Polyphenol-Functionalized Plant Viral-Derived Nanoparticles Exhibit Strong Antimicrobial and Antibiofilm Formation Activities

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ABSTRACT: (–)-Epigallocatechin gallate (EGCG) is a polyphenolic compound that shows a number of health-promoting effects, especially a broad antimicrobial activity. Virus-derived nanoparticles (VNPs) represent a promising drug carrier since they possess properties like biodegradability and their surface and interior are highly modifiable. Turnip mosaic virus (TuMV) VNPs offer an attractive number of conjugation sites on the external surface. EGCG-TuMV VNPs were synthesized by Mannich condensation, and their antimicrobial activities against the model bacteria *Sarcina lutea*, *Pseudomonas aeruginosa*, and *Dickeya dadantii* were tested. EGCG-TuMV VNPs did not only maintain TuMV structure but also showed an enhanced antimicrobial activity over that found with free EGCG for all of the bacteria tested. Biofilm formation by *P. aeruginosa* was also inhibited by EGCG-TuMV VNPs, contrary to free EGCG, which induced higher amounts of biofilm mass in a concentration-dependent manner. Taken together, our results open highly promising perspectives for the antimicrobial exploitation of EGCG-TuMV VNPs.

KEYWORDS: viral nanoparticles, epigallocatechin gallate, polyphenols, tannins, antimicrobial activity, virus-derived particles

INTRODUCTION

Microbial control is a growing global issue affecting human, animal, and environmental health. There are different aspects of this crisis that make it harder to overcome, such as the existence of multiple antimicrobial resistance mechanisms for survival, or the changing regulations for antimicrobial agents due to increasing environmental awareness. All these together cause a reduction in the effectiveness of current approaches for attacking infections, which leads to economic, environmental, and life losses. This reality marks the increasing need for novel, potent agents that can replace or boost the activity of the existing antimicrobials.¹

Antimicrobial resistance happens naturally in bacteria and other microbes. It is a dynamic threat since bacteria are capable of evolving, acquiring, and expressing resistance genes, which, ultimately, leads to the selection of resistant microbial clones.² Although antimicrobial resistance happens naturally, there are different reasons that accelerate its development. The misuse of antimicrobial drugs over time has led to the emergence and spread of multidrug-resistant bacteria. This is compounded by a scarcity of newer drugs attributable to restrictive regulatory requirements.³

Biofilm formation is another important area to consider when dealing with antimicrobials and antimicrobial resistance. Bacterial biofilms are ubiquitous, multicellular aggregates, usually attached to biotic or abiotic surfaces, within which

bacteria are embedded in an extracellular matrix of self-produced polymers.⁴ Biofilm bacteria usually display higher resistance to antimicrobials than planktonic bacteria due to low penetration and accumulation of antimicrobials in the biofilm and to conditions within the biofilm environment that inactivate antimicrobials. As a result, bacterial biofilms are refractory to antibiotic treatment and to immune responses; therefore, biofilms of pathogenic bacteria play a crucial role in chronic infections.

Antimicrobial control is also an issue in the agricultural sector. Due to a number of factors such as the increasing food demand, climate change, and regulatory limitations in plant protection products, we are witnessing an emergence of new plant diseases that cause significant crop losses.⁵ Current control measures involve the preventive use of traditional chemicals, copper compounds, or antibiotics that are, nonetheless, losing their efficacy due to bacterial resistance. The environmental effects of these control methods are also to be considered. For instance, the traditional use of copper

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compounds raises concerns, since 95% of Cu released into the environment will accumulate in soil and aquatic sediments to potentially toxic levels (>50 – $500\text{ }\mu\text{g/mL}$).⁶ These reasons highlight the need for the development of new strategies for plant disease control. These strategies need to be not only effective but also have minimal effects on the environment.

Plant-derived polyphenolic compounds have been reported to have a wide range of biological activities, among which are their antimicrobial and antibiofilm activities against a wide range of microorganisms, including bacterial, fungal, and viral pathogens.⁷ Studies conducted over the last 20 years have shown that the green tea polyphenolic catechins can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency. (–)-Epigallocatechin gallate (EGCG) is the most abundant of these, comprising about 50% of the green tea catechin pool, while (–)-epigallocatechin (EGC) accounts for around 20%, (–)-epicatechin gallate (ECG) for 13%, and (–)-epicatechin (EC) for 6%.⁸ Among these, EGCG is the most bioactive and the most studied. A cup of green tea may contain 100–200 mg EGCG, while catechin and gallic acid are present in lower amounts.⁹

Since EGCG is sensitive to pH, temperature, and oxygen^{10,11} there are limitations on how this molecule can be used in commercial applications. The design of a vehicle that maintains EGCG's attributed effects is an attractive strategy to overcome its lack of stability.

