

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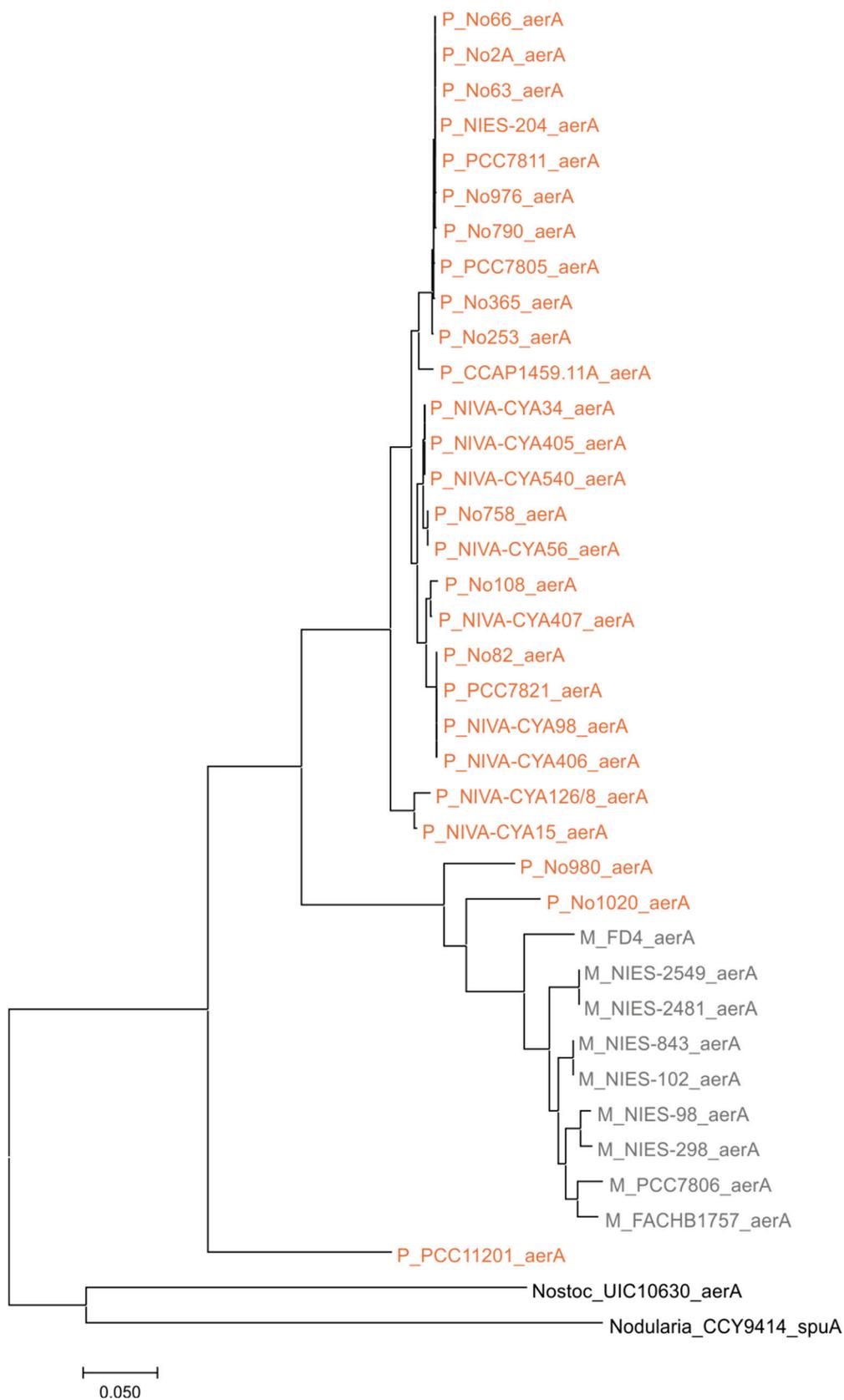
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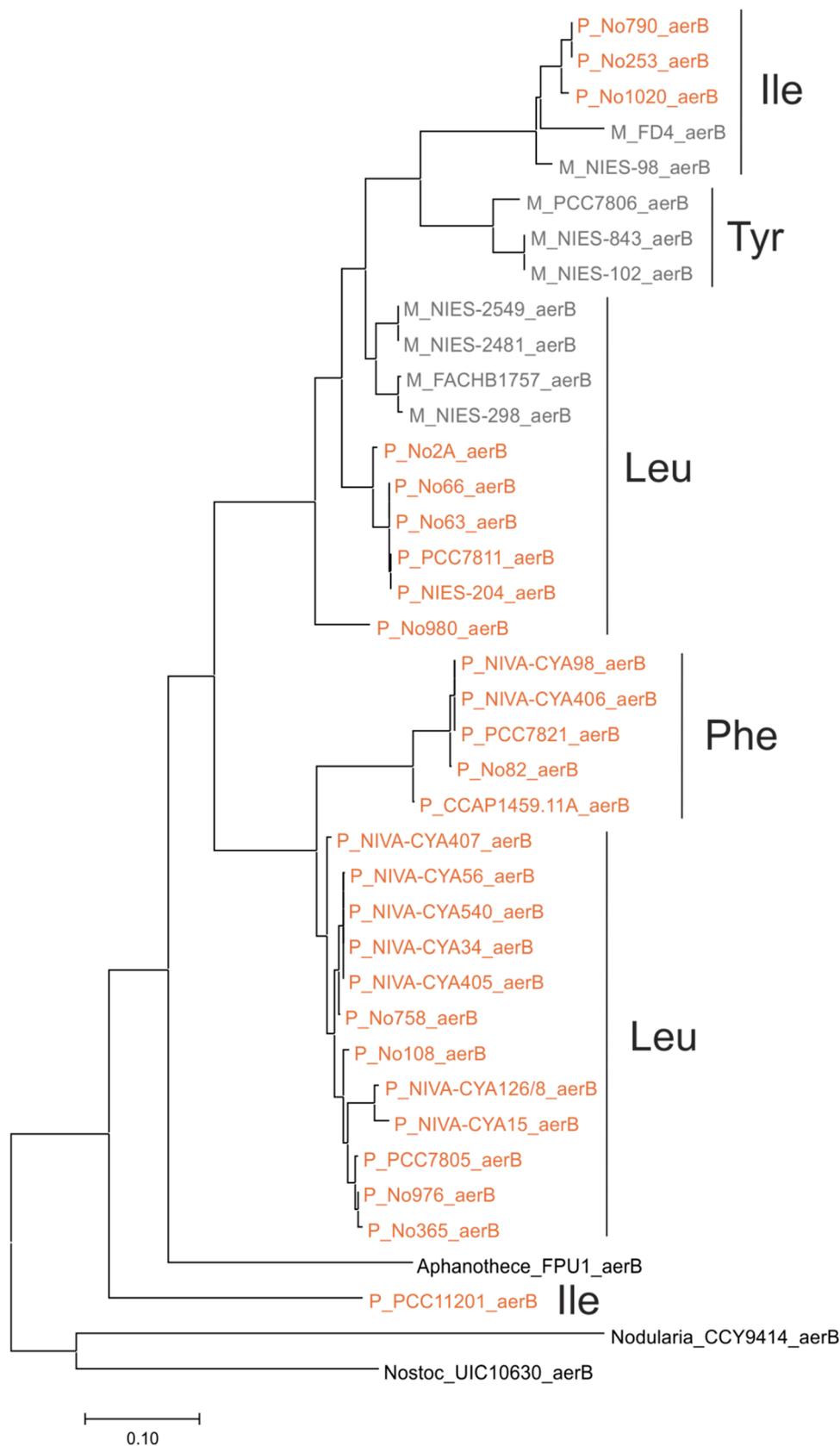
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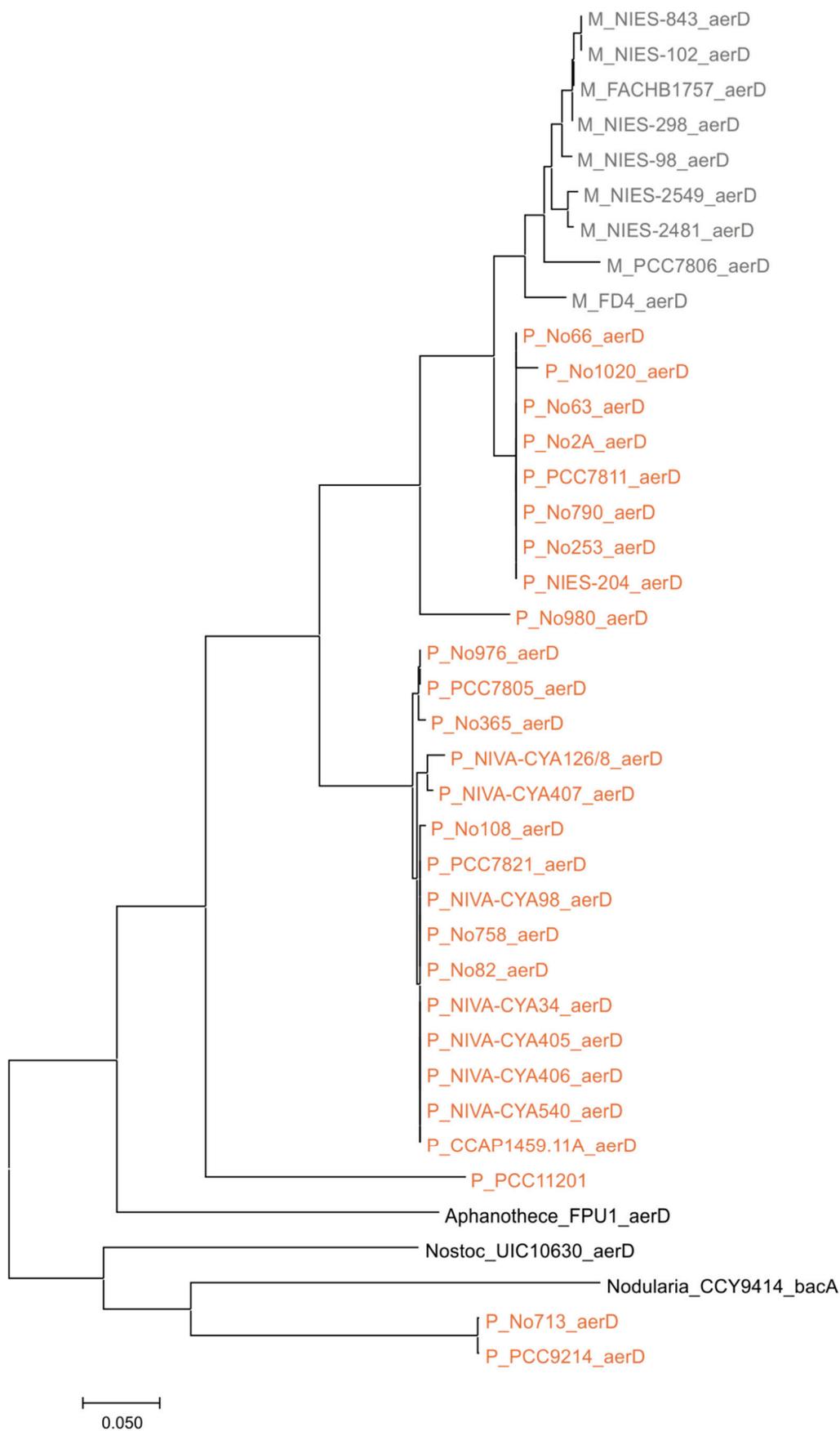
