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Allelopathic activity of the *Synechococcus* sp. (Cyanobacteria, Chroococcales) on selected cyanobacteria species

Introduction

Allelopathy may be one of the factors contributing to the formation and maintenance of cyanobacterial blooms, which strongly affect coastal marine ecosystems and cause economic problems for commercial aquaculture (Gross, 2003). Furthermore, some species of cyanobacteria are able to produce and release secondary metabolites that may be harmful to microorganisms, phyto- and zooplankton, crustaceans, fish and even humans (Stal et al., 2003; Mazur-Marzec et al., 2015). Cyanobacteria are known to produce a wide range of secondary metabolites with various biological actions. Some of them, termed allelopathic compounds, have been shown to play a role in allelopathy (Leflaive, Ten-Hage, 2007).

The precise mode of action of allelopathic compounds remains relatively poorly known due to methodological difficulties. It is believed that the allelopathic compounds may be responsible for the natural selection of organisms, competition and ecological succession (Legrand et al., 2003). Moreover, it was indicated that some cyanobacteria are able to produce and release allelopathic compounds that affect the growth and development of other organisms (Gross, 2003; Żak, Kosakowska, 2015). This can be important for many areas of science and industry. Secondary metabolites isolated from cyanobacteria can be used in medicine, agriculture, as herbicides or insecticides, and maybe even in the creation of drugs (Berry et al., 2008; Hernández-Carlos, Gamboa-Angulo, 2011). Generally, the blooms of cyanobacteria that develop each summer in the freshwater and brackish ecosystems are composed of two different groups: the large, colony-forming, filamentous cyanobacteria and small-sized picocyanobacteria from the genus *Synechococcus* and *Synechocystis*. Picocyanobacteria fraction may comprise as much as 80% of the total cyanobacterial biomass and contribute as much as 50% of the total primary production of a cyano-

bacterial bloom (Stal et al., 2003). Picocyanobacteria strain of the genus *Synechococcus* are very important organisms in the world's oceans, however, the information about allelopathic interactions between pico- and filamentous cyanobacteria in aquatic ecosystems are scarce. The main aim of this work was to estimate the allelopathic interaction of picocyanobacterium *Synechococcus* sp. on selected cyanobacteria. In this study, the influence of allelopathic compounds on the growth, cell-morphology, photosynthetic pigments, chlorophyll fluorescence and performance of photosynthesis in the analysed species was investigated by single and multiple addition of cell-free filtrate obtained from picocyanobacterium *Synechococcus* sp. In this experiment we investigated the effect of single and multiple addition of cell-free filtrate obtained from *Synechococcus* sp. on selected pico- and filamentous cyanobacteria: *Synechocystis* sp., *Geitlerinema amphibium*, *Nodularia spumigena* and *Nostoc* sp. Future studies should examine the allelopathic activity of different strains of picocyanobacteria, including *Synechocystis* and *Aphanocapsa*.

Material and methods

The experiments were conducted on the picocyanobacterium *Synechococcus* sp. (BA-124) and the cyanobacteria *Geitlerinema amphibium* (BA-13), *Nodularia spumigena* (BA-15), *Nostoc* sp. (BA-81) and *Synechocystis* sp. (BA-153). The strains were isolated from the coastal zone of the Gulf of Gdańsk (southern Baltic Sea) and are maintained as unialgal cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk, Poland (Latała et al., 2006). The tests on batch cultures were carried out in 25 mL glass Erlenmeyer flasks containing sterilized f/2 medium (Guillard, 1975). The media were prepared from Baltic water with a salinity of about 8 psu, which was filtered through Whatman GF/C glass fiber filters, and autoclaved. Analyzed cyanobacteria were grown 7 days in constant conditions of 20°C and 8 psu, under a 16:8h light : dark cycle at 10 $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ and this were the control treatment conditions. Fluorescent lamps (Cool White 40W, Sylvania, USA) were used as source of irradiance. The intensity of PAR was measured using a LI-COR quantum-meter with a cosine collector. The donor and target cyanobacteria were acclimated to these culture conditions for 7 days; afterwards, actively growing cultures were used for the establishment of the allelopathic experiment.

