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ABSTRACT

Biofouling on material surfaces is a ubiquitous problem in a variety of fields. In aqueous environments, the process of biofouling initiates with the formation of a layer of macromolecules called the conditioning layer on the solid-liquid interface, followed by the adhesion and colonization of planktonic bacteria and the subsequent biofilm development and maturation. In this study, the extracellular polymeric substances (EPS) secreted by *Bacillus subtilis* were collected and used to prepare conditioning layers on inert surfaces. The morphologies and antifouling performances of the EPS conditioning layers were investigated. It was found that the initial adhesion of *Escherichia coli* was inhibited on the surfaces precoated with EPS conditioning layers. To further explore the underlying antifouling mechanisms of the EPS conditioning layers, the respective roles of two constituents of *B. subtilis* EPS (γ-polyglutamic acid and surfactin) were investigated. This study has provided the possibility of developing a novel interfacial antifouling additive with the advantages of easy preparation, nontoxicity, and environmental friendliness.

I. INTRODUCTION

Biofouling on material surfaces is a worldwide ubiquitous problem in a variety of fields. In the marine industry, adhesion of fouling organisms on the bottom of vessels substantially increases the frictional resistance during sailing, resulting in higher fuel consumption and waste emission.[1–3] Biofouling on marine facilities also brings about severe corrosion, higher maintenance costs, and reduced service duration.[4–6] In the medical field, adhesion of pathogenic micro-organisms on implants and surgical instruments can cause lethal infections.[7–9] In the food and water treatment industry, biofouling also poses a massive threat to public health.[10] Therefore, taking effective measures to prevent biofouling is of great importance for the well-being of humanity.

Presently, the majority of antifouling techniques are based on the incorporation of bactericidal agents such as copper and silver into coatings or membranes.[11–15] Nevertheless, these bactericidal agents are usually harmful to the ecosystem when released into the environment.[16,17] Moreover, the antifouling efficacies of these coatings diminish over time as the amounts of remaining bactericidal agents decrease, limiting their effective service duration. Recently,

research attention has been turned to the influence of surface topography on bacterial adhesion.[18] Inspired by natural antifouling surfaces such as insect wings, animal skin, and plant leaves,[19–23] surfaces with microscale and nanoscale topographical features were produced to deter bacterial attachment or kill adherent bacteria by pure physical interactions.[24–28] However, the technologies required for the precise patterning of surfaces are usually expensive and complicated, making mass production almost impossible. There is thus an urgent need to develop a novel antifouling technique with the advantages of easy preparation, nontoxicity, and cost effectiveness.

In an aqueous environment, the process of biofouling initiates with the adsorption of a layer of macromolecules called the conditioning layer on the solid-liquid interface.[29,30] Conditioning layers directly interact with planktonic bacteria and mediate their subsequent adhesion and colonization. The influence of conditioning layers on bacterial adhesion takes effect in both specific and nonspecific ways. Nonspecifically, conditioning layers can alter the surface physicochemical properties such as surface charge, wettability, and topography, which have an unignorable impact on bacterial behaviors.[31,32] On the other hand, some macromolecules present in conditioning layers exhibit antibacterial activities against certain bacterial species. These macromolecules are generally of biological origin, including antibacterial proteins, bacteriocins, and biosurfactants.[33–37] *Bacillus subtilis* is a Gram-positive, rod-shaped bacterial species that has been reported to inhibit the growth and colonization of a variety of bacteria including *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella sp.*[38–40] Previous studies have proved the safety of *B. subtilis* for humans and animals, and the addition of *B. subtilis* in food has been acknowledged in the United States, Japan, and Europe.[41] Kim *et al.* found that the addition of *B. subtilis* to feed promoted the health of pigs preinfected by *E. coli*, evidencing the beneficial role of *B. subtilis* in treating *E. coli* infections.[42] Precoating surfaces with a conditioning layer consisted of the functional molecules secreted by *B. subtilis* may be an effective method to prevent bacterial adhesion and biofilm formation of *E. coli*. *B. subtilis* is capable of producing extracellular polymeric substances (EPS) comprised of polysaccharides, proteins, phospholipids, nucleic acids, lipopeptides, etc. Therefore, in this work, the EPS secreted and released into the culturing media by *B. subtilis* were extracted to prepare conditioning layers on smooth, inert surfaces. The morphologies of the preadsorbed EPS conditioning layers were characterized using atomic force microscopy (AFM). The subsequent adhesion and growth of *E. coli* on EPS-conditioned inert surfaces were investigated. To further explore the underlying mechanism of how EPS conditioning layers affected *E. coli* adhesion and biofilm formation, the respective roles of two major constituents of *B. subtilis* EPS [γ-polyglutamic acid (γ-PGA) and surfactin] were investigated.

II. EXPERIMENT

A. Substrates

For bacterial adhesion tests, smooth silicon wafers (Zhejiang Lijing Optoelectronics Technology Co., Ltd) were used as substrates. The silicon wafers were cut into rectangular pieces of approximately $1 \times 1\ cm^2$ and ultrasonically cleaned, first in ethanol for 15 min and then in de-ionized water for 15 min in order to remove contaminants. Before EPS preadsorption and bacterial adhesion, the silicon wafers were air-dried and sterilized under ultraviolet light for 30 min. For EPS layer morphology characterization with AFM, freshly cleaved mica slices were used as substrates.

B. EPS extraction

For EPS extraction, *Bacillus subtilis* CMCC(B)63501 was used as the producer of EPS. The culturing medium for *B. subtilis* was prepared by dissolving 5 g peptone and 1 g yeast extract into 1 l artificial seawater (ASW). The ASW used in this study was prepared according to ASTM D 1141-98 (2003). The chemical composition is described in Table I. The bacteria-containing medium was shaken at 120 rpm under 25 °C for 24 h. EPS extraction was performed when the bacteria reached the exponential growth phase after 24 h of incubation. The bacteria-containing medium was centrifuged at 12 000g for 30 min and the supernatant was filtered twice through 0.22 μm pore size syringe filters to remove remaining bacteria. The filtered supernatant was then dialyzed for 48 h using 3.5 kDa dialysis membranes in order to remove salts and metabolites of low molecular weight. EPS powders were obtained by lyophilizing the supernatant at −68 °C using a freeze drier.

