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Screening of microalgal culture media for the presence of algicidal compounds and isolation and identification of two bioactive metabolites, excreted by the cyanobacteria *Nostoc insulare* and *Nodularia harveyana*

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Abstract

Culture medium extracts obtained from 115 culture media of 35 different microalgae species were screened for the presence of algicidal compounds, in particular for compounds which are cytotoxic to *Arthrospira* (Spirulina) *laxissima*. In agar plate diffusion tests and in a test system combining thin layer chromatography (TLC) with the use of an aqueous suspension of living *A. laxissima* cells as spray reagent, 14 microalgae species were found with cytotoxic activity of different intensity to *A. laxissima*. In a so-called TLC plate diffusion test, using *A. laxissima* and other microalgae as test organisms, the culture medium extracts of *Nodularia harveyana* and *Nostoc insulare* possessed the highest strength and range of algicidal activity. The algicidal compound in the culture medium extracts of *Nodularia harveyana* was shown to be norharmane (9H-pyrido(3,4-b)indole), a known indole alkaloid. The main algicidal compound in culture medium extracts of *Nostoc insulare* was identified as 4,4′-dihydroxybiphenyl. The possible applicability of both compounds as therapeutics or as useful agents for removing cyanobacterial water blooms or for developing new antifouling systems is discussed.

Abbreviations: TLC, thin layer chromatography

Introduction

Biologically active compounds, produced by cyanobacteria, particularly the cyanotoxins, are of manifold interest. Some of them, such as the well-investigated hepatotoxic microcystins and nodularins, as well as the neurotoxic anatoxins and saxitoxins, present a toxicological problem, both for livestock and humans (Carmichael, 1992; Rinehart et al., 1994). Cyanobacterial metabolites which are cytotoxic to other algae are of special interest: On the one hand such compounds could possibly be helpful in combating (toxic) algal blooms or in the development of environmentally friendly and tributyltin-free antifouling paints for ships (Bagchi et al., 1990; Bhadury & Wright, 2004), on the other hand the knowledge about such compounds has a beneficial effect on a better understanding of interactions between competing organisms of the same habitat. Furthermore, cyanobacterial biogenic and particularly cytotoxic compounds could provide leads for future development of new therapeutic agents for a variety of diseases or for the development of new antibiotics (Falch, 1996; Patterson et al., 1994).

Several screening studies have been carried out over the past years with the aim to discover new antibiotic or cytotoxic metabolites of microalgae (green algae and cyanobacteria) (Falch et al., 1995; Ördög et al., 2004; Piccardi et al., 2000) as well as to discover cyanobacteria which were toxic to other cyanobacteria or green algae (Flores & Wolk, 1986; Schlegel et al., 1998). In

these studies either extracts of microalgal biomass or living test species (using a special double layer agar technique) were investigated. The present work was undertaken to search for biological active secondary metabolites which were excreted by cyanobacteria and other microalgae into their environment during growth and which could serve as new antialgal agents or as chemical leads useful in facilitating the development of new therapeutic or commercial agents in the future. The screening of 115 culture media of 35 selected microalgae (26 cyanobacteria, 1 Chlorophyceae, 1 Eustigmatophyceae, 5 Phaeophyceae and 2 Xanthophyceae) for the presence of algicidal compounds is described and the structures of two identified algicidal compounds are presented.

Materials and methods

Microalgae

Originally, the microalgae screened here were cultivated to examine their production of substances of pharmaceutical medical interest such as carotenoids, phycobiliproteins and exopolysaccharides (Fischer, 1996; Fischer et al., 1997; Jander, 2001; Schulze, 2000; Volk, 1996). In these studies the culture media were left over as by-products. Thus different numbers of cultures of each strain found their way into the screens presented here. After harvesting of the cultures, the media were extracted immediately and the extracts were freeze-dried and stored cold until examination.

The investigated microalgae are listed in Table 1, including the number of culture media of each species examined. All tested microalgae were part of an own culture collection of our institute; their original source is also presented in Table 1.

Cultivation

The microalgae were grown in different culture media as outlined in Table 1. The composition of the media derived from Pohl et al. (1987) and are described in Table 2. Cultivation was carried out in 10 L conical shoulder flasks containing 8 L of the corresponding culture medium under axenic conditions, continuous aeration (22 °C and 27 °C) and continuous illumination (25–30 μmol photon m^{-2} s^{-1}). Cultivation times ranged between 40 and 60 days.

