

Insecticidal Compounds of the Biofilm-Forming Cyanobacterium *Fischerella* sp. (ATCC 43239)

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Received 22 June 2004; accepted 10 January 2005

ABSTRACT: Cyanobacterial biofilms are grazed by many different benthic invertebrates. In particular, larvae of insects are often present in great numbers and exert strong grazing pressure on cyanobacteria. Along with morphological adaptations, allelochemicals may have been developed as defense mechanisms against insect larvae. To investigate the chemical defense of biofilm-forming cyanobacteria, larvae of *Chironomus* sp., a widely distributed genus in this habitat, were used. Ten artificial biofilms of axenic and non-axenic cyanobacteria were screened for insecticidal activity against *Chironomus* sp. *Fischerella* 43239 was the cyanobacterium that exhibited the highest acute toxicity. A bioassay-guided isolation procedure was used to study the compounds responsible for toxicity in more detail. A toxic fraction was obtained when the 60% methanolic extract of *Fischerella* 43239 was separated on a C18 HPLC column. Electrospray mass spectrometry indicated the presence of several compounds in this fraction. The successful separation into individual compounds was achieved by HPLC on a cyanopropyl column. The heavily clustered quasimolecular ions observed on an electrospray mass spectrometer and the absorption spectra of the separated compounds were indicative of indole derivatives. The existence in the benthic cyanobacterium *Fischerella* 43239 of strong insecticidal metabolites that serve as chemical protection agents against insect larvae is supported by the data. © 2005 Wiley Periodicals, Inc. *Environ Toxicol* 20: 363–372, 2005.

Keywords: cyanobacterial biofilms; insecticidal activity; *Chironomus* bioassay; bioassay-guided isolation; *Fischerella* 43239

INTRODUCTION

The ability of cyanobacteria to produce bioactive compounds recently has attracted much attention (Carmichael, 1992; Codd, 1995; Sivonen and Jones, 1999). One possible function of these bioactive compounds in nature is the protection of cyanobacteria against grazers. In particular, the well-documented allelochemical-mediated effects of planktonic cyanobacteria on crustaceans are believed to have a strong impact on the pelagic community (Lampert, 1982; DeMott and Moxter, 1991; Haney et al., 1994; Agrawal et al., 2001).

Less information is available for the benthic community of the littoral zone. In this habitat insects are the dominant taxa, and larvae of midges, in particular, can become highly abundant. Epilithic biofilms are the nurseries for these larvae and are subjected to a high grazing pressure. Therefore, biofilm-forming cyanobacteria can be expected to have defense mechanisms to reduce the feeding efficiency of insects.

Feeding mechanisms that are different in planktonic and benthic invertebrates might influence the adaptation of defense mechanisms in cyanobacteria. In contrast to filter-feeding zooplankton, many benthic invertebrates have strong mouthparts. Insect larvae possess strong mandibles for biting and chewing food, and gastropods have rasplike radulae that are used to scrape or cut off small pieces of biofilms. For these reasons, we assume that defense mechanisms such as formation of colonies, filaments, mucilage,

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Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.20113

and spines that disturb the ingestion of pelagic filter feeders are less efficient than chemical defense mechanisms for biofilm-forming cyanobacteria. Consequently, we expect the presence of insecticidal compounds in biofilm-forming cyanobacteria.

To test this hypothesis, biofilm-forming cyanobacteria were screened for toxicity against the larvae of chironomids. The axenic strain of *Fischerella* 43239 caused high mortality in juvenile chironomids in a 48-h assay and therefore was studied in more detail. In this article we report on the separation of different insecticidal compounds of *Fischerella* 43239 by bioassay-guided isolation.

MATERIALS AND METHODS

Cyanobacterial Cultures

Nostoc sp. (Taiwan), *Oscillatoria formosa*, and *Phormidium retzii* were isolates from cyanobacterial biofilms collected in Taiwan (Jüttner and Wu, 2000). *Anabaena doliolum* and *Anabaena* sp. (78-12-A; identical with *Nostoc* sp. ATCC 43238) were obtained from C. P. Wolk, Michigan State University (East Lansing, MI, USA). *Nostoc* sp. (ATCC 53789), *Fischerella* sp. (ATCC 29114), *Fischerella* sp. (ATCC 43239), and *Oscillatoria* sp. (ATCC 29134) were obtained from ATCC (Rockville, MD). *Calothrix anomala* (SAG 1410-4) was obtained from SAG (Göttingen, Germany).