C. Conditioning layer preparation and characterization

For conditioning layer preparation, the EPS powders were resuspended in ultrapure water at concentrations of 0, 100, 200, and 300 mg/l. These concentrations were chosen based on previous studies.[43–45] Before AFM and bacterial adhesion tests, 200 μl of each EPS solution was added onto the substrate surfaces by creating a droplet that covered the majority of the sample surface. After a preadsorption process of 30 min, the EPS solutions were removed and the samples were ready for further tests. Preparation of γ-PGA and surfactin conditioning layers was achieved following the same protocol described above. γ-PGA and surfactin powders were purchased (from Sinopharm Chemical Reagent Co., Ltd., and Zhejiang Tianyuan Pharmaceutical Technology Co., Ltd, respectively) and suspended in ultrapure water at concentrations of 0, 100, 200, and 300 mg/l. The solutions were added and remained on silicon surfaces for 30 min and then removed before bacterial adhesion tests.

TABLE I. Chemical composition of the ASW used to culture *B. subtilis*.[a]

Solution A		Solution B	
Chemical	Concentration (g/l)	Chemical	Concentration (g/l)
NaCl	24.53	KCl	0.695
Na_2SO_4	4.09	$NaHCO_3$	0.201
$MgCl_2{\cdot}6H_2O$	11.10	KBr	0.101
$CaCl_2$	1.16	H_3BO_3	0.027
$SrCl_2{\cdot}6H_2O$	0.0351	NaF	0.003

[a]Solution A and Solution B were mixed together 24 h after preparation.

AFM (Bioresolve, Bruker, Germany) was used in the peak-force tapping mode in air to characterize the morphologies of the EPS conditioning layers on freshly cleaved mica surfaces. The SCANASYST-AIR probe with a spring constant of 0.4 N/m was used. At least eight images were captured per condition. The acquired images were analyzed with NANOSCOPE ANALYSIS 1.8 software.

D. Bacterial adhesion tests

For bacterial adhesion tests, *E. coli* ATCC25922 was used in this study. The Luria Bertani culturing medium for *E. coli* was prepared by dissolving 10 g NaCl, 10 g peptone, and 5 g yeast extract into 1 l de-ionized water. Before inoculation, the media for bacteria culturing were autoclaved at 120 °C for 20 min. Inoculated bacteria-containing media were shaken at 120 rpm under 37 °C for 24 h. After incubation, the bacteria suspended in the culturing media were washed three times with phosphate buffer solution (PBS) via centrifugation (1500 rpm, 10 min), and the cell pellets were then resuspended in PBS. The number of bacteria was measured based on standard calibration with an assumption that an $OD_{600\,nm}$ value of 1.0 is equivalent to 10^9 cells/ml.[46,47] The samples were placed into sterile 24-well cell culture plates, and 1 ml bacterial culture with an initial concentration of 10^8 cells/ml was added into each well. The bacteria were incubated at 37 °C under aerobic conditions for up to two days.

E. Biofilm characterization

E. coli biofilm formation was investigated using field emission scanning electron microscopy (FEI Quanta FEG 250, the Netherlands). After removing the culturing media, the samples were washed three times with de-ionized water and then immersed into 2.5% glutaraldehyde solutions for no less than 4 h to immobilize bacterial cells. After removing the glutaraldehyde solutions, the samples were dehydrated by immersion in 25% (5 min), 50% (5 min), 75% (5 min), 90% (5 min), and 100% (2 × 10 min) ethanol solutions and eventually air-dried. Before SEM tests, the samples were sputtered with platinum. At least eight images were taken for each sample. IMAGEJ software was utilized to process the acquired images and calculate the percentage of surface area covered by bacteria.

Biofilm quantification was achieved using a method described previously.[48] Briefly, the bacteria-containing media were removed from the 24-well plates and the samples were washed three times with sterile de-ionized water to remove planktonic bacteria. 0.1% crystal violet solution was added into the wells to stain the biofilms for 5 min. The crystal violet solution was then removed and the samples were washed three times with de-ionized water. Finally, the samples were immersed into ethanol for 30 min to detach and resuspend the stained biofilms. Biofilm quantification was conducted by measuring the $OD_{570\,nm}$ values. Three independent replicates were used per condition to ensure repeatability.

