

Supplementary Information

High structural diversity of aeruginosins in bloom-forming cyanobacteria of the genus *Planktothrix* as a consequence of multiple recombination events

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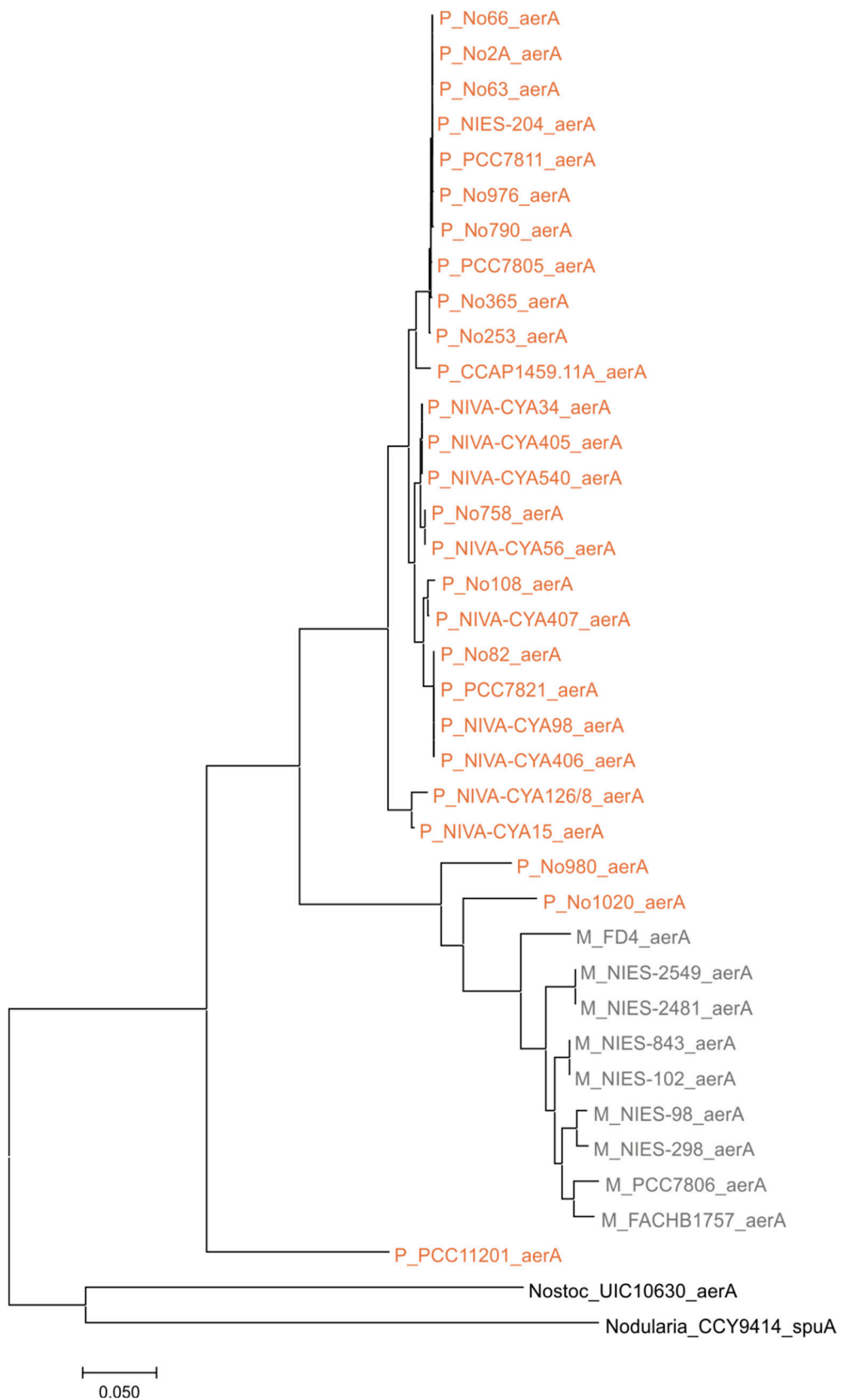


Figure S1. Maximum likelihood phylogenetic tree based on *aerA* (nucleotide) sequence (alignment: 4407 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.

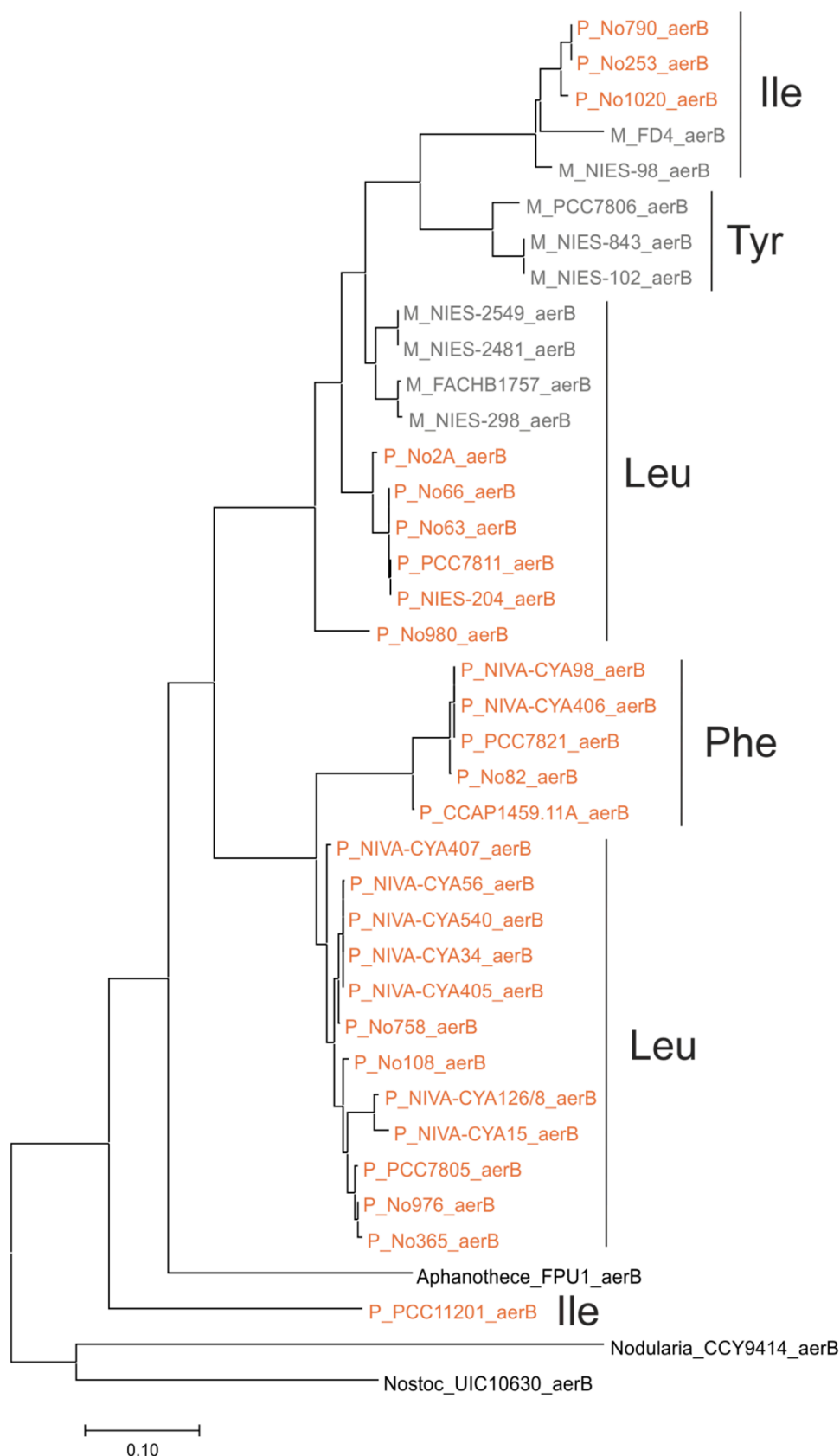


Figure S2. Maximum likelihood phylogenetic tree based on *aerB* (nucleotide) sequence (alignment: 4857 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups. Using the NRPS prediction tool [26] four different specificity-conferring codes for the adenylation domain of AerB were identified: Leu, Ile, Phe and Tyr.

