Highly Sensitive Detection and Quantification of Dissolved Free Extracellular DNA Using Colloid Adsorption and Foam Concentration

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Supplementary material

Calculation of recovery rates

The exDNA and target gene recovery rates were calculated using equations (1) and (2). In sections 2.3.3 and 2.3.4, the recovery rate was calculated using equation (1). Here C_1 is the DNA concentration after enrichment, C_2 is the DNA concentration in the artificial river water after adding exDNA stock solution, V_1 is the volume after enrichment (60 µL), and V_2 is the volume before enrichment (10 mL). In section 2.4, the recovery rate was calculated using equation (1). Here, C_1 is the DNA concentration after enrichment, C_2 is the DNA concentration in the exDNA stock solution, V_1 is the volume after enrichment (400 µL), and V_2 is the volume of exDNA stock solution added (100 µL).

In section 2.3.5, the recovery rate was calculated using equation (2), in which C_3 is the copy number of *uidA* after enrichment, C_4 is the copy number of *uidA* in the artificial river water after adding exDNA stock solution, V_3 is the volume after enrichment (30 µL), and V_4 is the volume before enrichment (10 mL). In section 2.4, the recovery rate was calculated using equation (2), where C_3 is the copy number after enrichment, C_4 is the copy number in the artificial river water after adding the exDNA stock solution, V_3 is the volume after enrichment (400 µL), and V_4 is the volume before enrichment (1,000 mL).

$$\text{exDNA recovery rate (\%)} = \frac{C_1}{C_2} \times \frac{V_1}{V_2} \times 100 \qquad (1)$$

$$\text{Target gene recovery rate (\%)} = \frac{C_3}{C_4} \times \frac{V_3}{V_4} \times 100 \qquad (2)$$

Text S1

Recovery of exDNA with the foam concentration method (referencing Jikumaru et al. (2020))

100 µL of exDNA stock solution was added to 1,000 mL of sterile artificial river water. The sterile artificial river water was rapidly mixed with ferric chloride (5 mg Fe/L) at 150 rpm for 3 min using a jar tester. Casein stock solution (10 g/L) was added to the water at 10 mg/L and rapidly mixed at 150 rpm for 1 min. Foam separation of the water sample was performed by transfer into a cylindrical column. Dispersed air was supplied from the bottom of the column via a glass-ball filter at 0.3 L/min for 5 min to generate foam. The foam generated on the water surface was drawn into a trap bottle using an aspirator. A vortex mixer defoamed the recovered foam. The recovered foam was transferred into a 50-mL centrifuge tube and centrifuged at 4,000 rpm for 5 min. Iron colloids that formed at the bottom of the tubes were recovered. exDNA was extracted from the recovered colloids using the DNeasy PowerWater Kit (Qiagen). The DNA concentration of the recovered solution (100 µL) was measured with a Quantus Fluorometer (Promega) to calculate the exDNA recovery rate.

Text S2

Recovery of exDNA from iron colloids (referencing Suzuki et al. (2016))

100 µL of exDNA stock solution was mixed with 10 mL of sterile artificial river water. Ferric chloride was added at a concentration of 10 mg Fe/L and mixed for 3 min. The iron hydroxide colloids were centrifuged at 4,000 rpm for 5 min using a tabletop centrifuge. After discarding the supernatant, the precipitated colloids were dissolved using DFOM (100 µL, 0.076 M). DNA concentrations in 10-fold diluted lysates were measured with a Quantus Fluorometer and dPCR to calculate the exDNA recovery rate. In dPCR, the target gene was uidA, and the recovery rate of the uidA gene was calculated. These experiments were performed in triplicate.

Text S3

Adjustment of each colloidal solution.

Ferric chloride (39% FeCl3, Takasugi Pharmaceutical, Fukuoka, Japan) was adjusted with 0.01 M HCl to a final concentration of 10,000 mg/L. Aluminum sulfate (Wako Pure Chemical Industries) was adjusted in 0.01 M HCl to a final concentration of 25 g Al2O3/L. PAC (AsahiKASEI Advance, Miyazaki, Japan) and aluminum hydroxide (Wako Pure Chemical Industries) were adjusted with distilled water to a final concentration of 10,000 mg Al/L.