III. RESULTS AND DISCCUSSION

A. Morphologies of EPS conditioning layers

The morphologies of the EPS conditioning layers preadsorbed on mica surfaces were characterized using AFM, and the results are shown in Fig. 1. Figures 1(a) and 1(e) show the surface morphologies of smooth mica surfaces, i.e., the mica surfaces treated with ultrapure water without EPS addition. No significant height variation was observed on the smooth mica surfaces, indicating that the inherent surface topography of mica surfaces is negligible. Figures 1(b) and 1(f) show the surface morphologies of the sample treated with 100 mg/l EPS solution. The majority of the sample surface was found to be covered with a porous layer, and the average diameter of the pores was 265 ± 71 nm. The pores distributed quite evenly on the entire layer, and an interwoven network of small fibrillar structures was found around the pores. Figures 1(c) and 1(g) show the surface morphologies of the sample treated with 200 mg/l EPS solution. An entangled network of intersecting fibrillar structures was found on the sample surface. The diameters of the fibrillar structures ranged from tens of nanometers to hundreds of nanometers. The heights of the intersections of the fibrillar structures were greater than those of the other parts of the layer, as indicated by the brighter dots in Figs. 1(c) and 1(g). This entangled network was similar to that of the alginate conditioning layers observed by He *et al.*[49] The fibrillar structure might be a mixture of polysaccharides, proteins, glutamic acids, and other macromolecules bound together. The pores and the empty spaces in-between fibers might be water detained by the highly hydrated matrix. The surface morphologies of the sample treated with 300 mg/l EPS solution are shown in Figs. 1(d) and 1(h). A porous EPS layer similar to that shown in Figs. 1(b) and 1(f) was observed, but this layer was more intact as the number and density of pores were notably lower. The average diameter of the pores was 1.07 ± 0.69 μm, and numerous nanometer-sized dots were found inside these pores, as shown in the magnified image in Fig. 1(h). Moreover, these pores seemed to be connected by threadlike structures that were slightly higher than other areas of the layer. Although none of the EPS conditioning layers were able to provide full coverage of the surfaces, the pores and empty spaces could hardly provide any space for bacterial cells to colonize. The approximate size of a typical *E. coli* cell is 2–3 μm in length and ~1 μm in diameter, whereas the pores could hardly reach this size. Therefore, the EPS conditioning layers did not obtain full coverage of surfaces but left little space for bacteria to fit in, and therefore adequate conditioning film formation was achieved.

Despite the distinction of surface morphology among the different EPS conditioning layers, bacterial adhesion was not to be affected by the surface topographies in this scenario. Surface topography affects bacterial adhesion mainly by increasing/decreasing the contact area between bacterial cells and surfaces[50,51] or by inducing the stretching/rupture of the bacterial cell membrane.[52,53] As a result, surface topography may significantly impact bacterial adhesion if the heights of the surface features exceed or approximate the size of bacterial cells, i.e., a few hundred nanometers or several micrometers. From the height information of the conditioning layers given on the right side of each small graph, it is clear that the height of the surface features present on the conditioning layers did not even exceed 10 nm. Surface features of very low heights are unlikely to significantly change the contact area or stretch the cell membrane, and they are unable to provide shelters that protect bacteria from shear forces. Moreover, the EPS secreted by *E. coli* itself could easily cover the low surface features of conditioning layers. Therefore, the surface topographies of the layers were not expected to have any effect on bacterial adhesion.

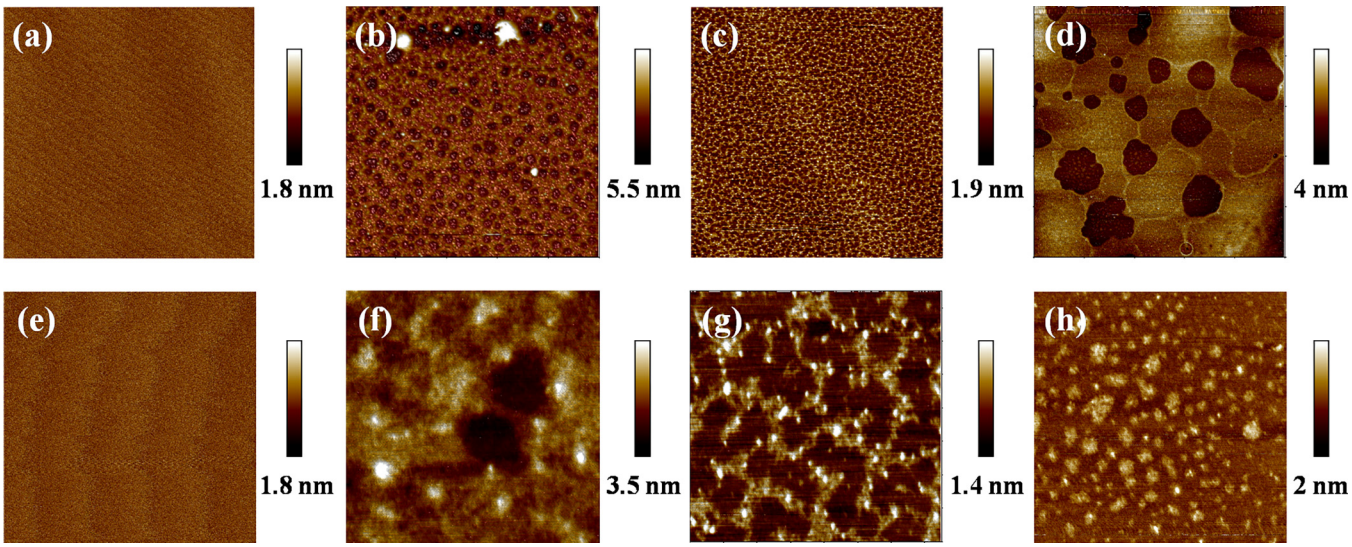

FIG. 1. AFM images showing the representative surface morphologies of mica treated with 0 [(a) and (e)], 100 [(b) and (f)], 200 [(c) and (g)], and 300 mg/l [(d) and (h)] EPS solutions. (a)–(d): $10 \times 10\,\mu m^2$. (e)–(h): $1.5 \times 1.5\,\mu m^2$. Height information is shown on the right side of each small graph.