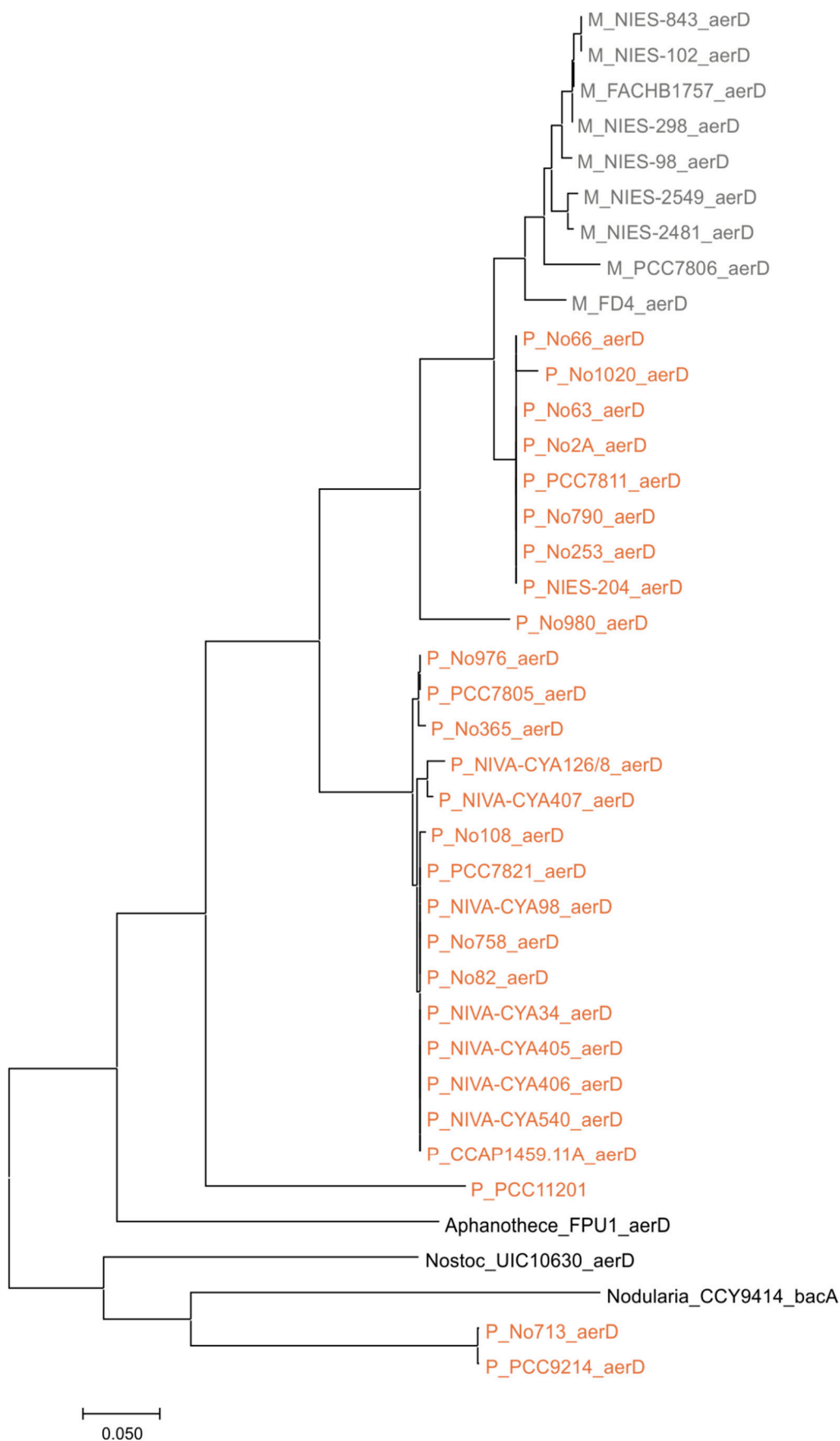


Figure S3. Maximum likelihood phylogenetic tree based on *aerD* (nucleotide) sequence (alignment: 633 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.

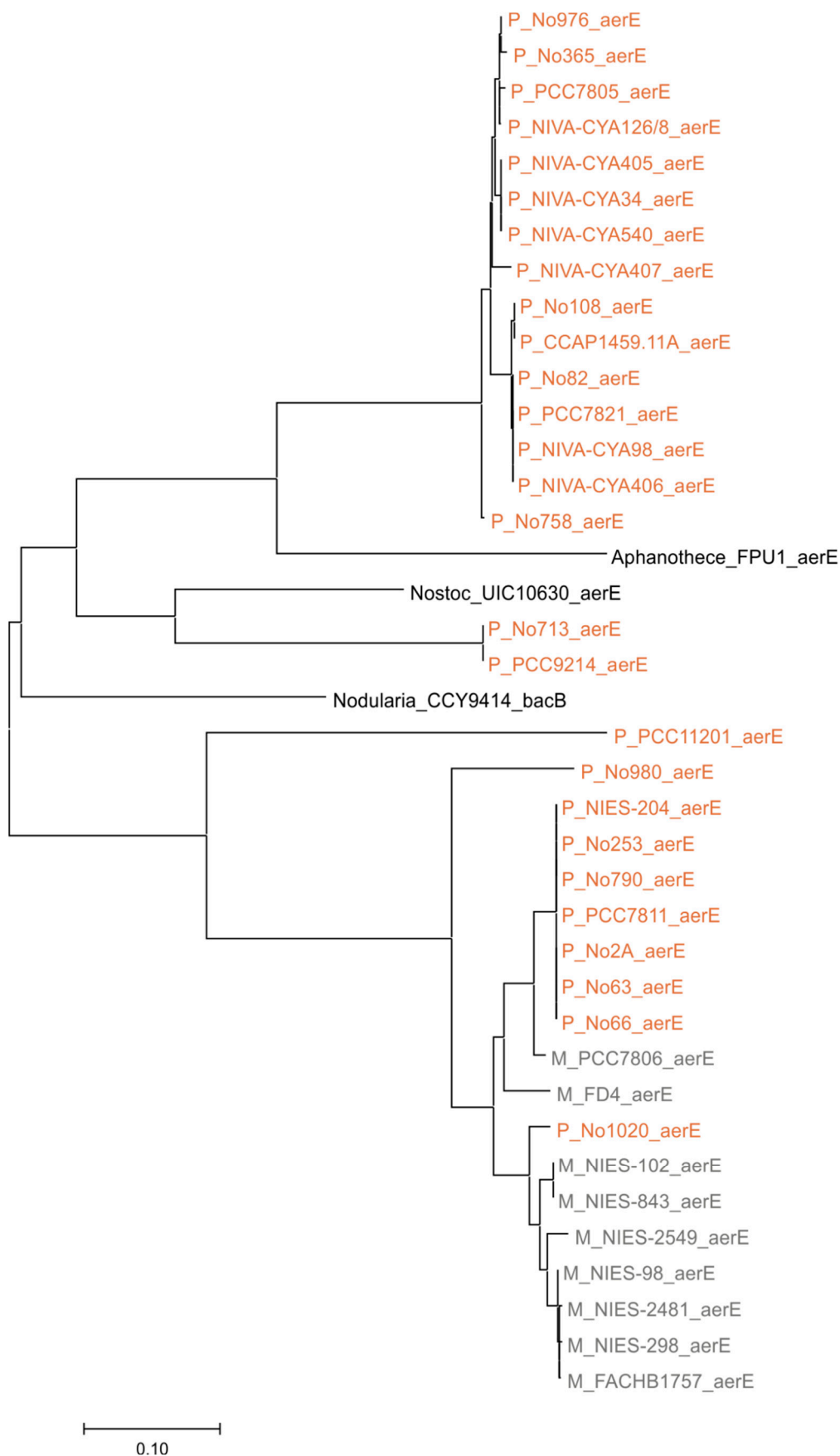


Figure S4. Maximum likelihood phylogenetic tree based on *aerE* (nucleotide) sequence (alignment: 756 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.

