EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

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CYANOCOST – ES 1105 Action

Cyanobacterial blooms and toxins in water resources: Occurrence, impacts and management.

Short Term Scientific Mission (STSM)

Bioactive properties of cyanobacteria and microalgae isolated from brackish rock pools

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Objectives

Enzyme inhibition and antibacterial properties of crude extracts of rock pool cyanobacteria and microalgae were studied. The crude extracts were analysed with liquid chromatography-mass spectrometry (LC-MS) and genetic studies of the cyanobacteria were performed. The work is part of a PhD-thesis.

Methodology

Enzyme inhibition assays were performed as colorimetric assays in 96-well plates. Inhibition of carboxypeptidase A, chymotrypsin, elastase, protein phosphatase 1, thrombin and trypsin was tested. Potential inhibition of the crude extracts on four pathogenic bacteria was tested in broth dilution assays. The inhibitory effect of a crude extract from the cyanobacterium *Cylindrospermum* sp. was tested on ten pathogenic bacteria. Cyanobacteria DNA was extracted, the 16S rRNA gene region amplified with PCR (Figure 1) and the products sent for gene sequencing. The crude extracts were analysed with LC-MS to scan for known potential bioactive cyanopeptides.

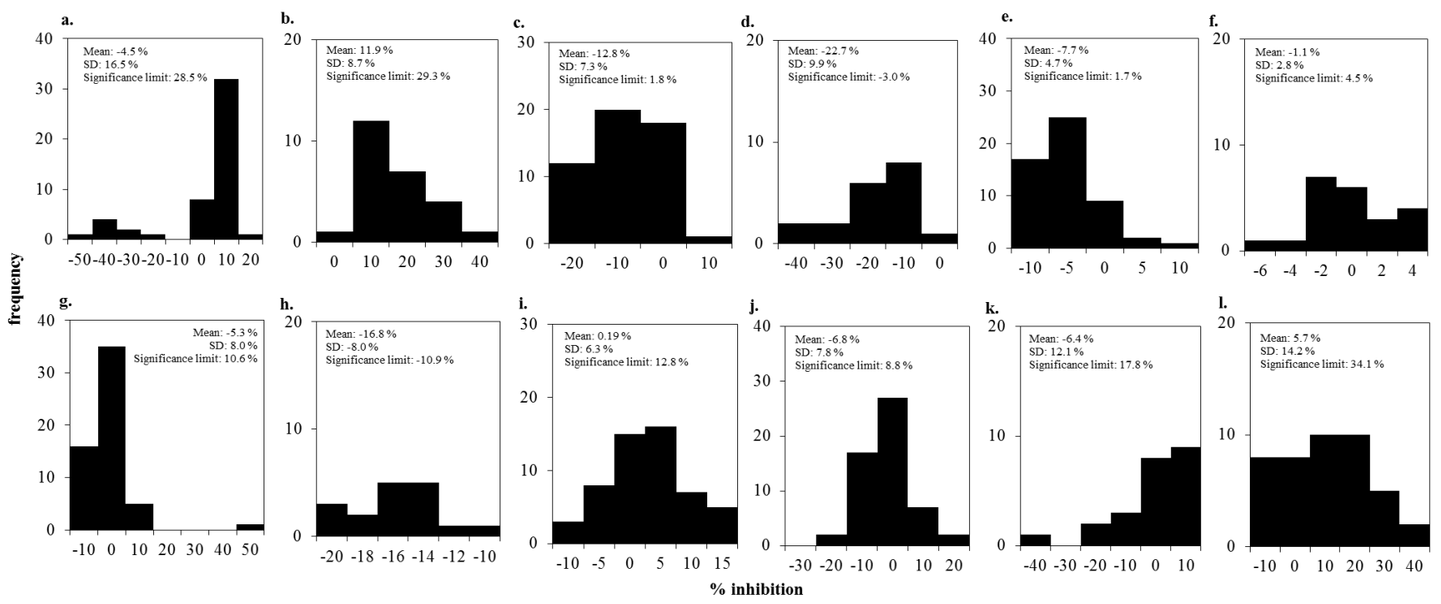

Figure 2. Distribution of percent inhibitions in the assays. **a.** Carboxypeptidase A (plate 1), **b.** carboxypeptidase A (plate 2), **c.** elastase (plate 1), **d.** elastase (plate 2), **e.** protein phosphatase 1 (plate 1), **f.** protein phosphatase 1 (plate 2), **g.** trypsin (plate 1), **h.** trypsin (plate 2), **i.** thrombin (first assay), **j.** thrombin (second assay, plate 1), **k.** thrombin (second assay, plate 2), **l.** chymotrypsin. The significance limit corresponds to a 95% confidence level, SD: standard deviation.

Results

The majority of the crude extracts did not inhibit the tested enzymes (Figure 2). Three of the crude extracts significantly inhibited bacterial growth. Four of the crude extracts significantly stimulated the growth of tested gram positive bacteria, and the diatom *Melosira arctica* significantly stimulated the growth of the gram negative *Vibrio cholera*. The crude extract of *Cylindrospermum* sp. did not significantly inhibit any of the tested bacteria. Anabaenopeptins (e.g. anabaenopeptin A, anabaenopeptin B) and oscillamide Y were identified in the crude extract of *Woronichina* sp. Ion fragmentation patterns characteristic of cyanopeptolins occurred in *Phormidium tenue*. Some of these peptides have previously been shown to inhibit enzymes. The lack of inhibition in the conducted enzyme assays implies some masking effect in the compound rich crude extracts.

Highlights

- crude extracts of cyanobacteria and microalgae did not significantly inhibit any of the tested enzymes
- three diatom crude extracts significantly inhibited *Enterococcus faecium*
- the 16S rRNA gene was successfully amplified and sequenced in ten cyanobacteria strains
- at least three of the cyanobacteria crude extracts contained potential bioactive cyanopeptides

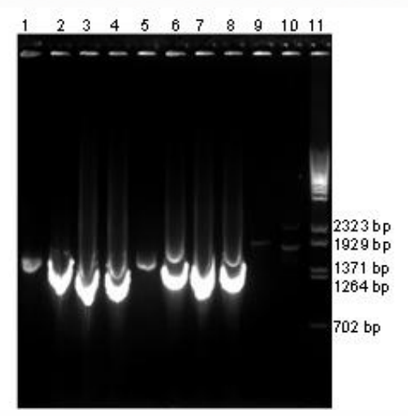

Figure 1. Presence of PCR products after amplification of the 16S rRNA gene in cyanobacteria was confirmed with 1% agarose gel electrophoresis. Well 1: *Chroococcus dimidiatus*, 2: *Merismopedia warmingiana*, 3: *Phormidium tenue*, 4: *Cylindrospermum* sp., 5: *Woronichinia* sp., 6,7,8: *Phormidium breve*, 9,10: *Spirulina subsalsa*, 11: DNA Marker λ/BstEII.

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