**Table S1.** Strains used for mock community, concentration of isolated DNA, and percentage contribution of used DNA.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| № | Taxa | Strain information | Strain ID | DNA concentration, ng/µl | % of total  DNA in MIX |
| 1 | *Phormidium autumnale (Microcoleus autumnalis)* | benthic, isolated from microbial mat (Eastern Pamir) | L01 | 142.5 | 23.9 |
| 2 | *Phormidium autumnale (Microcoleus autumnalis)* | benthic, isolated from microbial mat (Eastern Pamir) | L02 | 21.2 | 3.6 |
| 3 | *Phormidium sp.* | benthic, isolated from microbial mat (Eastern Pamir) | L03 | 53.1 | 8.9 |
| 4 | *Geitlerinema ionicum* | benthic, isolated from microbial mat (Eastern Pamir) | L04 | 16.5 | 2.8 |
| 5 | *Geitlerinema ionicum* | benthic, isolated from microbial mat (Eastern Pamir) | L05 | 50.5 | 8.5 |
| 6 | *Oscillatoria sp.* | benthic, isolated from microbial mat (Eastern Pamir) | L06 | 52.6 | 8.8 |
| 7 | *Nostoc* sp. | benthic, isolated from microbial mat (Eastern Pamir) | L07 | 13.4 | 2.3 |
| 8 | *Hillbrichtia pamiria genus novum species novum* | benthic, isolated from microbial mat from the hotspring (Eastern Pamir) | L08 | 56.5 | 9.5 |
| 9 | *Nostoc paludosum* | benthic, isolated from microbial mat (Eastern Pamir) | L09 | 18.8 | 3.2 |
| 10 | *Anabaena sp.* | benthic, isolated from microbial mat (Eastern Pamir, Tajikistan) | L10 | 17.3 | 2.9 |
| 11 | *Nostoc edaphicum* | benthic, isolated from microbial mat (Eastern Pamir ,Tajikistan) | L11 | 12.1 | 2.0 |
| 12 | *Calothrix* sp*.* | lithobionthic, isolated from calcite (Eastern Pamir, Tajikistan) | L12 | 79.7 | 13.4 |
| 13 | *Synechocystis* sp*.* | planktonic  (Baltic Sea) | L13 | 15.6 | 2.6 |
| 14 | *Microcystis aeruginosa* | SAG 14.85 | L14 | 10.25 | 1.7 |
| 15 | *Synechococcus*sp*.* | planktonic (lake, Poland) | L15 | 35.4 | 6.0 |

**Table S2**. The reds obtained for studied samples before and after filtering.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample-id** | **Input reads** | **Filtered reads** | **Percentage of input passed filter** |
| Cyx15a\_V3-V4 | 74752 | 63107 | 84.4 |
| *Cyx15a\_V3-V4* | *116810* | *102931* | *88.2* |
| Cyx15a\_V6 | 116713 | 105513 | 90.4 |
| Cyx8\_V3-V4 | 71955 | 64286 | 89.3 |
| *Cyx8\_V4-V6* | *97293* | *84287* | *86.6* |
| Cyx8\_V6 | 91616 | 83944 | 91.6 |
| Cyx9\_V3-V4 | 97344 | 85560 | 87.9 |
| *Cyx9\_V4-V6* | *99722* | *87687* | *87.9* |
| Cyx9\_V6 | 93486 | 84433 | 90.3 |
| Mix\_V3-V4 | 74943 | 64641 | 86.3 |
| *Mix\_V4-V6* | *117286* | *103375* | *88.1* |
| Mix\_V6 | 104716 | 96093 | 91.8 |

**Table S3.** The number of bacterial and cyanobacterial ASVs with the percentage contribution   
of cyanobacterial ASVs using studied primer pairs.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **ASV**  **bacteria** | **ASV cyanobacteria** | **% of cyanobacteria** |
| **Cyx15a V3-V4** | 8919 | 667 | 7% |
| ***Cyx15a V4-V6*** | 68024 | 66917 | 98% |
| **Cyx15a V6** | 75844 | 73340 | 97% |
| **Cyx8 V3-V4** | 10631 | 6306 | 59% |
| ***Cyx8 V4-V6*** | 83276 | 81677 | 98% |
| **Cyx8 V6** | 84149 | 83128 | 99% |
| **Cyx9 V3-V4** | 20572 | 855 | 4% |
| ***Cyx9 V4-V6*** | 62777 | 60711 | 97% |
| **Cyx9 V6** | 70235 | 67951 | 97% |
| **Mix V3-V4** | 9391 | 5996 | 64% |
| ***Mix V4-V6*** | 47514 | 46473 | 98% |
| **Mix V6** | 72762 | 71460 | 98% |

