

Supplementary Materials

Table S1. Oligonucleotide primers.

The parts which serve as PCR primers for amplification of the cassette are underlined.

Description	Primer	Oligonucleotide sequence (5' to 3')	Fragment size (bp)
Fragment 1	pRed N part SacI-F	ATGAGCTCGCCATCAGCTCAACCTGTTGAT	469
	pRed N part Cm ^R -R	GATTTTTTTCTCCATACACGGTGCCTGACTGCGT	
Fragment 2	pRed N Cm ^R connected-F	GTCAGGCACCGTGTATGGAGAAAAAATCACTGG	688
	Cm ^R pRed C-R	CAACGCGGGGAGGCTTACGCCCCGCCCTGCCAC	
Fragment 3	Cm ^R pRed C connected-F	GCGGGGCGTAAGCCTCCCCGCGTTGCGTCG	253
	pRed C EcoRV-R	ATATGATATCATTCCTCCTGATCTCGAACCC	
Homology arms for blc1	FRT blc1 (upper oligo)	CTGGGTGTGGCATTGATTAACACAGCAGATAATTC GCAAATACTTTATCGA <u>AATTAACCCTCACTAAAGGGCG</u>	-
	FRT blc1 (lower oligo)	TAAACGTCGGCTCGGTACGGTCGAGACGGAACGT TTCGTCTCCCAGCTGTTAATACGACTCACTATAGGGCTC	
Homology arms for ompC	FRT ompC (upper oligo)	CTGACAACAAAGATGTAGATGGCGACCAGACCTAC ATGCGTCTTGGCTTCA <u>AATTAACCCTCACTAAAGGGCGG</u>	-
	FRT ompC (lower oligo)	TACTGAACAGCAAAGTTCAGGCCGTCAACCAGACC GAAGAAGTCAGTGTTAATACGACTCACTATAGGGCTCG	
Homology arms for ompF	FRT ompF (upper oligo)	CGTAACTACGGTGTGGTTTATGATGCACTGGGTTAC ACCGATATGCTGCCA <u>AATTAACCCTCACTAAAGGGCG</u>	-
	FRT ompF (lower oligo)	CAGGTAGATGTTGTTTCGCGTCATACTTCAGACCAGT AGCCCACTGTTTCAGTAATACGACTCACTATAGGGCTC	

Table S2. The MIC of β -lactam antibiotics against the β -lactam-susceptible *Escherichia coli* (RC85).

Antibiotics	MIC
	($\mu\text{g/mL}$) ^a
Ampicillin	8
Cefotaxime	$\leq 1/4$
Cefoperazone	$\leq 1/4$
Amoxicillin	4
Cefazolin	1
Cefalexin	4

^aMIC indicates minimum inhibitory concentration

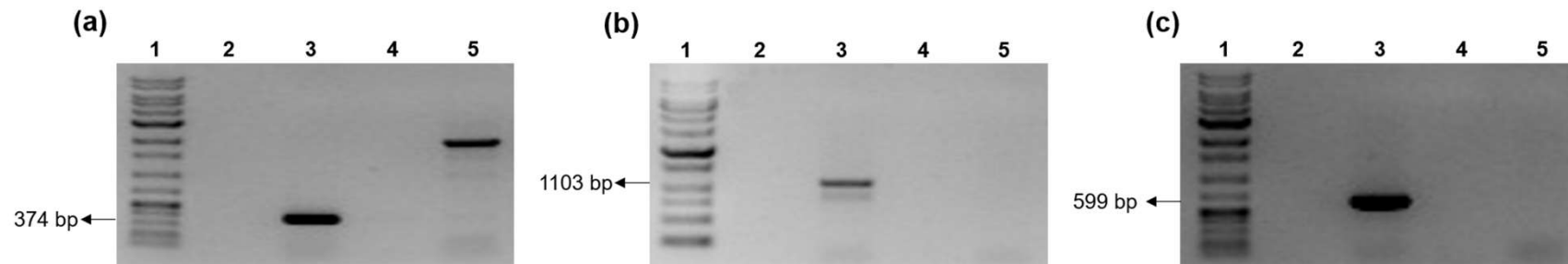


Figure S1. Gene deletion profile analysis in *blc1* (a), *ompC* (b), and *ompF* (c) using colony PCR followed by 1% agarose gel electrophoresis.

Lane 1: molecular weight marker; lane 2: DNA blank; 3: RC85⁺; 4: DNA blank; 5: mutant strain.