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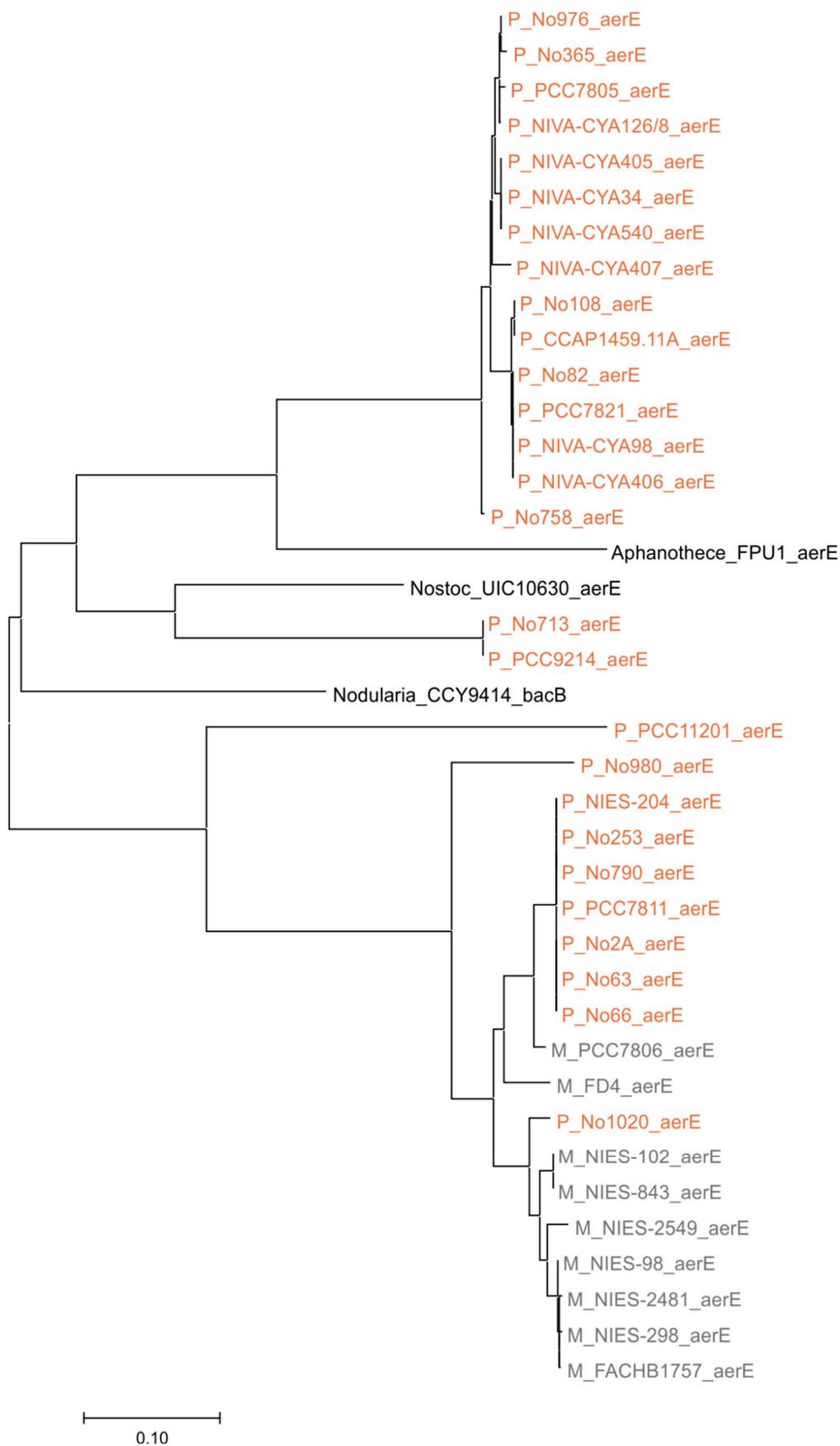
**Figure S1.** Maximum likelihood phylogenetic tree based on *aerA* (nucleotide) sequence (alignment: 4407 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.