Incorporation of EGCG into nanoparticles (NPs) has been increasingly reported as a strategy to promote the biological activities of the compound. Inorganic nanoparticles, especially metallic ones (silver, copper, gold, and zinc oxide), have been explored as carriers for antimicrobials.^{12–15} These types of nanoparticles suffer from a number of limitations related to their environmental effects. Absorption of metals may have a negative effect on the structure and function of the photosynthetic apparatus.¹⁶ Metallic nanoparticles may have an ease for oxidation and aggregation and are difficult to extract and purify after green synthesis.^{17,18} Also, they tend to accumulate in tissues and interact with proteins and DNA, which contributes to the toxicity that affects different organs within the human body. Metallic nanoparticles are also known to produce stimulation of cytokines that contributes to cytotoxicity, immunotoxicity, and genotoxicity.¹⁹ To prevent chronic toxicity elicited by metallic NPs, organic nanoparticles have been developed as drug delivery carriers. For the above reasons, organic nanoparticles have been favored for biological applications; hence, a number of organic nanoparticles have been tried as carriers for EGCG in different biological systems. These include lipidic,^{20–22} polysaccharide,^{23,24} or proteinaceous nanoparticles.^{25–27} Proteinaceous nanoparticles have received relatively lesser attention, although casein-based nanoparticles have been explored, especially in the nutraceutical area.^{27,28} In one form or another, underlying reasons to try nanoparticle formulations for EGCG relate to the lack of stability of the compound in its free form.

Viral plant-derived nanoparticles (VNPs) are a self-assembly-competent protein structure, the capsid, made out of multiple copies of one or a few types of protein subunits. VNPs have a series of advantages that make them suitable for nanoscale applications. Their proteic nature makes them biodegradable and capsids can be produced in large quantities by infection of plants, hence their production is affordable and

scalable. Finally, their surface is highly modifiable to target different molecules.²⁹

Specifically, Turnip mosaic virus (TuMV) particles are of interest since their architecture allows for different types of functionalizations. TuMV is a flexuous, filamentous plant virus that belongs to the Potyvirus genus. It is about 700 nm long, 12 nm in diameter, and comprises approximately 2000 identical copies of 33 kDa CP protein subunits that cover a molecule of viral RNA of approximately 10 kb size.³⁰ VNPs can be modified genetically or chemically to expose peptides or small molecules (like catechins) at the surface of the virus. There are 21 lysyl residues present in the CP protein, which are potential targets for chemical functionalization.³⁰ This multimeric presentation of EGCGs on the VNPs surface might boost its antimicrobial and antibiofilm activity against several Gram-positive and Gram-negative bacteria.

TuMV-VNPs constitute a versatile tool for the generation of a technological platform with the potential to impact a vast array of areas. Given the pressing need for creating new antimicrobial agents, VNPs are promising, since they have the potential to transport molecules with antimicrobial activity in a more efficient manner. In this study, we show that EGCGs can be efficiently conjugated to VNPs and that these EGCG-VNPs display enhanced antimicrobial activities, as compared to that of the free antimicrobial.

■ EXPERIMENTAL SECTION

Production and Purification of TuMV VNPs. Turnip mosaic virus (TuMV, isolate UK 1) was produced and purified as described by Sánchez and Ponz.³¹

Chemical Conjugation of EGCG. EGCG was chemically bound to VNPs through a Mannich reaction. Previously, a buffer exchange was required to eliminate the amines present in TRIS buffer. VNPs were centrifuged (50 min, $80\,000\times g$, $4\text{ }^{\circ}\text{C}$) and resuspended in 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES), pH 7.5, to a final concentration of 2 mg/mL. EGCG (Sigma-Aldrich, Germany) was dissolved in 2-morpholinoethanesulfonate (MES), pH 6.7, to a final concentration of 5 mg/mL. Different amounts of EGCG were added to 100 μg of VNPs and 5 μL of 37% formaldehyde, and the final volume was adjusted to 115 μL . The reaction was stirred and incubated at room temperature for 72 h. The excess EGCG was removed by centrifugation (50 min, $80\,000\times g$, $4\text{ }^{\circ}\text{C}$), the pellet was washed twice, and resuspended in 10 mM HEPES, pH 7.5, to a final concentration of 1 mg/mL of VNPs.