To examine cultures for possible contamination with other organisms, samples were taken during cultivation and after harvesting and were examined microscopically. Contaminated cultures were excluded from subsequent investigations.

Preparation of culture media extracts

Biomass and culture media were separated by centrifugation (10000 × g) and stored at −20 °C until further study.

The defrosted culture media were extracted with Amberlite^{TM} XAD-1180 (Rohm and Haas Company, Philadelphia, USA). 4 litres of culture medium were pumped through 120 g XAD resin in a closed cycle at room temperature for 24 h using a Soxhlet apparatus. The adsorbed compounds were removed from the XAD resin by extraction with methanol p.a. in two steps: first at room temperature (to extract adsorbed compounds which were possibly sensitive to heat) by shaking the resin with 500 mL methanol for 24 h; second with 600 mL hot methanol for another 24 h in a Soxhlet apparatus to complete extraction of all adsorbed compounds. The methanol fractions were combined and evaporated to dryness. The residue was dissolved in 50 mL ethanol, filtered and evaporated to dryness, dissolved in 25 mL demineralized water and finally freeze-dried.

Agar plate diffusion test using Arthrospira laxissima *as test organism*

To prepare sterile agar plates, 50 mL of DSN-10 culture medium (Pohl et al., 1987), containing 1.5% agar, were autoclaved in a glass dish (diameter 17 cm). After cooling and hardening of the agar, 10 mL of *Arthrospira laxissima* suspension was pipetted evenly onto this agar plate under sterile conditions and the plate was incubated at 27 °C under continuous illumination (25–30 μmol photon m^{-2} s^{-1}) provided by fluorescent tubes (Philips TL 65 W/25; white). After 2–3 weeks the *Arthrospira* was growing as an evenly thin green layer on the surface of the agar plate. Other microalgae, used in preliminary studies (with the aim to find the most suitable test organism for this purpose) were handled as described for *Arthrospira laxissima*.

For the test, 20.0 mg of culture medium extract was dissolved in 0.5 mL sterile demineralized water. 40 μL of this solution was pipetted under sterile conditions onto round 10 mm filter paper disks and these paper disks were placed on the agar plates. The plates were then incubated as described above. Inhibition zones of culture medium extracts, containing algicidal

Table 1. Investigated microalgae species.

Species	Source	Medium[a]	Test Strain (Yes/No)	# of media tested
Cyanobacteria				
Anabaena constricta[b]	3	DSN-10	No	6
Anabaena cylindrica 1403-2	1	DSN-10	No	5
Anabaena inaequalis 1403-10	1	DSN-10	No	2
Anabaena lutea 25.79	1	DSN-10	Yes	0
Anabaena species 16 Ms	2	DSN-10	No	2
Anabaenopsis siamensis B 11.82	1	DSN-10	No	5
Arthrospira (Spirulina) *laxissima* B 256.80	1	SP-5	Yes	3
Arthrospira (Spirulina) *maxima* B 84.79	1	SP-5	Yes[c]	2
Arthrospira (Spirulina) *platensis* B 85.79	1	SP-5	Yes[c]	0
Arthrospira (Spirulina) *subsalsa*[b]	3	DSN-50	No	2
Chroococcus minutus B 41.79	1	DSN-10	Yes	8
Nodularia harveyana 44.85	1	DSN-10	No	7
Nodularia spumigena HÜ 280	4	DSN-10	No	1
Nostoc carneum[b]	6	DSN-10	Yes	10
Nostoc commune B 1453-3	1	DSN-10	Yes[c]	5
Nostoc insulare 54.79	1	DSN-10	Yes	13
Nostoc species 12 Sn	2	DSN-10	No	2
Nostoc species 61 Sn	2	DSN-10	No	3
Oscillatoria agardhi-rubescens[b]	5	DSN-10	No	1
Oscillatoria amoena B 1459-7	1	DSN-10	Yes[c]	0
Oscillatoria brevis[b]	5	DSN-10	Yes[c]	1
Oscillatoria chalybea B 1459-2	1	DSN-10	Yes[c]	0
Oscillatoria geminata B 1459-8	1	DSN-10	Yes[c]	1
Oscillatoria nigro-viridis[b]	3	DSN-50	No	5
Oscillatoria redekei HUB 051	4	DSN-10	No	1
Oscillatoria sancta B 74.79	1	DSN-10	No	4
Phormidium foveolarum B 1462-1	1	DSN-10	Yes[c]	4
Phormidium species II 212.80	1	DSN-10	Yes[c]	1
Pseudoanabaena species 01 Sn	2	DSN-10	No	2
Synechocystis aquatilis B 90.79	1	DSN-10	Yes	2
Chlorophyceae				
Chlamydomonas mexicana 11-60 a	7	DSN-10	No	1
Coelastrum astroideum 65.81	1	DSN-10	Yes	0
Ulothrix minuta 386-1	1	DSN-10	Yes[c]	0
Eustigmatophyceae			No	
Eustigmatos vischeri 860-1	1	DSN-10	No	1
Phaeophyceae				
Dictyota dichotoma B 207.80 female strain, B 208.80 male strain	1	PHAE-100	No	1
Ectocarpus siliculosus B 63.81	1	PHAE-50	No	1
Halopteris scoparia B 211.80	1	PHAE-100	No	1
Spermatochnus paradoxus B 10.82	1	PHAE-100	No	2
Spacelaria rigida B 7.92	1	PHAE-100	No	6
Xanthophyceae				
Bumilleriopsis filiformis 809-2	1	DSN-10	No	1
Ophiocytium maius 855-1	1	DSN-10	No	3

