**SUPPORTING INFORMATION for**

# Cyanobacterial extracellular antibacterial substances could promote the spread of antibiotic resistance: impacts and reasons

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## Materials and methods

**Supporting material Text 1.** The method for CES extraction

The extraction method was referred to previous studies of Niu et al., with a little modification [1]. Briefly, after culturing *Nodosilinea* in MN liquid medium in a 14/10 h L/D cycle (light intensity $16 \pm 4\ \mu Em^{-2}\ s^{-1}$, approx.) for 21 days [2], approximately 100 L medium of *Nodosilinea* was collected by centrifugation (4 ℃, 6000 g, 15 min). Thereafter, the pH was adjusted to approximately 5 by adding dilute hydrochloric acid. The medium samples were passed through Strata strong anion exchanger (SAX) cartridges, followed by extraction with Oasis hydrophilic–lipophilic balance (HLB). Six milliliter methanol was used to elute the extracted material for each HLB. Twenty HLBs were used in this step. About 120 mL of eluate was collected, and concentrated to (35 ℃) to about 5 ml by rotary evaporation, which was dried in a gentle nitrogen stream. The collected extract was about 0.5 g. Eventually, the CES was dissolved in methanol with the final concentration about 100 mg/mL and stored at -20 ℃.

**Supporting material Text 2.** The method for construction of qPCR standard curve

The standard curves were constructed according to the plasmid dilution method. The plasmid for establishing the standard curve was synthesized at UW Genetics (Beijing, China), and was made by synthesizing the target gene, i.e., the product sequence of qPCR on a pMV vector (sequence list below). The sequences of the qPCR products were found on the NCBI primer blast website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) and the sequence for the PMV vector was listed below. The steps of copy number calculation for the standard curve were as follows: firstly, obtain the synthetic plasmid sequence (including vector and target gene), paste it into the website of Deoxyribonucleic Acid Calculator (https://molbiotools.com/dnacalculator.php), and set the parameters as DNA, double-stranded, and cyclic, calculate its molecular weight (Dalton), and then convert the molecular weight unit to grams in UnitConverters.net (https://www.unitconverters.net/). The copy number of the plasmid was obtained by dividing the molecular weight of the plasmid by the mass of the labelled plasmid dry powder. The plasmid dry powder was dissolved by sterile dd$H_2O$ and then diluted to $10^9$ copies/μL. The plasmid was sequentially 10-fold diluted from $10^9$ copies/μL to $10^2$ copies/μL, and the plasmid concentrations of $10^8$, $10^7$, $10^6$, $10^5$, $10^4$, $10^3$ and $10^2$ copies/μL were selected to construct the qPCR standard curve. The amplification efficiencies of all standard curves were in the range of 90-110% with $R^2 \geq 0.99$. All experiments were performed in triplicate, and sterile dd$H_2O$ was used as the blank control.

