



# Synthesis, antibacterial evaluation, and safety assessment of Se@PLA as a potent bactericide against *Xanthomonas oryzae* pv. *oryzae*

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## ABSTRACT

A novel biodegradable material, Se@PLA, was designed and prepared *via* the selenization reaction of polylactic acid using NaHSe as the selenization reagent. This material shows excellent antibacterial activity ( $EC_{50} = 13.38 \mu\text{g/mL}$ ) against *Xanthomonas oryzae* pv. *oryzae*, which is a highly destructive pathogen responsible for rice bacterial blight. Se@PLA induces oxidative stress in bacteria, leading to the rupture of bacterial cell membranes and eventual death. Moreover, Se@PLA can significantly inhibit the motility of bacteria and is low toxic to soil and aquatic organisms. This work provides an effective method for preventing and controlling rice bacterial blight, and reveals the great potential of using Se@PLA as an alternative next generation plant bactericide.

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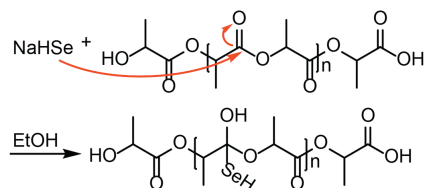
The chemistry of chalcogen elements have shown promising applications [1–3]. In the field, selenium-containing compounds and materials attract much attention due to their unique chemical properties [4–8]. Selenium possesses a large atomic radius and it easily undergoes oxidation due to its loosely bound valence electrons. However, the weak  $\pi$  bond of Se=O makes it easy to be reduced [9]. Selenium acts as a “cleanser” in the body through the above reversible redox reactions, reducing the production of reactive oxygen species, which is the culprit of certain chronic diseases. It is also an oxygen-carrier to enhance the oxidative stress in bacteria, leading to the rupture of bacterial cell membranes and eventual death. Therefore, selenium-containing chemicals are widely employed in agricultural and medicinal chemistry [9–17]. In the field of pesticide research and development, selenium-containing alternatives are highly concerned [16–20]. As an essential trace element, selenium can participate in metabolic processes in the form of selenoproteins [11], offering higher safety compared to other heavy metals [21]. With abundant selenium resources in China [22], selenium chemicals can be easily synthesized with relatively low cost under mild conditions. Previous studies have demonstrated that the application of selenium chemicals in agricultural production significantly increases the selenium content in products. For instance, selenized glucose is a soluble material being

synthesized *via* the selenization reaction of glucose with NaHSe [23], and it has been employed as feed additive and fertilizer to enhance the selenium levels in meat [24], eggs [25], and crops [26,27]. These advances highlight the potential of selenium chemicals to simultaneously prevent and control plant diseases while enriching agricultural products with selenium, achieving the integration of both pesticide and fertilizer. This is a new developing trend in the field of agricultural selenium chemistry.

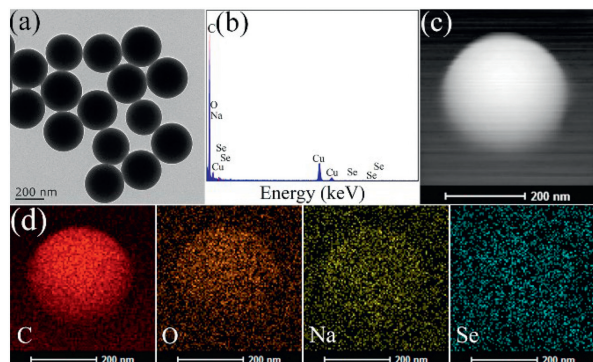
Yet, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a notorious pathogenic bacterium responsible for severe yield losses, reaching up to 80% in rice [28,29]. *Xoo* can infect rice and cause bacterial leaf blight, a serious disease that is widely distributed in many rice-growing regions around the world. This disease is particularly difficult to manage and exhibits increasing pathogenicity and diffusivity [30–32]. Currently, the options for bactericides used to control bacterial diseases in rice are limited, and the high frequency application of bismethiazol (BT) and thiodiazole copper (TC) [33] resulted in the emergence of drug-resistant strains [34,35]. Recognizing the unique biological activity of selenium, efforts have been made to develop new bactericides utilizing this element, with promising results in the field of pesticides [16–20]. In accordance with these progresses, we successfully synthesized selenized polylactic acid (Se@PLA) through the selenization of PLA using NaHSe. Further tests demonstrated that Se@PLA exhibited superior antibacterial activity against *Xoo* compared to the commercially available thiodiazole copper (TC) suspension, while maintaining higher safety levels. This suggests the potential of using

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**Scheme 1.** Diagram of the synthetic route of Se@PLA.



