The following supplement accompanies the article

Bacterial enhancement of gel particle coagulation in seawater

Yosuke Yamada, Hideki Fukuda, Yuya Tada, Kazuhiro Kogure, Toshi Nagata*

*Corresponding author: nagata@aori.u-tokyo.ac.jp

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SUPPLEMENT

Preparation of gel particles

The gel particles were prepared according to Nakamura et al. (2008) with modifications as described by Yamada et al. (2013). Fucoidan was extracted from brown seaweed, *Kjellmanialla crassifolia* (Gagomekombu). Gagome-kombu powder (10 g; Toyonaka Matumae Konbu Honpo Corp., Osaka, Japan) was washed with 100 mL 70% (v v^{-1}) ethanol containing 0.1 M acetic acid and boiled in 200 mL 8.8 µM $CaCl_2$ solution for 30 min. The suspension was centrifuged (2330 × g) and the supernatant was collected. After the addition of 2 × volume of 99.5% ethanol to the supernatant, the precipitated materials were collected on polycarbonate (PC) filters (10 µm pore size, 47 mm diameter; Whatman, Maidstone, UK). Next, the precipitates were dissolved in 0.9% NaCl (w v^{-1}) solution containing 10 mM EDTA-2Na. After the removal of undissolved precipitates by filtration, the filtrate was mixed with 2 × volume of 99.5% ethanol and centrifuged (2330 × g) again. Finally, the precipitates (fucoidan extracts) were air-dried in a flow hood. Fucoidan solution (2.1% w v^{-1}) was prepared by dissolving 16.6 mg fucoidan extract in 790 µL Milli-Q water (Millipore Corp., MA, USA). Chitosan solution (3.8% w v^{-1}) was prepared by dissolving 1.25 g chitosan (LL-40, extracted from snow crab, *Chionoecetes opilo*, nominal deacetylation rate: >80%; Yaizu Suisankagaku Industry, Shizuoka, Japan) in 33.1 mL 190 mM HCl.

The fucoidan and chitosan solutions were centrifuged at 1200 × g for 10 min at 23°C and successively filtered through 0.8 µm and 0.2 µm syringe filters (25 mm diameter, Acrodisc syringe filter with Supor® membrane; Pall Corp., NY, USA) to remove residual particles. Then, 210 µL chitosan and 790 µL fucoidan solutions were added to 50 mL Aclv-$FSW_{0.2}$ and mixed in a 50 mL polypropylene tube (BD Falcon Conical Centrifuge tube; Thermo Fisher Scientific Inc., MA, USA). At this stage, the formation of gel particles was observed. After storage of the suspension at 4°C for 3 h, gel particles that settled at the bottom of the tube were collected by removing the supernatant by aspiration (~10 mL solution remaining). Then, 40 mL Aclv-$FSW_{0.2}$ was added to the tube. The gel particle suspension was filtered through a nylon mesh (Nytal 7XX-200, mesh size 200 µm; Sefar AG, Heiden, Switzerland) to remove large particles. A 50 mL aliquot of gel particle suspension was diluted 10-fold by Aclv-$FSW_{0.2}$, autoclaved for 15 min at 120°C, and stored for up to 12 h at 20°C until their use in incubation experiments (in the main text, this suspension is referred to as “gel particle suspension”).

Incubation experiments using *Pseudoalteromonas* spp. isolates

The design of the incubation experiments was similar to that described for Exps. 1–5, except for the following aspects: (1) The gel particle suspension enriched with phosphorus (P) was prepared using artificial seawater (Cavanaugh 1975) rather than coastal seawater. (2) To prepare bacterial inocula, the following pretreatments were conducted: Isolates were grown on marine broth agar plates at 20°C. Bacterial cells were transferred from each plate to 1 mL of the gel particle suspension enriched with P. This bacterial suspension (100 µL) was transferred to a 50 mL gel particle suspension enriched with P and