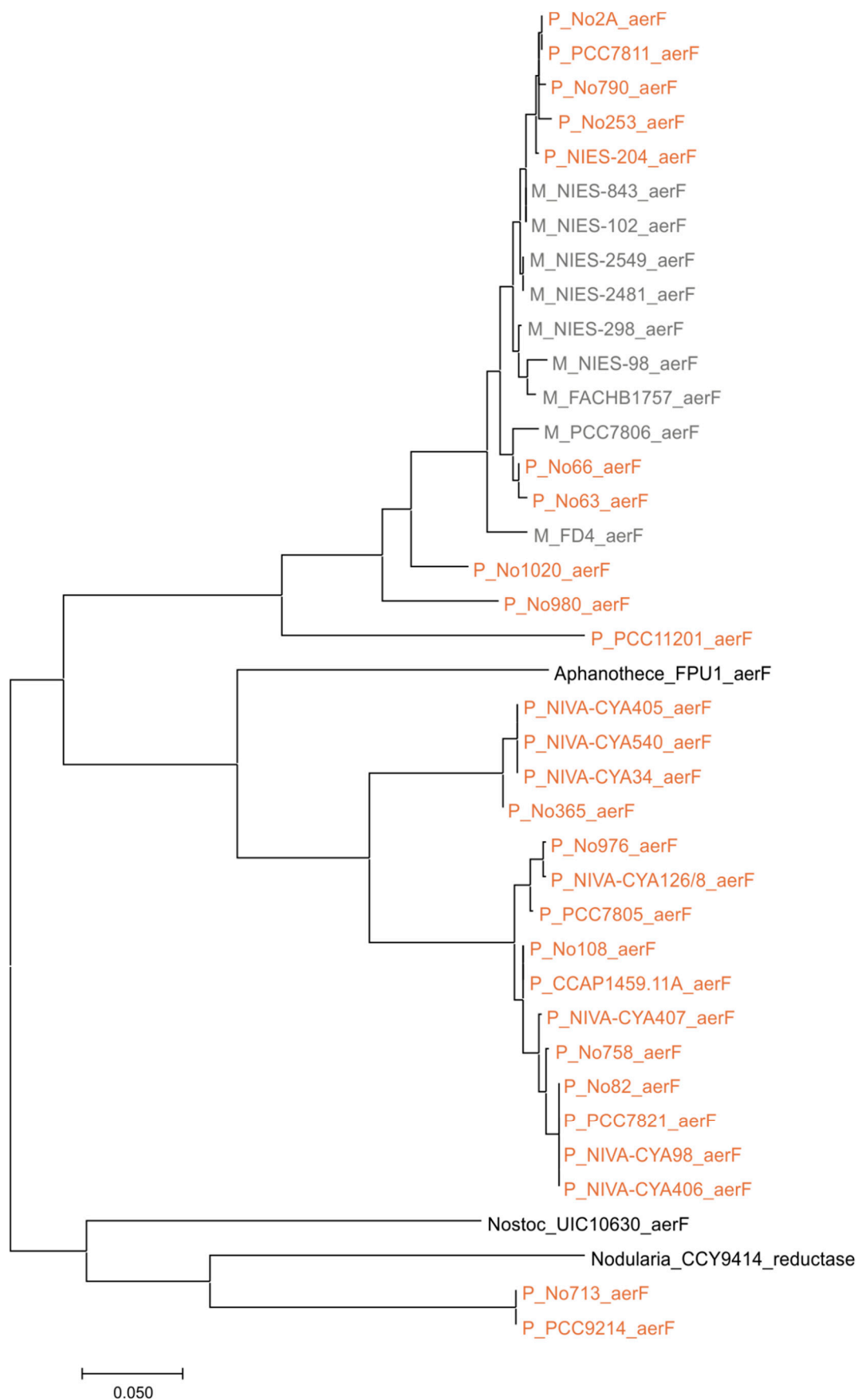


Figure S5. Maximum likelihood phylogenetic tree based on *aerF* (nucleotide) sequence (alignment: 801 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.

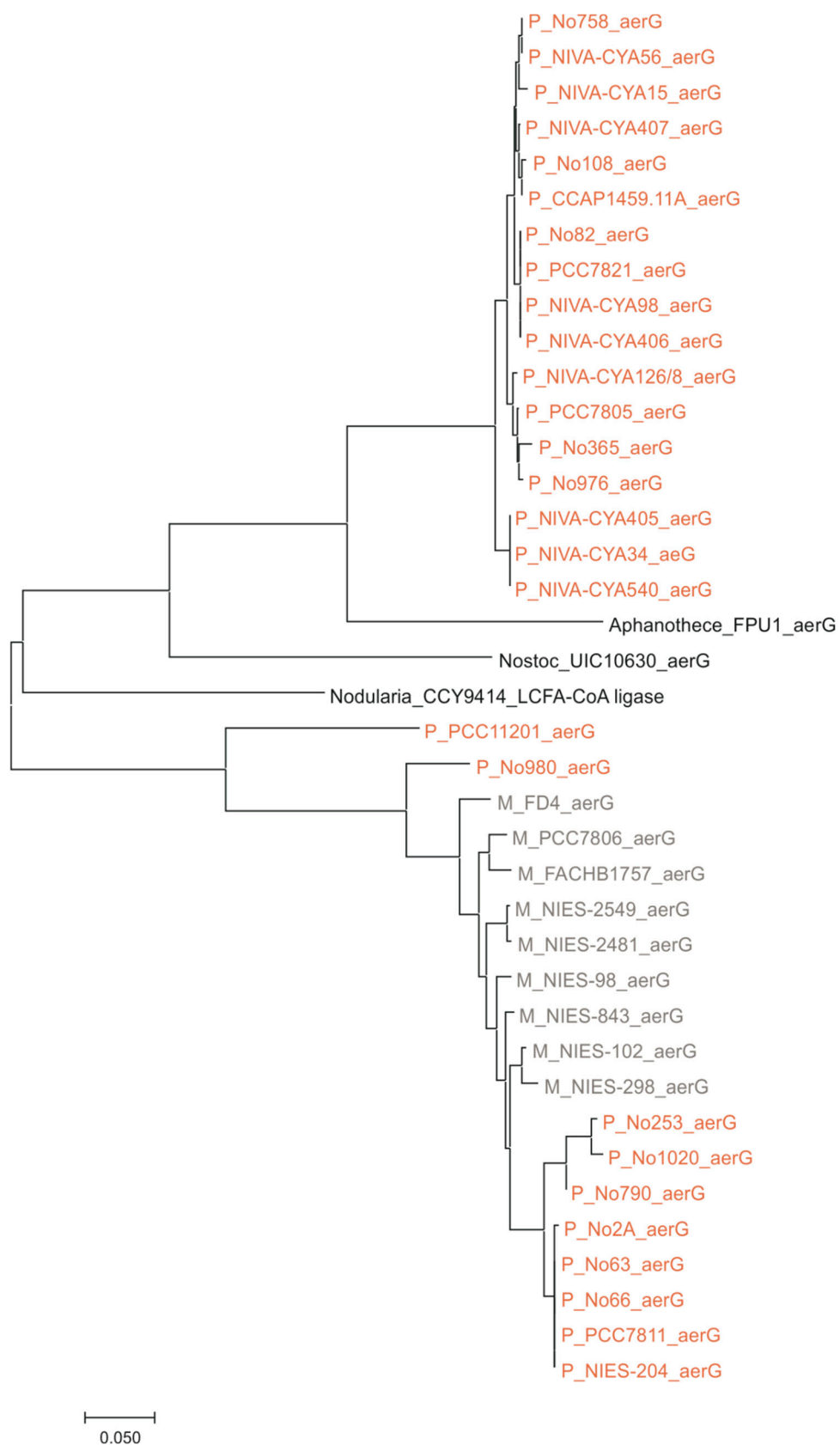


Figure S6. Maximum likelihood phylogenetic tree based on *aerG* (nucleotide) sequence (alignment: 3366 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.