Allelopathic interactions were determined by using the method proposed by Śliwińska-Wilczewska et al. (2016a). Allelopathic interaction was studied by adding the single and multiple cell-free filtrate obtained from picocyanobacterial culture to tested cyanobacteria. The culture of *Synechococcus* sp. was filtered through 0.45 μm pore size Macherey-Nagel MN GF-5 filters. The cell-free filtrate ($V = 2$ mL) was added to 25 mL Erlenmeyer flasks containing the tested cyanobacteria ($V = 20$

mL). In all experiments, the ratio of picocyanobacterium to target species in Erlenmeyer flasks was adjusted to 1:1 based on the chlorophyll *a* content (final chlorophyll *a* concentration in the experimental cultures was $0.8 \mu\text{g chl } a \text{ mL}^{-1}$). Control samples were prepared by adding mineral medium f/2 with a volume equal to the added cell-free filtrate. To simulate the effects of continuously released picocyanobacterial allelochemicals on target species, picocyanobacterial filtrates were added daily to the target cultures for one week. The first addition was made as described above. Subsequent additions were made by removing 2 mL of test volume, used for cell counts each day and replacing it with an equal volume of fresh filtrate or control medium. Tests were conducted in triplicate and all analysed species were obtained from early exponential growth phase. The identification of allelopathic compounds is a difficult and time-consuming task and will need further investigation.

Culture density was determined by the number of cells and optical density (OD). The number of cells was counted using Bürker chamber and OD was measured spectrophotometrically at 750 nm with a Multiskan GO Thermo Scientific UV-VIS spectrophotometer. The results of cell counts and respective OD measurements were then used to determine the linear correlation between them for each species. Determined relationships were then used to estimate the number of cells in the experimental cultures after 1st, 3rd and 7th day of the cyanobacteria exposure to the picocyanobacterial filtrate. In addition, the morphological changes of target cyanobacteria were documented using a Nikon Eclipse 80i microscope with a camera Nikon DSU2.

In this work the concentration of photosynthetic pigments of target organisms was measured by spectrophotometric method after one week of exposure to the picocyanobacteria cell-free filtrate. Chlorophyll *a*, carotenoids and phycobilins were determined with a Thermo Scientific spectrophotometer UV-VIS Multiskan GO using 1 cm glass cuvette. The concentration of pigments was calculated according to equations provided by Jeffrey and Humphrey (1975), Strickland and Parsons (1972) and Bennett and Bogorad (1973).

Chlorophyll *a* fluorescence was measured with a Pulse Amplitude Modulation (PAM) fluorometer (FMS1, Hansatech), using 594 nm amber modulating beam with 4 step frequency control as a measuring light. Analysed species were taken for chlorophyll fluorescence analysis after 7th day of exposure to the filtrate. Before measurements, each sample taken from the culture was filtered through 13 mm glass fiber filters (Whatman GF/C). Before starting the experiment, the filter sample was adapted in the dark for about 15 minutes. Fluorescence parameters such as maximum PSII quantum efficiency (F_v/F_m) and effective PSII quantum efficiency (Φ_{PSII}) were calculated (Campbell et al., 1998).

The measurements of oxygen evolution were carried out on the 7th day of the experiment by using Clark-type oxygen electrode (Chlorolab 2, Hansatech). Temperature was controlled at 20°C with a cooling system LAUDA (E100, Germany). Illumination was provided by a high intensity probe-type light array with 11 red LED's centered on 650 nm. Irradiance was measured with a quantum sensor (Quantitherm, Hansatech). Dark respiration was estimated from O₂ uptake by cells incubated in the dark. Experimental data were fitted to the photosynthesis-irradiance curve using equation (Jassby, Platt, 1976).

Analysis of variance (ANOVA) was used to test for differences in analysed parameters between the target cyanobacteria cultures treated with picocyanobacterial cell-free filtrates and the control over the experimental period. a post hoc test (Tukey's HSD) was used to show which experiments of picocyanobacterial filtrate affected the growth of target cyanobacteria differently. Data is reported as mean ± standard deviation (SD). Levels of significance were: * $p < 0.05$. The statistical analyses were performed using the Statistica® 12 software.

