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Fresh water, marine and terrestrial cyanobacteria display distinct allergen characteristics

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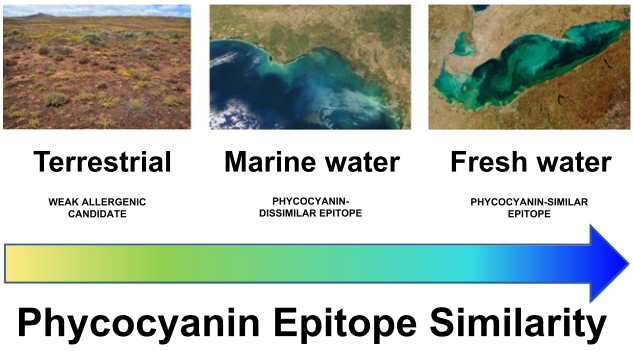
HIGHLIGHTS

- Cyanobacteria increase in abundance and usage, yet the allergenicity remained elusive.
- Allergenic potential of different taxa is demonstrated through three immunoassays.
- Similarities are observed between freshwater cyanobacteria and C-phycocyanin.
- *Nostoc* sp. shows anti-inflammatory and unique immune-reactive characteristics.
- Increase in public awareness would enable mitigation of the potential health risk.

GRAPHICAL ABSTRACT



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ABSTRACT

During the last decades, global cyanobacteria biomass increased due to climate change as well as industrial usage for production of biofuels and food supplements. Thus, there is a need for thorough characterization of their potential health risks, including allergenicity. We therefore aimed to identify and characterize similarities in allergenic potential of cyanobacteria originating from the major ecological environments. Different cyanobacterial taxa were tested for immunoreactivity with IgE from allergic donors and non-allergic controls using immunoblot and ELISA. Moreover, mediator release from human FcεR1-transfected rat basophilic leukemia (RBL) cells was measured, allowing in situ examination of the allergenic reaction. Phycocyanin content and IgE-binding potential were determined and inhibition assays performed to evaluate similarities in IgE-binding epitopes. Mass spectrometry analysis identified IgE-reactive bands ranging between 10 and 160 kDa as phycobiliprotein compounds. Levels of cyanobacterial antigen-specific IgE in plasma of allergic donors and mediator release from sensitized RBL cells were significantly higher compared to non-allergic controls ($p < 0.01$). Inhibition studies indicated

Abbreviation list: BSA, bovine serum albumin; C-PC, C-phycocyanin; DC, dendritic cell; HRP, horseradish peroxidase; FcεR1, human high-affinity receptor for IgE; KLH, keyhole limpet hemocyanin; LCM, linker core-membrane; LPS, lipopolysaccharides; PBS, phosphate buffered saline; PL, plasma; RBL, rat basophilic leukemia; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBS-T, Tris-buffered saline + 0.05% Tween 20.

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Immunoassay
Inhibition assay

cross-reactivity between IgE-binding proteins from fresh water cyanobacteria and phycocyanin standard. We further addressed IgE-binding characteristics of marine water and soil-originated cyanobacteria. Altogether, our data suggest that the intensive use and the strong increase in cyanobacterial abundance due to climate change call for increasing awareness and further monitoring of their potential health hazards.

1. Introduction

Cyanobacteria are considered to be the most ancient photosynthetically-active life form, having played a key role in Earth history and the evolution of life on Earth (Knoll, 2008; Lenton and Daines, 2016). Thus they are found in all illuminated environments on this planet, including salty lakes, ice fields, hot springs, soils, fresh and marine waters (Whitton, 2012).

Various cyanobacteria serve as main biocatalysts in the nitrogen cycle (Issa et al., 2014), and they may be responsible for about 50% of global nitrogen fixation (Elbert et al., 2012). Some species are used for biofuel production, e.g., *Synechococcus* spp. (Nozzi et al., 2013), and others serve as food additives, e.g., *Arthrospira platensis* (*Spirulina*), *Nostoc* spp., *Synechocystis* spp. due to their high nutrient value (Borowitzka, 2013) and anti-inflammatory properties (Ku et al., 2013). Increased and expended marine cyanobacterial blooms have been reported due to environmental change, e.g., increase in temperature and CO_2 (Gobler et al., 2017), which may lead to an extended aerosolization of such species, carried toward the coastal areas (Lang-Yona et al., 2014).

Human exposure to cyanobacteria can occur through skin contact, consumption of food or food supplements, contaminated water, or inhalation of aerosolized cyanobacteria (Genitsaris et al., 2011; Bernstein and Safferman, 1970; Mittal et al., 1979; Schlichting, 1974; Sharma and Rai, 2008). Numerous cyanobacterial species can produce dermatotoxic, neurotoxic or hepatotoxic agents (van Apeldoorn et al., 2007), which may cause animal and, rarely, human deaths (Osborne et al., 2001). In addition to toxins, lipopolysaccharides (LPS) present in the outer membrane of cyanobacteria, have been reported to induce influenza-like illnesses (Annadotter et al., 2005) and were detected in air samples (Lang-Yona et al., 2014).