EGCG Coupling Estimation. The amount of EGCG bound to TuMV was analyzed by modifying a previously described method for the determination of tannin–protein complexes.^{32,33} To microwells from a 96-well plate, 15 μL of portions of EGCG-VNPs solution was added and incubated with 15 μL of 10 mM HEPES, pH 7.5, 90 μL of SDS-trietanolamine solution (SDS 1% (w/v), trietanolamine 7% (v/v)), and 30 μL of a ferric chloride reagent (0.01 M ferric chloride in 0.1 M HCl) for 30 min, after which absorbance at 510 nm was recorded. A calibration curve was constructed by plotting absorbances for different, known amounts of EGCG.

Electron Microscopy. Electron microscopy grids (400 mesh nickel, carbon-coated) were prepared by gently placing them, film-down, on top of the antiserum drop and incubated for 10 min at room temperature. Grids were then removed with tweezers and rinsed with the same buffer. They were later floated on a 10 μL drop of VNPs (0.05 mg/mL), incubated at room temperature for 15 min, and washed with buffer. Finally, the grids were rinsed with distilled water and stained with 2% uranyl acetate for 2 min. The samples were examined on a transmission electron microscope (JEM JEOL 1010, Japan).

Assay for Antimicrobial Activity of TuMV-EGCG VNPs. The antimicrobial activity of EGCG-VNPs was evaluated against *Dickeya dadantii*, *Sarcina lutea*, and *Pseudomonas aeruginosa* by disk

susceptibility tests. Overnight bacterial cultures were grown in Mueller–Hinton media (MH), adjusted to an OD₆₀₀ of 0.1, and inoculated on Muller–Hinton agar (MHA) plates. Antibiotic disks containing 10 µg of EGCG-VNPs or 5 µg of kanamycin (as control) were placed on the plates and incubated for 24 h at 37 °C.

To evaluate the ability of EGCG-VNPs to inhibit bacterial growth in liquid media, overnight cultures were grown on MH (for *S. lutea*), or minimal M9 medium containing 48 mM Na₂HPO₄, 22 mM KH₂PO₄, 9 mM NaCl, 19 mM NH₄Cl, pH 7, supplemented with 2 mM MgSO₄, 100 µM CaCl₂, and 20 mM glucose (for *D. dadantii* and *P. aeruginosa*). The overnight cultures were adjusted to OD₆₀₀ of 0.1 and 150 µL portions were placed in microwells from a 96-well plate. Different amounts of EGCG-VNPs and free EGCG were added. The plates were incubated in a shaker (200 rpm) for 24 h at 37 °C. After incubation, absorbance was recorded at 600 nm and a single plate-serial dilution spotting (SP-SDS) was performed.³⁴ The results of three plates with three replicates corresponding to a particular sample were averaged, and this value was regarded as the minimal inhibitory concentration (MIC) of EGCG-VNPs against each microorganism.

Biofilm Formation Assays. For the evaluation of the inhibition of biofilm formation by *P. aeruginosa*, the OD₆₀₀ of an overnight culture was adjusted to of 0.1 with minimal M9 medium. One hundred fifty microliter portions were placed in microwells from a 96-well plate. The plates were incubated in a shaker (200 rpm) for 24 h at 37 °C. After incubation, microwells were carefully washed three times to remove planktonic cells. One hundred eighty microliters of crystal violet 2% (w/v) in methanol were added and incubated at room temperature for 10 min. Wells were washed three times to remove excess dye, 180 µL of ethanol was added, and absorbance was recorded at 550 nm.

RESULTS AND DISCUSSION

Chemical Conjugation of EGCG. Although a large number of health-promoting benefits have been attributed to EGCG, their transfer toward specific applications has proven limited. The incorporation of EGCG into nanoparticles is a promising strategy to help the bioavailability and, as a result, opens the path for the exploitation of its many beneficial activities. Protein-based nanoparticles, in particular VNPs, could represent a promising alternative, in view of their lack of such environmental disadvantages and of their highly modifiable surface, which allows high flexibility on the design of VNPs. The shape of TuMV makes it an attractive candidate to be used as a nanocarrier. As previously reported, lysine residues from the TuMV capsid protein are mainly exposed on the surface and can be used in the functionalization of the virion.³⁵ Through lysine conjugation, EGCG molecules bound to VNPs will be likewise exposed to the outside environment (Figure 1). This EGCG exposure toward the exterior of the nanoparticle is important since it has been suggested that the negatively charged EGCG binds to the positively charged external surface of cell membranes, thus damaging or fragmenting the lipid bilayer.³⁶ This configuration would allow for a high number of available EGCG molecules concentrated within a relatively small area (high effective concentration). To obtain such EGCG-TuMV nanoparticles, a Mannich condensation was used to target both the polyphenolic compound and primary amines from lysine residues, as shown in Figure 2.

