## SUPPLEMENTARY MATERIALS

## MATERIALS AND METHODS

**Bacterial Isolation, Identification and Growth Condition.** To isolate cultivable heterotrophic bacteria from different water treatment environments, single colonies on R2A agar plates were isolated and transferred to new plates based on their appearance and growth rate. Single colonies on R2A agar plates were then inoculated into R2A broth and shaken at 180 rpm at 28 °C overnight. Then the cultures were stocked with glycerol at -80 °C for further use. More than 70 bacterial strains were isolated from these water treatment environments, including source water, tap water, biofilms attached to the activated granular carbon in a full-scale drinking water biofilter (Pinghu, China), drinking water biofilms attached to the pipeline in a drinking water distribution system (Ningbo, China), and granule sludge in a simulated reactor treating artificial wastewater.

The identification of isolated strains was performed through determining the 16S rDNA sequence. After DNA extraction of the isolates, 16S rDNA was amplified with universal primers 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-TAC GGH TAC CTT GTT ACG ACT T-3'). PCR was performed in a thermocycler (Eppendorf Mastercycler ep Systems, Eppendorf AG, Hamburg, Germany) and the reaction occurred in 35 cycles with 1 min denaturation at 95 °C, 50 sec annealing at 50 °C and 50 sec extension at 72 °C, after a previous step of denaturation (95 °C for 5 min) and followed by a final extension step (72 °C for 5 min). The PCR products were purified using gel extraction Kit (Axygen, USA) and the 16S rDNA sequence was done using an auto-sequencer (ABI model 377A, Perkin-Elmer, Foster City, CA, USA). For each strain, the partial 16S rDNA sequence was assembled using BioEdit Sequence Alignment Editor (version 6.0.5), and then the sequences were compared with National Centre for Biotechnology Information GenBank entries using BLAST algorithm.

Thirteen strains, including the inhibition bacterial strain (*Bacillus* sp. SW9) that cannot form biofilm, and twelve bacteria strains with strong biofilm-forming ability, were used in this study (Table 1). When the stains were tested, glycerol stocks of the isolates were first streaked on R2A agar plates. Then, a single colony was inoculated into R2A broth, and cultures were incubated at 28 °C on an orbital shaker at 180 rpm overnight.

**Culture Supernatants and Capsular EPS Preparation.** *Bacillus* sp. SW9 was grown in R2A broth with shaking at 28 °C. After overnight incubation, cultures were in stationary phase ($OD_{600}$ of 0.35). Bacteria cells were separated from the culture broth by centrifugation at 4,000 g for 20 min at room temperature. The supernatant was then filtered through a 0.22-μm filter and kept at -20 °C. The cell pellets were then washed twice and resuspended in 0.9 % NaCl solution. Capsular EPS was chemically extracted according to the method described by Liu and Fang (2002) with some modifications. Sample was first combined with 1 N NaOH for 1 hr at 4 °C to raise the pH to 11. It was then centrifuged at 20,000 g for 20 min at 4 °C, and filtered through a 0.22-μm membrane to remove microbial cells. The obtained supernatant was the solution of the capsular EPS; it was kept at -20 °C.

**Liu H, Fang HHP.** 2002. Extraction of extracellular polymeric substances (EPS) of sludges. J. Biotechnol. **95** (3):249-256.

**Biofilm Formation Experiments.** Static biofilm formation in the wells of microtiter plates was performed as previously described (Stepanović *et al.*, 2000). In brief, following their overnight cultures, all biofilm-forming bacteria and inhibitor *Bacillus* sp. SW9 were diluted with fresh