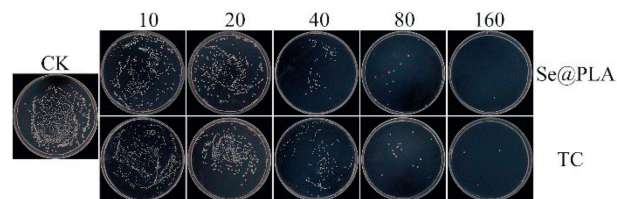
**Fig. 1.** Composition analysis of Se@PLA: (a) TEM image; (b) EDX spectra; (c) the HAADF-STEM image and (d) elemental mapping.

Se@PLA as an alternative fungicide. Herein, we would like to report our findings.

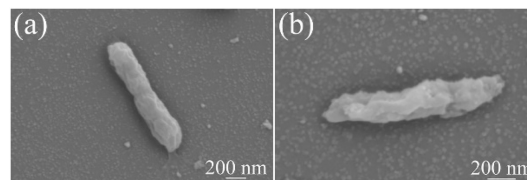
Poly(lactic acid) (PLA) is a biodegradable material that can be synthesized from lactic acid, which is a product derived from corn straw *via* fermentation [36]. Thus, using PLA as the support of Se is an environment-friendly approach. NaHSe was obtained *in situ* by reducing selenium powder with NaBH<sub>4</sub> in ethanol. The PLA particles were prepared *via* the solvent evaporation method, followed by stirring them in NaHSe solution for 24 h. After 2–3 times of ultrasonic washing, the product was separated by centrifugation at 8500 rpm for 7 min and dried in a freeze-drying machine for 24 h to obtain a pink powder (Se@PLA). During the process of selenization, the selenized reagent attacks the carbonyl group to produce -SeH, and Se<sup>2-</sup> attributed to the -SeH moieties anchored onto the material surface *via* Se-C bond (Scheme 1).

Inductively coupled plasma-mass spectrum (ICP-MS) analysis revealed that the material contained *ca.* 0.27% of selenium (w/w%). X-ray photoelectron spectroscopy (XPS) analysis attested that selenium in the material existed in the form of Se<sup>2-</sup> as expected (Fig. S1 in Supporting information). The X-ray diffraction (XRD) patterns of PLA before and after selenization were compared, as shown in Fig. S2a (Supporting information). The diffraction peaks of PLA appeared at 12.42°, 14.73°, 16.68°, 19.06°, and 22.29°, corresponding to the (103), (104), (200), (203), and (210) planes respectively. This suggests that the prepared PLA possess typical characteristics of the  $\alpha$ -crystal form. The introduction of selenium did not significantly change the crystal structure of PLA. No diffraction peak related to selenium was observed because the content of this element was too low [37]. Fourier-transform infrared (FT-IR) spectra of the two materials exhibited similar absorption peaks, indicating that there were no differences for the major components of the materials at molecular structure level (Fig. S2b in Supporting information).

The structure and morphology of Se@PLA were then characterized by transmission electron microscopy (TEM) and high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) (Fig. 1). TEM images reveal that the material is composed of spherical particles with an average size of 270 nm (Fig. 1a). The elemental composition was analysed through energy



**Fig. 2.** Colony growth inhibition of Se@PLA ( $\mu\text{g/mL}$ ) and thiodiazole copper (TC) against *Xoo*.



**Fig. 3.** Morphologies of *Xoo*: (a) control and (b) treated by Se@PLA.

dispersive X-ray spectroscopy (EDX), indicating the presence of C, O, Na, and Se elements (Fig. 1b).

Elemental mapping images were employed to further analyse the distribution of elements in Se@PLA. The HAADF-STEM image, which is sensitive to variations in atomic numbers (*z*-contrast image) [38], displayed consistent brightness throughout the Se@PLA, suggesting the homogeneous distribution of elements (Fig. 1c). As shown in Fig. 1d, the main structure of the particles comprised the C and O, with trace amounts of Se uniformly dispersed on the C-O framework, confirming that Se is combined with PLA through chemical bonds. There was also Na<sup>+</sup> involved in the material, but Na<sup>+</sup> is harmless to *Xoo*, as being reflected by the fact that these bacteria can be cultured under a high concentration of Na<sup>+</sup>. Thus, the involved Na<sup>+</sup> does not affect the bioactivity of the materials.

