A novel fungus *Penicillium canescens* LS-4.2 with algicidal activity against the toxic cyanobacterium *Microcystis aeruginosa*

Nadezda Medvedeva*, *Tatyana* Zaytseva, and *Irina* Kuzikova

St. Petersburg Federal Research Center of the Russian Academy of Sciences (SPC RAS), Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences, 18, Korpusnaya st., St. Petersburg, 197110, Russia

Abstract. In this study, the fungus strain LS-4.2 isolated from the bottom sediments of Lake Lower Suzdalskoe was tested for its algicidal activity. We identified the strain LS-4.2 as *Penicillium canescens* summing its morphological characteristics with the reported DNA sequence. We revealed that the filtrate of a 7-day culture of the fungus suppressed the cell growth of toxic *Microcystis aeruginosa*. Our results showed that the filtrate caused rapid *M. aeruginosa* growth inhibition up to complete cell lysis recorded after 4 days. Living fungal mycelia did not suppress the growth of cyanobacterium. According to the results of this study we suppose that strain LS-4.2 may be a potential bioagent in the control of cyanobacterial blooms.

1 Introduction

Harmful cyanobacterial blooms (HCBs) are currently being recorded in freshwater worldwide [1]. *Microcystis aeruginosa* is among the most common species of the blooming cyanobacteria, it should be noted that many strains of the genus *Microcystis* are able to form toxic metabolites – microcystins [2]. It was also reported about the cyanobacterial bloom from water bodies located in the St. Petersburg region, including Lake Lower Suzdalskoe (Suzdal). The surface area of Suzdal is 97 ha and the mean depth is 3.0 m. The citizens of St. Petersburg region use the water of this lake for recreational purposes.

The cyanobacteria *Microcystis spp.* and *Planktothrix agardhii*, a number of strains of which are known to produce cyanotoxins, dominate the lake Suzdal water for most of the summer season. In eutrophic and shallow Suzdal nine microcystins were found [3]. Microcystins (MCs) are cyclic heptapeptides that inhibit enzyme activity in hepatocytes. High doses of MCs cause extensive hemorrhages in the liver, and long-term exposure to low doses of MCs leads to malignant formations [4-5].

Many approaches have been proposed to combat HCBs, including physical and chemical methods [6-7]. However, most of the physical and chemical methods are

* Corresponding author: ngmedvedeva@gmail.com

expensive, and potentially hazardous to the environment. Biological methods are considered the most economical and environmentally friendly for inhibiting cyanobacteria. Many aquatic organisms have been studied for their ability to limit the growth of cyanobacteria. Viruses, bacteria, fungi, actinomycetes, protozoa, zooplankton organisms, mollusks have been investigated as potential agents of biological control in various studies. The activity of potential biocontrol agents varies from highly specific parasitism and predation to nonspecific forms of action, such as filtration, and competition for resources and the release of metabolites that inhibit cyanobacterial growth [2].

Many of these methods involve the use of microorganisms with anticyanobacterial activity [8]. Most of the known anticyanobacterial microorganisms are bacteria, belonging mainly to *Proteobacteria*, *Actinomycetes*, *Bacteroidetes*, *Firmicutes* and *Thermus* [2, 9].

However, the degradation of cyanobacteria cells by fungal strains is currently insufficiently explored. Only 15 fungal strains belonging to genera *Ascomycetes* (9 strains) and *Basidiomycetes* (6 strains) have been found to have the anticyanobacterial effects [2, 10].

In this study, we investigated the ability of novel fungus *Penicillium canescens* LS-4.2 to suppress the growth of the toxic cyanobacterium *Microcystis aeruginosa*.

2 Materials and methods

Fungal isolation, identification and cultivation.

The fungal strain LS-4.2 was isolated from bottom sediment collected from eutrophic Lake Lower Suzdalskoe on a Czapek's Agar with streptomycin (100 U mL^{-1}) as selective agent [11].