The cyanobacteria were cultivated at 20°C in sterile 300-mL Erlenmeyer flasks filled with approximately 120 mL of mineral medium (Jüttner et al., 1983). The amount of light provided was 5 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Larger amounts of *Fischerella* 43239 were cultivated as batch cultures in glass-tube reactors aerated with CO₂-enriched air (0.1% v/v). Detailed culture and harvest conditions for large-scale batch cultures are given by Jüttner and Wessel (2003).

Culturing of *Chironomus riparius* (Meigen)

An OECD guideline for the testing of chemicals (OECD, 2001) was used to establish a culture of *Chironomus riparius*. The first clutches of *Chironomus riparius* were kindly supplied by M. Vervliet-Scheebaum (Syngenta, Basel, Switzerland). Standard synthetic freshwater (moderately hard; Weber, 1993) was used as a culture medium. The quartz sand used as sediment in the culture vessels was kindly supplied by T. Ritter (Saint-Gobain Weber GmbH, Amstetten, Germany).

Extraction and Isolation

For the analysis of *Fischerella* 43239, fresh (1 g) or lyophilized (Lyovac GT2, Finn Aqua) biofilms were extracted with 10 mL of 60% aqueous methanol for 24 h at 3°C in

the dark. After centrifugation the supernatants were dried with a centrifugal vacuum concentrator (Speed Vac Plus, Savant, Runcorn, England) or a rotary evaporator at 40°C. Residues were dissolved in 50% methanol (1 mL of solvent per 1 g of fresh biomass) and filtered through 0.2- μm nylon filters (Semadeni, Ostermündingen, Switzerland). HPLC fractionation was performed on a Shimadzu 10AVP system equipped with a photodiode array detector (PDA) and an autosampler. Crude separation was achieved on a reversed-phase column (C18 Grom-Sil 120 ODS-4 HE, 4.6 \times 250 mm, 5 μm , Grom, Rottenburg-Hailfingen, Germany). Solvents A (UV-treated H₂O) and B (acetonitrile) were applied in the following gradient: solvent B from 50% to 100% in 12 min, 15 min isocratic. The flow rate was 1 mL min⁻¹. The toxic peak was found to elute at 16.3–17.0 min (UV₂₂₀). The toxic peak was collected, the solvent evaporated, and the residues were taken up in 70% methanol for further separation. For a second HPLC separation the sample was applied to a cyanopropyl column (CN Grom-Sil 120 Cyano-3 CP, 4.6 \times 250 mm, 5 μm , Grom). UV-treated H₂O was used as solvent A and methanol as solvent B. The applied gradient was: solvent B from 70% to 100% in 20 min. The obtained peaks (UV₂₈₀) that eluted at 8.2–9.05, 9.1–10.15, 10.2–11.0, and 11.05–12.15 min were collected and tested in the bioassay. A fraction that showed no absorption at 280 nm (fraction 1, 7.15–8.15 min) served as the control.

Mass Spectrometry (MS)

Mass spectra (positive ionization mode) of the toxic C18 HPLC fraction were obtained by direct infusion of 5 μL on an electrospray mass spectrometer (LCQ Duo mass spectrometer; Thermoquest Finnigan). Aqueous methanol (50%) acidified with acetic acid (1%) was used as the solvent.

Fractions obtained on the CN column were applied on a small HPLC column (C18 Grom-Sil 120 ODS-4 HE, 2 \times 40 mm, 3 μm , Grom) for mass spectrometry. Aqueous acetonitrile (95%) was used as the solvent (flow rate 0.25 mL min⁻¹). Tandem mass spectrometry (MS/MS; isolation width $m/z = 1$; normalized collision energy 45%) was used to study the fragmentation patterns of the fractionated compounds.