Sources: 1: "Sammlung von Algenkulturen Göttingen", Albert-v.-Haller-Institut für Pflanzenwissenschaften, Germany (Schlösser, 1994); *Arthrospira* strains are listed as "*Spirulina*" in this culture collection; 2: The Blue-Green Algae Collection at the International Rice Research Institute, Soil Microbiology Division, Manila, Philippines; 3: University of Kuwait City, Kuwait; 4: University of Greifswald, Department of Pharmaceutical Biology, Germany; 5: Max-Planck-Institut für Limnologie, Plön, Germany; 6: Forschungsinstitut Senkenburg, Frankfurt a.M., Germany; 7: Dr. R Lewin, San Diego/USA.

[a]See Table 2.

[b]Strain without strain number at the respective organization.

[c]Species used as test organism merely in the preliminary investigation.

Table 2. Composition of culture media.

	Media				
Nutrient	DSN-10	DSN-50	SP-5	PHAE-100	PHAE-50
Seawater	10%	50%	5%	100%	50%
Salts					
$CuSO_4 \cdot 5H_2O$	0.01	0.01	0.01	0.01	0.01
$MnCl_2 \cdot 4H_2O$	0.4	0.4	0.4	0.4	0.4
$CoSO_4 \cdot 7H_2O$	0.1	0.1	0.1	0.1	0.1
$Na_2MoO_4 \cdot 2H_2O$	0.1	0.1	0.1	0.1	0.1
$ZnSO_4 \cdot 7H_2O$	0.1	0.1	0.1	0.1	0.1
$Na_2EDTA \cdot 2H_2O$	5.5	5.5	5.5	5.5	5.5
$FeCl_3 \cdot 6H_2O$	4.0	4.0	4.0	4.0	4.0
$K_2HPO_4 \cdot 3H_2O$	25.0	25.0	100.0	50.0	50.0
KNO_3	500.0	500.0	500.0	300.0	300.0
$NaHCO_3 \cdot 3H_2O$	0.0	0.0	13100.0	0.0	0.0
Na_2CO_3	0.0	0.0	400.0	0.0	0.0
Other nutrients					
Vitamin B_1	0.0	0.0	0.0	0.05	0.05
Vitamin B_{12}	0.0	0.0	0.0	0.0005	0.0005

Concentrations of salts are given in mg L^{-1}.

compounds, became visible after 2–3 days and increased over the following days.