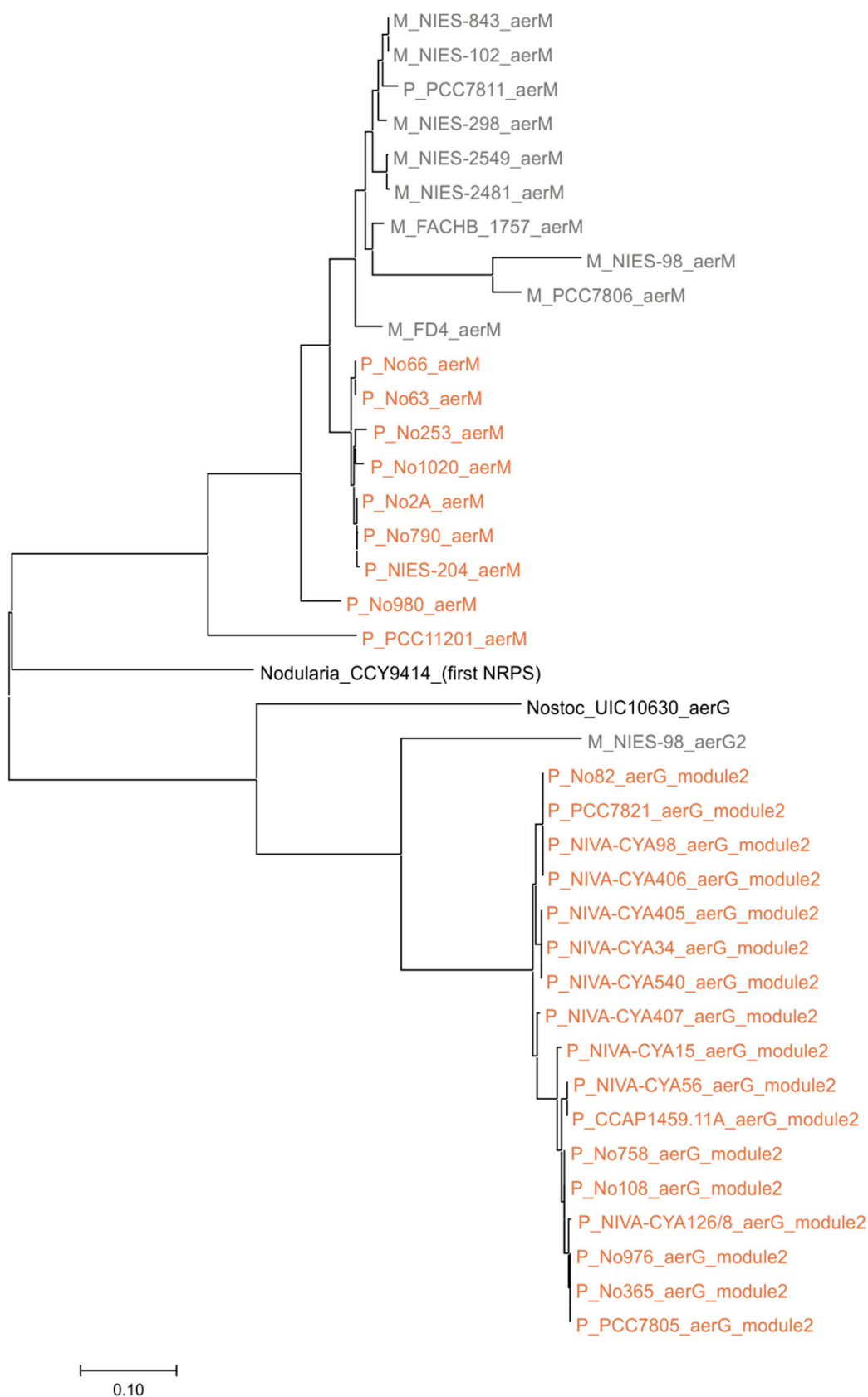


Figure S7. Maximum likelihood phylogenetic tree based on *aerG_module2* and *aerM* (nucleotide) sequence (alignment: 4533 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.

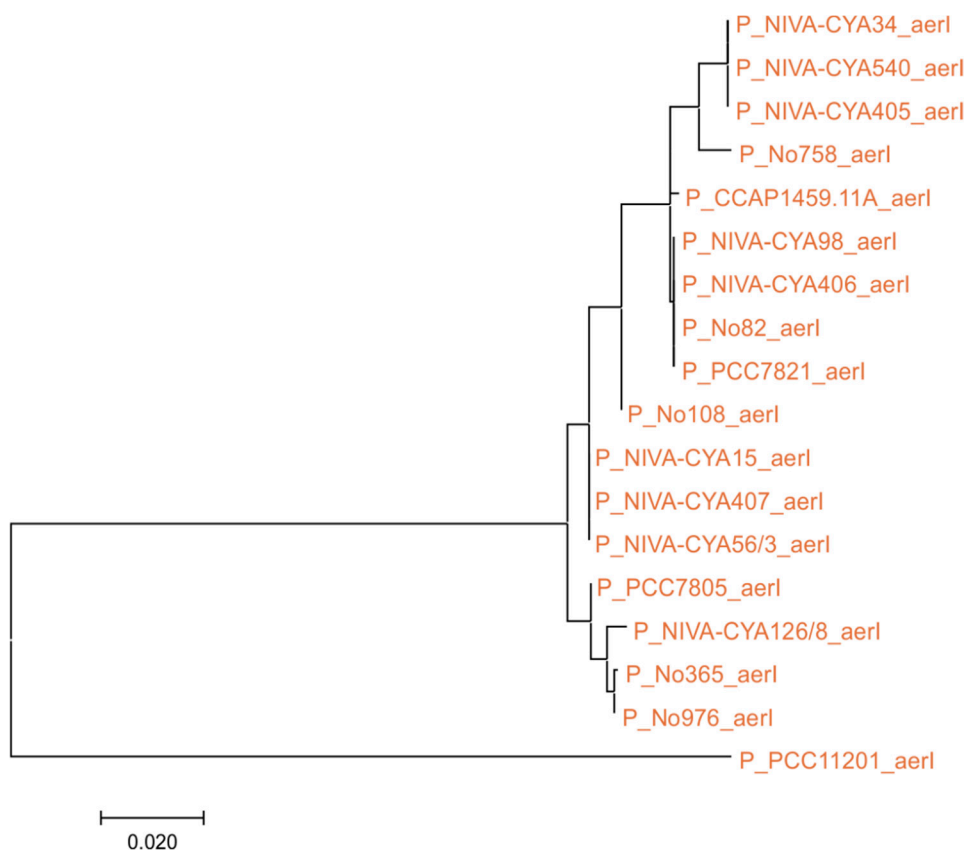


Figure S8. Maximum likelihood phylogenetic tree based on *aerI* (nucleotide) sequence (alignment: 1260 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each.