The fungal strain LS-4.2 was identified on the strength of morphological, cultural characteristics using determinant [12] and on ITS1-5.8S-ITS2 DNA region sequencing, which was amplified using universal primers pairs: ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' [13]. Isolation, amplification and sequencing of fungal genomic DNA were performed according to the previously described procedure [11]. The nucleotide sequence of the PCR products was determined using an ABI 3500xl genetic analyzer (Applied Biosystems, USA) at the Russian Collection of Agricultural Microorganisms (RCAM, WDCM 966). The sequences were compared with related sequences available in the GenBank databases using BLAST analysis (http://www.ncbi.nlm.nih.gov).

Fungus strain LS-4.2 was cultured in 250 mL Erlenmeyer flask with 50 mL of Czapek's liquid medium at 230 rpm (Certomat BS-1 rotary shaker) and 28±1°C. The fungal filtrate of a 7-day culture and living fungal mycelia were used for algicidal experiments.

Algal culture.

M. aeruginosa strain CALU 973 was acquired from Resource Center "Culture Collection of Microorganisms" of the Saint-Petersburg State University. The cyanobacterium was cultured under static conditions in 250-mL Erlenmeyer flasks with the BG11 liquid medium (100 mL) at a temperature 25±1°C, an illumination level of 1000 lux, a light/dark conditions of 12 h/12 h. Cell density of the culture was defined by OD_{680} (Genesys 10 UV scanning spectrophotometer).

Chlorophyll *a* was extracted from the biomass with 90% acetone at 4°C for 24 h. Optical densities of the acetone extract of chlorophyll *a* at wavelengths of 664, 647, and 630 nm were determined on a Genesys 10UV scanning spectrophotometer. The concentration of chlorophyll *a* was calculated by formular (1)

$$\text{chlorophyll } a \text{ (mg/l)} = 11.85 \times A_{664} - 1.54 \times A_{647} - 0.08 \times A_{630} \quad (1)$$

where A_{664}, A_{647}, A_{630} - optical densities of the acetone extract of chlorophyll *a* at wavelengths of 664, 647, and 630 nm, respectively.

Experimental designs.

An aliquot of algal culture was introduced into flasks with 100 mL BG11 medium to give an initial cell density OD_{680}=0.05. The living fungal mycelia (100 mg dw) and the fungal filtrate (10% (v/v)) were added into the flasks with algae. Algal culture with added BG11 medium (10% (v/v) for volume equalization and algal culture with Czapek's medium (10% (v/v) served as control 1 and control 2 respectively. All variants were cultivated under conditions for *M. aeruginosa*. The cyanobacterial growth was estimated daily. Three replicates were performed for treatment and controls.

Statistical analysis.

Statistical analysis and graphical presentation of the results were carried out using Microsoft Excel 2007 and Past 4.0 software. The statistical significance of differences between the variants was determined with one-way ANOVA (Mann-Whitney U-test ($p<0.05$). Data were presented as the arithmetic mean ± standard deviation (SD) of three independent biological replicates.

3 Results

The isolated strain LS-4.2 on solid nutrient Czapek-Dox medium on the 7th day of growth forms colonies consisting of a dense felt plexus of aerial mycelium, radially striated, with abundant conidial sporulation from grayish-greenish to smoky gray. Colonies on the 14th day at a temperature of 25°C reach a diameter of 21–25 cm. Reverse colonies is golden-yellow, than dark brown. ITS similarities between the strain LS-4.2 and other strains within genus *Penicillium – Penicillium arizonense* strain IBT: 122989T, *Penicillium canescens* NRRL910T, *Penicillium radiatolobatum* CBS 340.79T were 99.81% at 99-100% query cover. Strain LS-4.2 had the same ITS sequences as the type strains of *P. arizonense, P. canescens* and *P. radiatolobatum*. Though, based on macromorphological analysis, this strain is more likely to belong to the species *P. canescens*. Thus, based on the analysis of morphological characteristics and ITS sequencing, strain LS-4.2 was tentatively identified as *Penicillium canescens* Sopp.