Thin layer chromatography using Arthrospira laxissima *as a spray reagent*

TLC plates (carrier: 20 × 20 cm, glass; Merck silica gel 60 GF_{254}, 300 μm thin layer) were self prepared, using a CAMAG Automatic TLC Coater 21602. To prepare test solutions, 10.0 mg of culture medium extract was dissolved in 0.25 mL methanol (50%). 20 μL of this solution was applied to the TLC plates. Chromatography was carried out with a mixture of ethylacetate:methanol:water (100:16.5:13.5; v:v:v) (Hörhammer et al., 1963). After complete evaporation of the mobile phase and photographic documentation of the chromatograms (UV light, 254 nm as well as 366 nm) the plates were sprayed with 15 mL of an aqueous suspension of living *Arthrospira laxissima* which had been prepared in the following way: The optical density at 440 nm of an *A. laxissima* culture grown in a 10-L flask under the above described conditions was determined. Based on the measured density, the suspension was concentrated by centrifugation (5000 × *g*, 7 min) and adjusted to a theoretical optical density of 40. After spraying each TLC plate with 15 mL of this concentrated *A. laxissima* suspension the plates were kept moist in a TLC chamber at 27 °C under continuous illumination (25–30 μmol photon m^{-2} s^{-1}). Inhibition zones of extracts possessing algicidal activity became visible after 2–3 days (Figure 1).

TLC plate diffusion test

TLC plates were prepared as described above. To prepare test solutions, 10.0 mg of culture medium extract was dissolved in 0.25 mL methanol (50%). 20 μL of this solution was applied to the surface of a TLC plate. After complete evaporation of the solvent the plate was sprayed with 15 mL of a concentrated aqueous suspension of living microalgal test organism, prepared as described for *A. laxissima* in the TLC test. In the case of *Nostoc insulare*, however, it was necessary to remove gelatinous exopolysaccharides in the culture medium by washing the centrifuged biomass with fresh culture medium shortly before spraying. After spraying the TLC plates were kept moist in a TLC chamber at 27 °C under continuous illumination (25–30 μmol photon m^{-2} s^{-1}). Algicidal activities of extracts became visible within 2–3 days and increased in the following days.

Isolation and identification of 4,4′-dihydroxybiphenyl and norharmane

Cytotoxic substances, discovered in the TLC test of *Nostoc insulare* and *Nodularia harveyana* culture medium extracts, were isolated by preparative TLC

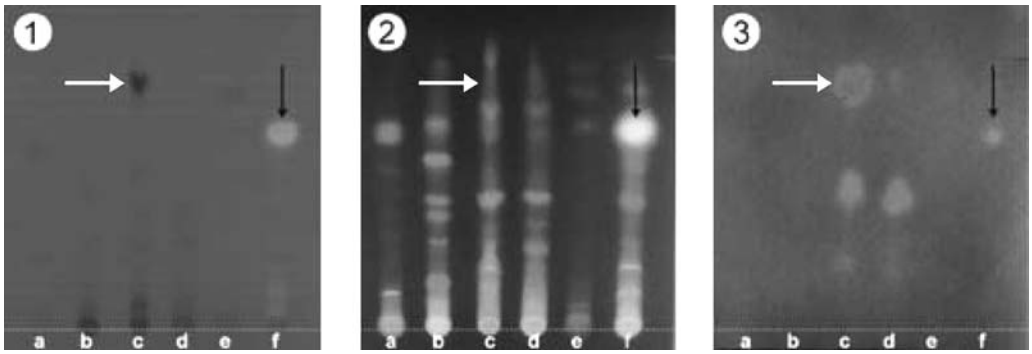

Figure 1. TLC of extracts obtained from the culture media of the following cyanobacteria: (a) *Nostoc* species 12 Sn, (b) *Phormidium* species II, (c) *Nostoc insulare*, (d) *Nostoc commune*, (e) *Anabaena constricta*, (f) *Nodularia harveyana*. (1) Plate in UV light 254 nm before spraying with *Arthrospira laxissima*; (2) Plate in UV light 366nm before spraying with *A. laxissima*; (3) Plate in daylight 3 days after spraying with living *A. laxissima*. In (1) and (2) white arrows mark 4,4′-dihydroxybiphenyl, constituent of the culture medium extract of *Nostoc insulare*; black arrows mark norharmane, constituent of the culture medium extract of *Nodularia harveyana*. In (3) the arrows indicate the resulting inhibition zones of these two algicidal compounds.