sterile medium to $OD_{600}$=0.001. Biofilm assays were conducted in two different experimental set-ups: (i) in 96-well polystyrene plates (Costar, Cambridge, MA, USA), where each well was filled with 100 μl diluted cultures and 100 μl medium or inhibitor cultures; (ii) in 24-well polystyrene plates (Costar, Cambridge, MA, USA) containing polyethylene terephthalate (PET) cell culture inserts of 0.4 μm or 8.0 μm pore size (Millipore, Billerica, MA, USA); each well was separated into upper and lower rooms, in which 800 μl diluted cultures were added to the bottom chambers and 200 μl medium or inhibitor cultures were added to the top chambers. The former set-up presented a simple and efficient method for quantification of biofilm biomass, and the latter ensured effective monitoring of the effect of cell-cell contact on biofilm formation. The plates were incubated at 28 °C for 24 h without shaking for biofilm growth, and non-adherent bacteria were washed twice with sterile phosphate buffer solution (pH 7.5). Biofilms on the 96-well or 24-well microtiter plates were stained with 200 μl or 800 μl of 0.1% (w/v) crystal violet solution for 20 min, respectively, and excess stain was washed twice with tap water. The crystal violet was dissolved with 100 μl or 600 μl of 95% (v/v) ethanol for 20 min, respectively, and biofilm mass was determined by measuring its absorbance at 590 nm using a microtiter plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA). At least four replicates were conducted for each sample, and each experiment was performed at least three times.

**Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M.** 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Meth. **40**(2):175-179.

**Scanning Electron Microscopy (SEM).** For scanning electron microscopy (SEM), biofilm formation was initiated on sterile plastic coupons in 24-well microtiter plates by dispensing a cell suspension that contained biofilm-forming bacteria and medium or inhibitor. The coupons were removed after 24 h and washed twice in sterile PBS. The biofilms were placed in fixative (2.5% (vol/vol) glutaraldehyde in PBS) for 1 h at room temperature and rinsed twice in sterile PBS. Samples were subsequently dehydrated in a series of ethanol washes (25% ethanol for 30 min, 50% ethanol for 30 min, 75% ethanol for 30 min, 95% ethanol for 30 min, and 100% ethanol for 1 hr). Finally, all samples were dried to critical point, coated with gold, and visualized under a Field Emission Scanning Electron Microscope (FE-SEM, S-4800, Hitachi, Japan).

**Statistical Analysis.** The data were analyzed using the one-way ANOVA with t-test. A *p* value of 0.001 or less was considered to be statistically significant.

## SUPPLEMENTARY TABLES

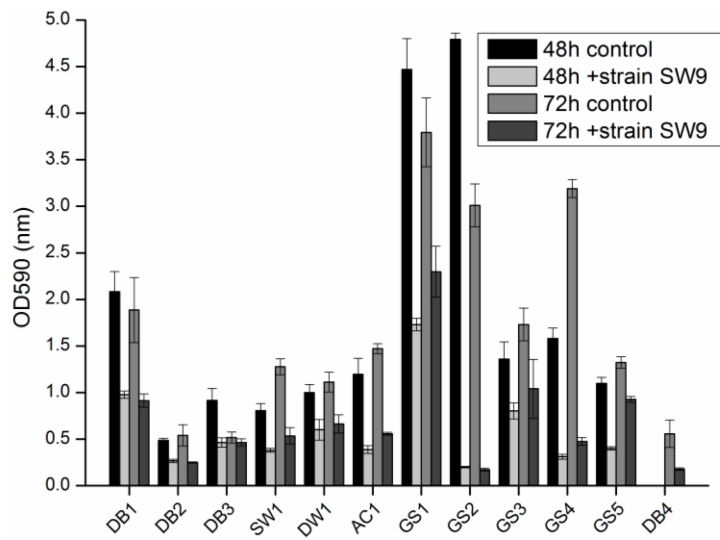
**Supplementary Table 1.** Biofilm Inhibition by *Bacillus* sp. SW9 and its Supernatant and Capsular EPS