>pMV vector complete sequence

AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC

TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGC

GTATCACGAGGCCCTTTCGTTGTAAAACGACGGCCAGTCGAACCACGCAA

TGCGTCTCGATCCGCAGTGTCTTGCGTCTCTTGAGACCTCGACAGCATCGA

CGTGCACGTGGAGCTGAAACACGAACTGCAGGGCCTCGACGCGGAACAG

CAGAAGCTGATCGGCAACGAACTGGGCAAGCAGATCAAGACCCATATCG

GCATCAGCGCGCAGGTGCTGATCCAGCCCTGCCACAGCCTCAAGCGCTCG

GAAGGCAAGGCCTGCCACGTCTACGACAAGCGCAACCAGGGTTGAGAGC

TCAAGAAGGAGATATACATATGGCGATGTTCACAACGACCGCTAAAGTTA

TTCAGCCGAAGATCCGTGGCTTTATTTGCACCACCACTCATCCTATCGGTT

GCGAGAAACGTGTTCAGGAAGAAATTGCCTATGCGCGTGCCCACCCGCCG

ACCTCTCCGGGTCCGAAGCGCGTACTGGTGATTGGGTGTAGCACAGGCTA

TGGCCTGAGCACCCGTATTACTGCCGCTTTCGGTTATCAGGCAGCCACGCT

GGGTGTGTTTCTGGCGGGTCCACCAACGAAAGGTCGCCCGGCGGCGGCTG

GTTGGTACAATACTGTAGCGTTCGAGAAAGCTGCTCTGGAGGCTGGCCTG

TATGCTCGCTCTCTGAACGGCGACGCATTTGATAGTACGACGAAAGCGCG

CACTGTTGAAGCTATTAAGCGCGATCTGGGTACAGTTGGTCTCAGAGACG

GAGTCACTGCCAACCGAGACGGTCATAGCTGTTTCCTGTGTGCCGCTTCCT

CGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCA

GCTCACTCAAAGGCGGTAATACGGTTACCCACAGAATCAGGGGATAACGC

AGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAA

AAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCAT

CACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT

AAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTC

CGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCG

TGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCG

TTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCT

GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGAC

TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA

TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACA

CTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCG

GAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC

GGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATC

TCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGA

AAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCA

CCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATAT

ATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCT

ATCTCAGCGATCTGTCTCTTTCGTTCATCCATAGTTGCCTGACTCCCCGTCG

TGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCA

ATAATACCGCGGGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAA

CCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCG

CCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGC

CAGTTAATAGTTTGCGCAACGTTGTTGCCATCGCTACAGGCATCGTGGTAT

CACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAA

GGCGAGTTACATGATCCCCCATGTTGCGCAAAAAAGCGGTTAGCTCCTTC

GGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCCGTGTTATCACTCATG

GTTATGGCAGCACTACATAATTCTCTTACTGTCATGCCATCCGTAAGATGC

TTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATG

CGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCC

ACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGC

GAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCC

ACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCT

GGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGG

GCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGA

AGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATT

TAG

**Figures**

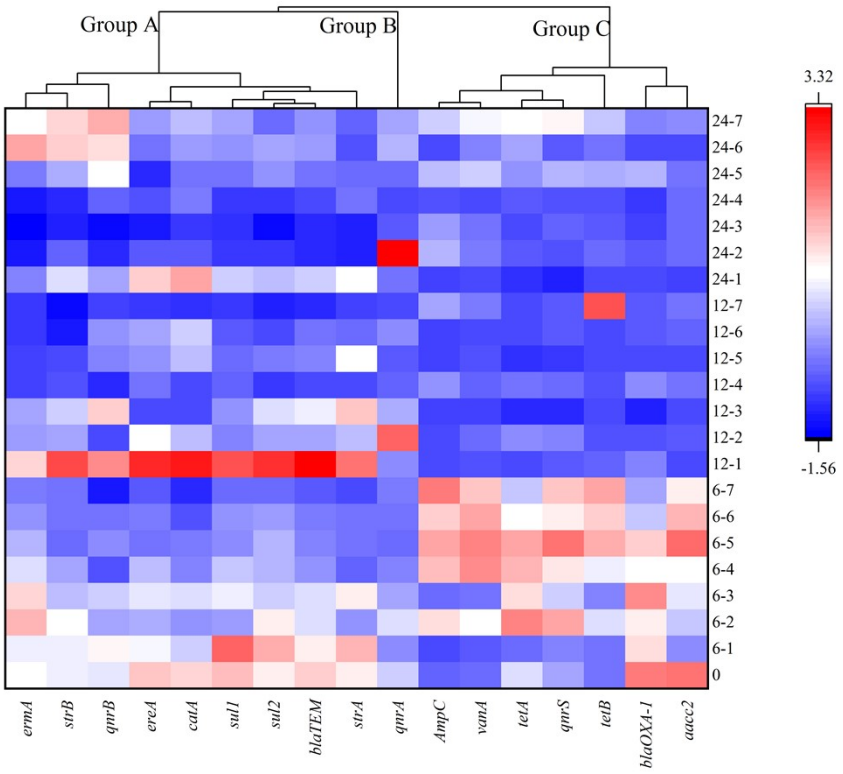


**Fig. S1** Heatmap for the abundance of ARGs in the samples. The values corresponding to the heat map are the Z-score normalised values of the ARGs across samples. The first number on the sample label represents time points and the second number represents different concentration groups, 1 to 7 representing the group with 1000, 100, 10, 1, 0.1, and 0 μg / mL of cyanobacterial substance, respectively. For example, 6-1 means the sample with 1000 μg / mL of cyanobacterial substance extracted at the 6 h time point. The meaning of the sample label in the other figures in this paper is the same.