B. Inhibition of *E. coli* adhesion by EPS conditioning layers

The SEM images of *E. coli* adherent on the blank/EPS-treated surfaces are shown in Fig. 2. On the sample surface without EPS addition, large multilayer biofilm "islands" were formed where bacteria and the underlying extracellular polymeric matrix completely covered the surface [Figs. 2(a) and 2(e)]. On the contrary, such pronounced biofilm formation and surface coverage were not observed on the EPS-treated sample surfaces [Figs. 2(b)–2(d) and 2(f)–2(h)]. Bacteria adhered in the form of individuals or small clusters. A similar

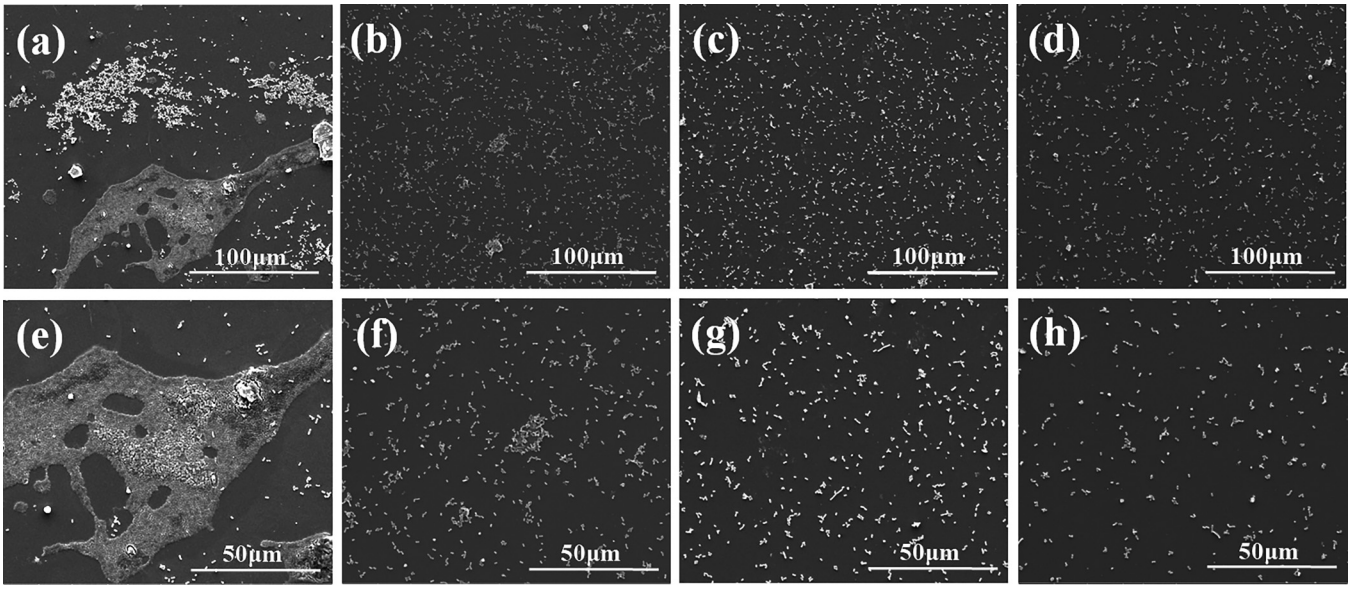

FIG. 2. SEM images of *E. coli* adherent on the samples treated with 0 [(a) and (e)], 100 [(b) and (f)], 200 [(c) and (g)], and 300 mg/l [(d) and (h)] EPS solutions after one day of immersion [(a)–d: ×500; (e)–(h): ×1000)].

phenomenon was observed on the samples after two days of immersion (Fig. 3). Bacteria formed "islands" on the smooth surface [Figs. 3(a) and 3(e)] but showed more random and dispersed distribution on the EPS-treated surfaces [Figs. 3(b)–3(d) and 3(f)–3(h)]. Large multilayer bacterial clusters were ubiquitous on the control surface but were barely found on the EPS-treated samples. It seems that preadsorption of EPS conditioning layers was detrimental to the adhesion of *E. coli*.

Calculation on the percentage surface coverage of bacteria on different samples was achieved using IMAGEJ software. The images were first converted to grayscale and the threshold was then adjusted to include all bacteria. Measurement was set to display the area fraction. The processed images are shown in Fig. 4. The results show that after one day of immersion, the EPS conditioning layers pronouncedly inhibited *E. coli* adhesion to silicon surfaces, averagely reducing surface coverage by 63.5%, 79.8%, and 71.0% [Fig. 5(a)]. After two days of immersion, the same tendency was observed, albeit with a less inhibition rate of 40.6%, 46.2%, and 42.6% [Fig. 5(b)]. No significant variation in surface coverage percentage was observed among samples treated with different concentrations of EPS. These data proved that *E. coli* adhesion to silicon surfaces was inhibited by the EPS conditioning layers.

C. Inhibition of *E. coli* adhesion by γ-PGA conditioning layers

Bacterial EPS are composed of polysaccharides, proteins, nucleic acids, phospholipids, lipopolysaccharides, and lipopeptides.[54–56] The exact composition of EPS varies among different bacterial species. γ-PGA is a polyanionic polypeptide secreted extracellularly by *B. subtilis* consisted of L- and D-glutamic acids linked by amide linkages between γ-carboxyl and α-amino groups. It is one of the major components of *B. subtilis* EPS maintaining the structural integrity and stability of *B. subtilis* biofilms.[57–59] Stanley and Lazazzera reported that γ-PGA is capable of promoting biofilm formation of *B. subtilis* by enhancing cell-surface interactions.[59] However, the influence of γ-PGA on the adhesion of other bacterial species has not been studied. To determine whether γ-PGA is a factor contributing to the bacterial inhibition of *E. coli*, we investigated the influence of preadsorption of γ-PGA conditioning layers on *E. coli* adhesion to silicon surfaces. Figure 6 shows the SEM images of *E. coli* adherent on the sample surfaces with/without γ-PGA conditioning layers after one day of immersion [Figs. 6(a)–6(d)], along with the processed images [Figs. 6(e)–6(h)]. There was no significant difference in the attachment pattern of bacteria among different samples. Bacteria were distributed evenly and dispersedly on all samples, but seemingly adhered in fewer numbers on the surfaces with γ-PGA conditioning layers. Calculation on the surface coverage of bacteria using IMAGEJ showed that γ-PGA conditioning layers averagely reduced surface coverage by 25.4%, 7.65%, and 34.2% [Fig. 7(a)]. Biofilm quantification using crystal violet staining showed that the average reduction rates of biofilm quantities by γ-PGA conditioning layers were 26.4%, 35.2%, and 20.3% [Fig. 7(b)]. Nevertheless, no correlation was observed between the concentration of γ-PGA and the amount of bacteria as was in the case of EPS. One possible explanation of the inhibition effect of γ-PGA is that bacterial cells and glutamic acids were both negatively charged in PBS because their isoelectric points were both lower than the *p*H of PBS, causing electrostatic repulsion between γ-PGA and bacteria.