First reports on allergic reactions to cyanobacteria have appeared around 1950 (Heise, 1951), and since then, several studies demonstrated a correlation between cyanobacteria crude extracts and cutaneous hypersensitivities (Sharma and Rai, 2008; Bernstein et al., 2011; Stewart et al., 2006b; Torokne et al., 2001; Stewart et al., 2006a). Few case studies have reported severe allergic reactions upon exposure to cyanobacteria, i.e. anaphylactic shocks after food supplement consumption (Le et al., 2014; Petrus et al., 2010) and after swimming in a lake during cyanobacterial bloom (Geh et al., 2016).

Phycocyanin, a blue pigment protein complex, is commonly found in cyanobacteria and serves as light-absorbing compound in their photosynthetic apparatus (Schirmer et al., 1985). This pigment is widely used in the industry as a natural fluorescent dye, in food and beverages as a natural coloring agent, or in pharmaceutical applications (Kuddus et al., 2013). Recent studies have identified this compound as a potential allergen in two cyanobacteria species, i.e., *A. platensis* (Petrus et al., 2010) and *Microcystis aeruginosa* (Geh et al., 2015). However, a wider characterization of cyanobacterial allergens is still needed to comprehensively understand their allergenic potential, as well as similarities or differences of the allergens present in the different species.

We therefore aimed to analyze and characterize the allergic reactivity of seven cyanobacterial taxa originating from terrestrial, fresh and marine water environments. We compared the allergenic behavior of these species to the previously characterized allergenic cyanobacterium *M. aeruginosa* (Geh et al., 2015), and searched for similarities through protein identification and IgE-binding inhibition patterns.

2. Materials and methods

2.1. Cultures of cyanobacteria

Eight cyanobacterial taxa as listed in Table 1 were obtained from the Culture Collection of Algae at the Göttingen University (international acronym: SAG; Göttingen, Germany). Five species originating from fresh water (*Microcystis aeruginosa*, *Cylindrospermum siamensis*, *Anabaena ambigua*, *Lyngbya lagerheimii*, *Planktothrix agardhii*), two from marine (*Synechocystis* sp. and *Phormidium* sp.), and one was from a terrestrial environment (*Nostoc* sp.). Cultures were grown in different media as detailed in Table 1 at 20 °C, 120 rpm, and a 16 h/8 h light/dark cycle (76 μmoles m^{-2} s^{-1} daylight lamps; Heraeus BK 6160 low temperature incubator, Thermo Fisher Scientific, Darmstadt, Germany). Growing cultures were screened by light microscopy (Axio Imager A2, Zeiss, Göttingen, Germany) for purity of the cultures and morphological validation.

2.2. Human plasma samples

Six plasma samples (PL 1–6) were obtained from PlasmaLab Ltd. (Everett, WA, USA) and four (PL 7, PL N1–N3) from the Department of Dermatology (University Medical Center of the Johannes Gutenberg University, Mainz, Germany). Donors of plasma PL 1–7 were sensitized to a variety of aeroallergens and food allergens as summarized in Table 2, (the complete list is detailed in Table S1). Plasma N1–N3 were from non-allergic donors with no sensitization against aeroallergens or food allergens, with low levels of total IgE. All plasma samples were aliquoted and stored at −80 °C until use. The study was approved by the local ethics committee (Landesaerztekammer Rheinland-Pfalz, no. 837.055.16 (10374)), and written consent was obtained from all subjects in advance.

2.3. Protein extraction and quantification

Crude extraction of total proteins from cyanobacteria was performed as previously described (Ivleva and Golden, 2007) with some modifications (Lang-Yona et al., 2016). In brief, cultures of cyanobacteria in the late exponential growth phase were transferred into 50 mL tubes and centrifuged at 10,000 × *g* for 10 min. The pellets were washed with sterile phosphate buffered saline (PBS; Sigma-Aldrich, Munich, Germany), and resuspended in 200 μL PBS buffer before overnight storage at −80 °C. After thawing at 37 °C, the samples were placed on ice and a protease inhibitor cocktail (Sigma-Aldrich) together with 0.5 mm acid washed glass beads (Sigma-Aldrich) were added to the samples. They were then homogenized for 30 s using the Fastprep 24 homogenizer (MP Biomedicals, Heidelberg, Germany), and additional 100 μL PBS were added. After 10 min centrifugation at 4 °C and 10,000 × *g*, the supernatant was collected. This step was repeated twice followed by filtration through sterile 5 and 0.45 μm syringe filters (Pall Corporations, Bad Kreuznach, Germany). *Phleum pratense* and *Betula pendula* pollen (Allergon AB, Ängelholm, Sweden) were used as positive controls for the allergic reaction assays, and the extraction of proteins followed the same procedure as described above.

Protein concentrations were determined with Pierce bicinchoninic acid assay (BCA, Thermo Fisher Scientific) according to the