Supporting Information:

Nanoplastics promote the dissemination of antibiotic resistance through conjugative gene transfer: implications from oxidative stress and gene expression

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Test 1. Detection of bacterial intracellular ROSs

ROS production of the donor and recipient bacteria were measured by using a ROS kit (St. Louis, MO, USA) according to the manufacturer's instructions. Briefly, the donor bacteria and recipient bacteria were resuspended in PBS and the OD600 of both bacteria solutions was adjusted to be 0.4. The value of OD600 was shown as the bacterial concentration. The donor bacteria or recipient bacteria were incubated with DCFH-DA at 37 ◦C for 30min in darkness. Then, the bacteria solutions were exposed to MNPs of different sizes. The concentrations of MNPs were 0.1, 1, 10 and 100mg/L, respectively. After incubation in darkness for 2h at 37 ◦C, the fluorescence intensity of samples was measured by a multifunctional microplate reader (synergy H1, BioTek, USA). The excitation wavelength was 488nm. The emission wavelength was 525nm. The ROS production was shown as the fluorescence intensity of the samples. Bacteria without MNPs were used as a control. All measurements were repeated for three times.

Test 2. Determination of cell membrane permeability

The donor bacteria and recipient bacteria were resuspended in PBS to a final concentration of 10^6 CFU/mL. Then, the bacteria solutions were exposed to different treatments. After incubation in darkness for 2 h at room temperature, 1 μL of PI (2 mM) was added into the above bacteria solution. Then samples were incubated in darkness for 15min. The cell membrane permeability was shown as the fluorescence intensity of the samples, indicating the ability of PI to enter the cells. The fluorescence intensity of each sample was measured at 488 nm excitation and 561 nm emission wavelength in a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA,

USA).

Test 3. Scanning electron microscopy

Before and after inactivation of *E. coli*, the cells were attached to the TiO_2 film slides and fixed by 3% glutaraldehyde for approximately 60 min at room temperature, then washed with 0.1 M cacodylate buffer (pH=7.4) for 10 min, and then fixed for 20 min in 1% osmium tetraoxide. Subsequently, the samples were dehydrated by a graded series of ethanol (70, 90 and 100%) and 100% amyl acetate 2 times each for 10 min, respectively. Finally, the cells on the substrates were critical point dried using critical point dryer (Denton Vacuum, Inc. USA), gold sputter coated on the substrates and were visualized using a field emission scanning electron microscope (FESEM, JEOL JSM-6300F).

Test 4. Detailed operation procedures for qPCR and the calculation of mRNA expression

The qPCR mixtures consisted of 12.5 μL of 2 × SYBR Premix Ex Taq (TaKaRa, Dalian, China), 0.75 μL of each primer (10 μM final concentrations), 1 μL of cDNA template, and 10 μL of distilled H_2O. The total volume was 25 μL. The thermocycling profile for the amplifications was 95 °C for 30 s, followed by 40 cycles of 95 °C for 45 s, 60 °C for 45 s, a melting curve analysis at 95 °C for 15 s, and, finally, annealing at 60 °C for 1 min. Each experiment was conducted at least in triplicate. The qPCR instrument was LightCycler® 96 qPCR System (Roche, Swiss). In addition, the mRNA expression levels of conjugation related-genes were calculated using the $2^{-\triangle\triangle CT}$ method as follows:

$\triangle Ct = Ct_{(Functional\ genes)} - Ct_{(16S\ rRNA\ gene)}$

$\triangle\triangle Ct = \triangle Ct_{(treatment)} - \triangle Ct_{(Control)}$

$FC(fold\ change) = 2^{-\triangle\triangle Ct}$

where the Ct value is the cycle threshold.