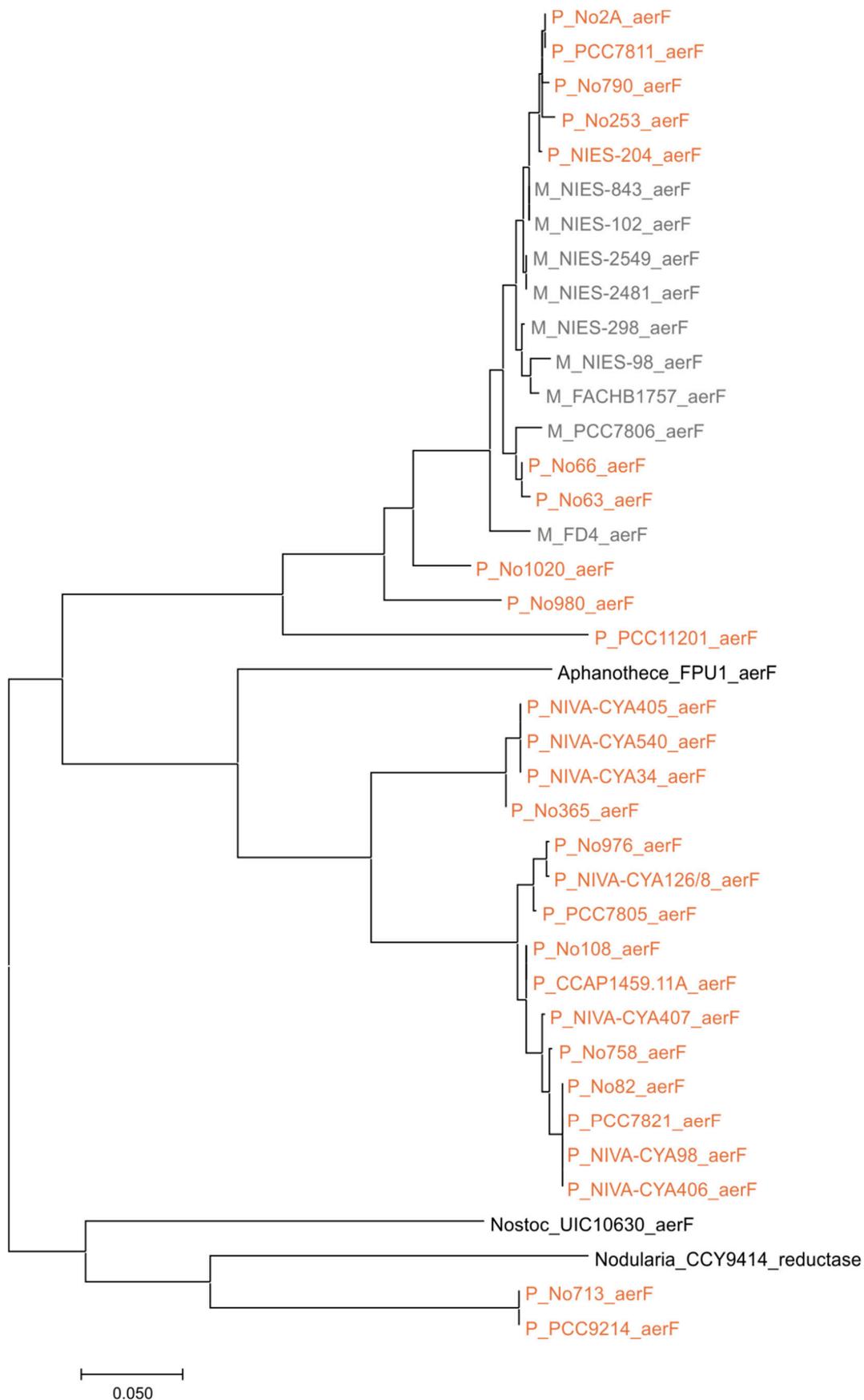
**Figure S2.** Maximum likelihood phylogenetic tree based on *aerB* (nucleotide) sequence (alignment: 4857 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups. Using the NRPS prediction tool [26] four different specificity-conferring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



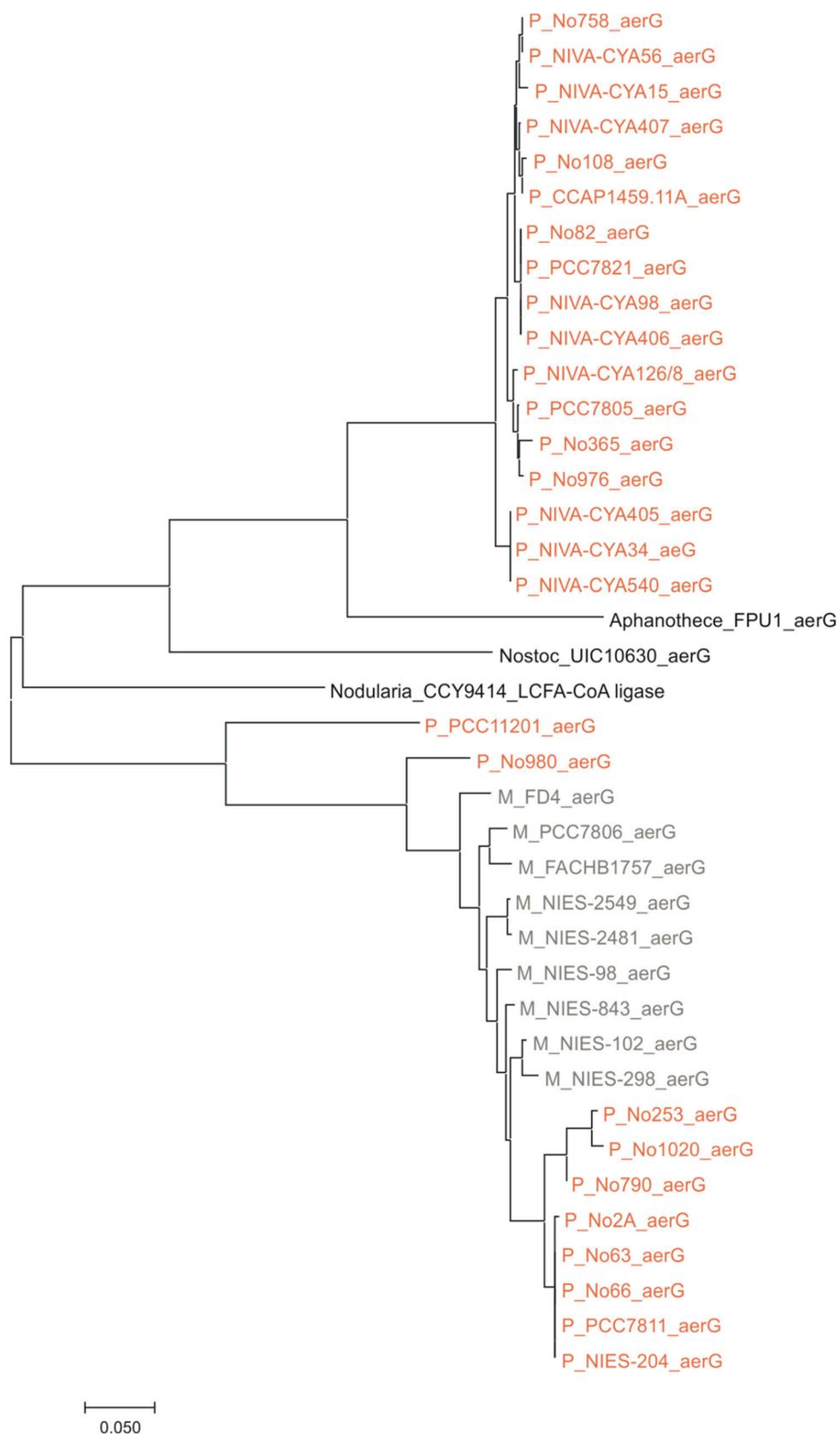
**Figure S3.** Maximum likelihood phylogenetic tree based on *aerD* (nucleotide) sequence (alignment: 633 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.