The ratio of EGCG to VNP in the reaction was optimized by testing different concentrations of EGCG. After reacting, unbound EGCG was removed by ultracentrifugation and the resulting conjugated EGCG was estimated. The TuMV concentration was kept constant at 0.87 µg/µL. As depicted in Figure 3, an EGCG concentration of 2.27 mM in the reaction yielded a high amount of conjugated EGCG. One

Figure 1. Schematic representation of EGCG-TuMV nanoparticle synthesis. The step of discarding the excess of reagents not incorporated into particles is not represented.

higher initial concentration of initial EGCG in the series was tested (2.66 mM), but this was too close to concentrations resulting in a high degree of protein precipitation (results not shown), a situation to be expected since aggregation from the binding at high EGCG loading capacities has been described.²⁸ Thus, we decided to work at 2.27 mM. At these chosen working conditions, the estimated amount of EGCG coupled to TuMV was 1.1 ± 0.1 µg EGCG/µg TuMV. These conjugated VNPs were used in subsequent biological assays.

Although EGCG is known to interact noncovalently with proteins,³⁷ as seen in Figure S2, those VNPs that were produced using formaldehyde yielded on a higher amount of linked EGCG. Since polyphenols are known to be highly reactive with proteins and tend to aggregate,³⁸ an important consideration was the maintenance of TuMV particle structure. TEM images, shown in Figure 3, did not reveal significant changes in the VNP structure after EGCG conjugation. The characteristic filamentous shape of TuMV particles remained largely unmodified.

Antimicrobial Activity of EGCG-TuMV VNPs. Once EGCG-TuMV conjugation was completed, the maintenance of EGCG-derived antimicrobial activity was tested. First, a disk diffusion susceptibility test was performed on MHA plates. As shown in Figure 4, there was a good antimicrobial activity for both *D. dadantii* and *S. lutea*. In both cases, we found inhibition around disks containing 10 µg of EGCG-TuMV.

To establish the limits of the antimicrobial properties, tests in liquid media were performed. It has been previously reported that EGCG shows lower antimicrobial activity against Gram-negative bacteria due to their outer layer of liposaccharides.³⁹ *D. dadantii* and *P. aeruginosa* (Gram-negative) and *S. lutea* (Gram-positive) were compared. Experiments were repeated three times to ensure reproducibility with three replicates each. Cultures were performed in 96-well microtiter plates with minimal M9 medium supplemented with glucose

Figure 2. Mannich condensation between EGCG and TuMV.

Figure 3. EGCG-TuMV characterization. (a) Amount (μg) of EGCG conjugated to TuMV as a function of EGCG concentration in the initial condensation reaction. TuMV concentration was maintained constant in the reaction at 0.87 mg/mL. (b) Electron microscopy (TEM) micrographs of purified TuMV (i) or EGCG-TuMV particles (ii).

Figure 4. Inhibition of bacterial growth by EGCG-TuMV in solid media plates. Inhibition haloes were obtained for both *D. dadantii* and *S. lutea*. The inoculum was adjusted to an OD_{600} of 0.1 and cultured overnight on HMA. Kanamycin was used as a positive inhibition control.

for *D. dadantii* and *P. aeruginosa* and MH medium for *S. lutea*. For all strains tested, as shown in Figure 5, the absorbance of overnight cultures showed the same behavior. Culture growth was notably lower in the cases of higher concentration of EGCG-TuMV nanoparticles, suggesting that the antimicrobial effect of the nanoparticles is concentration-dependent. The

observed antimicrobial effect of the EGCG-TuMV nanoparticles can be attributed to the conjugation of the polyphenolic molecule, given that cultures exposed to purified TuMV did not show growth inhibition (Figure 5) and those VNP produced without the presence of formaldehyde showed very low antimicrobial activity (Figure S2b). A difference in the antimicrobial activity of free EGCG vs EGCG-TuMV particles was observed, with free EGCG moderately inhibiting tested cultures.

