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ORIGINAL INVESTIGATION

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# Cyanobacteria a potential source of antiviral substances against influenza virus

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**Abstract** Aqueous and methanolic extracts of cultured cyanobacteria of several genera, *Microcystis*, *Nodularia*, *Oscillatoria*, *Scytonema*, *Lyngbya* and *Calothrix* were evaluated for their in vitro antiviral activity against influenza A virus in Madin Darby canine kidney cells. None of the methanolic extracts showed cytotoxic effects. The inhibitory concentration ($IC_{50}$) of antiviral activity ranged between 20.0 μg to 79.0 μg extract/ml. The most active extract in this screening derived from genus *Microcystis*. The further analysis of methanolic extracts of cultured strains of genus *Microcystis* revealed a remarkable antiviral activity against influenza A virus for *M. aeruginosa*, *M. ichthyoblabe* and *M. wesenbergii*. The observed antiviral activity was associated with protease inhibitory activity of approximately 90% and suggest that protease inhibitory activity may be responsible for reducing virus replication. These results show that cyanobacteria are able to produce compounds with biological activity that may be of potential clinical interest.

**Keywords** Antiviral · Cyanobacteria · Influenza virus · Natural product

## Introduction

Another approach besides synthesis and screening of structural metabolic analogues for the development of antiviral is the search for viral inhibitors from natural sources. Extensive studies have shown that medicinal plants from several parts of the world contain active compounds against viruses that cause human diseases [1, 7, 9]. Nowadays, attention has also focused on cyanobacteria. The cyanobacteria produce a myriad array of secondary metabolites including alkaloids, polyketides and non-ribosomal peptides, which rarely have a role in primary metabolism, growth, reproduction, but have evolved to somehow benefit the producing organisms [4]. Antiviral activity of extracts and compounds of cyanobacteria against human immunodeficiency virus (HIV) and herpes simplex virus (HSV) has been reported [6, 8].

The current study was undertaken to evaluate the antiviral activity of aqueous and methanolic extracts of genera of cyanobacteria against influenza virus.

## Materials and methods

Methanolic and aqueous extracts of several genera, *Microcystis*, *Nodularia*, *Oscillatoria*, *Scytonema*, *Lyngbya* and *Calothrix*, were prepared as described previously [3, 5]. Antiviral activity was evaluated using influenza virus A/WSN/33 (H1N1) London in Madin Darby canine kidney (MDCK) cells by colorimetric assay [7]. Protease inhibitory activity was monitored with BAPNA (α-*N*-benzoyl-DL-arginine-*p*-nitroanilide) as a substrate for trypsin and colorimetric analysis at 405 nm. Fractionation and separation of active compounds was done by chromatographic and spectroscopic methods [5].

## Results and discussion

A total of 30 extracts of cultured cyanobacteria were screened. Seven extracts, six methanolic and one aqueous derived from seven species showed antiviral activity in presence of 100 μg dried material/ml, all of them at a concentration non-toxic to MDCK cells. Inhibitory concentration ($IC_{50}$) ranged between 20.0 μg and 79.0 μg/ml (Table 1). The most effective extract derived from genus *Microcystis*. Therefore, 26 extracts of strains of genus *Microcystis* were prepared and investigated for

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**Table 1** Summary of 50% inhibitory concentrations of aqueous and methanolic extracts of cyanobacteria against influenza A virus

| Cyanobacteria genus/species | Strain | Extraction solvent | 50% inhibitory concentration (μg/ml) |
|---|---|---|---|
| *Calothrix gracilis* | EMAU96 | MeOH | 36.0 |
| *Lyngbya sp.* | SAG3691 | MeOH | 49.0 |
| *Microcystis ichthyoblabe* | BMMi/13 | MeOH | 20.0 |
| *Nodularia spumigena* | EMAU280 | $H_2O$ | 79.0 |
| *Nodularia spumigena* | EMAU280 | MeOH | 55.0 |
| *Oscillatoria lutea* | SAG1459-3 | MeOH | 39.0 |
| *Scytonema bohnerii* | SAGB25580 | MeOH | 39.0 |

anti-influenza virus activity. Three methanolic extracts of *M. aeruginosa*, *M. ichthyoblabe* and *M. wesenbergii* showed remarkable antiviral activity against influenza A virus with survival rate of cells of 73.0%, 45.9% and 55.4%, respectively. This effect was associated with protease inhibitory activity of approximately 90%.

In former experiments a strong effect of the crude aqueous extract of the collected field strain of *Microcystis aeruginosa* was observed [5]. The concentration of 50% inhibition of virus replication in MDCK cells was 11.0 μg/ml. The virus-specific protein synthesis was decreased if the extract was present over the whole time of replication. A bioassay-guided fractionation resulted in an aqueous phase fraction and a basic compound fraction. Both these fractions showed antiviral activity with $IC_{50}$ of 21.0 μg/ml and 47.0 μg/ml, respectively. Further separation using gel chromatography resulted in five subfractions and antiviral activity was detected in subfractions G2 until G5. The analysis of subfractions G4 and G5 by chromatographic and spectroscopic methods revealed that adenosine and adenine are the main constituents. These compounds could contribute to the biological activity against influenza virus. In addition, our experiments for protease inhibitory effect suggest that the antiviral activity of cyanobacteria extract is linked to the inhibition of the proteolytic activation of influenza virus. Furthermore, it was found that influenza A virus did not become resistant against the extract of cyanobacteria after continuous incubation in several virus passages [3].

Several reports of in vivo experiments on the antiviral activity of medicinal plants against influenza virus postulate that the mode of action of plant extracts is based on enhancement of the host immune response [2]. Basing on the experimental design of a former study [10], we plan to evaluate the protective effect of active extracts in an in vivo study. The data demonstrate and confirm that cyanobacteria are able to produce compounds with biological activity. The extract of *Microcystis aeruginosa* with a selectivity index of $>90$ could be a potential source of antiviral compounds. To our knowledge, this is the first report of anti-influenza virus activity of cultured cyanobacteria.

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