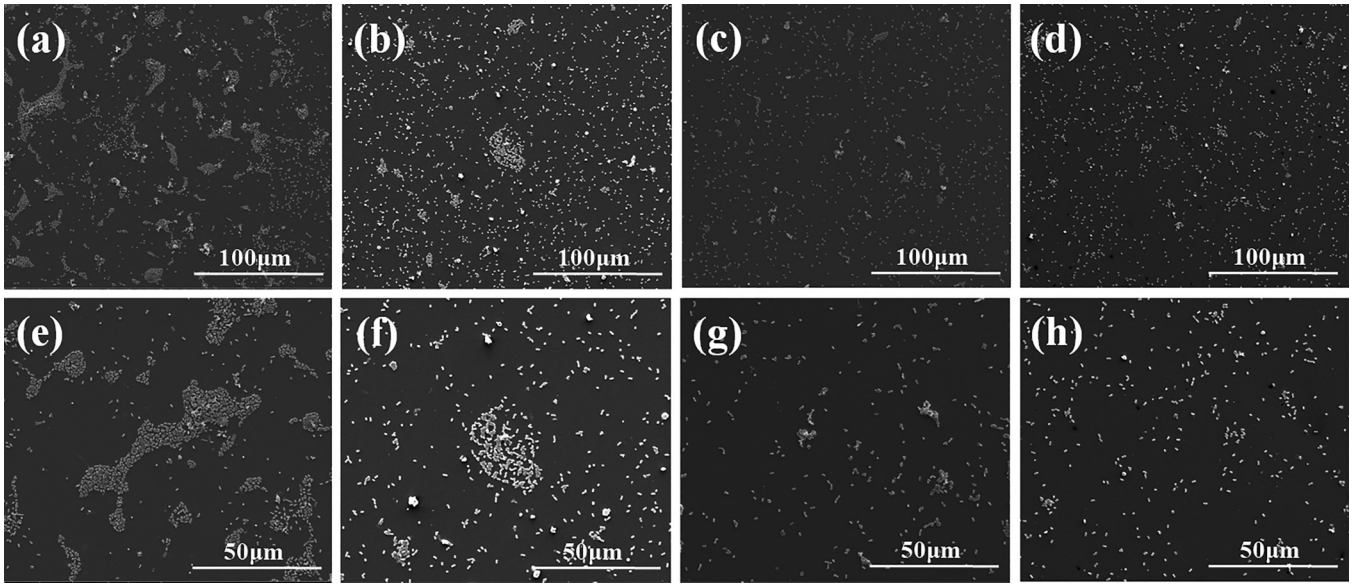

FIG. 3. SEM images of *E. coli* adherent on the samples treated with 0 [(a) and (e)], 100 [(b) and (f)], 200 [(c) and (g)], and 300 mg/l [(d) and (h)] EPS solutions after two days of immersion [(a)–d: ×500; (e)–(h): ×1000)].

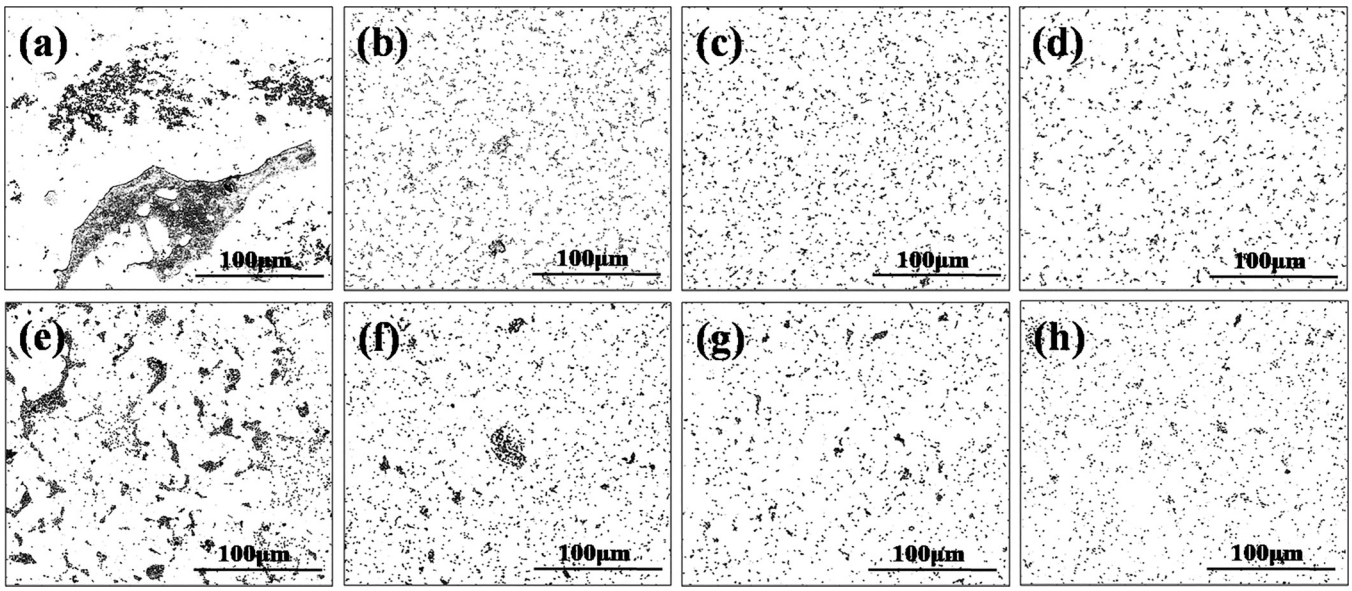

FIG. 4. Processed images of *E. coli* adherent on the samples treated with 0 [(a) and (e)], 100 [(b) and (f)], 200 [(c) and (g)], and 300 mg/l [(d) and (h)] EPS solutions after one day [(a)–(d)] and two days [(e)–(h)] of immersion (×500).