The minimum inhibitory concentrations (MICs) shown in Figure 6 were established as the minimum concentration at which 99.9% of bacterial growth was inhibited. First, cultures were performed in a 96-well microtiter plate with minimal M9 or MH media and incubated overnight at 37 °C. After incubation, a single plate-serial dilution spotting (SP-SDS) was performed. The MICs found for *D. dadantii*, *P. aeruginosa*, and *S. lutea* were 16.7, 50.0, and 24.8 $\mu\text{g}/\text{mL}$, respectively. Direct inoculation from these inhibited cultures did not yield any colonies, indicating that EGCG-TuMV nanoparticles are bactericidal, an observation that correlates with prior studies on the antimicrobial effects of free EGCG.⁴⁰ In view of this, values for MICs and minimum bactericidal concentrations (MBC) were very similar (Figure 6).

The MIC determinations confirmed that the inhibitory effects of EGCG-TuMV particles were higher than those of free EGCG for all bacteria tested. In the case of *P. aeruginosa*, a MIC of 400 $\mu\text{g}/\text{mL}$ has been reported for extracted EGCG,³⁹ which is still much higher than 50 $\mu\text{g}/\text{mL}$ we found for EGCG-TuMV.

Figure 5. Inhibition of bacterial growth by EGCG-TuMV in liquid cultures. Graphs represent the absorbance (OD₆₀₀ nm) of *P. aeruginosa*, *D. dadantii*, or *S. lutea* cultures after exposure o/n to increasing concentrations of free EGCG, TuMV, or EGCG-TuMV.

Figure 6. Quantification of inhibition through colony-forming unit (CFU) counting after bacterial growth in liquid media. CFU count of *P. aeruginosa* and *D. dadantii* after overnight incubation at 37 °C. The results for *S. lutea* are shown in a numerical form because in most cases, the results were too high to count (THTC).

Figure 7. Biofilm adhesion assay of *P. aeruginosa*. Cultures were grown for 24 h at 36 °C in 96-well microtiter plates containing M9 media. Biofilm formation, indicated by crystal violet staining, was measured at 550 nm.

Contrary to previous reports on a higher susceptibility to EGCG by Gram-positive bacteria, we found that *D. dadantii* (Gram-negative) had a lower MIC in comparison to *S. lutea* (Gram-positive). Given that similar results have also been found in other studies,⁴¹ it appears that generalizations regarding differential sensitivity to EGCG by Gram-positive and Gram-negative bacteria are still premature.

Within the concentration range tested, free EGCG did not show complete inhibition of culture growth for any of the bacteria tested. Since the same batch of purified EGCG was used both for these tests and for nanoparticle synthesis, other factors that affect free EGCG stability, such as pH, exposure to light, and susceptibility to redox reactions could play a role. It

has been reported that coupling EGCG to protein stabilizes the polyphenolic compound under physiologic conditions.²⁵ The latter, together with the exposed nature of EGCG molecules conjugated on the surface of the particle, might explain the difference in antimicrobial effectiveness observed in the present study. Finally, as seen in Figure S4, EGCG-VNP retained good antimicrobial activity after six months of being produced and stored at 4 °C in 10 mM HEPES.

Evaluation of Biofilm Formation. Bacterial biofilms are multicellular aggregates in which cells are embedded in an extracellular matrix, much more refractory to antimicrobials. This matrix encases bacterial cells and provides protection by performing different tasks. It confers structural stability and

flexibility, traps antimicrobial compounds, and maintains homeostatic conditions within the biofilm.⁴ Because of this biofilm-conferred infection resilience, the need of new antimicrobial agents addressing this problem has become evident. Biofilm-associated antimicrobial resistance is not acquired by classical antibiotic resistance such as mutations in antibiotic targets but is part of normal bacterial regulatory repertoire.⁴² Specifically, EGCG is an attractive candidate since EGCG-induced bacterial membrane damage deters the ability of the bacteria to bind to each other to form biofilms.⁴³

EGCG-TuMV's ability to inhibit biofilm formation was tested using cultures of *P. aeruginosa* in M9 supplemented with glucose exposed to different concentrations of EGCG and EGCG-TuMV and incubated for 24 h, after which the biofilm was quantitated with crystal violet. As shown in Figure 7, EGCG-TuMV VNP were capable to inhibit the *P. aeruginosa* biofilm formation at a concentration of 42 $\mu\text{g/mL}$. The biofilm inhibition was dependent on EGCG-TuMV VNP concentration and clearly different from the effects found with free EGCG and TuMV treatments.