Results

The effect of the cell-free filtrate addition obtained from *Synechococcus* sp. cultures on the growth of *Geitlerinema amphibium*, *Nodularia spumigena*, *Nostoc* sp. and *Synechocystis* sp. after 1, 3 and 7 days of exposition to the filtrates are shown in figure 1. The results showed that addition of cell-free filtrate from *Synechococcus* sp. decreased the number of cells of *G. amphibium*, *N. spumigena* and *Nostoc* sp. compared to their control. On the basis of the results it was found that the filtrate obtained from picocyanobacterium had the strongest effect on *G. amphibium*. After the 7th for a single and multiple filtrate addition obtained from *Synechococcus* sp. growth inhibition of *G. amphibium* expressed as a percent of culture density (% of control) constituted 48% and 56% respectively ($p < 0.05$). It was also observed, that the single addition of cell-free filtrate obtained from *Synechococcus* sp. significantly decreased the number of cells of *N. spumigena* and *Nostoc* sp. ($p < 0.05$). On the 1st, 3rd and the 7th day of the experiment, the minimum cell response of *N. spumigena* constituted 94%, 81% and 79% ($p < 0.05$) respectively, and for *Nostoc* sp. the percent of culture density constituted 87%, 81% and 82% ($p < 0.05$) respectively, in comparison to the control treatment. Moreover, on the 3rd and the 7th day of the experiment, the minimum cell response of *N. spumigena* after multiple addition of the cell-free filtrate, constituted 55% and 63%, respectively ($p < 0.05$). In addition, it was observed that the cell-free filtrate obtained from *Synechococcus* sp. did not affect the number of cells of *Synechocystis* sp. ($p > 0.05$).

The morphological changes of the target cyanobacteria after the picocyanobacterial cell-free filtrate addition was shown in figure 2. It was shown that the addition

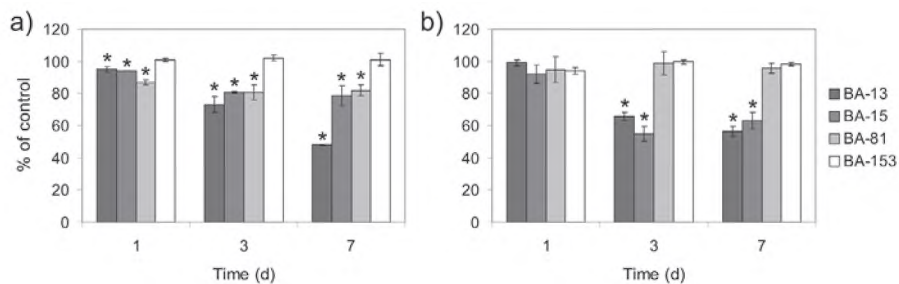


Fig. 1. The effect of the a) single and b) multiple additions of cell-free filtrate from *Synechococcus* sp. cultures on the growth of *Geitlerinema amphibium* (BA-13), *Nodularia spumigena* (BA-15), *Nostoc* sp. (BA-81) and *Synechocystis* sp. (BA-153) after 1, 3 and 7 days of exposition to the filtrates, expressed as a percent of culture density (% of control). The values refer to means ($n = 3$, mean \pm SD). Asterisk indicates significant difference compared with control

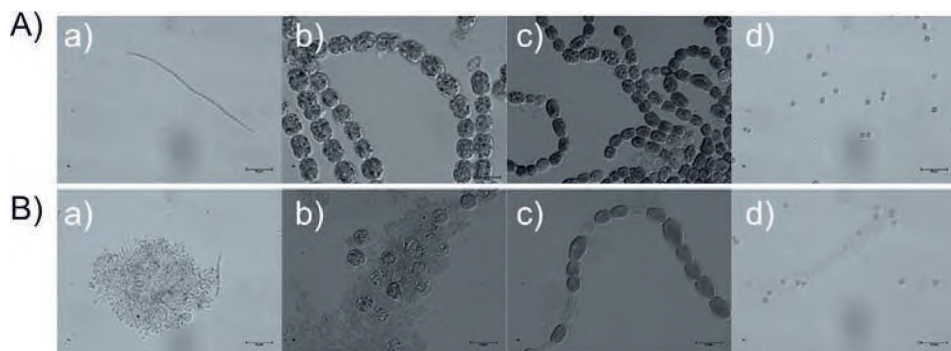


Fig. 2. The cells morphology of a) *Geitlerinema amphibium*, b) *Nodularia spumigena*, c) *Nostoc* sp. and d) *Synechocystis* sp. for A) control sample and B) in the experiments with the addition of cyanobacterial cell-free filtrate after 7 days of exposure

of cell-free filtrate caused a decline of pigmentation and cell lysis of *G. amphibium*, *N. spumigena* and *Nostoc* sp. compared to the control culture. In contrast, it was observed that the cell-free filtrate obtained from *Synechococcus* sp. had no effect on the picocyanobacterium *Synechocystis* sp.

The effect of the single and multiple additions of cell-free filtrate from *Synechococcus* sp. cultures on the pigment contents of *G. amphibium* after one week of exposition is shown in figure 3.