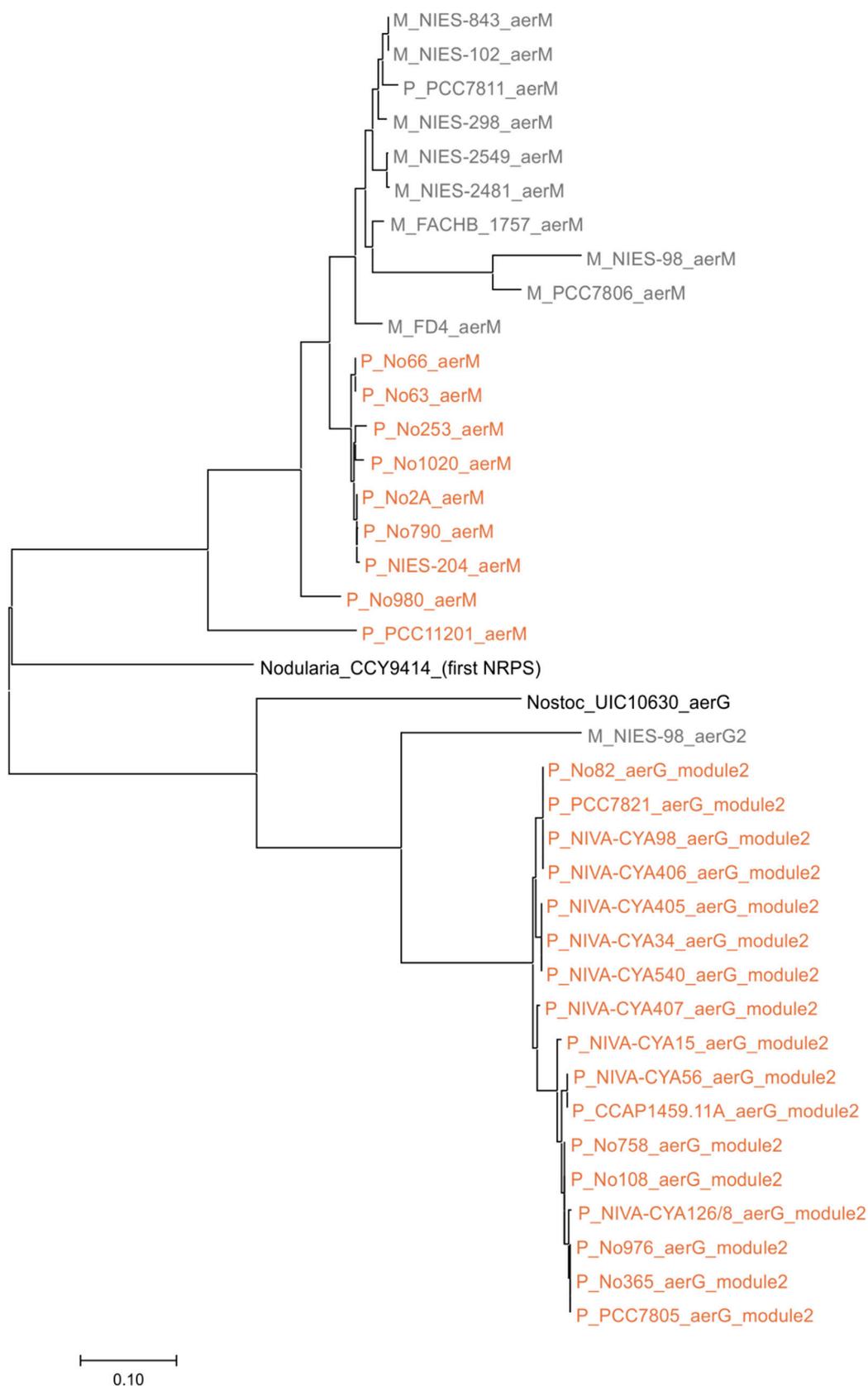
**Figure S4.** Maximum likelihood phylogenetic tree based on *aerE* (nucleotide) sequence (alignment: 756 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.



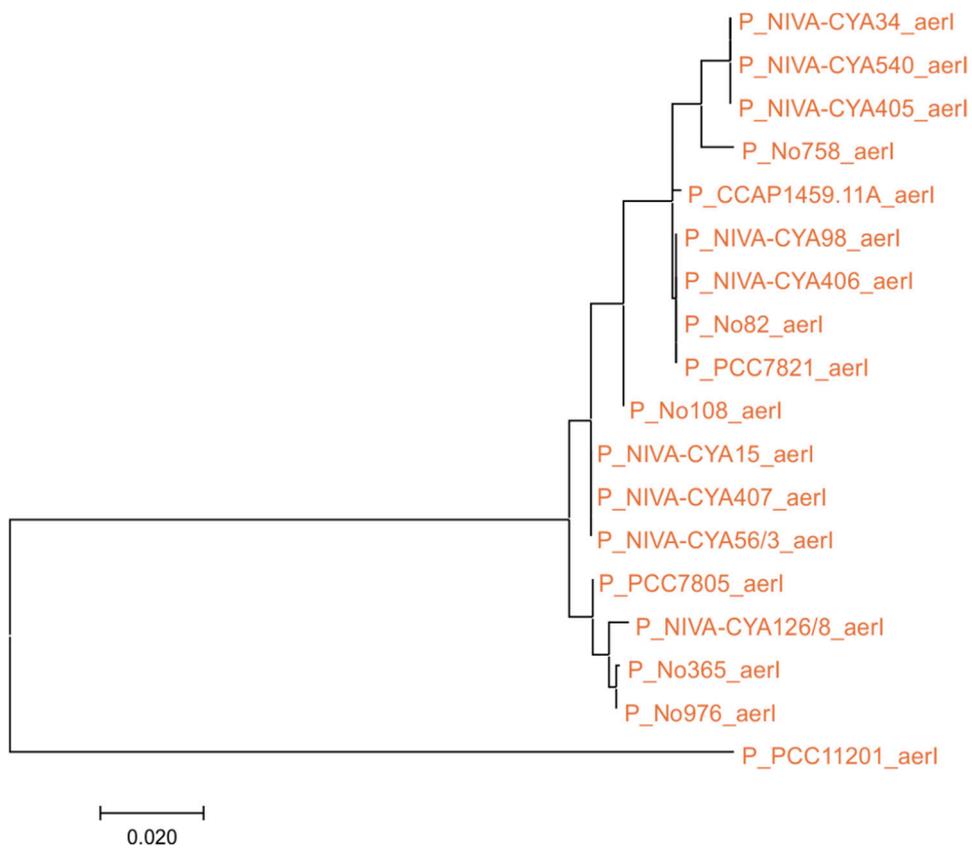
**Figure S5.** Maximum likelihood phylogenetic tree based on *aerF* (nucleotide) sequence (alignment: 801 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.