(stationary phase: Merck silica gel 60 GF_{254}; mobile phase: ethylacetate:methanol:water (100:16.5:13.5; v:v:v)). The substance-loaded silica gel areas were scraped off and extracted with methanol. The resultant compounds were purified by preparative HPLC (column: Aqua^{TM} C18 by Phenomenex; mobile phase (gradient): methanol and ammonium acetate/acetic acid buffer). Identification of the substances was achieved by structural analysis using HPLC-MS (HP 5989A-MS Engine Mass Spectrometer), ^{1}H-NMR (Bruker ARX 300, 300 MHz) and ^{13}C-NMR (Bruker ARX 300, 75 MHz). For NMR-analysis, the isolated substances were dissolved in D_2O. The identifications were verified by comparison with authentic norharmane and 4,4′-dihydroxybiphenyl (Fluka/Sigma-Aldrich) using TLC and HPLC. HPLC was combined with a Hitachi photo diode array detector L 3000 for detection of UV absorption spectra of both samples.

Results

Agar plate diffusion test with Arthrospira laxissima *as test organism*

Before screening of the culture media, a suitable test organism had to be found for the detection of algicidal activities of culture medium extracts in agar plate diffusion tests. For these preliminary studies, 15 microalgae were selected which were known to grow well in suspension culture according to earlier studies. These organisms, i.e. *Anabaena lutea*, *Arthrospira laxissima*, *A. maxima*, *A. platensis*, *Nostoc carneum*, *N. commune*, *N. insulare*, *Oscillatoria amoena*, *O. brevis*, *O. chalybea*, *O. geminata*, *Phormidium foveolarum*, *Phormidium* species II, *Synechocystis aquatilis* (all cyanobacteria) and *Ulothrix minuta* (Chlorophyceae), were tested against 19 culture medium extracts, obtained from the cultures of 15 different microalgae. Among these 15 test organisms, *Arthrospira laxissima* was the most suitable species because of its ability to grow fast and evenly on agar plates and its high sensitivity to algicidal activity in the culture medium extracts. Some of the other microalgae also were found to be suitable, but to a much lesser degree, i.e. *Nostoc carneum*, *N. insulare* and *Anabaena lutea*. Thus, *A. laxissima* was selected as test organism for subsequent screening.

Extracts of 115 culture media obtained from the culture of 35 different microalgae were investigated in agar plate diffusion tests for the presence of compounds toxic to *A. laxissima*. Culture medium extracts of the following microalgae were found to possess high (+) or very high (++) cytotoxic effects: *Anabaena cylindrica* (+), *Anabaena inaequalis* (++), *Anabaenopsis siamensis* (+), *Arthrospira laxissima* (++), *Arthrospira maxima* (++), *Chroococcus minutus* (++), *Nodularia harveyana* (+), *Nostoc carneum* (+), *Nostoc commune* (+), *Nostoc insulare* (++), *Oscillatoria sancta* (++) and *Synechocystis aquatilis* (+). Remarkably, *Arthrospira laxissima* excretes substances which are toxic to itself.

TLC of culture medium extracts using suspensions of living Arthrospira laxissima *cells as spray reagent*

In this test system, the cytotoxic effects of the investigated culture medium extracts coincided with those

obtained by the preceding agar plate diffusion tests. In addition, the culture medium extracts of two more cyanobacteria, *Nostoc* 61 Sn and *Phormidium foveolarum*, possessed reproducible cytotoxic effects to *Arthrospira laxissima*. Therefore, microalgae test organisms may possess a higher sensitivity when they are used as spray reagents in such assays. This assumption confirmed when *Anabaena lutea* was used as test organism (spray reagent) in the later TLC plate diffusion test.

TLC plate diffusion tests with different test organisms

To detect the cytotoxic activity of selected microalgal culture medium extracts to microalgae other than *Arthrospira laxissima*, this easy procedure which combines the experiences gained during agar plate diffusion tests and TLC tests was used. Besides *A. laxissima*, microalgae of known sensitivity (*Anabaena lutea*, *Nostoc carneum* and *Nostoc insulare*, see earlier) were selected as test organisms as well as some other microalgae (*Chroococcus minutus*, *Synechocystis aquatilis* (cyanobacteria) and *Coelastrum astroideum* (Chlorophyceae)) which had not been investigated before but formed fine suspensions appropriate for spraying.

Culture medium extracts which had shown good cytotoxic activities to *A. laxissima* and which were available in sufficient quantities were selected for testing. An extract from pure culture medium and two culture medium extracts from cultures of *Anabaena constricta* and *Oscillatoria brevis* which had shown no cytotoxic activities to *A. laxissima* were used as controls.