The results showed that allelochemicals released by picocyanobacterium *Synechococcus* sp. significantly decreased the chlorophyll *a* and phycobilins of *G. amphibium* compared to their control ($p < 0.05$). After one week of exposition, it was noted that the single addition of the filtrate resulted in decrease of chlorophyll *a* and phycobilins in the cells of analysed cyanobacterium, which was lower by 28% and 50%, respectively, compared to a control ($p < 0.05$). Based on the results, it was found that the multiple addition of cell-free filtrate obtained from *Synechococcus* sp. also caused

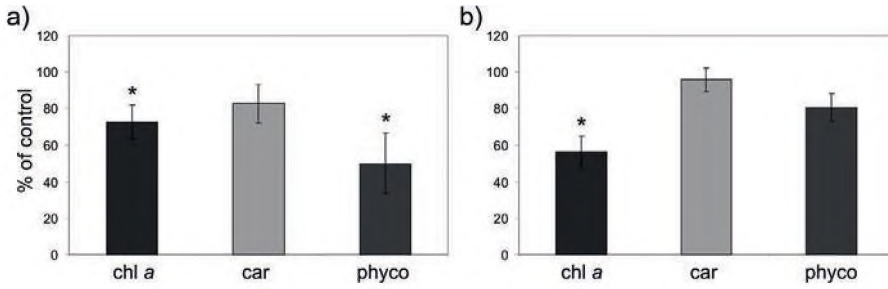


Fig. 3. The effect of the a) single and b) multiple additions of cell-free filtrate from *Synechococcus* sp. cultures on the pigment contents: chlorophyll *a* (chl *a*), carotenoids (car) and phycobilins (phyco) for *Geitlerinema amphibium* (BA-13) after 7 days of exposure. The values refer to means ($n = 3$, mean \pm SD). Asterisk indicates significant difference compared with control

a significant change in the pigment contents of *G. amphibium* cells. After one week of the experiment, chlorophyll *a* of this cyanobacterium was lower by 44% compared to control ($p < 0.05$). In addition, in this study it was observed that the cell-free filtrate obtained from *Synechococcus* sp. did not affect the carotenoids content of analysed cyanobacterium ($p > 0.05$).

The effects of picocyanobacterial cell-free filtrate on chlorophyll *a* fluorescence after 7 days of incubation are shown in figure 4. It was observed, that the single addition of cell-free filtrate from *Synechococcus* sp. significantly stimulated the values of Φ PSII ($p < 0.05$). Moreover, the multiple additions of cell-free filtrate from *Synechococcus* sp.

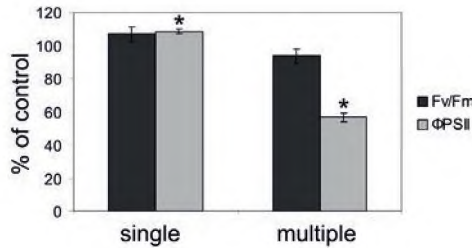


Fig. 4. The effect of the single and multiple additions of cell-free filtrate from *Synechococcus* sp. cultures on the fluorescence parameter F_v/F_m and Φ PSII for *Geitlerinema amphibium* (BA-13) after 7 days of exposure. The values refer to means ($n = 3$, mean \pm SD). Asterisk indicates significant difference compared with control

inhibited the values of the analysed parameters F_v/F_m and Φ PSII which amounted to 94% ($p > 0.05$) and 57% ($p < 0.05$) in comparison to the control, respectively.

P-E curves for analysed *G. amphibium* treated with single and multiple cell-free filtrate obtained from *Synechococcus* sp. are presented in figure 5. It was demonstrated that the investigated cyanobacterium was sensitive to the picocyanobacterial cell-free

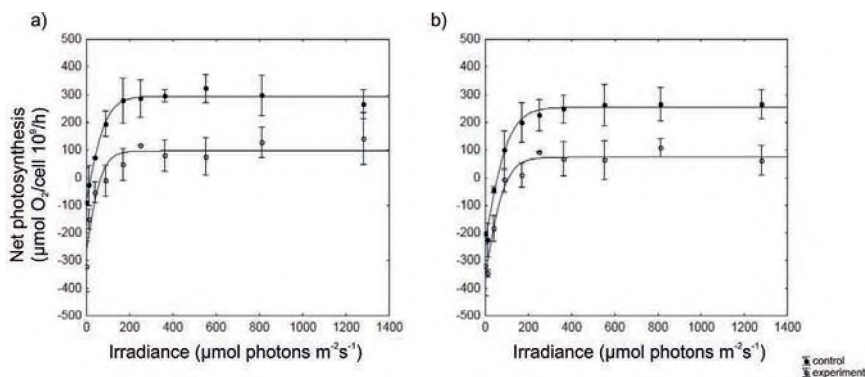


Fig. 5. P-E curves for *Geitlerinema amphibium* (BA-13) for control and a) single and b) multiple additions of cell-free filtrates obtained from *Synechococcus* sp. cultures after 7 days of exposure. The values refer to mean \pm SD ($n = 3$)

filtrate. In this study the influence of picocyanobacterial allelochemicals on the maximum photosynthesis (P_m) of the tested cyanobacterium was noted and its value constituted 31% and 27% ($p < 0.05$), respectively, in comparison to the control treatment.