**Table S4.** The results of the amplicon sequencing of the mock community (MIX). The taxa names of the ASVs were verified based on the Cydrasill package with our matched 16S sequences from the mock community. The interactive tree is available under the link iTOL, https://itol.embl.de/personal\_page.cgi login: CyanoMIX, password: CyanoMIX2021

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Strain ID | V3-V4-MIX | V4-V6-MIX | V6-MIX |
| Calothrix\_PCC6303 L12 Calothrix sp. (Calothrix klad 0.999999) | L12 | 338 | 8122 | 0 |
| Cyanobacteriaceae L08 | L08 | 8 | 0 | 0 |
| Cyanobacteriales L12 Calothrix sp. (Calothrix klad 0.999911) | L12 | 0 | 0 | 15014 |
| Cyanobacteriales | ? | 16 | 35 | 3448 |
| Cyanobacteriales L08 | L08 | 457 | 0 | 0 |
| Cyanobacteriia | ? | 964 | 0 | 2205 |
| Cyanobacteriia MG652616 Oculatella kazantipica KZ-19-s-2 | ? | 0 | 0 | 261 |
| Cyanobacteriia L08 0.999869 | L08 | 59 | 41 | 53 |
| Cyanobacteriia\_Chloroplast | ? | 66 | 19 | 0 |
| Cyanobium\_PCC6307 | L15 | 70 | 760 | 0 |
| Geitlerinema\_L0D9 Geitlerinema clad (Geitlerinema 0.9592812992) | 4? | 0 | 652 | 0 |
| Geitlerinema\_L0D9\_uncultured\_Antarctic Y423710 Geitlerinema carotinosum AICB 37 | 5? | 218 | 4344 | 0 |
| Geitlerinema\_uncultured\_Antarctic | 4,5? | 2 | 0 | 0 |
| Leptolyngbya\_VRUC\_135 | ? | 0 | 209 | 0 |
| Leptolyngbya\_VRUC\_135\_Oculatella\_mojaviensis MG652616 Oculatella kazantipica KZ-19-s-2 | ? | 0 | 204 | 0 |
| Nodosilinea\_PCC7104 | ? | 0 | 85 | 77 |
| Nodosilinea\_PCC7104 Leptolyngbya sp KIOST-1 0.987916 | ? | 0 | 0 | 62 |
| Nodosilinea\_PCC7104 Nodosilinea nodulosa PCC 7104 Leptolyngbya sp RG Leptolyngbya PCC7104 (0.995741) | ? | 24 | 0 | 0 |
| Nodularia\_PCC9350 | L10 | 46 | 0 | 0 |
| Nodularia\_PCC9350 L10 Nodularia 1 (Nodularia spumigena CCY9414) | L10 | 56 | 0 | 0 |
| Nostoc\_PCC73102 | L7 | 95 | 730 | 36472 |
| Nostocaceae L08 | L08 | 0 | 9162 | 3508 |
| Oxyphotobacteria\_Incertae\_Sedis\_Unknown\_Famil | ? | 52 | 0 | 9 |
| Phormidiaceae | 2? | 0 | 13139 | 0 |
| Sedis\_Unknown\_Family\_uncultured\_uncultured\_organism | ? | 39 | 0 | 0 |
| Synechococcus\_nidulans | L15 | 14 | 0 | 0 |
| Synechocystis\_PCC6803\_uncultured\_bacterium L13 (Aphanocapsa cf rivularis UAM 390 JQ070058 0.3343881702) | L13 | 0 | 391 | 1139 |
| Trichocoleus\_sociatus Geitlerinema sp PCC 7105/Planktothrix agardhii NIVA-CYA 98 G Planktothrix rubescens NIVA-CYA 98/Planktothrix agardhii NIVA-CYA 34 G Planktothrix agardhii NIVA-CYA 34/Planktothrix agardhii NIVA-CYA 15 G Planktothrix agardhii NIVA-CYA 15 0.986649 | L06 ? | 79 | 0 | 0 |
| Tychonema\_CCAP\_145911B |  | 7704 | 21596 | 0 |
| Tychonema\_CCAP\_145911B L03 Oscillatoria princeps PCC 10802/Uncultured cyanobacterium | L03 | 0 | 0 | 9719 |
| other bacteria | ? | 3559 | 0 | 653 |
| Sum |  | 13866 | 59489 | 72620 |