The results are shown in Table 3. *Anabaena lutea* was found to be too sensitive in this TLC plate diffusion test. Even the control extract from pure culture medium inhibited *A. lutea* in the same way as the other culture medium extracts. Therefore this test organism was excluded from this assay.

Of the 12 selected culture medium extracts those from cultures of *Nodularia harveyana* and *Nostoc*

Table 3. Algicidal activities of culture medium extracts obtained from 14 selected microalgae and one blank value (control) using TLC plate diffusion tests.

	Test organisms						
	Filamentous cyanobacteria				Unicellular cyanobacteria		Chlorophyceae
Origin of culture medium extracts	A	B	C	D	E	F	G
Controls							
DSN culture medium	++	−	−	−	−	−	−
Filamentous cyanobacteria							
Anabaena constricta	++	−	−	−	−	−	−
Oscillatoria brevis	++	−	−	−	−	−	−
Filamentous cyanobacteria							
Anabaena cylindrica	++	+	++	−	+	−	−
Anabaenopsis siamensis	++	++	++	−	−	−	−
Arthrospira (Spirulina) *laxissima*	++	+	+	+	−	−	−
Arthrospira (Spirulina) *maxima*	++	++	++	+	−	+	−
Nodularia harveyana	++	++	++	+	+	+	−
Nostoc carneum	++	++	+	−	−	−	−
Nostoc commune	++	++	++	+	−	−	−
Nostoc insulare	++	++	++	+	+	+	−
Nostoc species 61 Sn	++	++	++	+	−	−	−
Oscillatoria sancta	++	++	++	+	−	−	−
Phormidium foveolarum	++	+	+	−	−	−	−
Unicellular cyanobacterium							
Chroococcus minutus	++	++	++	+	−	−	−

Test organisms: A: *Anabaena lutea*, B: *Arthrospira* (Spirulina) *laxissima*, C: *Nostoc carneum*, D: *Nostoc insulare* (all filamentous cyanobacteria); E: *Chroococcus minutus*, F: *Synechocystis aquatilis* (both unicellular cyanobacteria); G: *Coelastrum astroideum* (Chlorophyceae).
++: diameter of inhibition zone > 2 cm; +: diameter of inhibition zone < 2 cm; −: no activity.

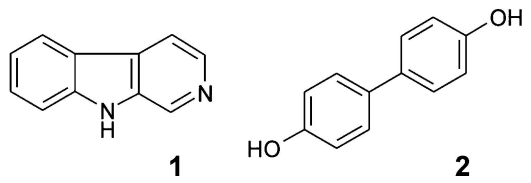

Figure 2. Structures of identified algicidal compounds. (1) Norharmane (9H-pyrido(3,4-b)indole), algicidal exometabolite of *Nodularia harveyana*, (2) 4,4′-dihydroxybiphenyl, algicidal exometabolite of *Nostoc insulare*.

insulare possessed the highest strength and range of algicidal activity. For *Nostoc insulare* a low self inhibiting activity was found, as well as for *Nostoc carneum* and *Arthrospira laxissima*.

Because of the strength and wide range of algicidal activity of the culture medium extracts obtained from the cultures of *Nodularia harveyana* and *Nostoc insulare* (Table 3), these extracts were investigated in detail. TLC showed that the culture medium extracts of *Nodularia harveyana* were dominated by one substance, characterised by an intensive blue fluorescence in UV light (254 nm as well as 366 nm), whereas the chromatograms of the culture medium extracts of *Nostoc insulare* were dominated (UV light 254 nm) by a substance located on the top of the chromatogram (Figure 1). The cytotoxic effect of the total culture medium extracts, discovered in the agar plate diffusion test, could be assigned to these compounds by detecting the chromatograms with living *A. laxissima* (Figure 1). The isolation of these substances was carried out by preparative TLC.

In the case of *Nodularia harveyana*, the active substance was identified as norharmane (9H-pyrido(3,4-b)indole) which belongs to a group of indole alkaloids, the β-carboline alkaloids. For *Nostoc insulare*, the cytotoxic substance was identified as 4,4′-dihydroxybiphenyl (Figure 2). The identification of both substances was confirmed by comparison with authentic norharmane and 4,4′-dihydroxybiphenyl, using TLC and HPLC.