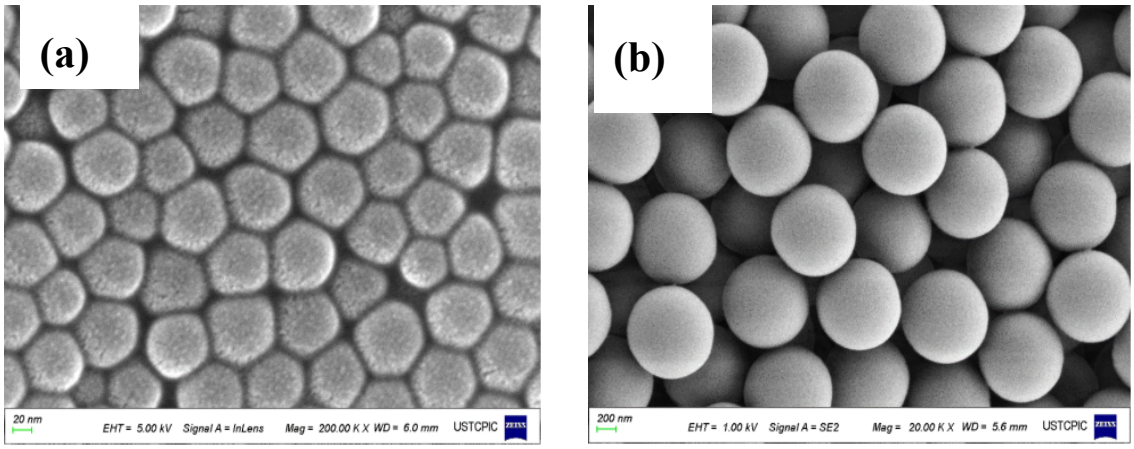

Fig S1. Images of scanning electron microscope (SEM) for polystyrene nanoplastics with a diameter of 80 nm (a) and 800 nm (b), respectively.