Discussion

Allelopathic interaction may play a significant role in an aquatic ecosystem (Gross, 2003). Allelopathy is considered one of the factors promoting and maintaining cyanobacterial blooms in freshwater, brackish and marine ecosystems around the world. Although the allelopathy phenomenon is common in aquatic ecosystems, the mode of action of allelopathic compounds produced by picocyanobacteria on cyanobacteria remains poorly investigated.

Inhibition of growth of the target organism by production of allelopathic compounds is relatively widespread and the most frequently reported mode of action of cyanobacteria (Issa, 1999; Schagerl et al., 2002). In this study it was demonstrated that the addition of cell-free filtrate from *Synechococcus* sp. decreased the number of cells of filamentous cyanobacteria *Geitlerinema amphibium*, *Nodularia spumigena* and *Nostoc* sp. compared to their control. The results also showed that the allelochemicals produced by the picocyanobacterium had the strongest effect on *G. amphibium*. It was also observed, that for *G. amphibium*, the effect of single cyanobacterial filtrate additions was stronger than the effect of multiple additions. Surprisingly, it was observed that the cell-free filtrate obtained from *Synechococcus* sp. did not affect the number of cells of picocyanobacterium *Synechocystis* sp. Picocyanobacteria of the genus *Synechococcus* plays an important role in aquatic ecosystems but not much is known about its allelopathic activity. Information about the ability of allelopathic interactions of picocyanobacterium *Synechococcus* sp. was described by Śli-

wińska-Wilczewska et al. (2016a). In this work the authors showed that addition of the cell-free filtrate obtained from the picocyanobacterium *Synechococcus* sp. had a significant inhibitory effect on *N. spumigena*. Authors described that the longer the exposure time, the slower the growth of the analysed filamentous cyanobacterium, and on the 7th day of experiment the minimum cells response constituted about 60% in comparison to control treatment. In another work Schagerl et al. (2002) also demonstrated growth in inhibition of cyanobacteria *Anabaena cylindrica* and *Microcystis flos-aquae* after adding the filtrate from the cyanobacterium *Anabaena torulosa*. Also Issa (1999) investigated the effect of allelopathic compounds produced by *Oscillatoria angustissima* and *Calothrix parietina* on *Microcystis aeruginosa*, *Synechococcus* sp., *Scytonema hofmanni*, *Anabaena spiroides*, *Phormidium mölle*, *Nostoc muscorum*, *Oscillatoria angustissima* and *Calothrix parietina*. Author noted that cyanobacteria of the genus *Oscillatoria*, *Calothrix*, *Nostoc* and *Anabaena* were resistant to allelopathic compounds released by analysed cyanobacteria. Moreover, the recovery of *G. amphibium* growth after a multiple filtrate additions may have been due to, e.g., its ability to metabolise allelochemicals, as suggested by Suikkanen et al. (2004). It is believed that selective inhibition of growth of the target organism may affect the succession of selected cyanobacteria in aquatic ecosystem (Legrand et al., 2003).

There are only several reports indicating that the chemical compounds can cause structural and morphological changes in target cells (Gantar et al., 2008). In the present study it was shown that the tested allelopathic compounds obtained from *Synechococcus* sp. caused restriction of pigmentation and cell lysis of all analysed filamentous cyanobacteria. Gantar et al. (2008) also noted that after being exposed to the crude extract from *Fischerella* sp., cells of *Chlamydomonas* sp. showed distinctive morphological and structural changes. The electron microscopy revealed degeneration of thylakoids and disappearance of other cell structures including the nucleus. The results may in part explain the reasons for achieving competitive advantage of picocyanobacterium *Synechococcus* sp. over other filamentous cyanobacteria in many aquatic ecosystems.