Text S4

Adsorption and dissolution of exDNA with iron colloids

100 µL exDNA stock solution was mixed with 10 mL sterile artificial river water. Ferric chloride was added at a concentration of 10 mg Fe/L and mixed for 3 min. The formed iron hydroxide colloids were centrifuged at 4,000 rpm for 5 min with a tabletop centrifuge (Kokusan, Saitama, Japan). After discarding the supernatant, the precipitated colloids were dissolved in HCl (0.01 M, 2,000 µL; 0.1 M, 200 µL; 0.5 M, 100 µL; 1.0 M, 50 µL), acetic acid (300 µL), and ascorbic acid (300 µL). After confirming that all colloids were dissolved, DFOM (200 µL) or EDTA (30 µL) was immediately added. Fe^{3+} was masked to prevent re-colloid formation due to increased pH. The pH was adjusted to nearly neutral with 10× TE and NaOH, and all samples were purified with the ReliaPrep DNA Clean-Up and Concentration System (Promega, Madison, WI, USA). The DNA concentration of the purified solution was measured with a Quantus Fluorometer (Promega), and the exDNA recovery rate was calculated. These experiments were performed in triplicate.

Text S5

Adsorption and dissolution of exDNA with aluminum colloids

100 μL exDNA stock solution was mixed with 10 mL sterile artificial river water. PAC or aluminum sulfate was added to a final concentration of 10 mg Al/L and 50 mg Al_2O_3/L, respectively. After mixing for 3 min, the formed aluminum hydroxide colloids were centrifuged at 4,000 rpm for 5 min with a tabletop centrifuge (Kokusan). After discarding the supernatant, the precipitated colloids were dissolved in HCl (0.1 M, 200 μL) and NaOH (0.1 M, 200 μL; 1.0 M, 50 μL). After confirming that all colloids were dissolved, EDTA buffer (10 μL), CyDTA (50 μL), TTHA (200 μL), and DTPA (100 μL) was immediately added. The pH was adjusted to nearly neutral with 10× TE, HCl, and NaOH. All samples were purified with the ReliaPrep DNA Clean-Up and Concentration System (Promega). The DNA concentration of the purified solution was measured with a Quantus Fluorometer (Promega), and the exDNA recovery rate was calculated. These experiments were performed in triplicate.

Text S6

exDNA elution from aluminum colloids

100 μL exDNA stock solution was mixed with 10 mL sterile artificial river water. PAC was added at a concentration of 10 mg Al/L, and the mixture was combined for 3 min. Aluminum hydroxide was added at a concentration of 100 mg Al/L, and the mixture was mixed at 100 rpm for 10 min. The formed colloids were centrifuged at 4,000 rpm for 5 min. After discarding the supernatant, 1 mL of eluent was added, and the mixture was vortexed for 2 min. All eluents were filtered through a 0.45-μm PES syringe filter, and the filtrates were purified with the ReliaPrep DNA Clean-Up and Concentration System. The DNA concentration of the purified solution was measured with digital PCR (dPCR), and the exDNA recovery rate was calculated. These experiments were performed in triplicate.

Text S7

Elution of exDNA from iron colloids using EDTA as a chelating agent

100 μL of exDNA stock solution was mixed with 10 mL of sterile artificial river water. Ferric chloride was added at a concentration of 10 mg Fe/L and combined for 3 min. The iron hydroxide colloids were centrifuged at 4,000 rpm for 5 min using a tabletop centrifuge. After discarding the supernatant, the precipitated colloids were dissolved using HCl (1.0 M: 50 μL). After confirming that all colloids were dissolved, EDTA was immediately added (3.34–33.4 μL). The quantity of EDTA was set to 1–10 times the quantity of iron in molar mass. The pH was adjusted to near neutral by adding 10× TE (11.5–14.8 μL) and NaOH (1.0 M: 50 μL). The samples were purified using the ReliaPrep DNA Clean-Up and Concentration System (Promega). The DNA concentration of the purified solution was measured with a Quantus Fluorometer (Promega) to calculate the exDNA recovery rate. These experiments were performed in triplicate.

