Isolation of axenic cyanobacterium and the promoting effect of associated bacterium on axenic cyanobacterium

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Effects of antibiotics and lysozyme on the xenic cyanobacterium

The antibiotics (including tetracycline, cephalosporin, kanamycin, penicillin and streptomycin) and lysozyme were purchased from Wuhan Dingguo biological technology Co., LTD. The sensitivities of heterotroph to tetracycline, cephalosporins, kanamycin, penicillins and streptomycin were evaluated by the filtering paper method with the final concentration of 100 µg mL-1 for each treatment. The sensitivities of cyanobacterium to five antibiotics were carried out by adding antibiotic with the final concentration of 100 µgmL-1 in 250 mL sterilized Erlenmeyer flasks containing 100 mL xenic *Microcystis* 905 culture (the initial cyanobacterial cell number was 1.0 × 106 cell mL-1), and the controls (CK) were added without any antibiotic. Effects of lysozyme on the cyanobacterium and heterotrophs were performed by adding lysozyme (the lysozyme was dissolved with sterile distilled water and then ﬁltered through with a 0.22 μm membrane) at ﬁnal concentrations of 0, 1.0, 2.0, 5.0, and 10.0 mg mL-1 in 250 mL sterilized Erlenmeyer flasks containing 100 mL xenic *Microcystis* 905 culture (the initial cyanobacterial cell number was 1.0 × 106 cell mL-1). Heterotrophs and cyanobacterial cell densities were determined after incubation for 2 d. All the experiments were performed under aseptic conditions, the controls (CK) and the treatments were replicated three times, and the arithmetical means (± SD) were used as the ﬁnal results.

The sensitivities of the cyanobacterium and heterotrophs to antibiotics were shown in Table S1. It indicated that tetracycline, cephalosporin and streptomycin had obvious inhibiting effect on strain B905-1, while kanamycin and penicillin had no inhibiting effect. It was also demonstrated that *Microcystis* 905 was significantly inhibited by all of the tested antibiotics except penicillins. Although the associated heterotrophic bacteria could be inhibited by tetracycline, cephalosporin and streptomycin, the growth of *Microcystis* 905 was inhibited at the same time, hence, the antibiotics used in this study were unable to obtain axenic cyanobacterium.

Table S1 Effects of antibiotics on heterotrophs and cyanobacterium

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibiotics | Tetracycline | Cephalosporins | Kanamycin | Penicillins | Streptomycin |
| Halo of growth inhibition | 3.3 mm | 2.4 mm | — | — | 1.8 mm |
| Inhibition efficiency | 94.3 ± 3.5% | 88.5 ± 4.1% | 50.7 ± 2.4%\* | 5.6 ± 0.2%\*\*  | 81.2 ± 3.9%\*\* |

\* and \*\* represent a statistically significant difference of *p* < 0.05 and *p* < 0.01 when compared to the control.

The results of lysozyme on the cyanobacterium and heterotrophic bacteria demonstrated that both *Microcystis* 905 and heterotrophic bacteria were reduced with the increasing of lysozyme concentration; moreover, the reduction of *Microcystis* 905was much more obvious (Fig. S1). As the lysozyme concentration increased from 0 to 10.0 mg mL-1, the cell number of cyanobacterium decreased much stronger than that of the heterotroph. Hence, these approaches were ineffective in completely separating the *Microcystis* from the heterotrophs.

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Fig. S1 Effects of lysozyme on heterotrophs and *Microcystis* 905