Abstract

Cyanobacteria are one of the oldest known life forms on Earth. Through their existence in diverse and often challenging environments, they developed various adaptive strategies. One of which is the synthesis of a wide range of secondary metabolites that protect them from adverse environmental conditions and interactions with other organisms. From a human perspective, these compounds are highly valuable and can serve as a source of new pharmaceuticals. This study provides a detailed description of two classes of peptides – anabaenopeptins (APs) and cyanopeptolins (CPs) – produced by the Baltic cyanobacterium strain, *Nostoc edaphicum* CCNP1411 (here: CCNP1411).

Genetic studies of this strain revealed the existence of four gene clusters responsible for encoding the synthesis of: cyanopeptolins, anabaenopeptins, nostocyclopeptides, and one as-yet-undescribed class of compounds. To better understand the metabolites produced by the CCNP1411 strain, axenic cultures were grown to obtain biomass, which was then subjected to extraction in order to isolate these compounds. Metabolic analyses using mass spectrometry revealed that *N. edaphicum* CCNP1411 synthesises four anabaenopeptin analogs and ninety-three cyanopeptolin analogs. For the first time, in this study 3 APs and 89 CPs are described. To confirm the diversity within cyanopeptolins, parallel bioinformatic studies and structural analyses using nuclear magnetic resonance spectroscopy (NMR) were conducted, yielding consistent results.

The activity of three anabaenopeptins against five proteolytic enzymes was evaluated. *In vitro* studies revealed typical activity for this class of peptides, with the most pronounced effects observed against carboxypeptidase A.

Of the 93 cyanopeptolins, 34 analogues were isolated in sufficient quantities to assess their activity against serine proteases. These tests confirmed the significance of the amino acid in the second position of the compound. Cyanopeptolins with Arg^2 were active against thrombin with IC_{50} values ranging from 0.28 to 7.25 μM and weak or no activity against chymotrypsin. On the other hand, peptides with Tyr^2, Phe^2, and Leu^2 were strongly, and selectively active against chymotrypsin. Additionally, cyanopeptolins with Leu^2 were active against elastase. The selectivity of compounds containing Leu^2 was also observed in tests against the cervical cancer cell line HeLa. CP 919 reduced the cell viability by 97.6% applied at the highest tested concentration of 200 $\mu g \times mL^{-1}$. The study also examined the activity of some cyanopeptolins against the SARS-CoV-2 virus. Among the tested compounds, $Cp\text{-}Arg^2$ showed the strongest inhibition of Delta SARS-CoV-2 infection in $A549^{ACE2/TMPRSS2}$ cells. Functional assays indicated a direct interaction of CP 978 containing Arg with virions. CP 978 also caused a significant reduction in virus replication in primary human airway epithelial cells (HAE). Confocal microscopy and SARS-CoV-2 pseudovirus analyses showed that CP 978-mediated inhibition of viral infection resulted from direct binding of the cyanopeptolin to the coronavirus S protein.

Natural compounds, including peptides, are intensively studied for their potential use in the production of new drugs. The results described in this thesis reveal the significance of the Baltic cyanobacterium *Nostoc edaphicum* CCNP1411 as a potential source of many promising metabolites with pharmaceutical application.