Text S8

Damage rate of exDNA by HCl

In this method, exDNA is exposed to a strong acid solution during the dissolution of exDNA from iron colloids, which may damage the exDNA. Therefore, we investigated the damage rate of exDNA by colloidal dissolution using HCl. To exDNA (100 μL), 0.1, 0.5, and 1.0 M HCl (1,500, 400, and 200 μL) was added and mixed, then DFOM (840 μL), 10× TE (280, 190, and 140 μL), and 1.0 M NaOH (150, 200, and 200 μL) were added. All samples were purified with the ReliaPrep DNA Clean-Up and Concentration System (Promega). The DNA concentration of the purified solution was measured by Quantus Fluorometer to calculate the recovery rate. These experiments were performed in triplicate.

Table S1

Recovery rate of exDNA by previous methods. Following the method by Jikumaru et al., 2020 (top) and Suzuki et al., 2016 (bottom).

	exDNA recovery efficiency（n = 1）
Fluorometer	1.02 %

	exDNA recovery efficiency（mean ± SD, n=3）
Fluorometer	0.43±0.31 %
dPCR	0.035±0.0054 %

Table S2

Primers, probes, and temperature for target genes

Bacteria	Target genes	primers, probes	Sequence (5'-3')	μM	Thermal conditions	References
E. coli	*uidA*	*uidA* - f	CGGAAGCAACGCGTAAACTC	0.9	95°C, 10 min > 95°C, 20 sec > 60°C, 1 min; 50 cycles	Silkie et al. (2008)
		uidA - r	TGAGCGTCGCAGAACATTACA	0.9		
		uidA - pro	FAM-CGCGTCCGATCACCTGCGTC-TAMRA	0.3		
E. coli	*tetA*	*tetA* - f	CTTCATGAGCGCCTGTTTCG	0.9	95°C, 30s > 95°C, 15 sec > 57°C, 1 min; 40 cycles	this study
		tetA - r	TGGACAACATTGCTTGCAGC	0.9		
		tetA - pro	FAM-CTCAACCCGCTCGCTT-TAMRA	0.3		

Table S3

Library preparation conditions and sequencing results.

	DNA concentration		Library preparation		Library concentration		Sequencing result			
	Concentration (ng/ul)	Volume (ul)	Input DNA (ng)	Cycle	Concentration (ng/ul)	Volume (ul)	Pair reads	Read length (bp)	>=Q20(%)	>=Q30(%)
Effluent	57.9	50	200	–	6.5	20	39,120,645	15,648,258,000	88.0	73.4

Table S4

Recovery rate of exDNA from iron colloids using EDTA as a chelating agent. The EDTA/Fe ratio was varied to improve the masking effect.

	Dosage of EDTA（The quantity of EDTA was set to N times the quantity of iron in molar mass）									
N	1	2	3	4	5	6	7	8	9	10
exDNA recovery rate (%)	0	0.0198	0.0095	0.0084	0.0129	0.2950	0.0183	0	0	0.0016

Table S5

The concentration of dissolved free extracellular DNA (exDNA) in the effluent from a wastewater treatment plant.

	DNA concentration (μg/L, mean ± SD, n = 3)	
exDNA	Fluorometer	Spectrometer
	1.57 ± 0.23	3.63 ± 0.45