| Type | strains | *Bacillus* sp. SW9 | supernatant | capsular EPS |
|---|---|---|---|---|
| | | Biofilm inhibition (%) | | |
| Type I | AC1 | 65.0 | 45.8 | -8.0 |
| | GS2 | 90.2 | 68.7 | -1.3 |
| | GS4 | 65.5 | 71.9 | -2.2 |
| Type II | DB1 | 75.2 | 6.2 | 54.2 |
| | GS1 | 89.9 | 7.3 | 68.9 |
| | GS5 | 61.8 | 21.0 | 64.6 |
| | DB4 | 61.7 | 7.4 | 46.0 |
| Type III | DB2 | 57.0 | -26.0 | -9.6 |
| | DB3 | 58.3 | -20.0 | 25.7 |
| | SW1 | 57.3 | 10.3 | -21.2 |
| | DW1 | 57.7 | 5.00 | -6.5 |
| | GS3 | 65.3 | 21.0 | -14.9 |

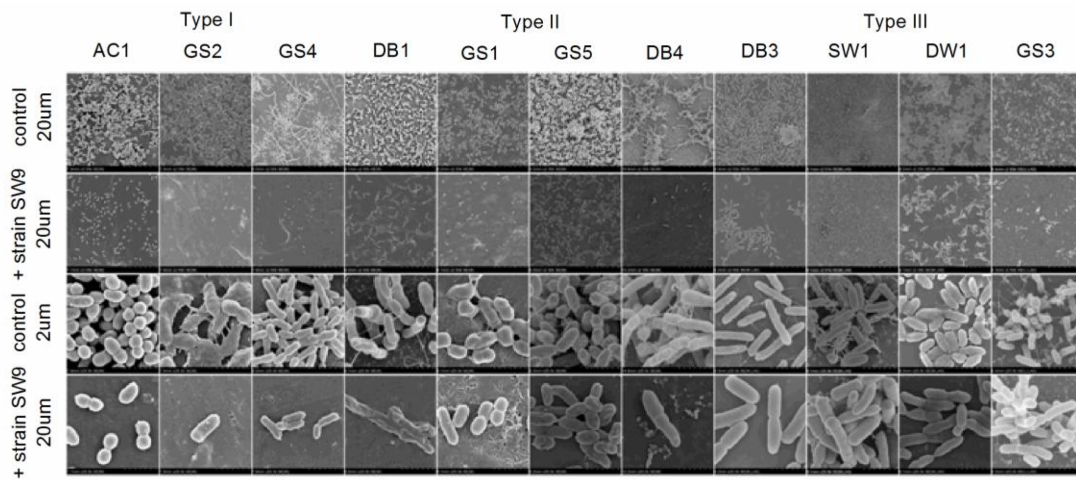
**Supplementary Table 2.** Biofilm Inhibition by *Bacillus* sp. SW9 with or without Polyethylene Terephthalate (PET) Membrane

| Type | strains | *Bacillus* sp. SW9 | 0.4 μm membrane | 8.0 μm membrane |
|---|---|---|---|---|
| | | Biofilm inhibition (%) | | |
| Type I | AC1 | 81.7 | 63.6 | 82.9 |
| | GS2 | 93.2 | 68.9 | 92.4 |
| | GS4 | 79.4 | 70.6 | 79.2 |
| Type II | DB1 | 67.1 | 40.7 | 69.6 |
| | GS1 | 64.6 | 35.5 | 73.8 |
| | GS5 | 83.0 | 85.3 | 84.3 |
| | DB4 | 46.8 | 39.7 | 46.3 |
| Type III | DB2 | 53.8 | -31.8 | 58.1 |
| | DB3 | 76.6 | 2.3 | 80.3 |
| | SW1 | 62.5 | 8.5 | 66.0 |
| | DW1 | 58.3 | 5.4 | 63.9 |
| | GS3 | 40.3 | -1.7 | 30.3 |

## SUPPLEMENTARY FIGURES




**Supplementary Figure 1.** Effect of *Bacillus* sp. SW9 on bacterial biofilm formation (48 or 72 h incubation).




**Supplementary Figure 2.** SEM micrographs of biofilms developed by biofilm-forming strains with or without *Bacillus* sp. SW9 on PVC surfaces.