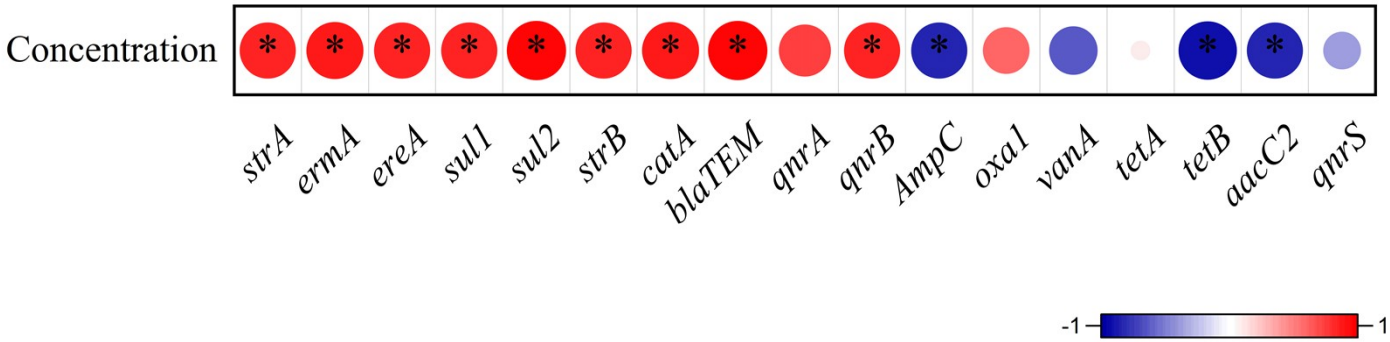


**Fig.S2** Correlation between CES concentration and ARGs. Red indicates positive correlation and blue indicates negative correlation. * represents $p < 0.05$.

**Table S1** qPCR primers used in this study.

| Gene | Primer | Sequence (3'-5') | Product size (bp) | Tm (℃) | Reference |
|---|---|---|---|---|---|
| *sul*1 | sul1-F | CGCACCGGAAACATCGCTGCAC | 162 | 65 | 3 |
| | sul1-R | TGAAGTTCCGCCGCAAGGCTCG | | | |
| *sul*2 | sul2-F | TCCGATGGAGGCCGGTATCTGG | 191 | 60 | 4 |
| | sul2-R | CGGGAATGCCATCTGCCTTGAG | | | |
| *tet*A | tetA-F | GCTACATCCTGCTTGCCTTC | 210 | 60 | 3 |
| | tetA-R | CATAGATCGCCGTGAAGAGG | | | |
| *tet*B | tetB-F | TACGTGAATTTATTGCTTCGG | 206 | 53 | 3 |
| | tetB-R | ATACAGCATCCAAAGCGCAC | | | |
| *erm*A | ermA-F | ATGTCTGCATACGGACACGG | 185 | 55 | 5 |
| | ermA-R | ACTTCAACTGCCGTTATCGC | | | |
| *ere*A | ereA-F | CCGACAGTCCTCTTGGATTCT | 215 | 57 | 6 |
| | ereA-R | GCTTGGAACACAGACGATGG | | | |

| | | | | | |
|---|---|---|---|---|---|
| *qnr*A | qnrA-F | AGGATTTCTCACGCCAGGATT | 124 | 60 | 7 |
| | qnrA-R | CCGCTTTCAATGAAACTGCAA | | | |
| *qnr*B | qnrB-F | CAGATTTYCGCGGCGCAAG | 134 | 54 | 7 |
| | qnrB-R | TTCCCACAGCTCRCAYTTTTC | | | |
| *qnr*S | qnrS-F | GTATAGAGTTCCGTGCGTGTGA | 189 | 55 | 7 |
| | qnrS-R | GGTTCGTTCCTATCCAGCGATT | | | |
| *cat*A | catA-F | ATGGCAATGAAAGACGGTGAGC | 122 | 64 | 5 |
| | catA-R | TGCCGGAAATCGTCGTGGTATT | | | |
| *van*A | vanA-F | TCTGCAATAGAGATAGCCGC | 376 | 60 | 8 |
| | vanA-R | GGAGTAGCTATCCCAGCATT | | | |
| *str*A | strA-F | CCAGTTCTCTTCGGCGTTAG | 99 | 60 | 9 |
| | strA-R | ACTCTTCAATGCACGGGTCT | | | |
| *str*B | strB-F | CGGCTGGCTGGTGATAGAT | 238 | 60 | 6 |
| | strB-R | GCGTTGCTCCTCTTCTCCA | | | |
| *aacC*2 | aacc2-F | GTATGAGATGCCGATGCTTGG | 256 | 58 | 6 |

| | | | | | |
|---|---|---|---|---|---|
| | aacc2-R | GAGTGGCTCCGAAGTGCTT | | | |
| *bla*$_{TEM}$ | blaTEM-F | GCKGCCAACTTACTTCTGACAACG | 247 | 61 | 5 |
| | blaTEM-R | CTTTATCCGCCTCCATCCAGTCTA | | | |
| AmpC | AmpC-F | CCTCTTGCTCCACATTTGCT | 189 | 58 | 5 |
| | AmpC-R | ACAACGTTTGCTGTGTGACG | | | |
| *bla*$_{OXA-1}$ | OXA-1-F | GCAAATGGCACCAGATTCAAC | 177 | 55 | 6 |
| | OXA-1-R | TGCGAAACCCAAACAACAGAA | | | |
| *intI1* | intI-F | CCTCCCGCACGATGATC | 280 | 60 | 10 |
| | intI-R | TCCACGCATCGTCAGGC | | | |
| 16S | 16S-F | CGGTGAATACGTTCYCGG | 142 | 60 | 10 |
| | 16S-R | GGWTACCTTGTTACGACTT | | | |

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