EGCG has been reported to act against *P. aeruginosa* biofilms by interfering with the assembly of amyloid fibers and impairing quorum sensing.^{44,45} These effects produce biofilms that are less stiff and more susceptible to antibiotics. On the other hand, it has also been reported that EGCG interferes with *P. aeruginosa* swarming motility and enhances the biofilm formation.⁴⁶ The latter was also found in our tests. Free EGCG enhanced the biofilm formation in a concentration-dependent manner (Figure 7). However, nanoparticles loaded with EGCG clearly inhibited the formation of the biofilm of *P. aeruginosa*.

Perspectives. The conjugation of EGCG to TuMV does not seem to impair its capacity to act as an antimicrobial. Rather, it promotes it. Several mechanisms of action have been reported for EGCG, and the compound affects bacteria in different ways. It inhibits enzymes such as dihydrofolate reductase and DNA gyrase, which affect DNA.³⁶ It also causes oxidative stress by generating H_2O_2 ⁴⁷ and binds to both proteins and phospholipids from the lipid bilayer, damaging the cell membrane and increasing the permeability.⁴⁸ Since EGCG has a high affinity for cell membranes and different mechanisms of action have been reported at different levels in the cell, it appears that there is still a lot to be learned about the complete scope of its effects. Although we were able to demonstrate that EGCG-TuMV nanoparticles have a potential as an antimicrobial agent, the actual mechanisms of their inhibitory action are yet to be characterized. Because of the length of TuMV nanoparticles, ca. 700 nm, EGCG-TuMV nanoparticles are more likely to interact outside the cell membrane. Further studies are required to understand whether fragments or complete EGCG-TuMV particles are capable to pass through the cell membrane and to have an effect from within. Nevertheless, for both antimicrobial and antibiofilm properties, EGCG-TuMV VNP did not only maintain the EGCG activity but also showed a notable enhancement.

Antimicrobial resistance is a rising problem that needs to be addressed promptly. One of the major advantages of VNP is the versatility they provide. This technological platform may allow a rapid response to the increasing list of drug-resistant microbes. VNP not only have a number of sites that can be modified but also there is an array of preparation methods for genetic and chemical manipulations with a high flexibility of applications.⁴⁹ VNP could be used to address this problem in

a number of ways. For instance, an additional target molecule could be conjugated to the nanoparticle, which may increase the amount of antibiotic in an infected site. Likewise, the design of the VNP with an additional antimicrobial may boost their antimicrobial effect. Specifically, TuMV has proven to have a potential for multiple functionalization.³⁵ This versatility, in combination with the results obtained in this study, makes TuMV VNP good candidates to explore different arrays of molecules to act synergistically with EGCG.

CONCLUSIONS

The present study showed that EGCG-TuMV nanoparticles produced via Mannich condensation maintain the TuMV structure after the addition of the polyphenolic compound. The EGCG-TuMV nanoparticles proved to be functional, with excellent antimicrobial and antibiofilm properties. Higher antimicrobial susceptibility of Gram-positive bacteria over Gram-negative bacteria was not found. While free EGCG promoted biofilm formation within the range of concentrations tested, EGCG-TuMV showed the opposite effect of inhibiting its formation. In all bacteria tested, susceptibility to EGCG-TuMV was higher than to free EGCG. EGCG-TuMV VNP have the potential to act as antimicrobial agents that enhance EGCG's antimicrobial activities.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsabm.9b01161>.

Structural TuMV model (Figure S1); EGCG-VNP characterization in function of the presence of formaldehyde (Figure S2); inhibition after overnight liquid culture (Figure S3); and EGCG-VNP antimicrobial activity at different times after conjugation (Figure S4) (PDF)

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Author Contributions

F.P. and E.V.-L. conceived the presented idea. All assays were performed by E.V.-L., who also proposed the first drafts of the manuscript and contributed to its final form. J.I. suggested the

microbiological approaches to the assays and the bacteria to be used in them. He supervised the final manuscript versions. F.P. supervised the experiments and assays. He suggested the manuscript structure and supervised its final form.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

EGCG, (−)-epigallocatechin gallate; VNPs, viruslike nanoparticles; TuMV, Turnip mosaic virus; NP, nanoparticle

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