Toxicity Assay with *Chironomus* Larvae

To screen for the toxicity of the biofilms, larvae of chironomids were incubated in 1 mL of standard synthetic freshwater (moderately hard) with flakes of different cyanobacterial biofilms. The biotests were performed in 24-well tissue culture plates (TPP; Trasadingen, Switzerland). Small pieces of fresh biofilms were applied to *Chironomus* larvae for 8–11 days. Larvae were considered dead when a reaction to a

TABLE I. Insecticidal activity of benthic cyanobacteria in larvae of chironomids

Cyanobacterial Strain Assayed	Axenic Culture	Time of Incubation days	Test Animals	Mortality
No food (control)		8	CLZ	–
<i>Nostoc</i> sp. (Taiwan)	–	8	CLZ	–
<i>Oscillatoria formosa</i>	–	8	CLZ	–
<i>Anabaena doliolum</i>	+	8	CLZ	–
<i>Phormidium retzii</i>	–	8	CR	–
<i>Nostoc</i> sp. (ATCC 53789)	+	8	CR	–
<i>Fischerella</i> sp. (ATCC 29114)	+	8	CR	–
<i>Oscillatoria</i> sp. (ATCC 29134)	+	11	CLZ	–
<i>Calothrix anomala</i>	–	11	CLZ	+
<i>Anabaena</i> sp. (78-12-A)	+	11	CLZ	+
<i>Fischerella</i> sp. (ATCC 43239)	+	11	CLZ	+

Chironomids were isolated from the littoral zone of Lake Zürich (CLZ) or were from laboratory cultures (CR). Mortality (–), 0–2 dead animals; mortality (+), more than 2 of 6 animals dead.

mechanical stimulus with a pipette and motion of the inner organs could no longer be observed under a stereomicroscope (MZ FLIII, Leica). For the general screening, larvae of *Chironomus* sp. were collected from the littoral zone of Lake Zürich or were taken from a laboratory culture of *Chironomus riparius*. For the detailed analysis of *Fischerella* 43239, only larvae of the standardized laboratory culture of *Chironomus riparius* were used. To minimize the variability of the test animals, larvae of the same clutch were used for each series of assays.

The C18 HPLC fractions, equivalent to 1.2 g biofilm of *Fischerella* 43239, were dried and redissolved in 300 μ L of 50% aqueous methanol. In each well 50 μ L of this solution was added to 950 μ L of standard synthetic freshwater and one insect larva. Six wells were tested for each fraction over 48 h. Control wells with 2.5% methanol showed no toxicity.

The second HPLC separation on a CN column allowed the separation of the toxic C18 fraction into four peaks (fractions 2–5), which were collected. UV-absorption spectra of these four CN fractions were measured with a PDA. Assuming the same molar absorption coefficient, the maximum of 281 nm (measured on a photometer, Carry 3, Varian) was used to adjust the fractions to the same concentration. Fraction 2, which showed the lowest absorption, required the largest volume to obtain the final concentration. The same volume of the eluent also was used for testing fraction 1 as a control. The adjusted fractions were dried and dissolved in 50% methanol. In each well 50 μ L of the solution was added to 950 μ L of standard synthetic freshwater and 5 insect larvae. Three wells were tested for each fraction over 24 h.

RESULTS

In an initial screening, 10 strains of biofilm-forming cyanobacteria (Table I) were tested for insecticidal activity

against larvae of chironomids. Seven of the cyanobacterial strains exhibited only weak or no insecticidal activity (0–2 dead larvae), whereas three strains caused mortality (3–6 dead animals). Figure 1 shows the development and survival of larvae incubated on the nontoxic cyanobacterium *Oscillatoria* 29134 and the three bioactive cyanobacteria, *Calothrix anomala*, *Anabaena* 78-12-A, and *Fischerella* 43239. All animals incubated on *Oscillatoria* 29134 survived, some reaching later developmental stages. Some of the larvae incubated on *Calothrix anomala* and *Anabaena* 78-12-A died within a week, but others survived the whole