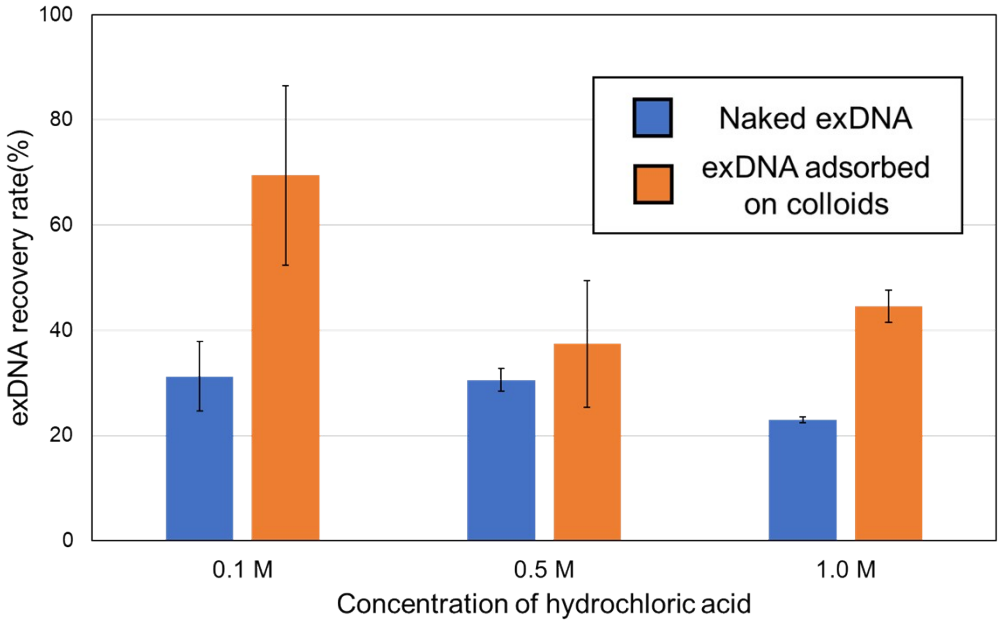

Fig. S1. Damage rate of exDNA by hydrochloric acid. Untreated exDNA (naked exDNA) and exDNA adsorbed on colloids were compared (The recovery of exDNA adsorbed on the colloids referring to Fig. 1 in section 3.1.).

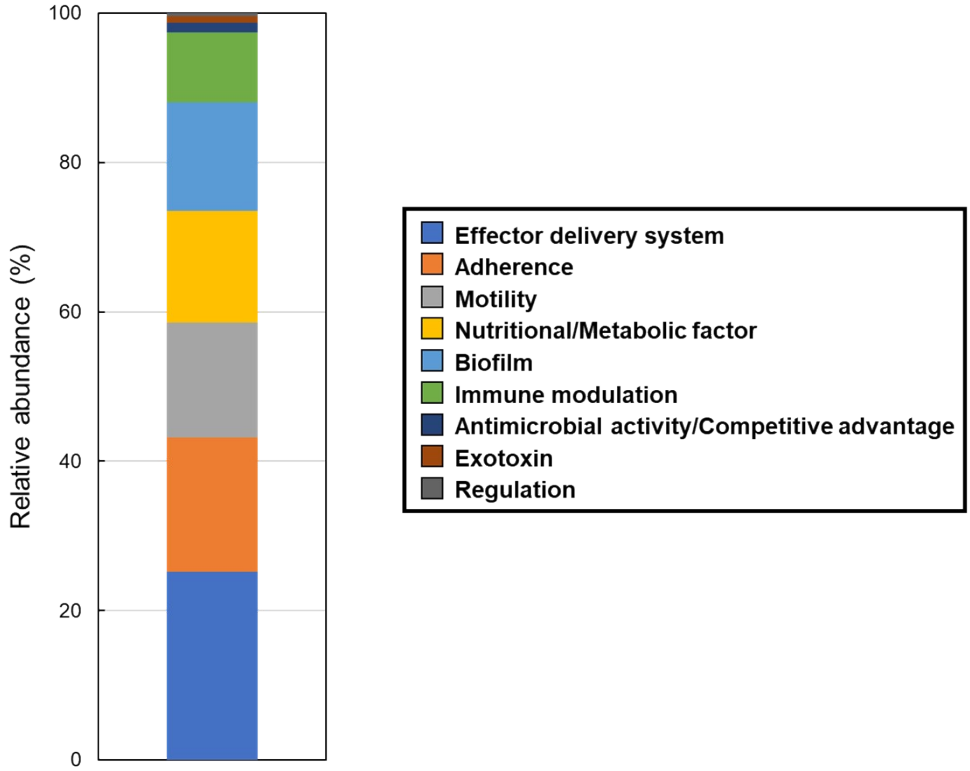

Fig. S2. Relative abundance of pathogenesis-related gene categories in exDNA.