were extensively used [4–6]. Despite their positive roles in maintaining soil fertility and increasing greenhouse production [7,8], the negative impact they generate on the soil has become a novel threat against environmental safety and human health.

As is well known, manure-originated fertilizer is a fairly perfect reservoir for antibiotics, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs), and its extensive usage has resulted in an increase of these mixed contaminants in greenhouse soil [6–10]. On the other hand, plastic film has been applied to maintain the humidity and temperature in greenhouse facilities, which promotes the vegetable growth in lower temperature season [4,6]. However, after harvest, the residual plastic film is commonly abandoned in the soil without any particular treatment [1,5,7]. Then after a long-term aging process, the plastic film generally degrades into microplastics (with a diameter <5 mm), and accumulates in the closed greenhouse soil as a consequence, becoming frequently-detected pollutants in greenhouse soil [1,2]. Therefore, the simultaneous application of organic fertilizer and plastic film has resulted in the mixed contamination of antibiotics, ARB, ARGs, and microplastics [1,3,9,10]. The interaction between these pollutants has become a crucial problem that remains uninvestigated.

In addition, the ARG dissemination among the mixed contaminants is especially concerning due to the potential threat to human health. Former research found that organic chemicals were able to enhance the abundance of ARGs in soil, and stimulate ARG transmission among bacteria [3]. Auta et al. reported that microplastics could alter the composition of microbial communities [11], however, whether their existence could impact the transmission of ARGs among microorganisms in the soil was still unclear.

It is widely accepted that ARG transmission in the environment is mainly achieved by conjugation among indigenous bacteria [12]. However, more evidence has shown that bacteriophages (phages) transduction might be an even more crucial way for ARG transmission [13–15]. As the most abundant biological entity on the earth, phages could well reach a population of 10^{31}, which is approximately ten-fold higher than that of any bacteria [16,17]. For both virulent and temperate phages, after completing the infection, reproduction, and lysis cycles, it is very likely for the phages to acquire partial gene fragments from their specific hosts, bacteria, or integrate their own gene fragments into the plasmids or chromosomes of the host bacteria [18–20]. Therefore, the frequency of horizontal transfer of ARGs between ARB and antibiotic sensitive bacteria, and among various bacteria species is much higher than has been thought previously.

Therefore, it has been hypothesized that the residual microplastics in antibiotic/ARG-contaminated greenhouse soil is likely to significantly inhibit the dissipation of ARGs harbored in the indigenous bacteria/phages. As a consequence, action needs to be taken to stimulate the TC/ARG dissipation with the presence of microplastic in the soil. Because of the capacity to improve the bioaccessibility of organic pollutants, including polyaromatic hydrocarbons and antibiotics in soils [24,25], environmentally-friendly biofactant was found to exert positive impact on organic pollutants' dissipation [21–23]. However, little is known about its interaction with microplstics. Moreover, whether its application could outweigh the negative impact caused by microplastic residue, and increase the dissipation of antibiotic/ARGs in the soil remains investigated.

To the best of our knowledge, this is the first study applying biosurfactant sophorolipids to stimulate the dissipation of ARG levels co-mediated by bacteria and phages in antibiotic-microplastic mixed contaminated soil. The objectives of this work included, i) determining the impact of microplastics on antibiotic dissipation and ARG transmission between bacteria and phages; ii) understanding sophorolopid's positive role in stimulating the dissipation of mixed contaminants; and iii) providing new information for controlling ARG dissemination in mixed contaminated greenhouse soil. Our pilot work proposed an applicable way to mitigate the transmission of ARGs in a closed vegetable greenhouse soil system.

2. Materials and methods

2.1. Test soil and microcosm design

Soils were sampled in November 2016 at Guli fruit and vegetable planting base in Nanjing, Eastern China (31°88′17′ N, 118°67′29′ E). It has a 20-year history of cultivating various greenhouse vegetables with polyolefin film (thickness between 0.08 and 0.10 mm), leading to the frequent detection of microplastics in the soil. The amount of microplastics in the greenhouse soil in this area was between 0.05% and 1.00% *w/w*. And the amount of microplastics in the soil used in this work was approximately 0.1% (*w/w*; among which fiber, transparent and small microplastics <1 mm were the most abundant type). In addition, our preliminary investigation found this area a typical tetracycline (TC) contaminated greenhouse soil due to the repetitive manure application to the soil to stimulate vegetable growth [1,2] (twice a year, 1500 kg ha^{-1} livestock-manure-originated organic fertilizer each time). This also resulted in the *tet* gene (*tet*C, *tet*E, *tet*G, *tet*M, *tet*O, and *tet*X) accumulation in the soil (Fig. S1).

Original TC and *tet* genes mixed contaminated soil samples were collected in the closed vegetable greenhouse described above (at the depth of 0–15 cm). After air drying, samples were grounded through 2-mm sieve, and mixed thoroughly for the subsequent microcosm trials. Detailed information about the physicochemical properties of the soil is presented in Table S1. TC detected in the original soil was $341.2 \pm 16.6\ \mu g\ kg^{-1}$.

For separately exploring the effect of microplastic and sophorolipid on the dissipation of soil antibiotics and ARGs, four treatments were set in this study, including, CK: original TC and *tet* genes mixed contaminated soil; M: CK + 0.5% (*w/w*) microplastics.; S: CK + 0.5% (*w/w*) sophorolipid; MS: CK + 0.5% (*w/w*) microplastics + 0.5% (*w/w*) sophorolipid. For each treatment, 2.0 kg soil was used, and was set triplicate. Throughout the 49 days of incubation, each treatment was maintained 70% maximum water capacity with the temperature of the greenhouse at 28 ± 2 °C. Fifty grams of soil samples were collected at the interval of 7 days from each treatment, and were placed in sealed plastic bags at 4 °C for subsequent analysis. Considering the original microplastic content of the soil was fairly low, extra micrplastic (0.5%, *w/w*) was added to simulate the impact of the external microplastic input on the antibiotic