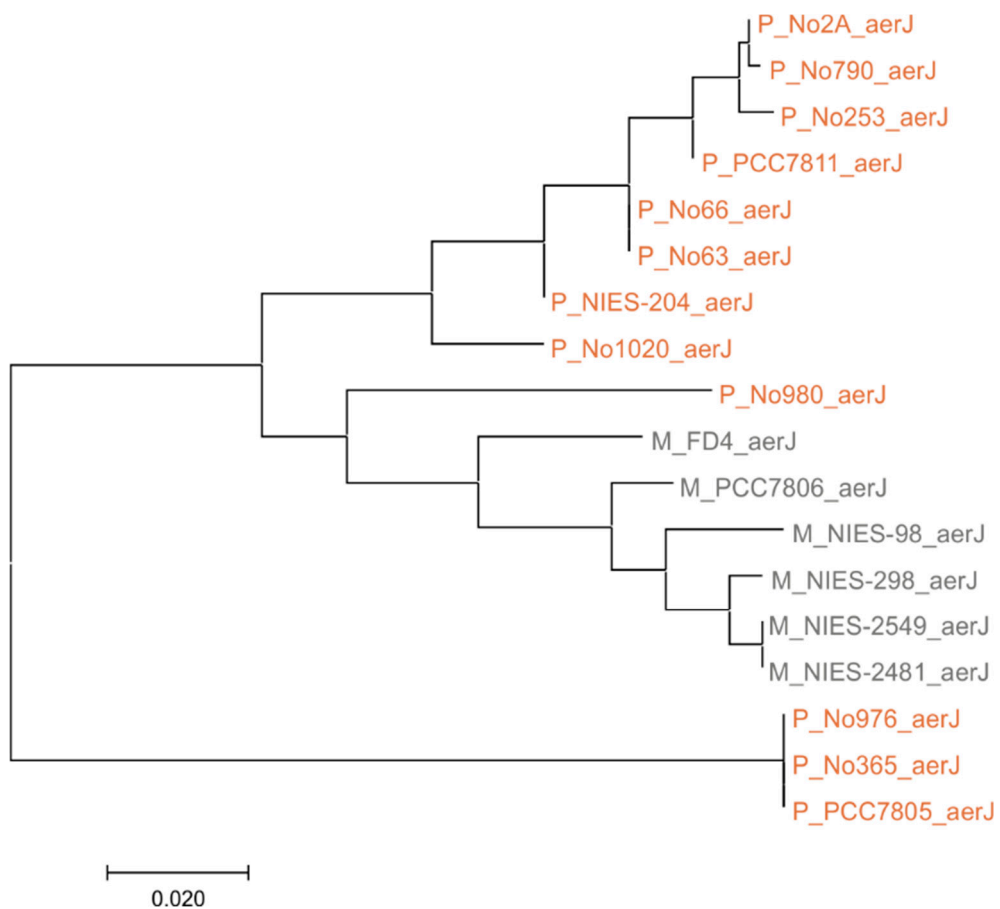


Figure S9. Maximum likelihood phylogenetic tree based on *aerJ* (nucleotide) sequence (alignment: 1905 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each.

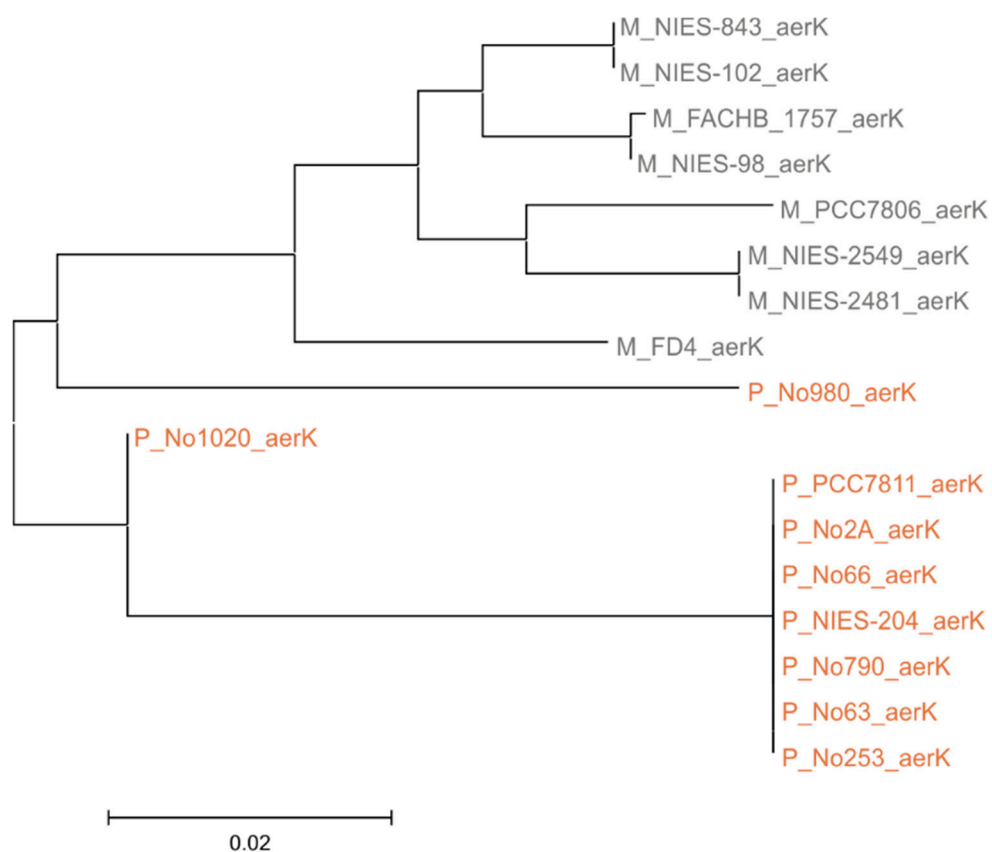


Figure S10. Maximum likelihood phylogenetic tree based on *aerK* (nucleotide) sequence (alignment: 1044 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each.

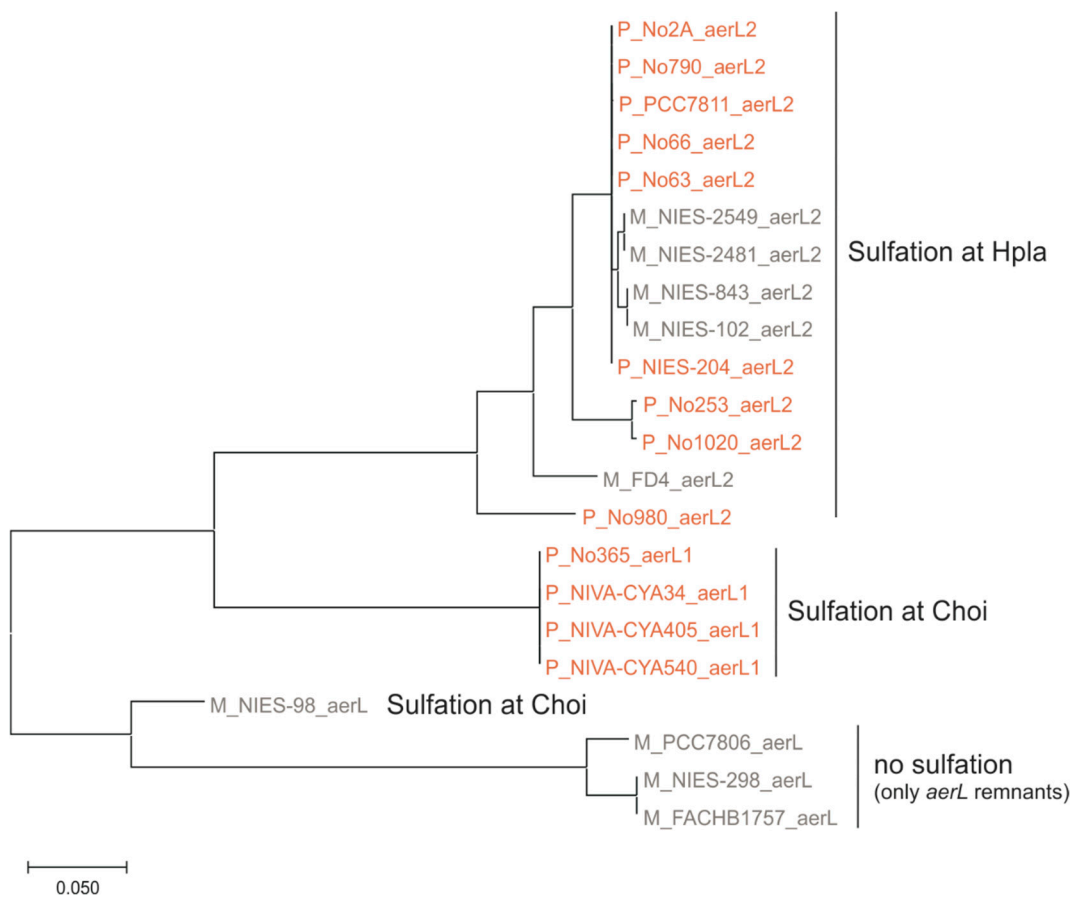


Figure S11. Maximum likelihood phylogenetic tree based on *aerL* (nucleotide) sequence (alignment: 1011 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. In addition the putative sulfation reactions are indicated (Figure 4).