Discussion

Cyanobacteria and other microalgae are known to produce a wide variety of biological active organic compounds. Most of those compounds are accumulated in the microalgal biomass, others are excreted during growth into the environment (Jaki et al., 2000, 2001). Therefore, in the present screening for algicidal metabolites, microalgal culture media were tested instead of microalgal biomass. The TLC of extracts obtained from microalgal culture media illustrates the complex composition of such extracts (Figure 1). Whereas in many cases the natural function of microalgal exometabolites so far is unknown, exometabolites which possess an algicidal activity in all likelihood are associated with the regulation of algal populations and with the interaction between competing microalgal organisms of the same habitat. However, current knowledge about such bioactive allelopathic chemicals and regulatory mechanisms in aquatic plant communities is low and more studies on this topic are required (Smith & Doan, 1999).

In previous screening studies, dealing with this subject, algicidal properties have been observed generally in a restricted number of cyanobacterial genera: *Anabaena*, *Calothrix*, *Fischerella*, *Nostoc*, *Oscillatoria* and *Scytonema* (Flores & Wolk, 1986; Schlegel et al., 1998; Smith & Doan, 1999). The results of the present work partially agree with this. Besides two *Anabaena*, four *Nostoc* and one *Oscillatoria* species algicidal species of other genera were discovered, too, namely one *Anabaenopsis*, two *Arthrospira*, one *Nodularia*, one *Phormidium* and furthermore one *Chroococcus* and one *Synechocystis* species. This is of special interest because the two last-mentioned species are unicellular. The proportion of bioactive species in previously published screening studies was about 10% (Schlegel et al., 1998); in the present study the proportion of algicidal species was 40%. This high value maybe due to the fact that culture media were tested instead of extracted microalgal biomass or living species.

In addition to this high proportion of bioactive species the autoinhibition, observed for three species (*A. laxissima*, *N. carneum*, *N. insulare*), must be emphasised. Whether this property is involved in a self regulation mechanism of populations of these species must be determined in the future.

The algicidal substance dominating the culture medium extracts from *Nodularia harveyana* was identified as norharmane (9H-pyrido(3,4-b)indole). Indole alkaloids, containing an intact β-carboline skeleton such as norharmane, are of widespread natural occurrence. Norharmane, the simplest example of β-carbolines, has been described to occur naturally in some higher plants (Gramineae, Sapotaceae and Zygophyllaceae) (Allen & Holmstedt, 1980; Bourke et al., 1992) and rarely for marine organisms, such as the dinoflagellate *Noctiluca miliaris* (Inoue et al., 1980). Larsen et al. (1994) described the isolation of three chlorine-containing β-carbolines (the so-called

bauerines A–C) from the cyanobacterium *Dichotrix baueriana*. Although various pharmacological effects of norharmane have been found, such as the inhibition of indoleamine 2,3-dioxygenase and nitric oxide synthase (Chiarugi et al., 2000; Connop et al., 1995) or an increase of insulin secretion from isolated human Islets of Langerhans (Cooper et al., 2003), the therapeutical benefit of norharmane is low because of its co-mutagenic activity (Totsuka et al., 1998, 2004). Searching for useful agents to control blooms of cyanobacteria, Kodani et al. (2002) isolated harmane (1-methyl-β-carboline) from the algicidal bacterium *Pseudomonas* sp. K44-1. The authors described the antagonistic activities of harmane and norharmane against bloom-forming cyanobacteria such as *Microcystis aeruginosa* and *M. viridis* and proposed the potential use of these alkaloids to combat algal blooms. Because of the various pharmacological and, in particular, the co-mutagenic effects of norharmane, the possibility of using this compound as an agent for removing algal blooms or as a commercial algicide in antifouling paints must be rejected. Furthermore, Kodani et al. (2002) found no activity of norharmane against green algae. This agrees with the results of the study presented here.

The algicidal substance dominating the culture medium extracts of *Nostoc insulare* was identified as 4,4′-dihydroxybiphenyl. This is the first isolation of this substance from cyanobacteria or their culture media. Based on its antialgal activity shown here, an antibacterial activity which is common to simple phenolic compounds appears probable. But up to now no data about such effects are available. To discover the pharmaceutical or commercial potential of this substance, or its potential as a lead in these application areas, further studies of the cytotoxity of 4,4′-dihydroxybiphenyl to other microorganisms, in particular bacteria, as well as its toxicity to eukaryotic cells, humans and animals needs to be determined.

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