D. Inhibition of *E. coli* adhesion by surfactin conditioning layers

B. subtilis is known to produce a variety of biosurfactants, among which surfactin is a type of bioactive lipopeptide with antimicrobial potential.[60,61] In the biofilm formation process, *B. subtilis* secretes surfactin to trigger extracellular matrix production. Surfactin is an amphiphilic lipopeptide consisted of a cyclic heptapeptide ring with the sequence Glu-Leu-D-Leu-Val-Asp-D-Leu-Leu and a C_{13-15} β-hydroxy fatty acid chain. Surfactin is able to create pores on the bacterial cytoplasmic membrane, causing potassium leakage that is in turn sensed as a quorum sensing signal to stimulate the differentiation of *B. subtilis* toward matrix-producing cells.[62] Nevertheless, for those bacterial species in lack of such survival strategies, the

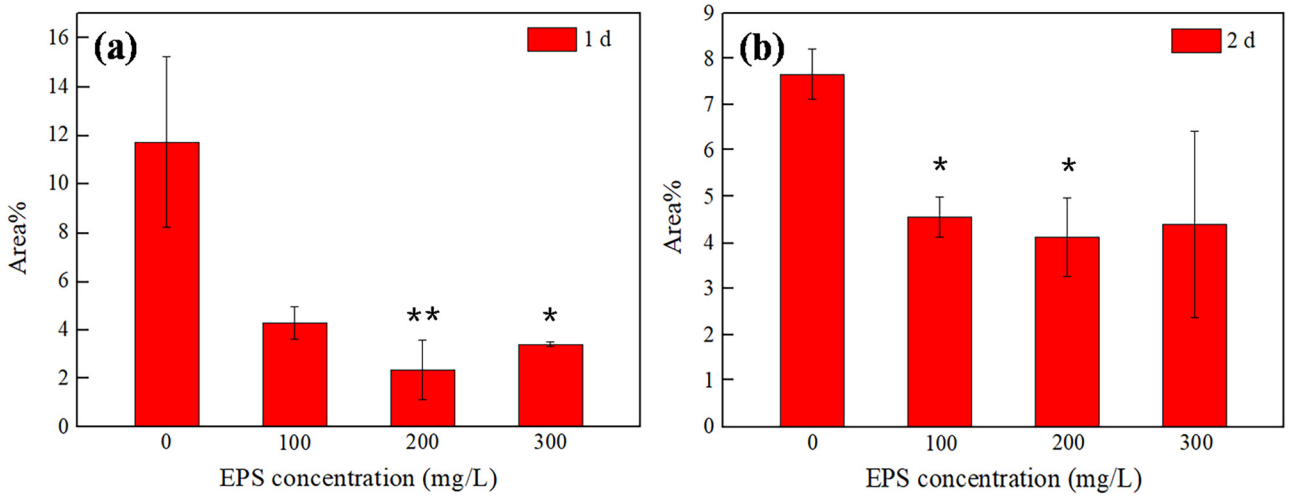

FIG. 5. Percentage of surface area covered by adherent *E. coli* on samples with/without EPS conditioning layers after one day (a) and two days (b) of immersion. Error bars are shown as ± SD. *$p < 0.05$; **$p < 0.01$.