The precise mode of action of allelopathic compounds remains relatively poorly known due to methodological difficulties. Therefore, there are only a few reports that allelopathic compounds may affect the pigments content, chlorophyll fluorescence and photosynthesis of target organisms (Gross et al., 1991; Śliwińska-Wilczewska et al., 2016b). In the present study it was shown that the tested allelopathic compounds obtained from *Synechococcus* sp. caused restriction of pigmentation and inhibition of photosynthetic activity of analysed *G. amphibium* compared to the control culture. Literature data indicates that allelopathic compounds produced by cyanobacteria can affect the photosynthesis, and detailed studies have shown that they act mainly on the photosystem II (PSII). Gross et al. (1991) showed that fischerellin a produced by cy-

anobacterium *Fischerella muscicola*, and the compounds released by *Trichormus dolium* and *Oscillatoria late-virens* inhibited activity of PSII in selected cyanobacteria and microalgae. In addition, Śliwińska-Wilczewska et al. (2016b) showed that the addition of cell-free filtrate from *Synechococcus* sp. cultures grown under varied light, temperature and salinity significantly inhibited the values of F_v/F_m of the diatom *Navicula perminuta*. Moreover, authors noted that the lowest values of P_m in *N. perminuta* were observed after addition of cell-free filtrate obtained from *Synechococcus* sp. grown at 25°C, and was 49% lower than for the control group. Inhibition of photosynthesis, the major physiological process of competing phytoplankton species, can be a defensive strategy of co-occurring cyanobacteria (Gross, 2003). Therefore, allelopathic interaction may result in inhibition of pigments content, photosynthesis and growth of target organisms which in part explain the domination of picocyanobacteria in many aquatic ecosystems.

Many studies indicated that cyanobacteria produced a wide spectrum of secondary metabolites, a source of a new bioactive and natural compounds, which can be used in medicine and pharmaceutical industry as antibacterial, antiviral and antifungal compounds and even against tumor cells (Berry et al., 2008; Hernández-Carlos, Gamboa-Angulo, 2011). The compounds produced by cyanobacteria can also be used in agriculture as herbicides, or insecticides, and may also have potential applications in biotechnology (Leflaive, Ten-Hage, 2007). It is also believed that allelopathy may be one of the important factors affecting the formation of massive cyanobacterial blooms in the aquatic environment (Gross, 2003; Legrand et al., 2003). Species forming harmful blooms in many places in the world are a serious problem, both ecologically and economically. Despite the seriousness of the problem, relatively little is known about the inhibitory effect of secondary metabolites of cyanobacteria on coexisting organisms in the Baltic Sea. Therefore, providing new information on the extent of the effect of allelopathic cyanobacteria, it may also be important for better understanding of the worldwide intensification of massive phytoplankton blooms in various aquatic ecosystems.

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References

- Bennett, A., Bogorad, L. (1973). Complementary chromatic adaptation in freshwater blue-green alga. *Journal of Cell Biology*, 58, 419–435.
- Berry, J.P., Gantar, M., Perez, M.H., Berry, G., Noriega, F.G. (2008). Cyanobacterial Toxins as Allelochemicals with Potential Applications as Algaecides, Herbicides and Insecticides. *Marine Drugs*, 6, 117–146. DOI: 10.3390/md6020117