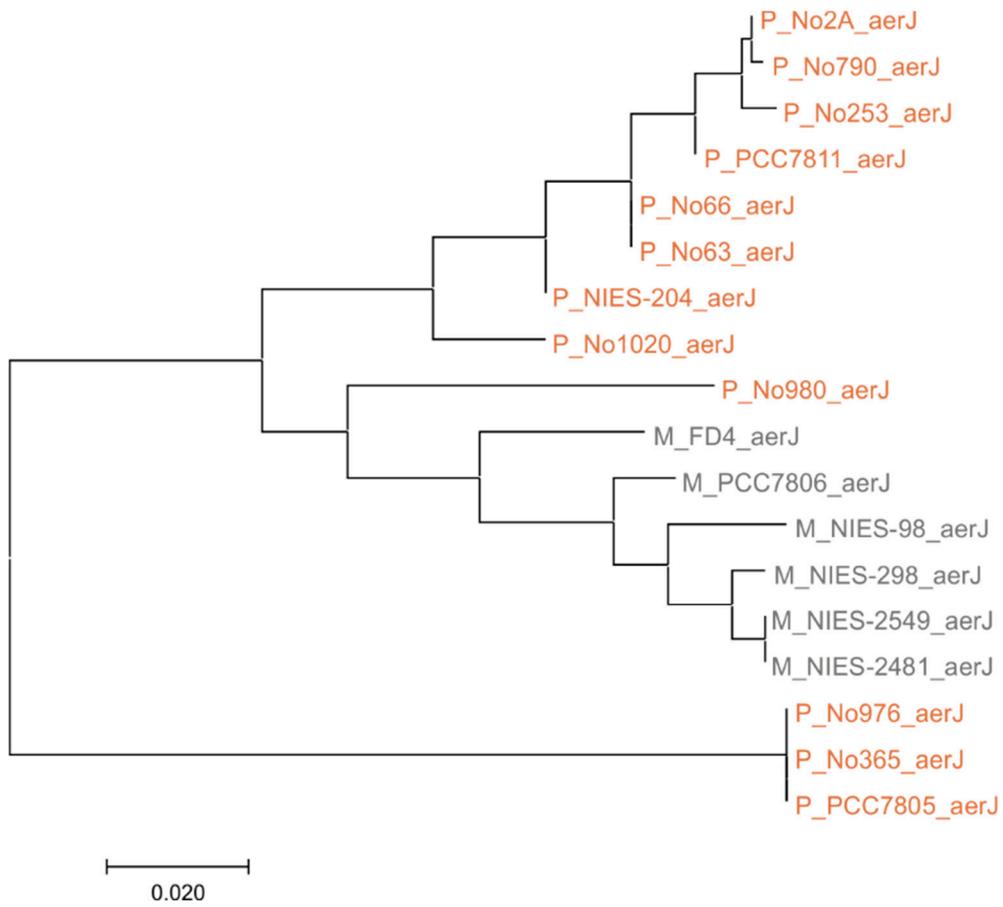
**Figure S6.** Maximum likelihood phylogenetic tree based on *aerG* (nucleotide) sequence (alignment: 3366 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.



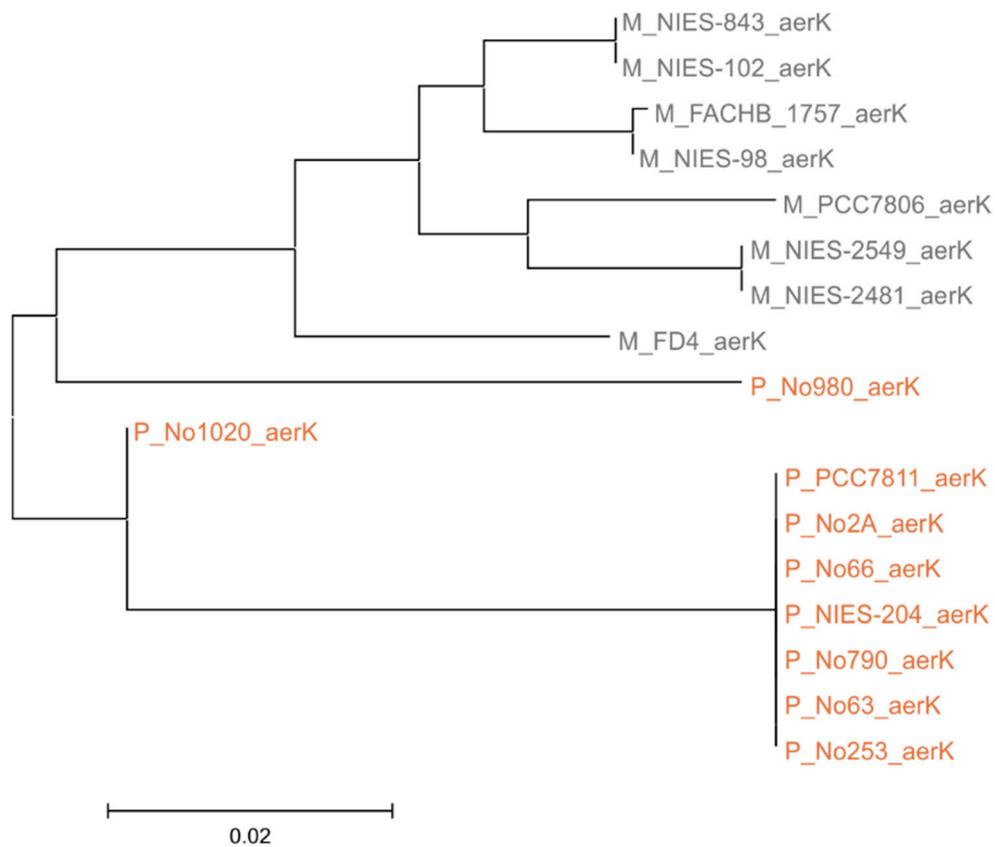
**Figure S7.** Maximum likelihood phylogenetic tree based on *aerG\_module2* and *aerM* (nucleotide) sequence (alignment: 4533 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nostoc* UIC10630 and *Nodularia* CCY9414 served as outgroups.



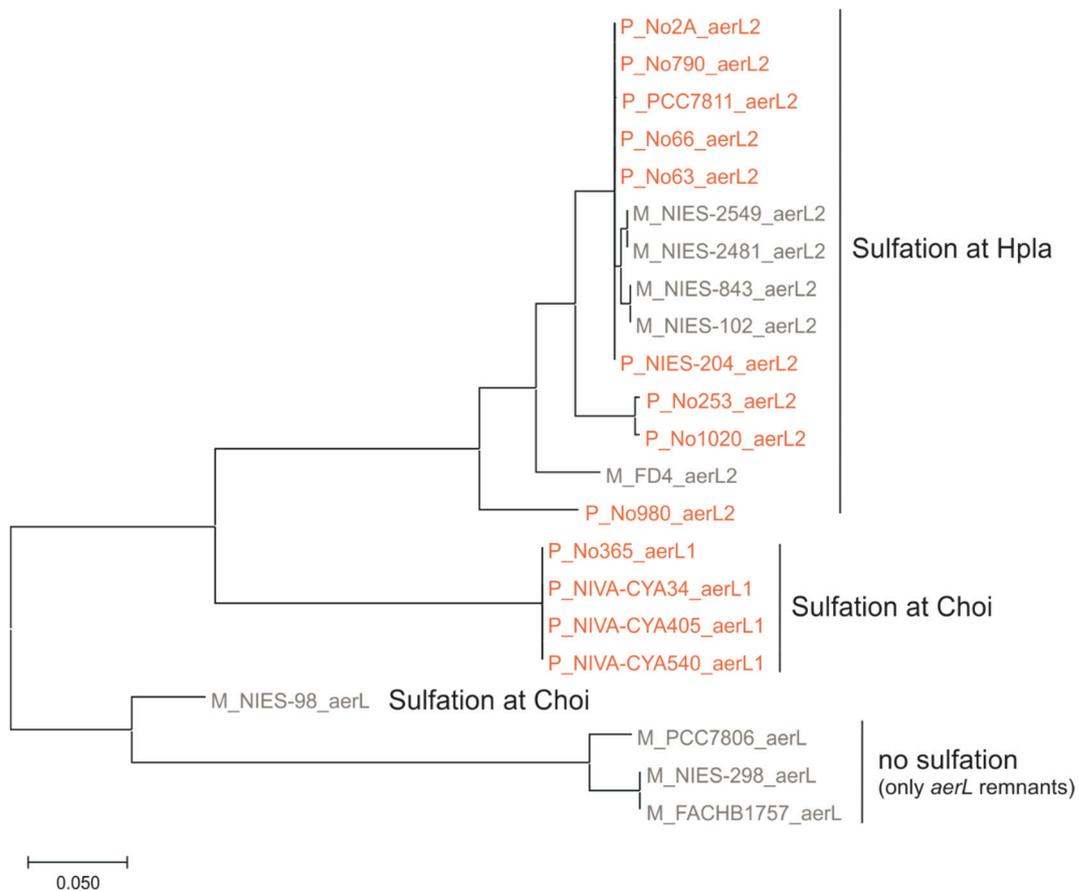
**Figure S8.** Maximum likelihood phylogenetic tree based on *aerI* (nucleotide) sequence (alignment: 1260 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each.