In our study the LS-4.2 fungal filtrate of the 7-day culture greatly suppressed the growth cells of *M. aeruginosa* (Figures 1 and 2).

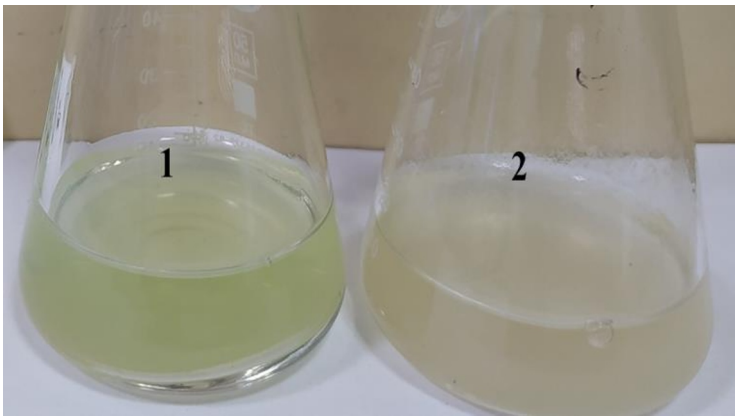

Fig. 1. Algicidal effect of *Penicillium canescens* LS-4.2 (4 days): 1 – *M. aeruginosa* cells; 2 – *M. aeruginosa* cells with fungal filtrate.

We observed that inhibitory effect was time-dependent (Figure 2).

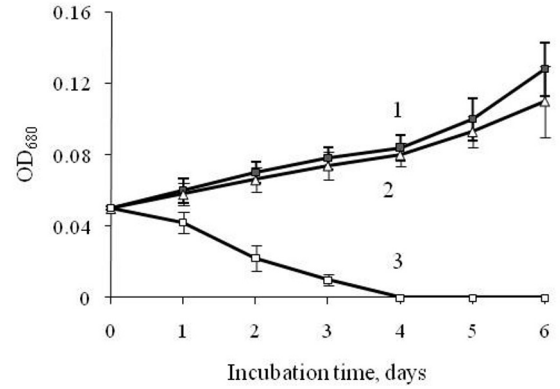

Fig. 2. The time-dependent effect of *Penicillium canescens* LS-4.2 filtrate on the *M. aeruginosa* growth: 1 – control 1; 2 – control 2; 3 – with fungal filtrate.

The insignificant (by 17%), but statistically significant level of *M. aeruginosa* cells lysis was observed already after the first day of incubation of cyanobacteria with fungal filtrate cells was observed. The cyanobacterial cells were almost completely lysed after 4 days. These results are consistent with the data on changes in the content of chlorophyll *a* in the medium (Figure 3). The living fungal mycelia did not suppress the growth of cyanobacterium (data not shown).

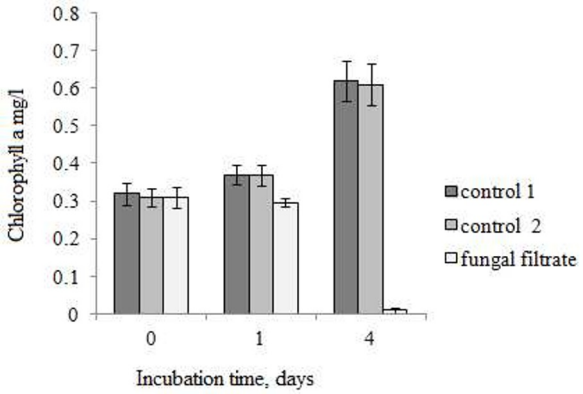

Fig. 3. The time-dependent effect of *Penicillium canescens* LS-4.2 filtrate on the chlorophyll *a*

4 Discussion

Many fungi of the genus *Penicillium* are destructors of toxic anthropogenic and natural environmental pollutants. It is known about the ability of the strain *Penicillium canescens* to decompose toxic polycyclic aromatic hydrocarbons [14]. Meanwhile, as far as with known, there is no information on the antialgal activity of *Penicillium canescens*.