- Campbell, D., Hurry, V., Clarke, A.K., Gustafsson, P., Öquist, G. (1998). Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiology and Molecular Biology Reviews*, 62(3), 667–683. DOI: 1092-2172/98/\$04.0010
- Hernández-Carlos, B., Gamboa-Angulo, M.M. (2011). Metabolites from freshwater aquatic microalgae and fungi as potential natural pesticides. *Phytochemistry Reviews*, 10(2), 261–286. DOI: 10.1007/s11101-010-9192-y
- Gantar, M., Berry, J.P., Thomas, S., Wang, M., Perez, R., Rein, K.S. (2008). Allelopathic activity among Cyanobacteria and microalgae isolated from Florida freshwater habitats. *Federation of European Microbiological Societies*, 64, 55–64. DOI: 10.1111/j.1574-6941.2008.00439.x
- Gross, E.M. (2003). Allelopathy of Aquatic Autotrophs. *Critical Reviews in Plant Sciences*, 22, 313–339. DOI: 10.1080/713610859
- Gross, E.M., Wolk, C.P., Jüttner, F. (1991). Fischerellin, a new allelochemical from the freshwater cyanobacterium *Fischerella muscicola*. *Journal of Phycology*, 27, 686–692. DOI: 10.1111/j.0022-3646.1991.00686.x
- Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In: W.L. Smith, M.H. Chanley (eds.), *Culture of Marine Invertebrate Animals*. New York: Plenum Press, 26–60.
- Issa, A.A. (1999). Antibiotic production by the cyanobacteria *Oscillatoria angustissima* and *Calothrix parietina*. *Environmental Toxicology and Pharmacology*, 8, 33–37. DOI: 10.1016/S1382-6689(99)00027-7
- Jakubowska, N., Szeląg-Wasielewska, E. (2015). Toxic picoplanktonic cyanobacteria – Review. *Marine drugs*, 13(3), 1497–1518. DOI: 10.3390/md13031497
- Jassby, A.D., Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography*, 21, 540–547. DOI: 10.4319/lo.1976.21.4.0540
- Jeffrey, S.W., Humphrey, G.F. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, *c1* and *c2* in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, 167, 191–194.
- Latała, A., Jodłowska, S., Pniewski, F. (2006). Culture Collection of Baltic Algae (CCBA) and characteristic of some strains by factorial experiment approach. *Archiv für Hydrobiologie*, 165, *Algological Studies*, 122, 137–154. DOI: 10.1127/1864-1318/2006/0122-0137
- Leflaive, J., Ten-Hage, L. (2007). Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of Allelopathic compounds and toxins. *Freshwater Biology*, 52, 199–214. DOI: 10.1111/j.1365-2427.2006.01689.x
- Legrand, C., Rengefors, K., Fistarol, G.O., Granéli, E. (2003). Allelopathy in phytoplankton – biochemical, ecological and evolutionary aspects. *Phycologia*, 42(4), 406–419. DOI: 10.2216/i0031-8884-42-4-406.1
- Mazur-Marzec, H., Błaszczuk, A., Felczykowska, A., Hohlfeld, N., Kobos, J., Toruńska-Sitarz, A., Devi, P., Montalvão, S., D'souza, L., Tammela, P., Mikosik, A., Bloch, S., Nejman-Faleńczyk B., Węgrzyn, G. (2015). Baltic cyanobacteria – a source of biologically active compounds. *European Journal of Phycology*, 50, 343–360. DOI: 10.1080/09670262.2015.1062563
- Schagerl, M., Unterrieder, I., Angeler, D.G. (2002). Allelopathy among cyanoprokaryota and other algae originating from Lake Neusiedlersee (Austria). *International Review of Hydrobiology*, 87, 365–374. DOI: 10.1002/1522-2632(200207)87:4<365::AID-IROH365>3.0.CO;2-B
- Stal, L.J., Albertano, P., Bergman, B., Bröckel, K., Gallon, J.R., Hayes, P.K., Sivonen, K., Walsby, A.E. (2003). BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea – responses to a changing environment. *Continental Shelf Research*, 23, 1695–1714. DOI: 10.1016/j.csr.2003.06.001
- Strickland, I.D.H., Parsons, T.R. (1972). a practical handbook of seawater analysis. *Journal of the Fisheries Research Board of Canada*, 167, 1–310.

- Suikkanen, S., Fistarol, G.O., Granéli, E. (2004). Allelopathic effects of the Baltic cyanobacteria *Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena lemmermannii* on algal monocultures. *Journal of Experimental Marine Biology and Ecology*, 308, 85–101. DOI: 10.1016/j.jembe.2004.02.012
- Słwińska-Wilczewska, S., Pniewski, F., Latała, A. (2016a). Allelopathic interactions between *Synechococcus* sp. and *Nodularia spumigena* under different light conditions. *Allelopathy Journal*, 37(2), 241–252.
- Słwińska-Wilczewska, S., Pniewski, F., Latała, A. (2016b). Allelopathic activity of the picocyanobacterium *Synechococcus* sp. under varied light, temperature and salinity conditions. *International Review of Hydrobiology*, 101, 1–9. DOI: 10.1002/iroh.201501819
- Żak, A., Kosakowska, A. (2015). The influence of extracellular compounds produced by selected Baltic cyanobacteria, diatoms and dinoflagellates on growth of green algae *Chlorella vulgaris*. *Estuarine, Coastal and Shelf Science*, 167, 113–118. DOI: 10.1016/j.ecss.2015.07.038