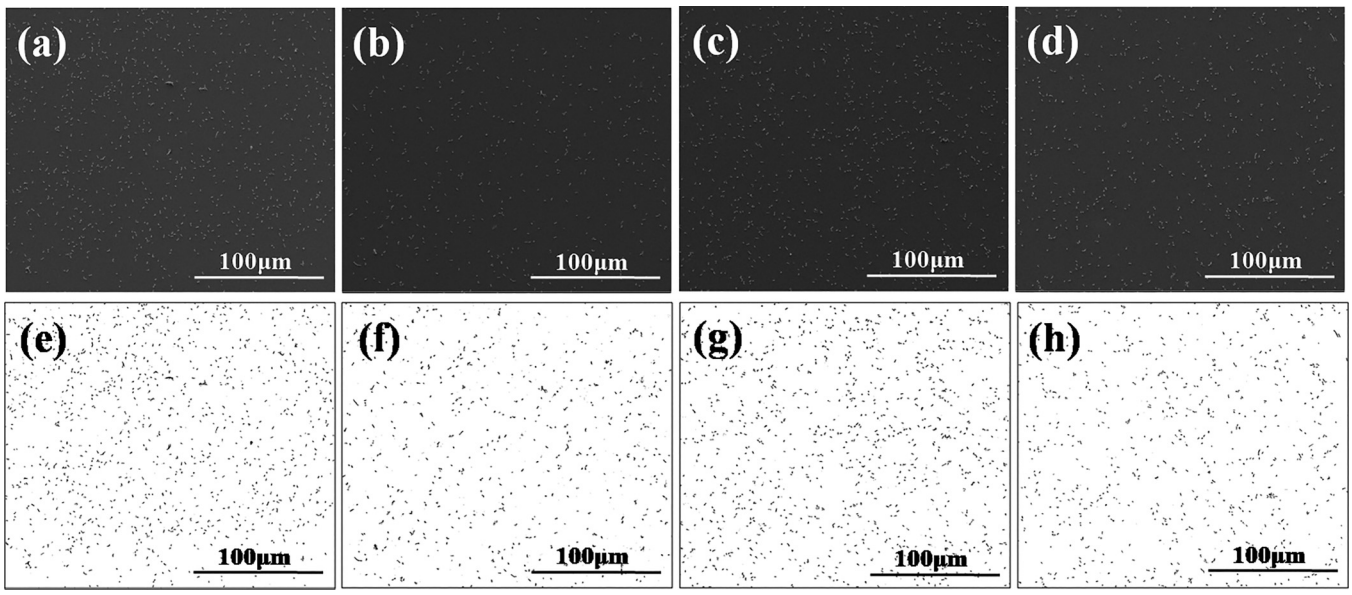

FIG. 6. SEM images [(a)–(d)] and processed images [(e)–(h)] of *E. coli* adherent on the samples treated with 0 [(a) and (e)], 100 [(b) and (f)], 200 [(c) and (g)], and 300 mg/l [(d) and (h)] γ-PGA solutions after one day of immersion (×500).

disruption of cytoplasmic membrane by surfactin can be lethal. To determine whether surfactin is a factor contributing to the bacterial inhibition of *E. coli*, we investigated the influence of preadsorption of surfactin conditioning layers on *E. coli* adhesion to silicon surfaces. The SEM images of *E. coli* adherent on the sample surfaces with/without surfactin conditioning layers are shown in Fig. 8. Large multilayer bacterial clusters were formed on all samples in which bacteria were embedded in the biofilm matrix [Figs. 8(a)–8(d)]. Close observation of the bacterial clusters disclosed the distinct biofilm morphologies among different samples [Figs. 8(e)–8(h)]. On the sample surface without surfactin conditioning layers, the bacterial clusters were thick and bacteria were tightly enwrapped in the biofilm matrix. Bacterial cells were hardly discernable in these bacterial clusters [Fig. 8(e)]. On the sample surfaces treated with surfactin solutions,

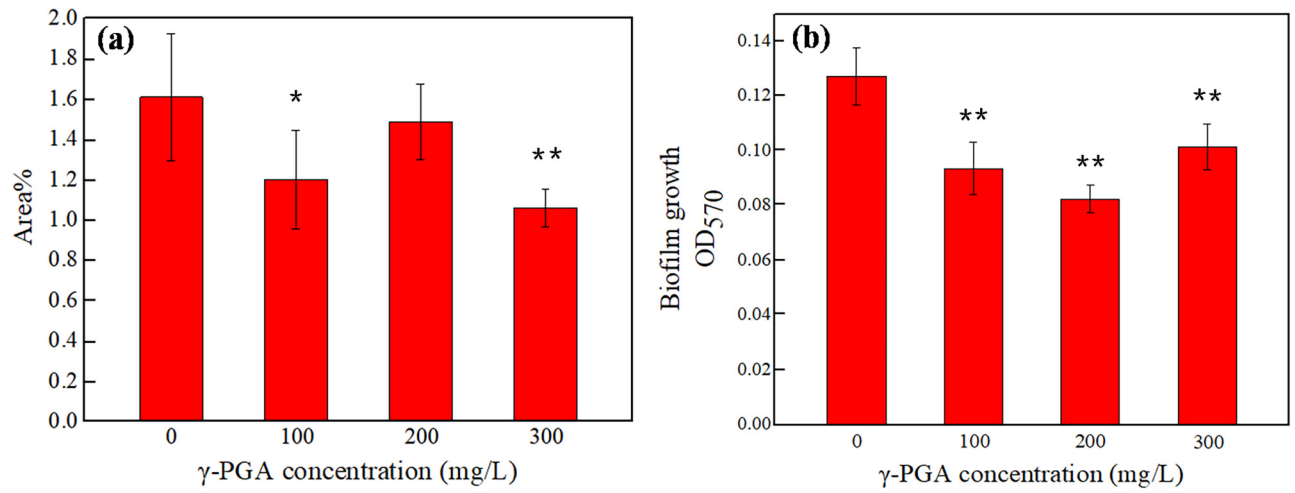

FIG. 7. Percentage of surface area covered by adherent *E. coli* (a) and biofilm quantity (b) on samples with/without γ-PGA conditioning layers after one day of immersion. Error bars are shown as ± SD. *$p < 0.05$; **$p < 0.01$.