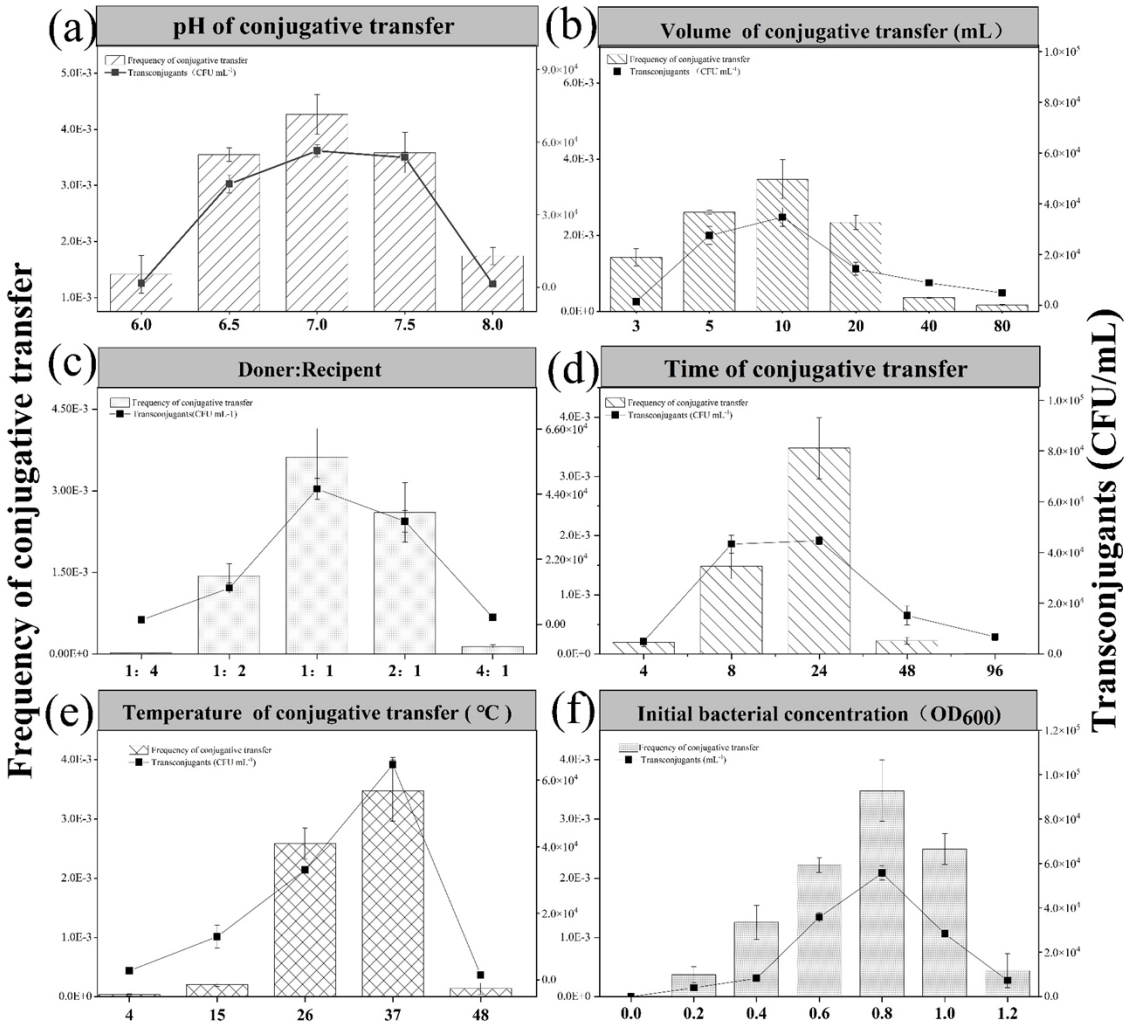

Fig. S2. Optimization of the conjugative transfer microcosms.

Table S1. Annealing temperature of 16S rRNA and 16 ARGs and other information

Gene	Primer	Sequence（5′-3′）	TM（℃）	Length of Products (bp)
16S rRNA	16S-F	CCTACGGGAGGCAGCAG	57.5	194
	16S-R	ATTACCGCGGCTGCTGG		
korA	*korA*-F	TCGGGCAAGTTCTTGTCC	60	147
	korA-R	GCAGCAGACCATCGAGATA		
korB	*korB*-F	CTGGTCGGCTTCGTTGTA	60	149
	korB-R	TGAAGTCACCCATTTCGGT		
trbA	*trbA*-F	TGGAAACTCCCCTACCTCTT	55	120
	trbA-R	CCACACTGATGCGTTCGTAT		
trbBp	*trbBp*-F	CGCGGTCGCCATCTTCACG	60	131
	trbBp-R	TGCCCGAGCCAGTACCGCCAATG		
traF	*traF*-F	GGCAACCTCGTCGCCTTTA	54.1	118
	traF-R	GCAAGTCGGCGTGTTTTCG		
trfAp	*trfAp*-F	GAAGCCCATCGCCGTCGCCTGTAG	55	183
	trfAp-R	GCCGACGATGACGAACTGGTGTG G		
traJ	*traJ*-F	GCCCGTGATTTTGTAGCCC	60	151
	traJ-R	TGAAACCAAGCCAACCAGGAA		
ompA	*ompA*-F	TGAGCCTGGGTGTTTCCTA	55	167
	ompA-R	CAGAGCAGCCTGACCTTCC		
ompF	*ompF*-F	GGTCTGCGTCCGTCCAT	60	99
	ompF-R	GGTTGCGCCCACTTCA		
ompC	*ompC*-F	AAGTAGTAGGTAGCACCAACATC A	50	163
	ompC-R	GGGCGAACAAAGCACAGAA		
rpoS	*rpoS*-F	TTTTACCACCAGACGCAAGT	60	184
	rpoS-R	GGAACTGTTATCGCAGGGAG		
oxyR	*oxyR*-F	AGCCAATGTTGCTGGCTATC	60	110
	oxyR-R	ACAGTGACCATCTTCCAGCA		
lexA	*lexA*-F	GGAAGAGGAAGAAGGGTTGC	60	103
	lexA-R	TCGGCTTGAATAAGGAAGGA		
recA	*recA*-F	CTACGCCTCTGTTCGTCTCG	60	100
	recA-R	TTTGTTCTTCACCACTTTCACG		
polB	*polB*-F	TTCTGATGCCAACCATTCG	60	153
	polB-R	GCTCCTGCTGAAACTGCTG		
RuvA	*RuvA*-F	AATGTCAGCGCAGCAGTTC	60	107

RuvA-R	TTTCAACAATCAAGCGTTCG