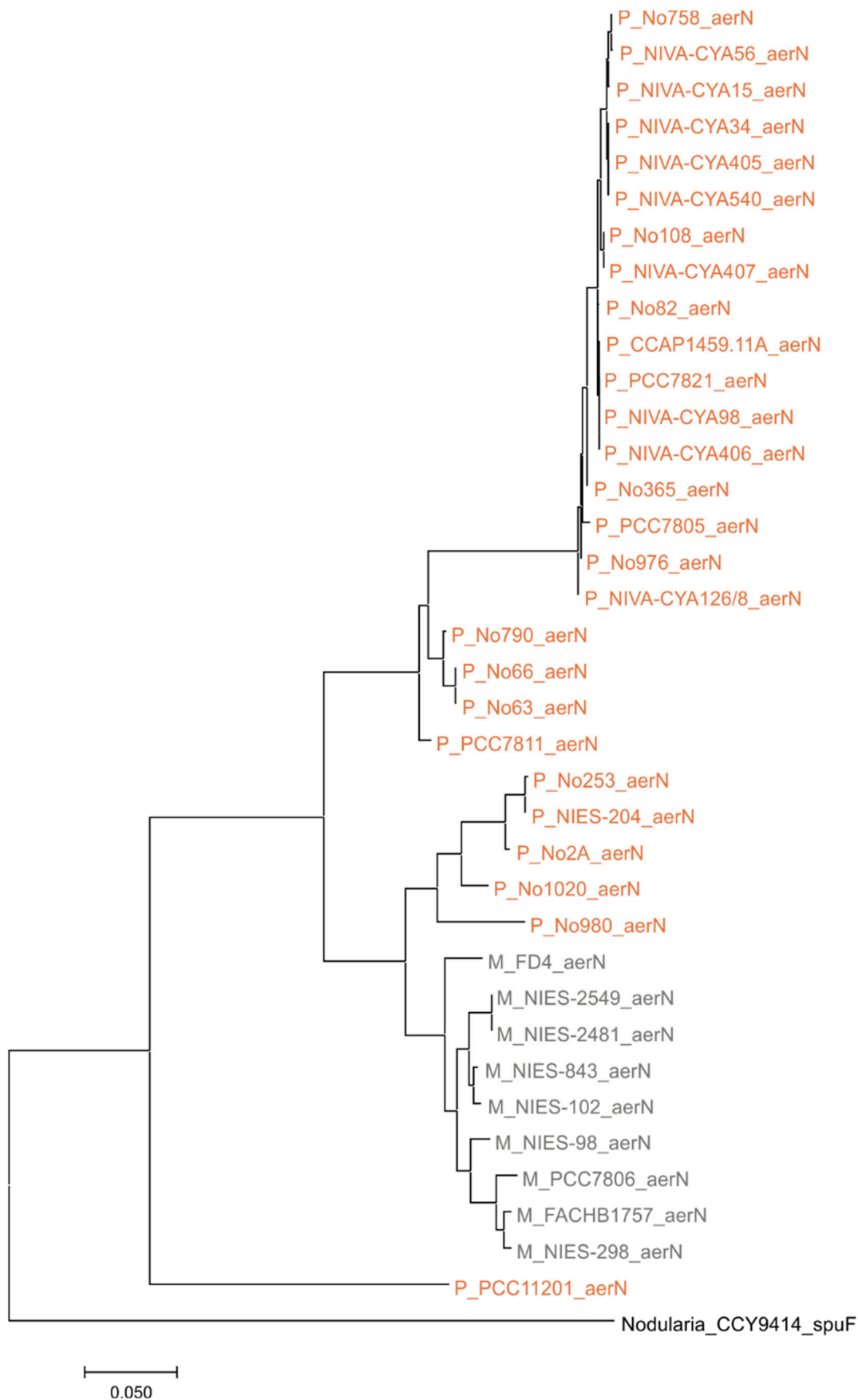


Figure S12. Maximum likelihood phylogenetic tree based on *aerN* (nucleotide) sequence (alignment: 2058 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each. *Nodularia* CCY9414 served as outgroup.

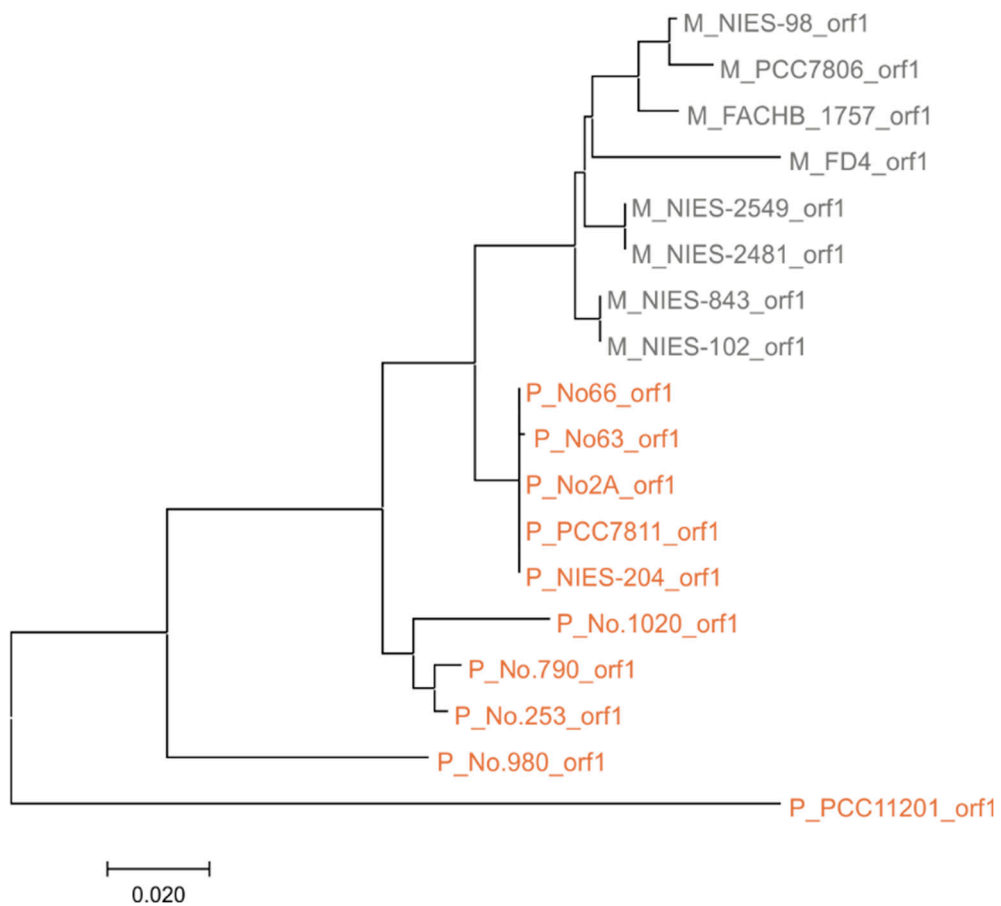


Figure S13. Maximum likelihood phylogenetic tree based on ORF1 (nucleotide) sequence (alignment: 786 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each.