Several studies have shown *Ascomycetes* including *Acremonium charticola* and *A. kiliense*, *Aspergillus niger*, *Aureobasidium pullulans*, *Emericellopsis minima* and *E. salmosynnemata*, *Penicillium chrysogenum, Trichoderma citrinoviride* and *T. deliquescens* are capable to inhibit the cyanobacterial growth [15–19].

In the present study, for the first time, the anticyanobacterial activity of *Penicillium canescens* was reduced. The fungal filtrate of strain LS-4.2 inhibited the cell growth of *M.*

aeruginosa and chlorophyll content in the medium. Our strain *P. canescens* LS-4.2 removed 100% of cyanobacterial cells within 4 days. This data suggest that strain LS-4.2 secrets antialgal substances into culture medium.

Most antialgal fungi have been found to have an indirect mechanism of action, releasing algicidal compounds that inhibit the growth of cyanobacteria [16, 18, 19]. An indirect mechanism of algicidal activity was found in the fungus *Trichoderma citrinov*iride against *M. aeruginosa*. The addition of the culture liquid filtrate of the ascomycete fungus *T. citrinoviride* to the medium with *M. aeruginosa* cells inhibited the growth of cyanobacteria after 48 h of incubation [19]. It is noted that the algicidal activity of this ascomycete exceeds the activity of some basidiomycetes.

Unlike ascomycetes, basidiomycetes exhibited algicidal activity upon direct contact with cyanobacterial cells [2, 10]. The species of fungi *Irpex lacteus* T2b, *Trametes versicolor* F21a and *Bjerkandera adusta* T1 with an initial dry weight of inoculum of 142, 97.6 and 78.4 mg, respectively, significantly inhibited the growth of *Microcystis aeruginosa* (initial concentration of chlorophyll a 600-700 µg/l) on average after 60 hours. The *T. versicolor* F21a strain showed the highest algicidal activity, complete inhibition of algae was observed after 30 hours of incubation. It was noted that neither inactivated fungal cells nor culture filtrates inhibited the growth of cyanobacteria, indicating that the algicidal activity of these fungi was due to a direct effect on cyanobacteria cells [20].

5 Conclusion

In summary an antialgal fungal strain identified as *Penicillium canescens* LS-4.2 was isolated from the bottom sediment from Lower Lake Suzdalskoe. The obtained results show that extracellular substances released from *P. canescens* LS-4.2 into medium lysed the toxic cyanobacterium *M. aeruginosa* cells. The fungus *P. canescens* could be used as cyanobacterial blooms control agent.

Acknowledgments

Present study was supported by the Russian Science Foundation (Grant No 23-24-00140).

References

1. Harke M J, Steffen M M, Gobler C J, Otten T G, Wilhelm S, Wood S A and Paerl H W 2016 A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis spp. Harmful Algae* **54** 4–20
2. Kong Y, Wang Y, Miao L, Mo S, Li J and Zheng X 2022 Recent Advances in the Research on the Anticyanobacterial Effects and Biodegradation Mechanisms of *Microcystis aeruginosa* with Microorganisms. *Microorganisms* **10** 1136
3. Chernova E, Russkikh I, Voyakina E, Zhakovskaya Z 2016 Occurrence of microcystins and anatoxin-a in eutrophic lakes of Saint Petersburg, Northwestern Russia. *Oceanol. Hydrobiol. Stud.* **45(4)** 466–476.
4. Drobac D, Tokodi N, Simeunovic J, Baltic V, Stanic D and Svircev Z 2013 Human exposure to cyanotoxins and their effects on health. *Archives of Industrial Hygiene and Toxicology* **64** 305–315
5. Svircev Z, Krstic S, Miladinov-Mikov M, Baltic V and Vidovic M 2009 Freshwater cyanobacterial blooms and primary liver cancer epidemiological studies in Serbia. *Journal of Environmental Science and Health* **27** 36–55
6. Xin H, Yang S, Tang Y, Wu M, Deng Y, Xu B and Gao N 2020 Mechanisms and