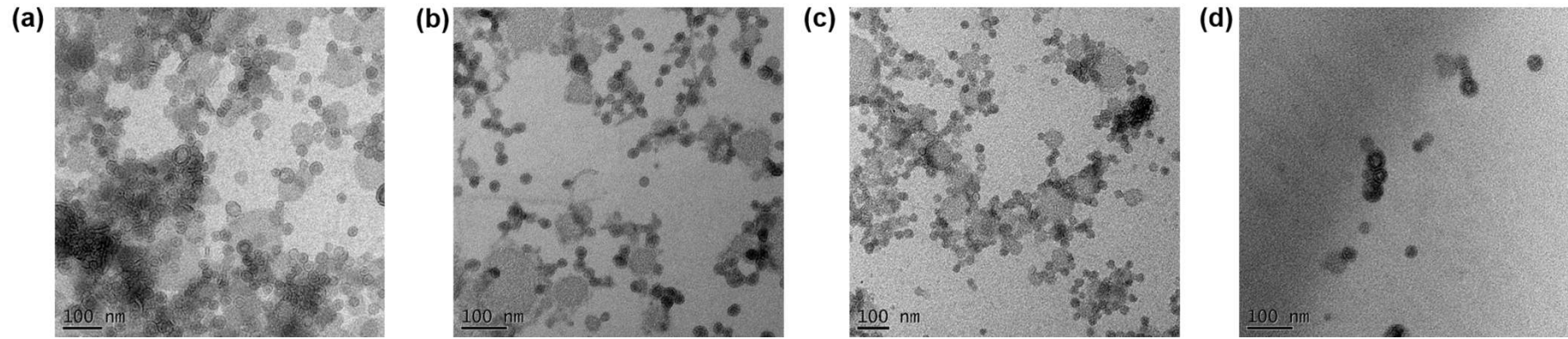


Figure S2. TEM images of OMVs released from RC85⁺ (a), Δ blc1 (b), Δ ompC (c), and Δ ompF (d) cells. Bar = 100 nm.

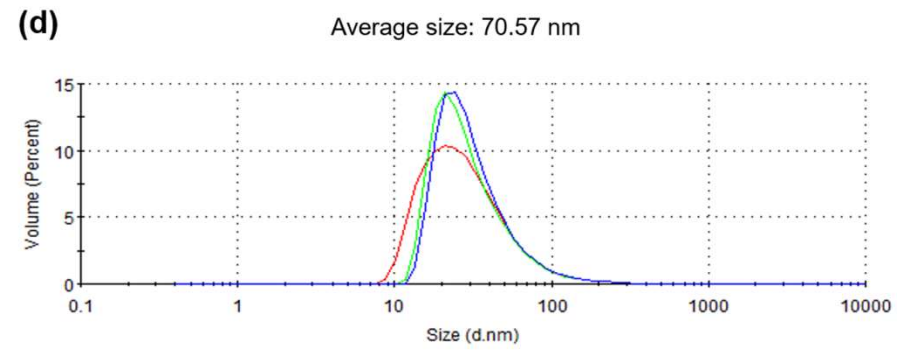
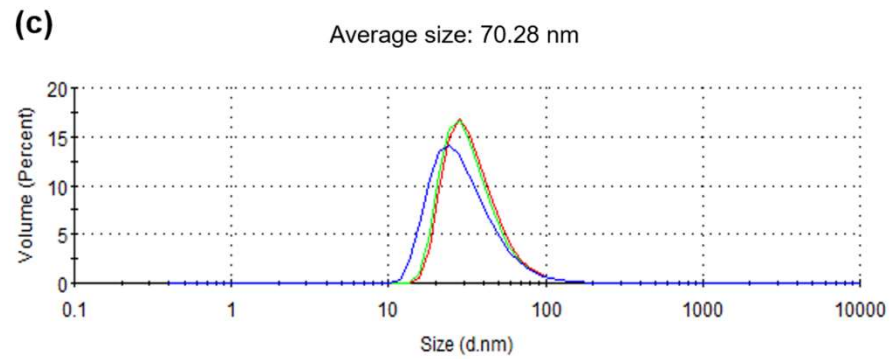
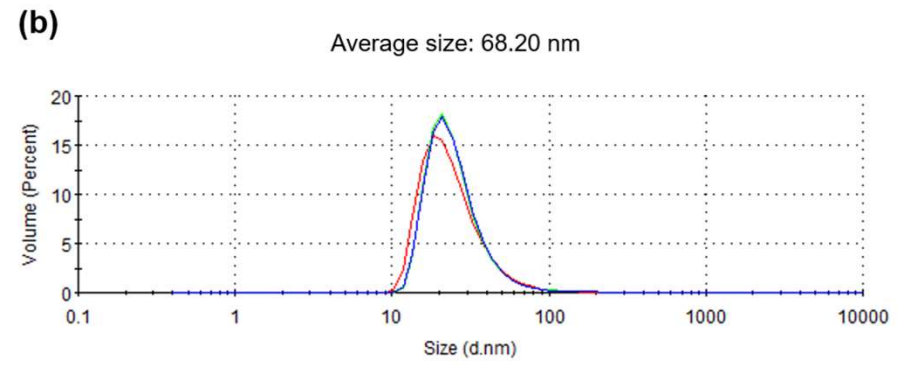
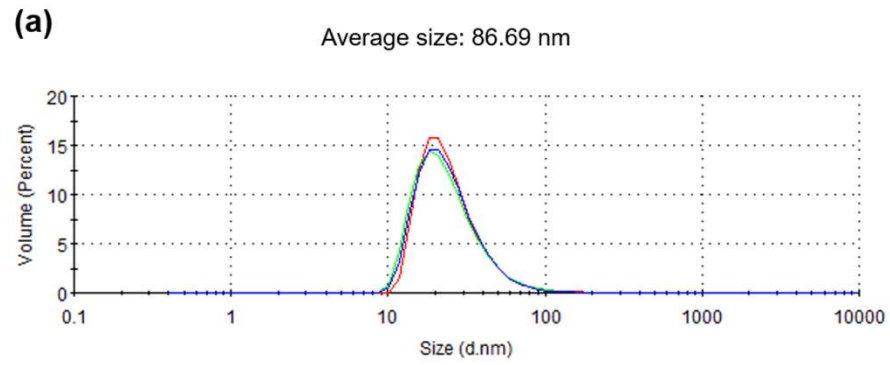


Figure S3. The size distribution OMVs derived from RC85⁺ (a), Δ blc1 (b), Δ ompC (c), and Δ ompF (d) cells, as assessed by Zeta-sizer. Three independent measurements were performed.