Abstract

Picocyanobacterium *Synechococcus* sp. is very important but still poorly understood component of marine and freshwater ecosystems. In this study, the effect of single and multiple addition of cell-free filtrate obtained from *Synechococcus* sp. on selected cyanobacteria *Synechocystis* sp., *Geitlerinema amphibium*, *Nodularia spumigena* and *Nostoc* sp. was investigated. The species present in this work are groups of aquatic phototrophs known to co-occur in the Baltic Sea. The study showed that the picocyanobacterial cell-free filtrate inhibits the growth and changes the cell morphology of filamentous cyanobacteria *G. amphibium*, *N. spumigena* and *Nostoc* sp. It was shown that the addition of cell-free filtrate caused a decline of pigmentation and cell lysis of *G. amphibium*, *N. spumigena* and *Nostoc* sp. compared to the control culture. In addition, it was observed that the filtrate obtained from *Synechococcus* sp. did not affect the *Synechocystis* sp. It was found that the filtrate obtained from picocyanobacterium had the strongest effect on growth of *G. amphibium*, therefore for this cyanobacteria additional experiments were performed to show whether the filtrate affected also photosynthetic pigments, chlorophyll fluorescence and photosynthesis. The study proved that the picocyanobacterial allelopathic compounds reduce the efficiency of photosynthesis, which results in the inhibition of growth of target organisms. This way of interaction may explain the formation of almost monospecific cyanobacterial blooms in many aquatic ecosystems, including the Baltic Sea.

Key words: allelopathy, cyanobacteria, fluorescence, growth, photosynthesis, photosynthetic pigments

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Zjawisko oddziaływania allelopatycznego sinicy *Synechococcus* sp. (Cyanobacteria, Chroococcales) na wybrane gatunki sinic

Streszczenie

Pikoplanktonowa sinica *Synechococcus* sp. jest bardzo ważnym, lecz nadal słabo poznany składnikiem wodnych ekosystemów. W przeprowadzonych badaniach określono wpływ pojedynczo i wielokrotnie dodawanego przesączu uzyskanego z *Synechococcus* sp. na wybrane gatunki pikoplanktonowych i nitkowatych sinic: *Synechocystis* sp., *Geitlerinema amphibium*, *Nodularia spumigena* oraz *Nostoc* sp. Badane gatunki sinic występują w tych samych ekosystemach i znane są z odgrywania istotnej roli w Morzu Bałtyckim. W pracy wykazano, że przesącz wpływał hamująco na wzrost nitkowatych sinic *G. amphibium*, *N. spumigena* oraz *Nostoc* sp. Nie zanotowano natomiast istotnych zmian liczebności u pikoplanktonowej sinicy *Synechocystis* sp. Dla wszystkich gatunków badanych sinic zostały wykonane analizy zmian morfologicznych, które zaszły w ich komórkach pod wpływem dodania przesączu uzyskanego z kultur *Synechococcus* sp. Na podstawie uzyskanych wyników wykazano, że przesącz powodował utratę pigmentacji i lizę komórek nitkowatych sinic. Ponadto dla sinicy *G. amphibium* zostały wykonane dodatkowe eksperymenty, na podstawie których stwierdzono, że dodany przesącz hamował fluorescencję chlorofilu *a*, tempo

fotosyntezy, a także wpływał znacząco na zawartość barwników fotosyntetycznych. W pracy wykazano, że związki allelopatyczne produkowane i uwalniane przez *Synechococcus* sp. ograniczały sprawność fotosyntezy, co skutkowało zahamowaniem wzrostu organizmów targetowych. Tego rodzaju oddziaływanie może wyjaśniać tworzenie się prawie monogatunkowych zakwitów sinic w wielu zbiornikach wodnych, w tym również w Morzu Bałtyckim.

Słowa kluczowe: allelopatia, sinice, fluorescencja, wzrost, fotosynteza, barwniki fotosyntetyczne

Information on the authors

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Currently she is researching the allelopathic activity of picocyanobacteria. Recently she has examined that the *Synechococcus* sp. reveals allelopathic activity on the photosynthesis and chlorophyll fluorescence, which results in the inhibition of growth of analyzed target species. She has also discovered that picocyanobacterium *Synechococcus* sp. produces and releases allelopathic compounds which have negative influence on different green algae, diatoms and filamentous cyanobacteria.

Kinga Gergella

The field of her interest is allelopathic interactions of phytoplankton, in particular of Baltic microalgae and cyanobacteria. She is investigating what influence allelopathic compounds have on those organisms. In her studies she uses innovative methods: analyzing chlorophyll *a* fluorescence and measuring rate of photosynthesis to determine what impact allelochemicals have on algae.

Adam Latała

He has wide experience in ecophysiology and ecotoxicology of marine benthic and planktonic algae. He is interested in influence of the main environmental factors, such as salinity, temperature and light, on the photosynthesis, photoacclimation, fluorescence, respiration and growth of algae from natural communities and cultured under laboratory conditions. He uses fluorescence techniques to determine algal and cyanobacterial ecophysiology and ecotoxicology.