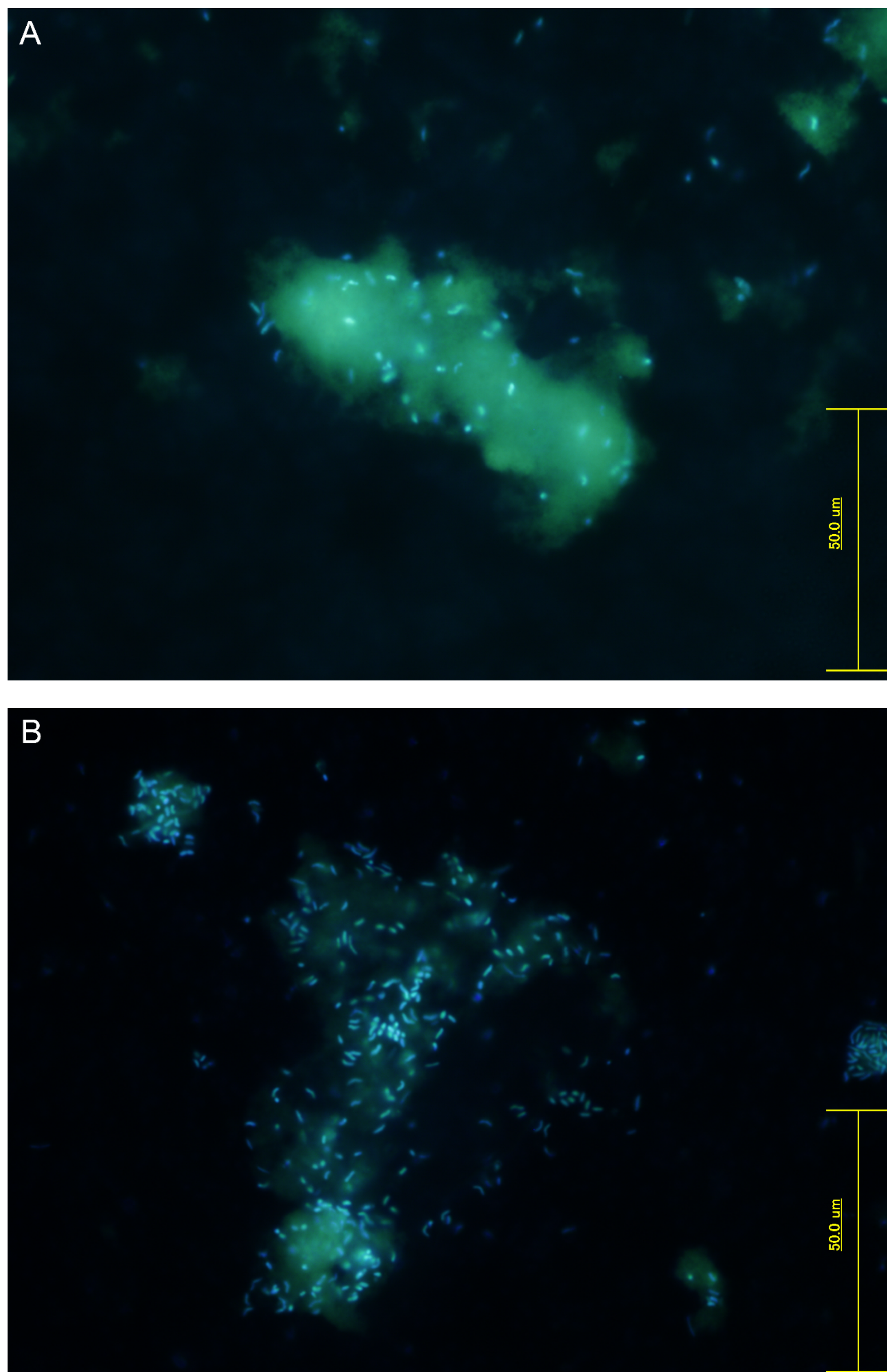
incubated at 20°C in the dark for 1–2 days. This pre-incubated bacterial suspension was used as an inoculum and was added to a rotating tube containing the gel particle suspension enriched with P. The abundance of bacterial cells was adjusted to lie in the range of 10^3–10^4 mL^{-1}. The initial cell concentrations varied presumably because we pre-incubated the cells in the gel particle suspension (as opposed to liquid media with dissolved organic substrate) with large spatial heterogeneities in bacterial cell distribution, which resulted in large variability in cell abundance in small volume inocula. However, we consider that this did not alter our general conclusion of aggregate formation by bacterial isolates because the incubation period (3–4 days) was sufficiently long and each bacterial strain was able to grow to reach its apparent maximum level (or plateau), except for *P. ruthenica*. (3) A sterile control was prepared using an autoclaved, pre-incubation culture of *P. citrea*. (4) Seawater subsamples were filtered through a 0.2-mm-pore-size PC filter to prepare slides for determination of total bacterial abundance. It should be noted that the abundances of isolates reached a maximum after 3 days of incubation, except for *P. undina* (which reached its maximum abundance after 4 days of incubation, data not shown), and that we judged the extent of their effects on gel particle enlargement until 4 days of incubation.

Table S1. Oligonucleotide sequences used as catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) probes (Loy et al. 2007).

Probe	Target	Probe sequence (5'–3')	FA* (%)
Eub338-I	Bacteria	GCTGCCTCCCGTAGGAGT	35
Eub338-II	Supplement to Eub338	GCAGCCACCCGTAGGTGT	35
Eub338-III	Supplement to Eub338	GCTGCCACCCGTAGGTGT	35
Non338	Negative control	ACTCCTACGGGAGGCAGC	20
Alf968	*Alphaproteobacteria*	GGTAAGGTTCTGCGCGTT	20
SAR11-441	SAR11 clade	GGACCTTCTTATTCGGGT	25
ROS537	*Roseobacter* clade	CAACGCTAACCCCCTCC	35
Gam42a†	*Gammaproteobacteria*	GCCTTCCCACATCGTTT	35
Alt1413	*Alteromonas/Colwellia*	TTTGCATCCCACTCCCAT	40
PSU730	*Pseudoalteromonas*	TTGACCCAGGTGGCTGCC	40
CF319a	*Bacteroidetes*	TGGTCCGTGTCTCAGTAC	35

*Formamide (FA) concentration in CARD-FISH hybridization buffer.
†Including an unlabelled competitor probe Bet42a (5'-GCCTTCCCACTTCGTTT-3') (Manz et al. 1992).

Figure S1. Bacteria attached on gel particles in Exp. 4 (bacteria-addition treatment [P-enriched]). Samples collected after the incubation period of 24 h (A) and 96 h (B) were examined under the epifluorescence microscope after DAPI-staining (see text for the method of sample preparation). The images were captured using a charge-coupled device camera (Olympus DP70; Olympus, Tokyo, Japan). Scale bars are 50 µm. Bright blue dots and rods are bacteria and pale yellow matrices are gel particles.



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