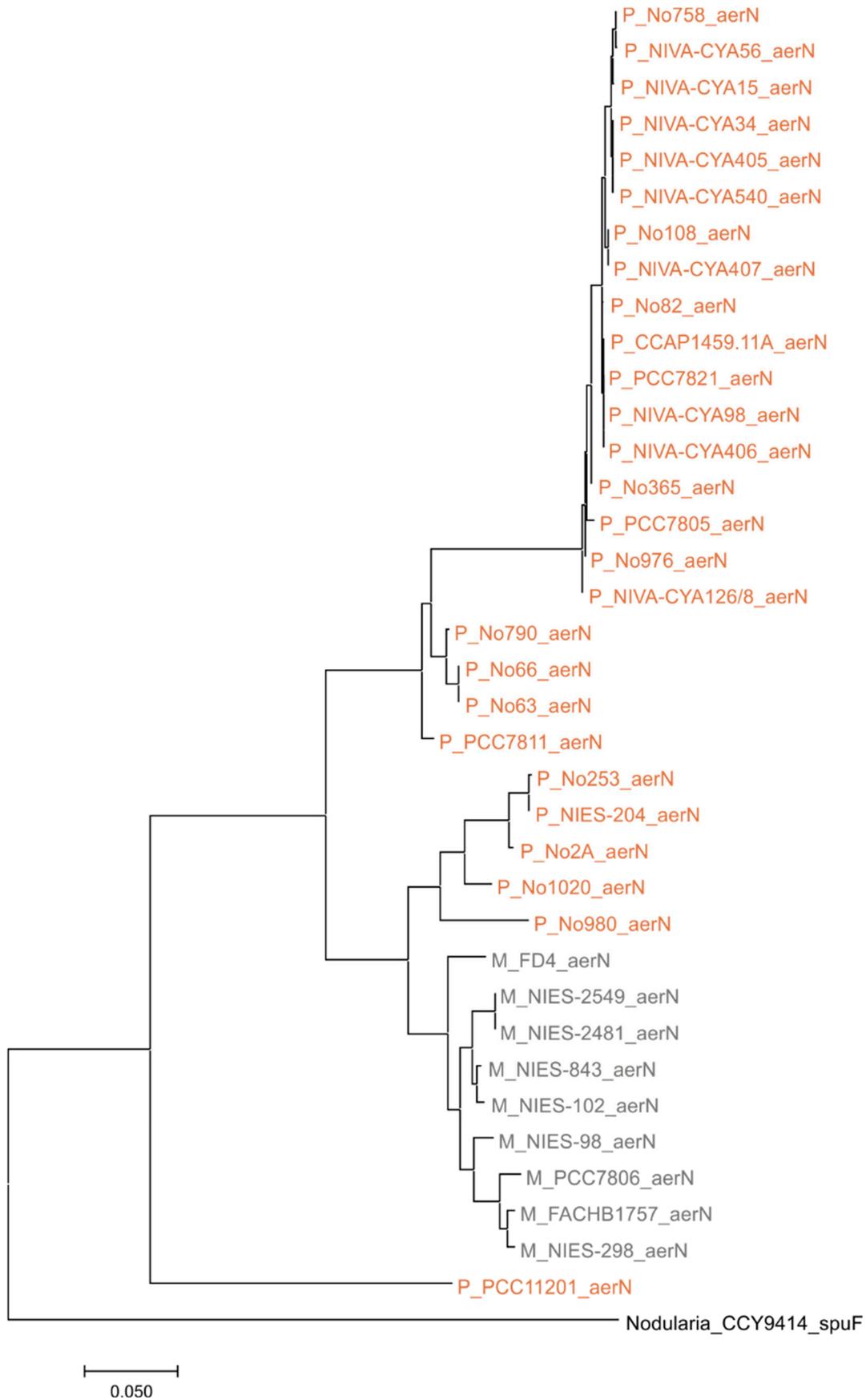
**Figure S9.** Maximum likelihood phylogenetic tree based on *aerJ* (nucleotide) sequence (alignment: 1905 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each.



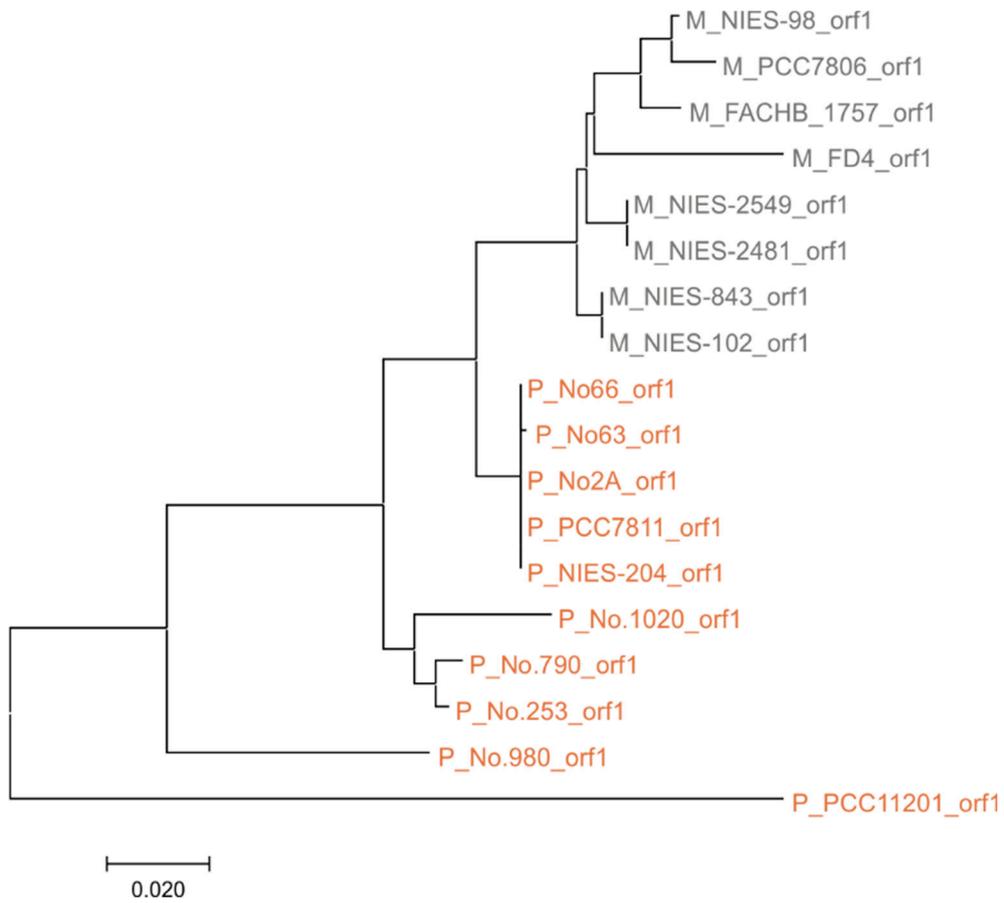
**Figure S10.** Maximum likelihood phylogenetic tree based on *aerK* (nucleotide) sequence (alignment: 1044 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each.