performance of calcium peroxide-enhanced Fe(ii) coagulation for treatment of *Microcystis aeruginosa*-laden water. *Environ. Sci. Water Res. Technol.* **6** 1272–1285
7. Chen Z, Li J, Chen M, Koh K Y, Du Z, Gin K Y-H, He Y, Ong C N and Chen J P 2021 *Microcystis aeruginosa* removal by peroxides of hydrogen peroxide, peroxymonosulfate and peroxydisulfate without additional activators. *Water Res.* **201** 117263
8. Sun R, Sun P, Zhang J, Esquivel-Elizondo S and Wu Y 2018 Microorganisms-based methods for harmful algal blooms control: A review. *Bioresour. Technol.* **248** 12–20
9. Zaytseva T B, Safronova V I and Medvedeva N G 2022 *Streptomyces geldanamycininus* Z374 – a novel strain with biocidal activity against cyanobacteria. *Theoretical and applied ecology* **1** 159–166
10. Mohamed Z A, Hashem M, Alamri S, Campos A and Vasconcelos V 2021 Fungal biodegradation and removal of cyanobacteria and microcystins: potential applications and research needs. *Environ Sci Pollut Res* 28 37041–37050
11. Kuzikova I, Safronova V, Zaytseva T and Medvedeva N 2017 Fate and effects of nonylphenol in the filamentous fungus *Penicillium expansum* isolated from the bottom sediments of the Gulf of Finland. *Journal of Marine Systems* **171** 111-119
12. Samson R A and Reenen-Hoekstra E S 1988 *Introduction to food-borne fungi. 3rd ed*. (Baarn: CBS) 297
13. White T J, Bruns T, Lee S and Taylor J 1990 *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications (*New York: Academic Press) 315–322
14. Veignie E, Rafin C 2022 Efficiency of *Penicillium canescens* in dissipating PAH in industrial aged contaminated soil microcosms and its impact on soil organic matter and ecotoxicity. *Processes* **10(3)** 532
15. Han S, Zhou Q, Lilje O, Xu W, Zhu Y, and van Ogtrop F 2021 Inhibition mechanism of Penicillium chrysogenum on Microcystis aeruginosa in aquaculture water. *Journal of Cleaner Production* **299** 126829
16. Redhead K and Wright J 1978 Isolation and properties of fungi that lyse blue-green algae. *Appl Environ Microbiol* **35(35)** 962–969
17. Safferman R S and Morris M-E 1962 Evaluation of natural products for algicidal properties. *Appl Microbiol* **10** 289–292
18. Mohamed Z A, Alamri S, Hashem M and Mostafa Y 2020 Growth inhibition of *Microcystis aeruginosa* and adsorption of microcystin toxin by the yeast *Aureobasidium pullulans*, with no effect on microalgae. *Environ Sci Pollut Res Int* **27** 38038–38046
19. Mohamed Z A, Hashem M and Alamri S A 2014 Growth inhibition of the cyanobacterium *Microcystis aeruginosa* and degradation of its microcystin toxins by the fungus. *Trichoderma citrinoviride*. *Toxicon* **86** 51–58
20. Han G, Feng X, Jia Y, Wang C, He X, Zhou Q and Tian X 2011 Isolation and evaluation of terrestrial fungi with algicidal ability from Zijin Mountain, Nanjing, China. *The Journal of Microbiology* **49(4)** 562–567
21. Lukyanova M, Kovshov V, Zalilova Z, Faizov N 2022 Modeling the Expansion of Agricultural Markets. *Montenegrin Journal of Economics* **18(2)** 127–141
22. Salimova G, Ableeva A, Lubova T, Zalilova Z, Sharafutdinov A 2020 The Role of Agriculture in Gross Added Value. *Montenegrin Journal of Economics* **16(1)** 183–191