Antibacterial activity enhanced nanoparticles loaded with MC and CAPE mixed proportion against *Ralstonia solanacearum*

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Propolis, a kind of natural food with very complex ingredients, is a sticky mixture formed by bees after collecting the secretions of plant branches, callus and buds. And propolis is rich in chemical components, containing more than 20 categories and 30 chemical components, including phenolic acid, flavonoids, amino acids and other substances (Osés et al., 2016). In recent years, the total flavonoid content is usually utilized as the main criterion for evaluating product quality of propolis health products, while a large number of caffeic acid and its ester derivatives with significant antibacterial activity remain in the propolis residue. And caffeic acid (CA), a phenolic acid compound, which is capable of anti-inflammatory, and anti-cancer, antiviral and antibacterial activity, widely found in the animal and plant kingdom (M et al., 2011). Then caffeic acid and its derivatives were employed to inhibit the bacterial wilt one of the most deadly disease for plant, such as the commercial crop, potato, mulberry and tobacco (Jiang et al., 2017). Therefore, caffeic acid and its ester derivatives in the propolis residue could be used as an effective bacteriostatic agent to inhibit bacterial wilt.

Bacterial wilt was caused by the infection of *Ralstonia solanacearum*, and it is called one of the most harmful soil-borne diseases in the world due to its wide distribution and host range (Kim et al., 2016). Furthermore, mulberry bacterial wilt, characterized by rapid onset, rapid spread, serious damage and difficulty in control, is one of the most important mulberry diseases with devastating damage to silkworm production. However, synthetic chemical pesticide was employed to prevent the crop and control bacterial wilt in daily agricultural production, despite the toxicity and pollution to environment. Therefore, it is urgent to produce a novel pesticide with less toxicity, environmental friendliness, sustainability and long-acting effects. A large number of reports have proved that propolis has an inhibitory effect on more than 20 pathogenic bacteria, such as *Staphylococcus aureus* (Afrouzan, 2012), *Bacillus subtilis* (Benhanifia et al., 2014), *Ralstonia solanacearum* (Abo-Elyousr et al., 2017). Furthermore, there is still a large amount of propolis residue remaining after the brass is extracted. After the previous research, caffeic acid and its derivatives which are contained in propolis residue have the potential possibility to be the novel pesticide. Among the derivatives of CA, caffeic acid phenethyl ester (CAPE) and Methyl caffeate (MC) shows an inhibitory effect on bacterial wilt, while the effects of CAPE on inhibition was more significant than MC. MC has a lot of pharmacological activities, moreover it was synthetic intermediates of many natural drugs and food additives, such as PC (Propyl caffeate,) and CAPE which could be prepared by transesterification of MC with the corresponding alcohol (Rani, 2013). CAPE, which was the main active ingredient isolated from propolis residue (Derman, 2015), has many pharmacological activities such as anti-oxidation, anti-virus, anti-bacterial, anti-inflammatory, anti-arteriosclerosis and immunity enhancement. It is one of the hot-spot compounds for the development of new drugs.

Although caffeic acid and its derivatives show many good pharmacological activities, its limited solubility in water and organic solvents limits its application. Thus, nanoparticles were employed to improve their solubility and dispersibility in water without affecting its efficacy (Arasoglu et al., 2016). Nanoparticles have many advantages as drug delivery and controlled release: (1) It can protect drugs effectively, avoid degradation and improve drug stability. (2) It can control drug release, prolong half-life, and improve bioavailability (Arasoglu et al., 2016; Rathor et al., 2017). Among the materials of nanoparticles, poly (lactic-co-glycolic acid) (PLGA) has a wide range of applications due to its good histocompatibility, degradability and adjustable degradation rate.

In this study, emulsion solvent evaporation technique (Hyo-Young et al., 2015) was employed to prepare the nanoparticles loaded with MC/CAPE (MC/CAPE-NPs), while the antibacterial rate, encapsulation efficiency and loading efficiency were selected as the evaluation standard. And the mixture ratio, PVA concentration, ultrasonic power, ultrasonic time and oil-water phase volume ratio were optimized. In order to evaluate the effect of MC/CAPE-NPs on the pathogenicity of *R. solanacearum*, RT-PCR was employed to detect the expression levels of eight pathogenic genes associated with the pathogenicity. In addition, the slow-release characteristics also been investigated.

First, mixed MC and CAPE in different ratios diluted them at different concentrations. After 24 hours, calculate the antibacterial effect of different ratios and different concentrations of the drug, and calculate the EC_{50} (concentration for 50% of maximal effect) of the mixed drug. When the drug volume ratio (MC/CAPE) was changed from 3:7 to 1:1, the value of EC_{50} reduced from 0.62 mg/mL to 0.25 mg/mL. And when the drug volume ratio (MC/CAPE) was changed from 1:1 to 7:1, the value of EC_{50} increased from 0.25 mg/mL to 0.51 mg/mL.

Therefore, the value of EC_{50} is the lowest when the drug volume ratio is 1:1 (MC/CAPE), and inhibitory effect of the mixed drug on bacterial wilt was the most significant.

Fig. 1A shows that the ultrasonic power increased from 65 W to 325 W, the encapsulation rate increased from 26.89% to 40.92%, and the drug loading rate increased from 9.62% to 11.08%. Due to the increase of power, the particle size of nanoparticles was decreased, therefore the encapsulation will be better. Fig. 1B shows that the higher the concentration of nanoparticles containing drugs, the better the inhibitory effect on bacterial wilt. Therefore, the antibacterial effect is the best when the power is 350W at the same concentration.

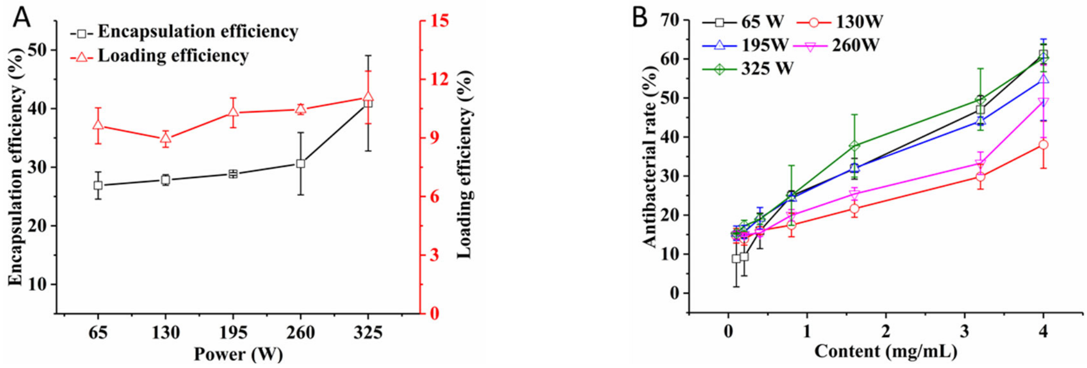

Fig.1 Effect of ultrasonic power on encapsulation efficiency, loading efficiency (A) and antibacterial rate (B).

In conclusion, considering the encapsulation efficiency, loading efficiency and antibacterial rate on nanoparticles, the single factor conditions will be optimized with the 350W power in the next step.

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