Targeted PLGA Nano-agents preparation and antibacterial activity evaluation against *Rastonia solanacearum*

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Bacterial wilt is a global disease that occurs most easily under high temperature and high humidity. It is one of the main diseases of mulberry and other solanaceous plants such as tomatoes and potatoes (Lebeau, A, et al., 2011). Soil could be used as a transmission medium, and it is easy to form a source of soil infection and cause cyclic pollution.

At present, there are mainly three effective methods for controlling bacterial wilt, including chemical control, biological control, and soilless cultivation (Yuliar, et al., 2015). Common chemical agents require large doses, high costs, and weak inhibitory effects on bacterial wilt. Meanwhile, it can't be accurately released to the strains of *Ralstonia solanacearum*. And those traditional pesticide may cause drug resistance and other problems such as excessive pesticide residues, and even could jeopardize the health of human-body. Therefore, it is urgent to prepare an agent with ability to target pathogenic bacteria, such as *R. solanacearum*.

In order to obtain this nanoparticle, a layer of degradable high molecular biological material poly (lactic-co-glycolic acid) (PLGA) was employed to constructed this novel nanoparticle. This kind of wall material is widely used in the development of antitumor agents (Danhier, F, et al., 2012), which can slow drugs release, prolong the holding period, and reduce the drug dosage (Dadong, et al., 2019). Furthermore, the antibody could be coupled to the surface of PLGA nanoparticles to give it a targeting property. In recent years, a new nano-pesticide formulation has been successfully developed (Yan, J, et al., 2006), which is safer and more efficient than traditional pesticides. Nano-pesticide preparation can significantly enhance the medicinal effect, extend the shelf life, improve bioavailability, eliminate harmful solvents and additives, thereby greatly saving the dosage of pesticides, reducing agricultural product residues and environmental pollution.

Caffeic acid phenethyl ester (CAPE) and caffeic acid methyl ester (MC) were loaded into PLGA nanoparticles, which were prepared by an emulsification solvent evaporation method. And it was considered to be naturally bacteriostatic agent. The liquid mixed with the organic phase and the aqueous phase was subjected to cell disruption to obtain nanoparticles. In order to conjugate the antibody to the surface of the nanoparticle to impart its targeting property, an EDC/NHS chemical coupling method was employed. This targeted nanoparticle was prepared by using magnetic stirring at room temperature for 40 min with PB buffer, and antibody dilution factor was 1000-fold.

Figure 1 shows the effect of different ratios of EDC to NHS, pH value of PB buffer solution, magnetic activation time, and room temperature connection time on the inhibition rate. Figure 1A shows when the concentration of the agent was 4 mg/mL, the ratio of EDC to NHS was 1: 1, and the bacteriostatic rate reached a maximum of 90.60%. And the highest inhibition rate 70.78% could be reached under pH 7.0(B) which shows as Figure 1B. Figure 4C shows that the bacteriostatic rate also changed with the activation time of magnetic stirring, reaching a maximum of 92.26% at 30 minutes, and then began to decrease. At the same time, Figure 1D shows when the connection time is 30 minutes and the concentration is 4mg / mL, it can also inhibit *R. solanacearum* to the greatest extent 84.59%. In general, the best antibacterial effect is when EDC: NHS is 1: 1, pH of PB buffer solution is 7.0, magnetic activation time is 30 minutes, and room temperature connection time is 40 minutes.

The inhibition rate of *R. solanacearum* could reach above 90% under the optimal conditions. Compared with the nanoparticles, the EC_{50} value of the targeted nanoparticles decreased from 0.285mg/mL to 0.045mg/mL which shows that it has a better antibacterial effect. In addition, the effectiveness of the pesticide was improved and an accurate drug delivery system has been constructed. Due to the targeting specificity of the antibody, the nanoparticles could be accurately adsorbed on the surface of *R. solanacearum* and released the drug-effective molecules, thereby strengthening the drug effect and greatly reducing the drug dosage. The novel targeted nanoparticle has good antibacterial and targeting properties provides a new idea for the prevention of *R. solanacearum*.

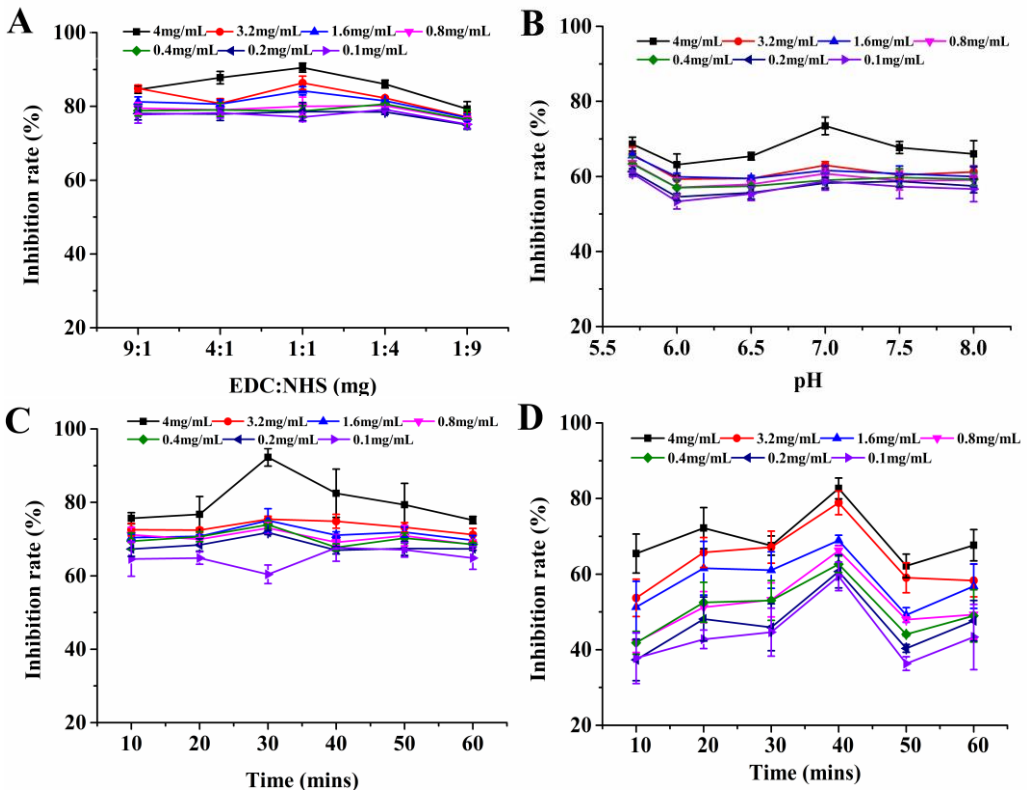

Figure 1 Effects of different ratios of EDC to NHS (A), pH value (B), magnetic activation time (C), and room temperature connection time of PB buffer solution (D) on inhibition rate.

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