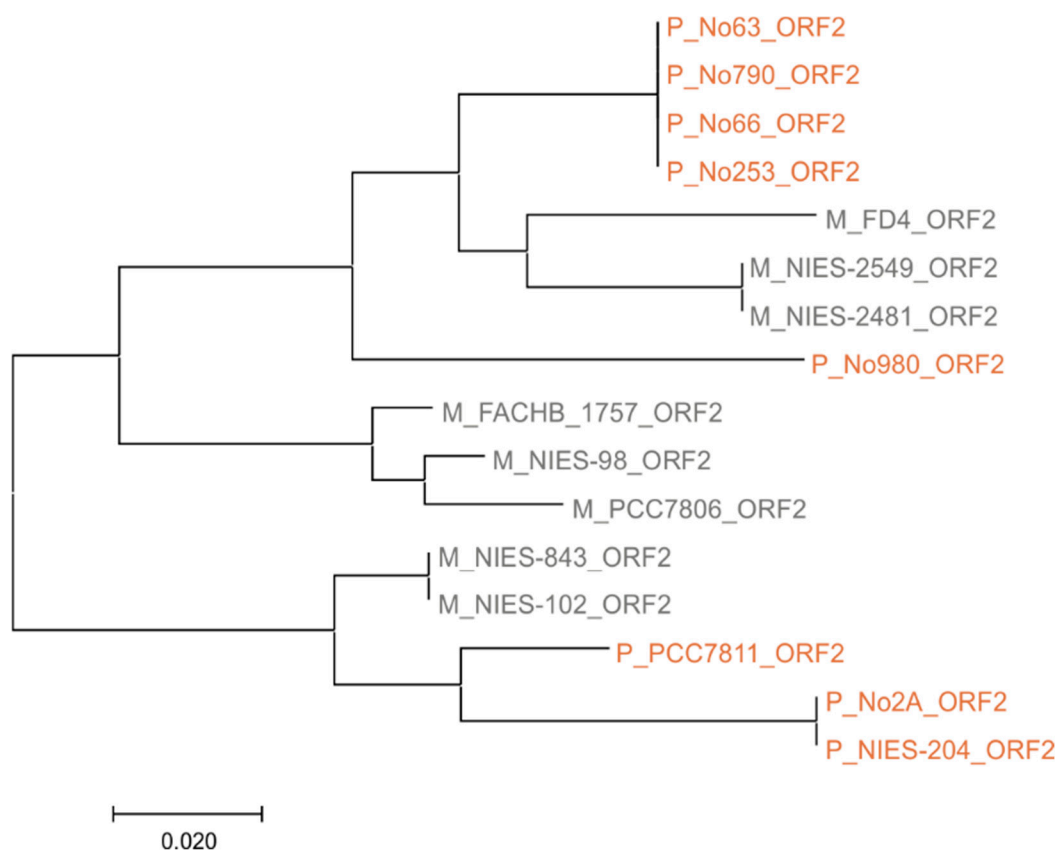


Figure S14. Maximum likelihood phylogenetic tree based on ORF2 (nucleotide) sequence (alignment: 627 bp). P_, M_ indicate genera *Planktothrix* or *Microcystis*, each.

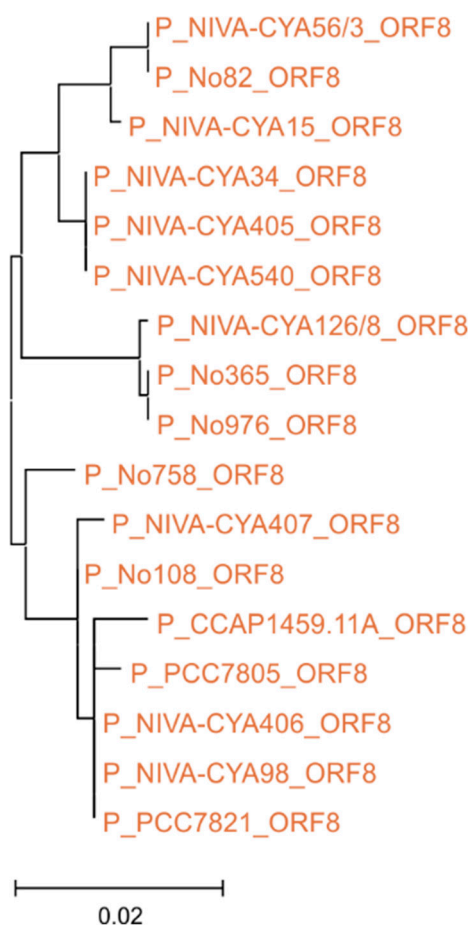


Figure S15. Maximum likelihood phylogenetic tree based on ORF8 (nucleotide) sequence (alignment: 1161 bp). P_ indicates genus *Planktothrix*.

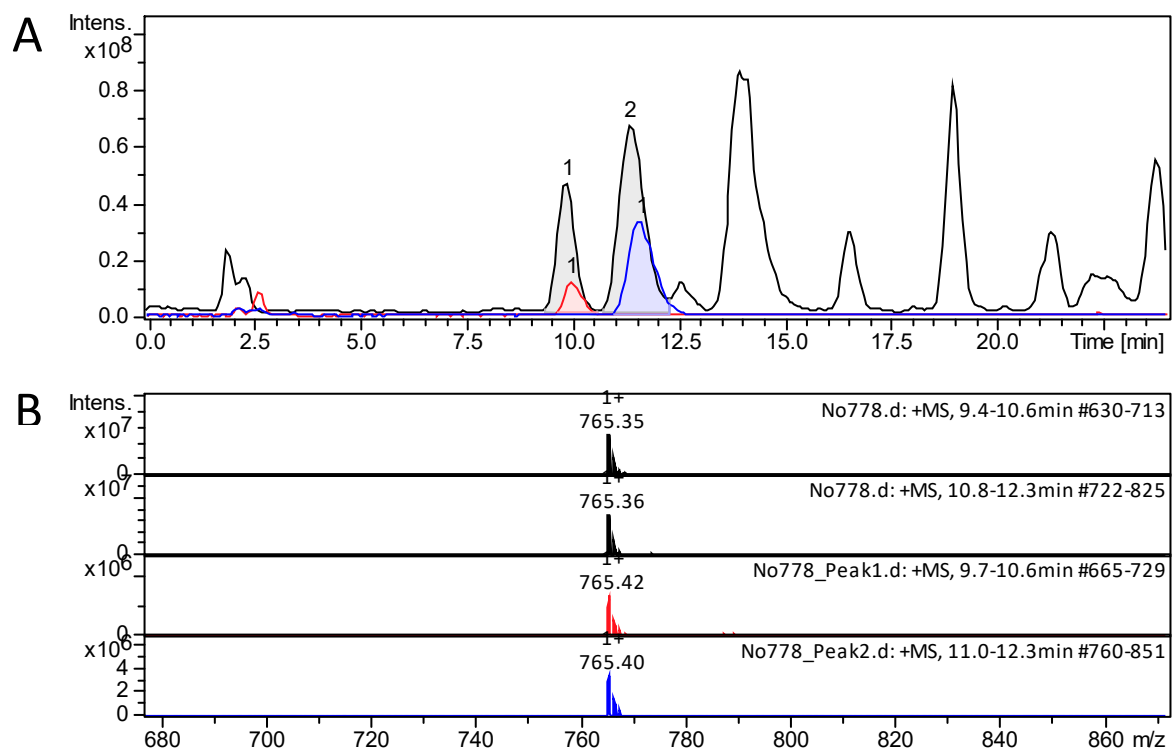


Figure S16. (A) Base peak chromatograms (BPC) of methanolic peptide extracts from *Planktothrix* strain No778 with first injection (black), re-injection of peak 1 (red) and re-injection of peak 2 (blue). **(B)** Mass peak chromatograms [m z⁻¹] of peak 1 and 2 from the first injection (black), re-injection of peak 1 (red) and re-injection of peak 2 (blue); [m z⁻¹] 765.3.