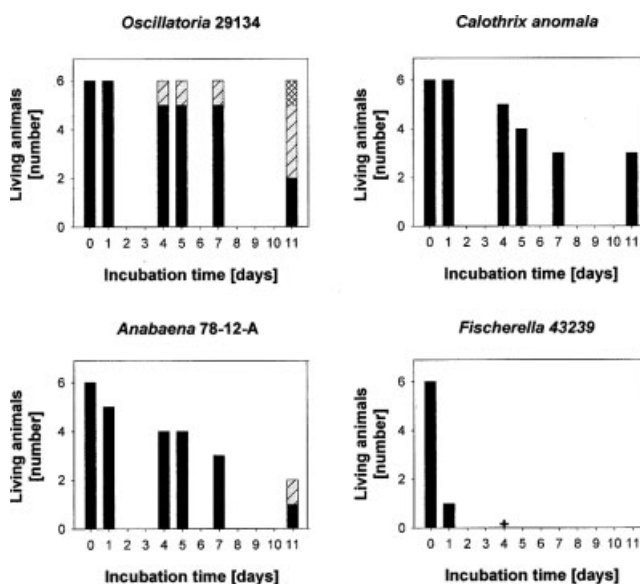


Fig. 1. Development and survival of *Chironomus* sp. larvae sampled from Lake Zürich and incubated on biofilms of different strains of cyanobacteria. The columns indicate the number of surviving larvae (solid black bars) and individuals that developed to the pupal (diagonal hatching) and adult (cross-hatching) stages. Examination showed that by the fourth day *Fischerella* 43239 had caused total mortality (+).

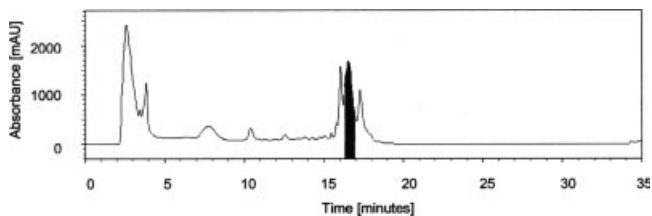


Fig. 2. C18 HPLC chromatogram (UV₂₂₀) of the crude extract of *Fischerella* 43239 used for the bioassay-guided isolation of the insecticides. The toxic fraction is labeled black.

incubation time of 11 days. We found a strong indication of insecticidal activity in *Fischerella* 43239. Acute toxicity was documented by the observation that only one individual of six survived after 24 h of incubation. On the next date of observation (2.5 days later), all animals were dead.

In a further experiment we tested biofilms of *Fischerella* 43239 on a larger number of larvae obtained from laboratory cultures of *Chironomus riparius* that were grown in standardized conditions. Again, high mortality was observed, with 46 of 54 larvae dying within 48 h compared to 6 dead larvae in the control without food.

To isolate the bioactive compounds of *Fischerella* 43239, the fresh biomass was extracted with 60% methanol and fractionated by HPLC. Figure 2 shows a chromatogram of the crude extract on a reversed-phase C18 column. The peak at 16.3–17.0 min contained all the toxic components. This peak was collected and further examined by electrospray mass spectrometry. The mass spectrum obtained (Fig. 3) showed the presence of quasimolecular ions at m/z 305.4 and 339.4/341.4. The latter pair is an indication of chlorine ($^{35}\text{Cl}/^{37}\text{Cl}$)–containing molecules. The MS analysis indicated the toxic fraction contained several different compounds. In addition, strong clustering of 2–3 molecules was observed.