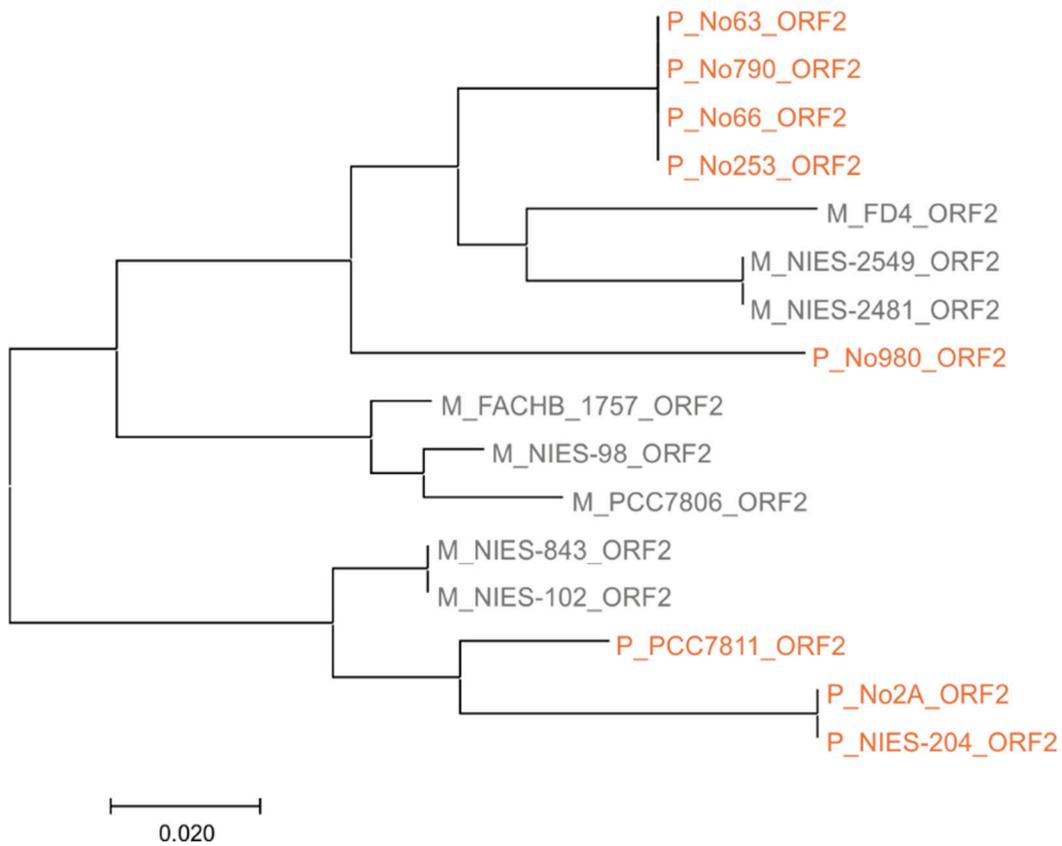
**Figure S11.** Maximum likelihood phylogenetic tree based on *aerL* (nucleotide) sequence (alignment: 1011 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. In addition the putative sulfation reactions are indicated (Figure 4).



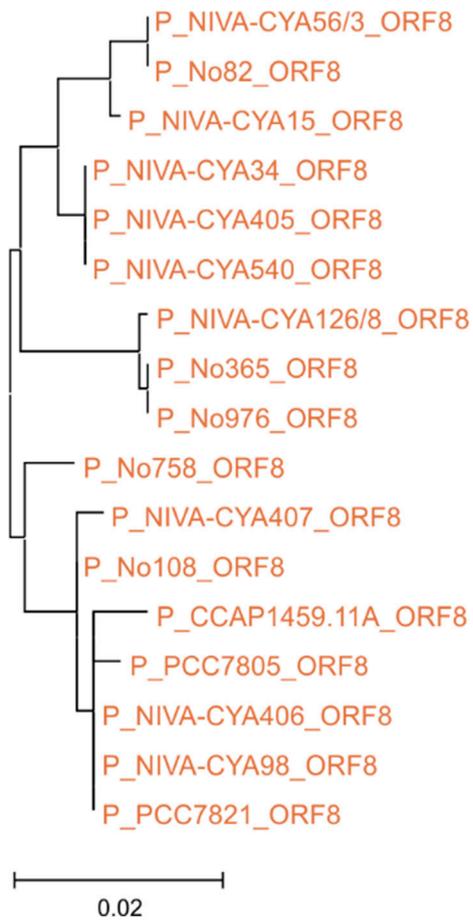
**Figure S12.** Maximum likelihood phylogenetic tree based on *aerN* (nucleotide) sequence (alignment: 2058 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each. *Nodularia* CCY9414 served as outgroup.



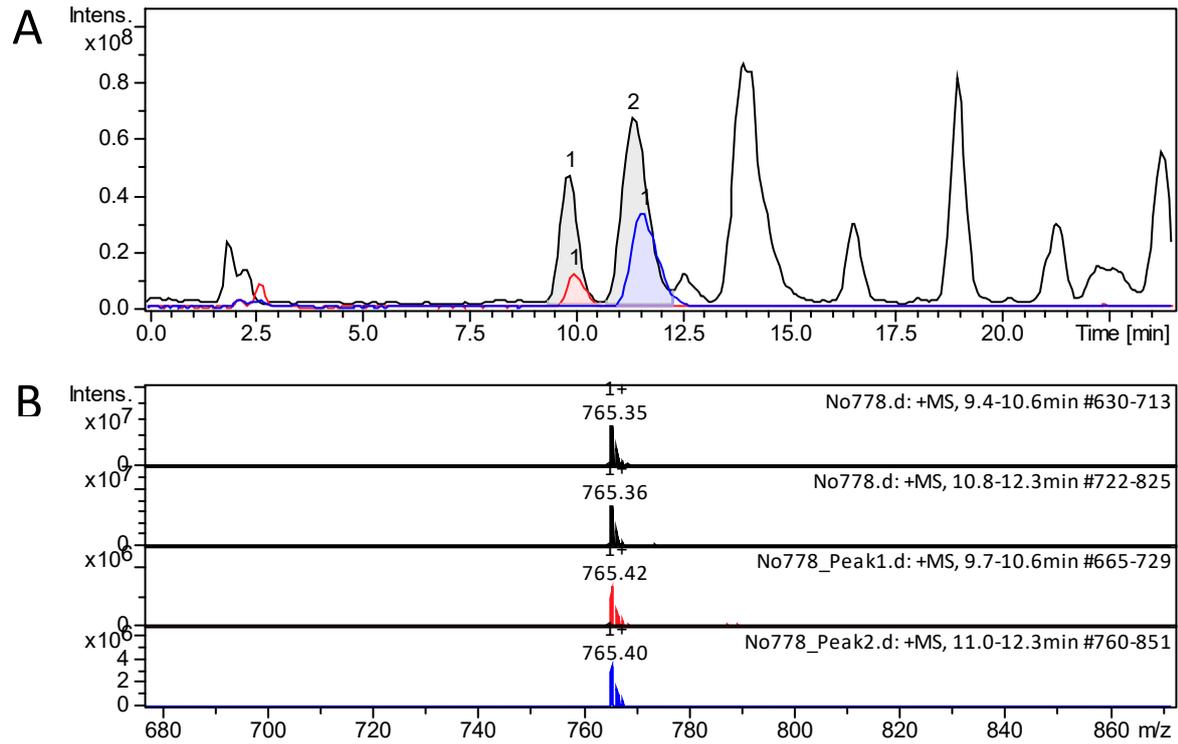
**Figure S13.** Maximum likelihood phylogenetic tree based on ORF1 (nucleotide) sequence (alignment: 786 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each.