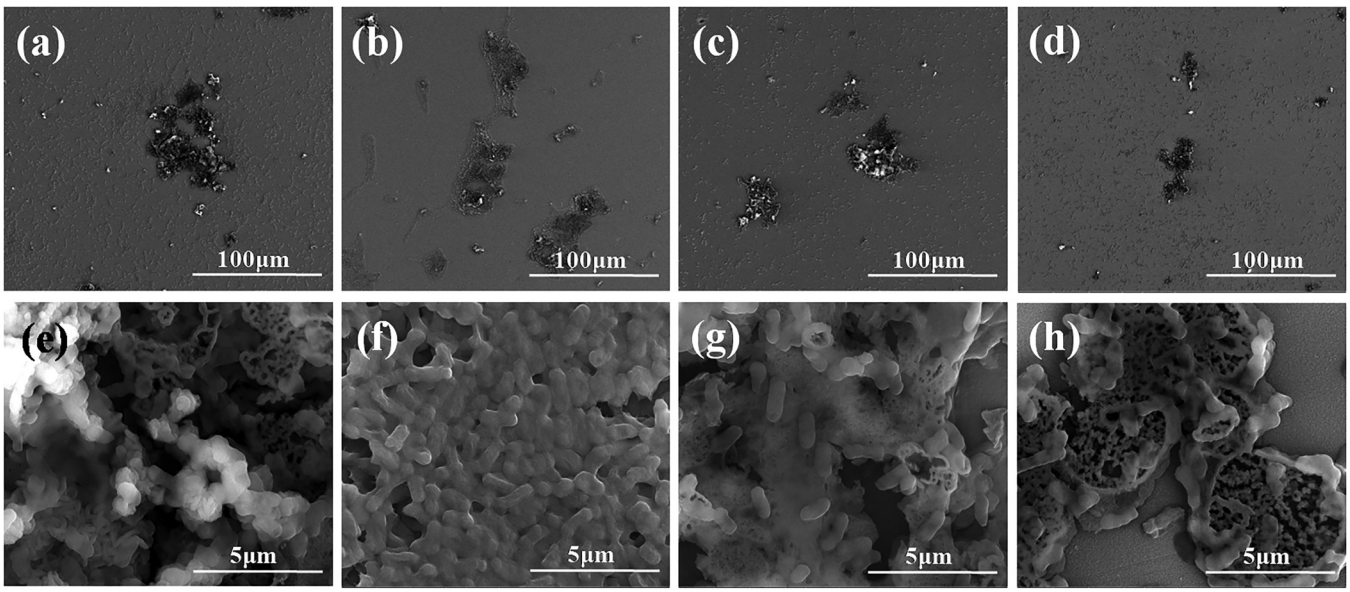

FIG. 8. SEM images of *E. coli* adherent on the samples treated with 0 [(a) and (e)], 100 [(b) and (f)], 200 [(c) and (g)], and 300 mg/l [(d) and (h)] surfactin solutions after one day of immersion [(a)–d: ×500; (e)–(h): ×1000)].

the biofilms were less thick and bacteria were partly embedded in the extracellular matrix [Figs. 8(f)–8(h)]. On the surface treated with 300 mg/l surfactin solution, it is clear that *E. coli* biofilms were disrupted and intracellular substances were exposed [Fig. 8(h)]. Biofilm quantification using crystal violet staining showed that surfactin conditioning layers effectively inhibited *E. coli* biofilm formation, with an average reduction of 51.4%, 28.8%, and 65.5%, respectively (Fig. 9). It is still unclear as to how the molecular structure of surfactin contributes to its antibacterial activities, but it is widely agreed that surfactin kills bacteria by disrupting the bacterial cell membrane. One possible mechanism is that the nonpolar fatty acid chain of surfactin inserts into the phospholipid bilayer while the heptapeptide ring remains in the liquid phase, causing a detergentlike permeabilization effect.[63,64] The phenomenon observed in Fig. 8(h) proved that surfactin ruptured the cytoplasmic membrane of *E. coli* and caused cell lysis.

From the results demonstrated above, it can be deduced that γ-PGA and surfactin both contributed to the inhibition of *E. coli* adhesion. However, these are not the only functional molecules accounting for the antifouling properties of *B. subtilis* EPS. For example, TasA is a major protein component of *B. subtilis* biofilm matrix with antibacterial properties.[33] *B. subtilis* can also produce a variety of antibacterial compounds including fengycin, iturin, mycosubtilin, and bacillomycin that may be present in EPS.[65] Further investigations are needed for the antibacterial tests of *B. subtilis* EPS against a wide spectrum of bacterial species, as well as the identification of other antibacterial components. *B. subtilis* has been widely applied as food and feed additives due to its superior antibacterial properties and biosafety.[41,42] However, using live bacteria for antifouling purposes is impracticable. Our study has demonstrated the antifouling properties of *B. subtilis* EPS against *E. coli* adhesion. Therefore, *B. subtilis* EPS have the potential to be developed into a novel interfacial antifouling additive with easy preparation, nontoxicity, and environmental friendliness.

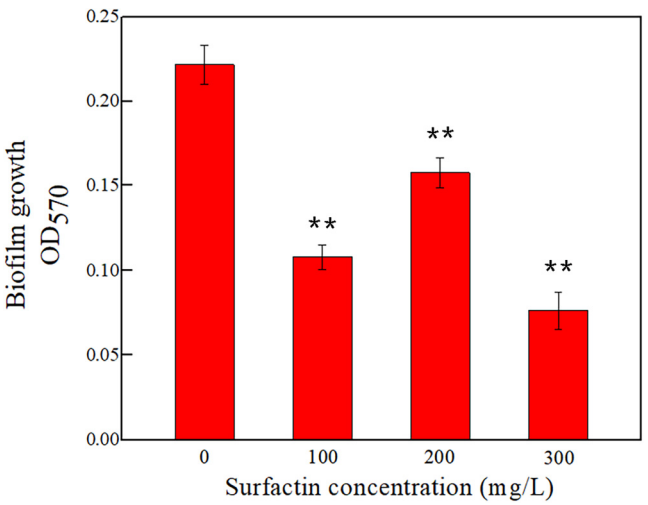

FIG. 9. Biofilm quantity of *E. coli* on samples with/without surfactin conditioning layers after one day of immersion. Error bars are shown as ±SD. *$p < 0.05$; **$p < 0.01$.

IV. CONCLUSIONS

In this study, the EPS secreted and released into the culturing media by *B. subtilis* were extracted to prepare conditioning layers on smooth, inert surfaces. The morphologies of the preadsorbed EPS conditioning layers were characterized using AFM. The subsequent adhesion and growth of *E. coli* on EPS-conditioned inert surfaces were investigated. To further explore the underlying mechanism of how EPS conditioning layers affected *E. coli* adhesion and biofilm formation, the respective roles of two major constituents of *B. subtilis* EPS (γ-PGA and surfactin) were investigated. The results show that the EPS of *B. subtilis* preadsorbed on smooth mica surfaces formed porous layers or interconnected networks, depending on the concentration of the EPS solutions. The EPS conditioning layers pronouncedly inhibited *E. coli* adhesion to silicon surfaces. Surfactin and γ-PGA both contributed to the inhibition of *E. coli* adhesion. Thus, *B. subtilis* EPS have the potential to be developed into a novel interfacial antifouling additive.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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