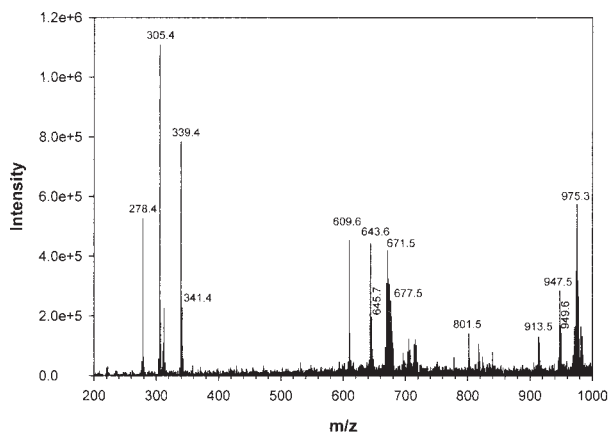
Several attempts to improve the separation of the compounds with the reversed-phase C18 column were not successful. Neither variation in the gradient (isocratic) and flow rate nor application of acidified solvents resulted in a better separation. No improvement was obtained by application of phosphate buffer containing hexanesulfonic acid (pH 2.5) as the eluent. This system was described as an optimized procedure for the separation of mixtures of indole alkaloids (Stöckigt et al., 2002). Several other types of HPLC column packings were tested, which also gave no satisfying separation. However, when cyanopropyl was used as the solid phase, the toxic fraction was separated into four peaks (Fig. 4, fractions 2–5). The mass spectra (Fig. 5) illustrate the separation of the different compounds. Fractions 2 and 3 show quasimolecular ions at m/z 305, whereas fractions 4 and 5 show quasimolecular ions at m/z 339/341. The mass spectra of the fractions show the easy formation of clusters between the molecules. The quasimolecular ions m/z 609, in fraction 2, and m/z 677, in fractions 4 and 5, indicate the clustering of the masses 304 and 338, respectively. In fraction 3 the interval of 304

masses between m/z 669 and 973 also indicates clustering. The quasimolecular ions m/z 609 (fraction 2) and 677 (fraction 4) were able to be fragmented by tandem mass spectrometry, which resulted in the ions m/z 305 (Fig. 6, fraction 2) and 339 (Fig. 6, fraction 4). Figure 7 shows the different mass spectra obtained by MS/MS fragmentations of the quasimolecular ions at m/z 305 (fractions 2 and 3), 339, and 341 (fractions 4 and 5). The fragment patterns of these spectra are a strong indication of the presence of chlorine ($^{35}\text{Cl}/^{37}\text{Cl}$) in quasimolecular ions m/z 339/341 (Table II). The absorption spectra of fractions 2–5 showed absorption maxima at 222 and 281 nm (Fig. 4), consistent with the presence of indole-containing compounds.

The peaks and a control fraction that showed no absorbance (fraction 1) were collected to test their insecticidal activities in a bioassay with *Chironomus riparius* larvae. Assuming the same molar absorption coefficient for the different compounds, fractions 2–5 were adjusted to the same concentration and tested for 24 h on *Chironomus* larvae (Fig. 8). Fraction 1, which was used as a control, showed no toxicity. All the other four fractions caused mortality, with fraction 3 showing the highest toxicity.

DISCUSSION

Few studies have documented the presence of insecticidal compounds in cyanobacteria, and the compounds described in the literature are very diverse in their chemical structures. Turrel and Middlebrook (1988) evaluated mosquitoes as test animals for toxin assays. Among several other biological toxins, the mosquitoes were sensitive to the cyano-



$[M+H]^+$	$[2M+H]^+$	$[3M+H]^+$
305 $[M_1+H]^+$	610 $[2M_1+H]^+$	914 $[3M_1+H]^+$
339 $[^{35}\text{Cl}M_2+H]^+$	644 $[M_1+^{35}\text{Cl}M_2+H]^+$	948 $[2M_1+^{35}\text{Cl}M_2+H]^+$
341 $[^{37}\text{Cl}M_2+H]^+$	646 $[M_1+^{37}\text{Cl}M_2+H]^+$	950 $[2M_1+^{37}\text{Cl}M_2+H]^+$

Fig. 3. Mass spectrum (ESI) of the toxic fraction. The masses in the table are the rounded results of the calculations with the masses from the spectrum.