To preliminarily assess the impact of Se@PLA on *Xoo* and investigate its antibacterial properties, *in vitro* experiments were conducted under controlled laboratory conditions. Throughout the entire experimental process, no activity of PLA was observed (Fig. S3 in Supporting information). The bacteriostatic effect of Se@PLA against *Xoo* is illustrated in Fig. 2. As the concentration of Se@PLA decreased (from 160  $\mu\text{g/mL}$  to 10  $\mu\text{g/mL}$ ), the number of bacterial colonies increased significantly, indicating the inhibitory effect was reduced. The positive control group with the commercial bactericide thiodiazole copper (TC) also exhibited comparable results, however its inhibitory effect on *Xoo* was relatively inferior to that of Se@PLA (Fig. 2). Notably, at moderate concentrations, the inhibition ratio in the Se@PLA treatment group of 40  $\mu\text{g/mL}$  was markedly higher than that in the TC treatment group.

The toxic regression equation of Se@PLA against *Xoo* was represented by  $y = 1.82 + 2.81x$ , as shown in Table S1 (Supporting information). The median effective concentration (EC<sub>50</sub>) was 13.38  $\mu\text{g/mL}$ , with the 95% confidence limit of 10.94–16.38  $\mu\text{g/mL}$ . By contrast, the EC<sub>50</sub> of TC against *Xoo* was 20.07  $\mu\text{g/mL}$ , with the 95% confidence limit of 17.26–23.34  $\mu\text{g/mL}$ . These results verify that the introduction of Se can significantly enhance the antibacterial activity of PLA, and the bacteriostatic effect of Se@PLA against *Xoo* was superior than that of TC, underscoring its potential as an alternative antibacterial agent.

To explore the antibacterial mechanism of Se@PLA, we analysed the SEM of bacterial morphology and measured the reactive oxygen species (ROS) and malondialdehyde (MDA) levels within *Xoo*. In Fig. 3a, it was obvious that untreated *Xoo* was a plump and rod-shaped bacterium with intact cell wall. Upon treatment with Se@PLA, serious damage to the cell wall of *Xoo* was observed, accompanied by sunken and incomplete cell structures (Fig. 3b).

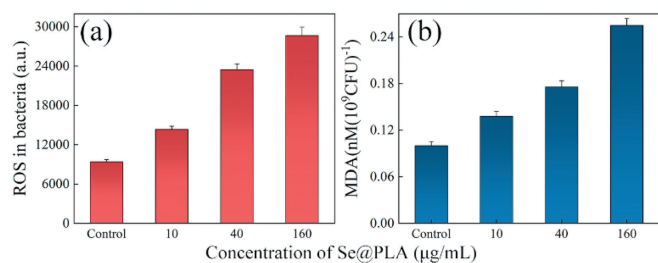


Fig. 4. Changes of (a) ROS content and (b) MDA content in *Xoo*.

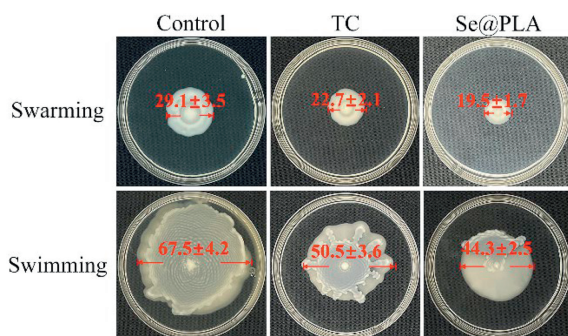


Fig. 5. Effects of Se@PLA (2 µg/mL) on the swarming and swimming motilities of *Xoo*.

Fig. 4a shown the impact of Se@PLA treatment on the fluorescence intensity of ROS in *Xoo*. At 10 µg/mL, the fluorescence intensity of ROS increased by nearly 50%, and at 40 µg/mL, it exhibited a 150% increase. When the concentration of Se@PLA reached 160 µg/mL, the ROS fluorescence intensity was found to be three times of the control group. The MDA assay revealed that the lipid peroxidation levels in *Xoo* treated with 160 µg/mL of Se@PLA increased by 150% (Fig. 4b). These results signify that Se@PLA induces excessive ROS production in *Xoo*, surpassing the intrinsic antioxidant capabilities of bacteria. Consequently, the imbalance between the oxidation and antioxidant systems arises, leading to cellular damage. The peroxidation of lipids in cell membranes and lipoproteins disrupts membrane permeability, impairs bacterial physiological activities, and ultimately resulted in bacterial cell death [39,40]. Hence, the antibacterial effect of Se@PLA may be attributed to the interaction between materials and *Xoo*, which induces the overproduction of reactive oxygen within bacterial cells. The oxidative stress resulted in cell membrane injury and bacterial death. A similar mechanism was also observed when treating *Xcc* with Se/C [16].