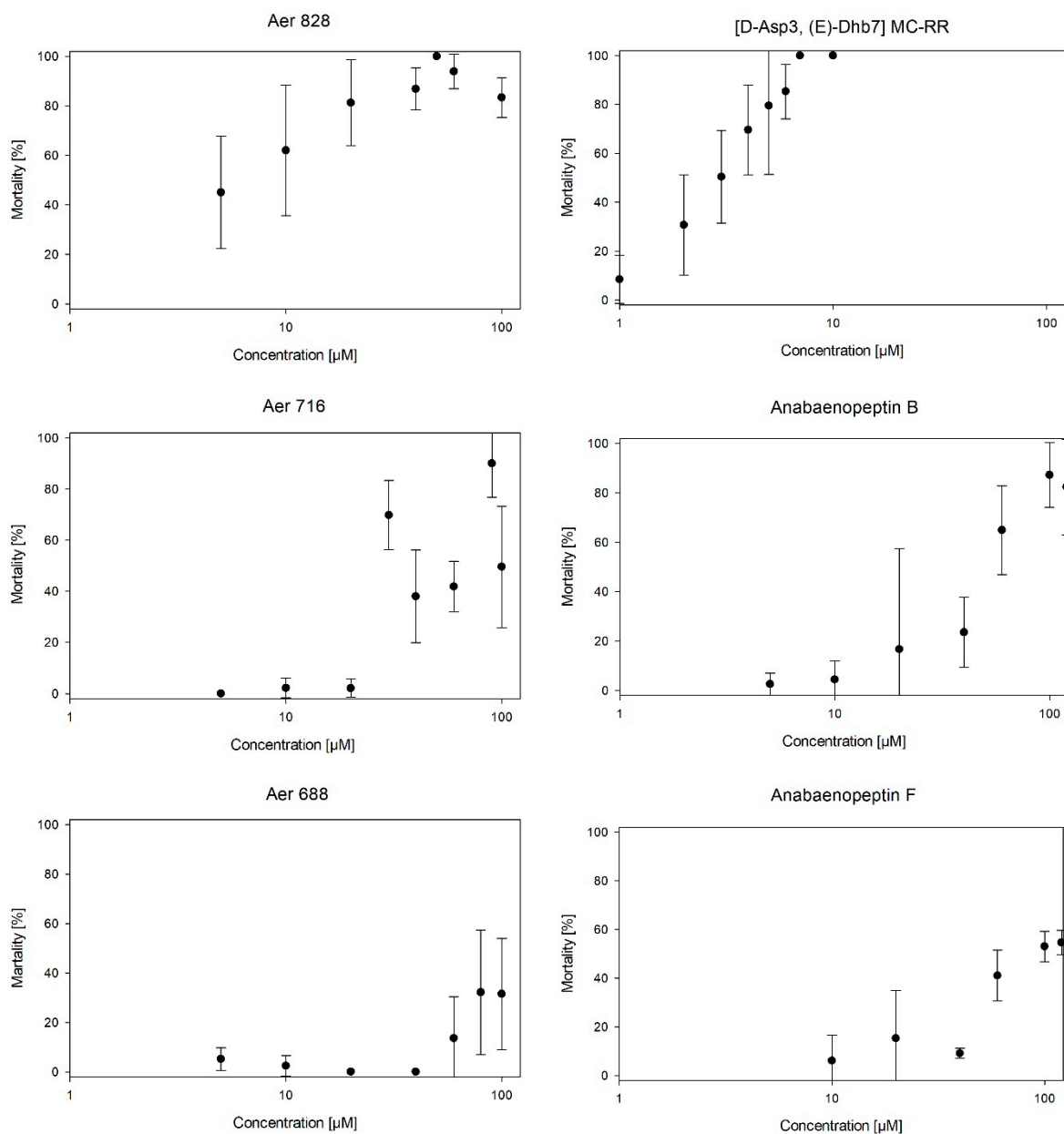


Figure S17. Percentage mortality (mean \pm 1SD) through purified aeruginosin variants Aer 828A ($[M+H]^+$ 829.3), Aer 89 ($[M+H]^+$ 717.3), Aer 688 ($[M+H]^+$ 689.3) as inferred using a standard toxicity assay (i.e. *Thamnocephalus platyurus*) and compared with that of purified [D-Asp3, (E)-Dhb7] microcystin-RR ($[M+H]^+$ 1024), and anabaenopeptin B ($[M+H]^+$ 837) and anabaenopeptin F ($[M+H]^+$ 851).

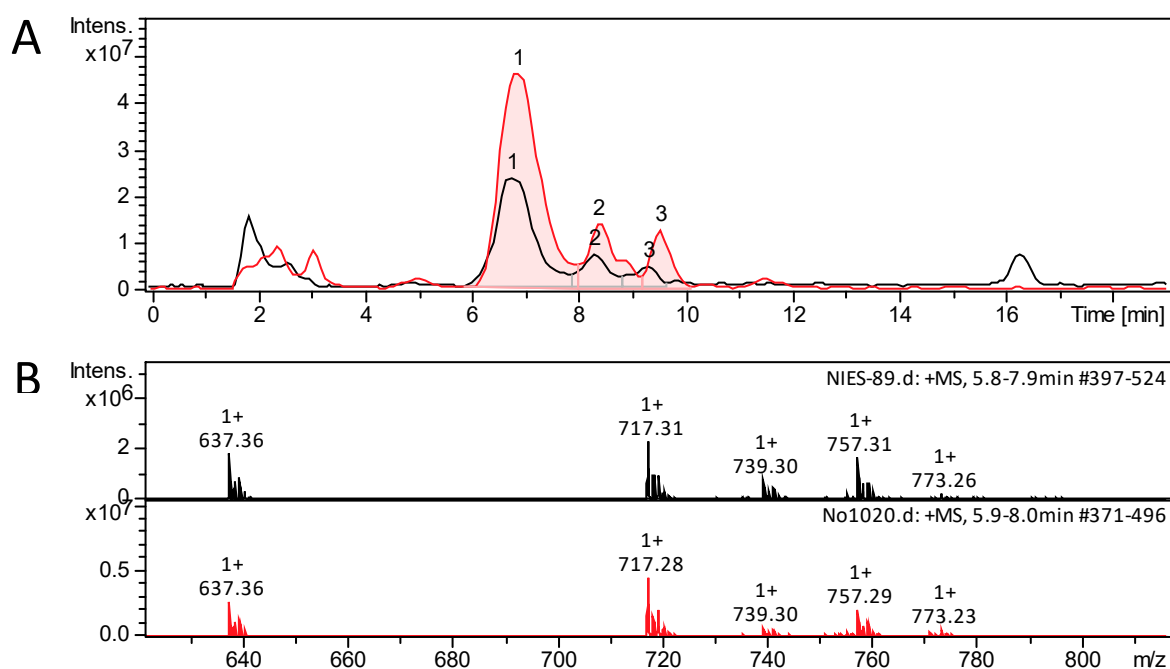


Figure S18. (A) Base peak chromatograms (BPC) of methanolic peptide extracts from *Microcystis* strain NIES-89 (black) and *Planktothrix* strain No1020 (red). **(B)** Mass peak chromatograms [m z⁻¹] of NIES-89_peak1 (black) and No1020_peak1 (red); [M-SO₃+H]⁺ 637.3; [M+H]⁺ 717.3; [M+Na]⁺ 739.3; [M+K]⁺ 757.3.

References

26. Röttig, M.; Medema, M. H.; Blin, K.; Weber, T.; Rausch, C.; Kohlbacher, O., NRPSpredictor2--a web server for predicting NRPS adenylation domain specificity. *Nucleic Acids Res* **2011**, 39, (Web Server issue), W362-7. doi: 10.1093/nar/gkr323.