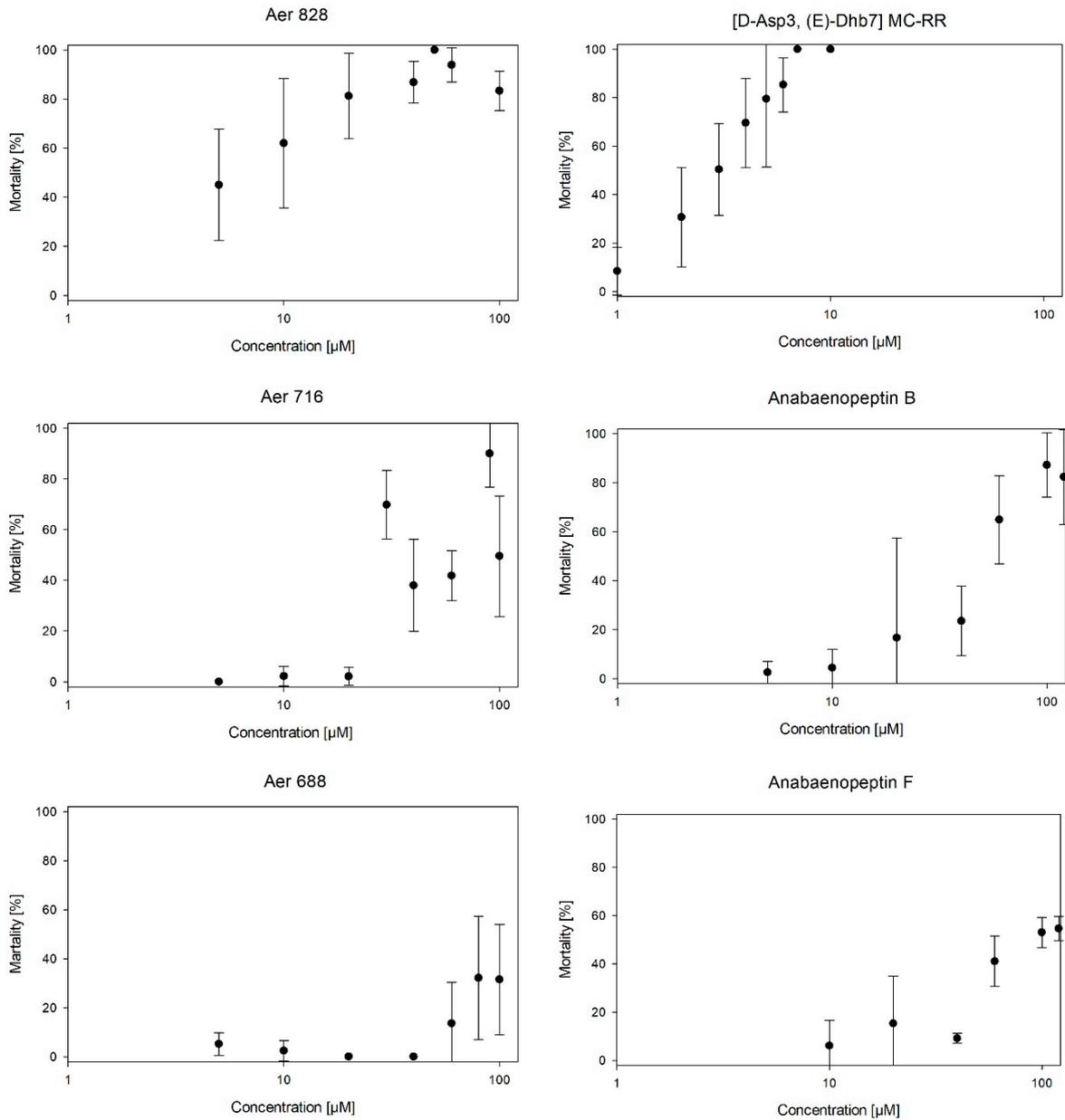
**Figure S14.** Maximum likelihood phylogenetic tree based on ORF2 (nucleotide) sequence (alignment: 627 bp). P\_, M\_ indicate genera *Planktothrix* or *Microcystis*, each.



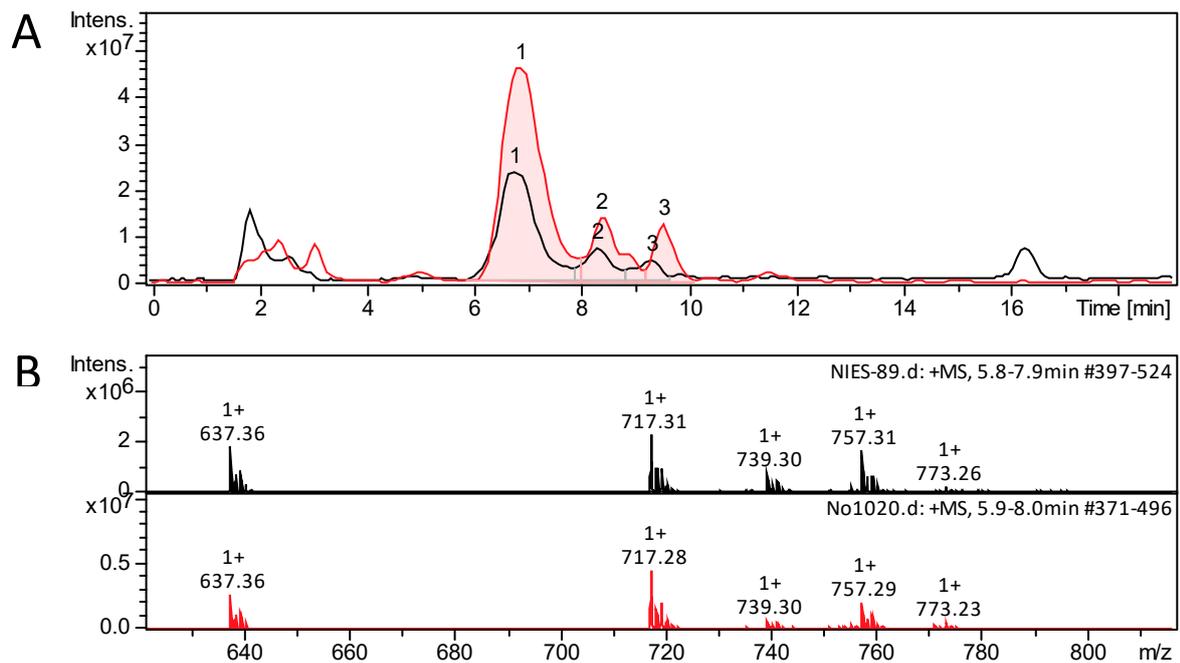
**Figure S15.** Maximum likelihood phylogenetic tree based on ORF8 (nucleotide) sequence (alignment: 1161 bp). P\_ indicates genus *Planktothrix*.



**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);  $[M-SO_3+H]^+$  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.