Motility is a crucial factor influencing the colonization of plant pathogens [41]. Flagella-mediated swimming serves as the primary mode of movement for individual bacteria in liquid environments, whereas swarming is a collective behaviour observed when high-density bacteria congregate on surfaces [42]. In Fig. S4 (Supporting information), it is evident that Se@PLA at 2 µg/mL does not exert any noticeable effect on cellular viability. Therefore, this specific concentration was employed to observe the impact of Se@PLA on the swimming and swarming motility of *Xoo* stimulated at 28 °C for 24 h (Fig. 5).

Following treatment with 2 µg/mL Se@PLA, the swarming motility of *Xoo* significantly decreased with a motion area measuring 19.5 ± 1.7 mm, compared to the diffusion range of 29.1 ± 3.5 mm in the untreated control group. Furthermore, the inhibition of swimming was like that of swarming, leading to the reduction of swimming range from 67.5 ± 4.2 mm to 44.3 ± 2.5 mm. The commercial bactericide TC also exhibited inhibition of *Xoo* movement, but its effectiveness was lower than that of Se@PLA. The swarm-

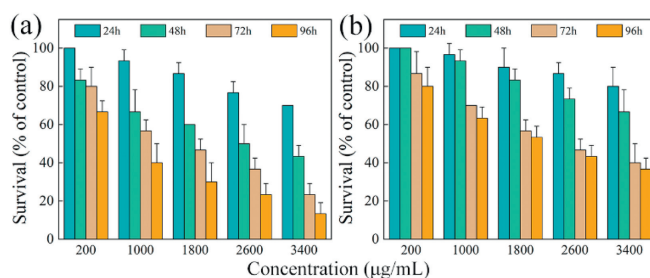


Fig. 6. Survival rates of zebrafish treated with (a) TC and (b) Se@PLA for 24, 48, 72, and 96 h.

ing and swimming movement zone of *Xoo* treated with TC were 22.7 ± 2.1 mm and 50.5 ± 3.6 mm, respectively. In related study, Yan *et al.* reported a remarkable inhibitory effect of CuS NPs on the movement of *Pcc* [43].

In practical applications, Se@PLA may potentially leach into the soil and affect soil organisms. Therefore, the acute toxicity of Se@PLA to earthworms was investigated to evaluate its safety. Earthworms were cultured in soil containing different concentrations of Se@PLA and TC for 7 and 14 days, respectively. Even at extremely high concentration (3400 µg/g), no mortality was observed in the earthworms treated with either Se@PLA or TC. These results indicate that the LC<sub>50</sub> of Se@PLA exceeds 3400 µg/g. Consequently, the toxicity of Se@PLA to earthworms is extremely low, suggesting its good safety to soil organisms.

Adult zebrafish were then employed to investigate the acute toxicity of Se@PLA to aquatic organisms. After conducting two sets of treatments (TC and Se@PLA), it was observed that the survival rate of zebrafish decreased as the concentration increased. The toxicity of TC to zebrafish was higher compared to Se@PLA (Fig. 6). Zebrafish in the control group remained alive over the entire experiment. After 96 h, the LC<sub>50</sub> values of TC and Se@PLA were 531.41 and 1817.17 µg/mL respectively. This indicates that the toxicity of Se@PLA to zebrafish was much lower than that of TC (Table S2 in Supporting information). These results collectively suggest that both TC and Se@PLA exhibit low toxicity levels to zebrafish (>10 µg/mL).

In conclusion, we successfully developed a new bactericide Se@PLA via the selenization of PLA with NaHSe. Compared to conventional bactericides, this material employs PLA as the biodegradable support for the low-loading bioactive Se and is friendly to the environment. It exhibits excellent antibacterial properties vs. *Xoo*, and is safe to both soil and aquatic organisms. It is surprising that even at trace amounts, the antibacterial activity of Se<sup>2-</sup> in Se@PLA surpasses that of commercial bactericide (TC), showing remarkable utilization efficiency of selenium. This work not only provides a promising solution for the prevention and control of rice bacterial blight, a severe agricultural disease, but also greatly expands the applications of selenium-containing materials. It might also inspire new insights for antibacterial materials design: the significance of more active organoselenium materials should be noticed to develop the pesticide/fertilizer dual effect reagent.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccllet.2024.109635.

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