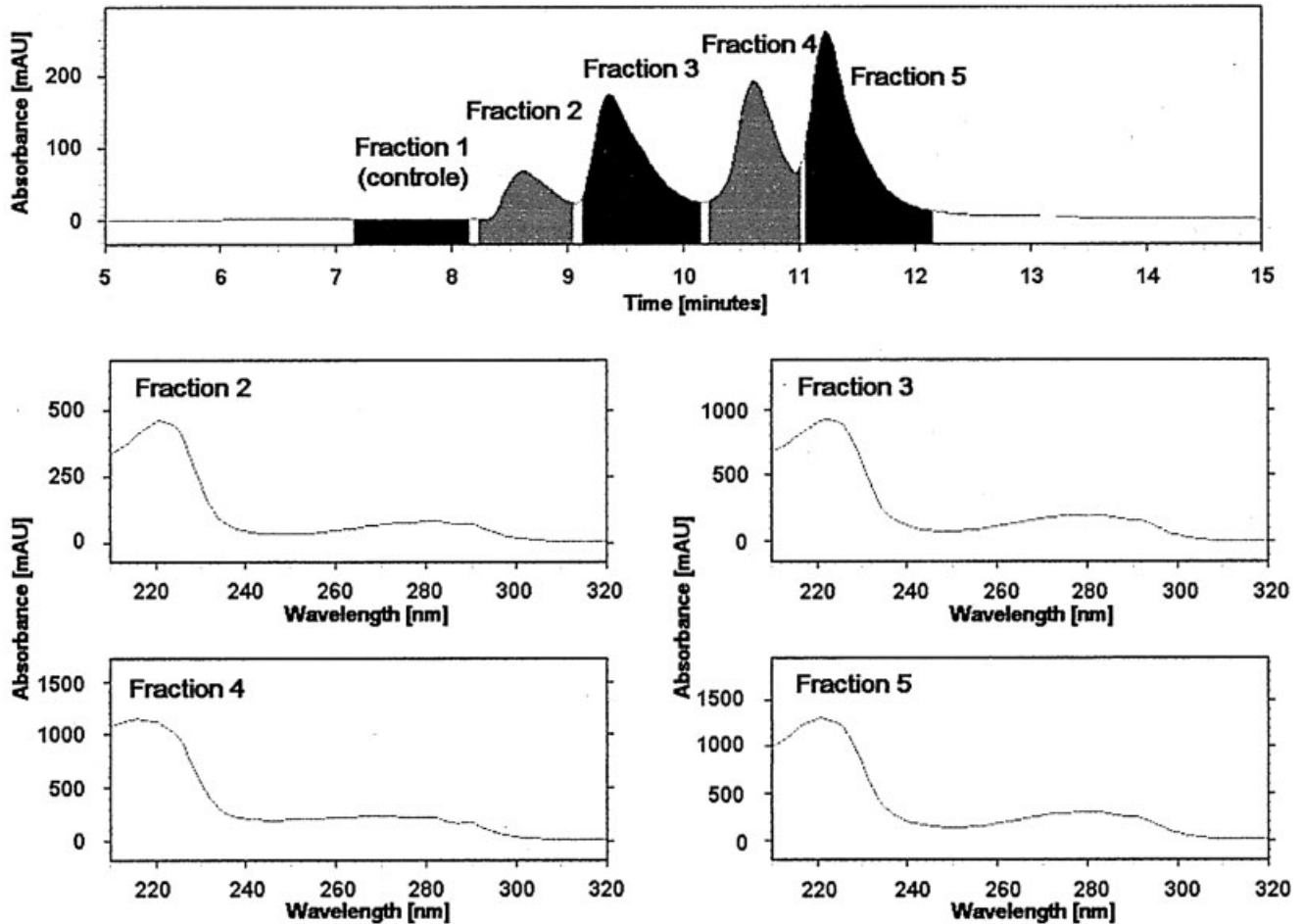


Fig. 4. HPLC (UV₂₈₀) of the toxic fraction on a cyanopropyl column and the absorption spectra of the resulting four peaks (fractions 2–5).

bacterial toxins saxitoxin and microcystin. Kiviranta and coworkers (1993) published an extensive study of insecticidal activity of 76 strains of planktonic cyanobacteria and showed that the mortality of the mosquito larvae *Aedes aegypti* was associated with the presence of neurotoxins and microcystins. *Microcystis aeruginosa* was the most toxic cyanobacterium found in this study, and [Dha⁷]microcystin-RR was determined as the major toxic compound (Kiviranta et al., 1992). Delaney and Wilkins (1995) determined microcystin-LR as a toxin against various insects and confirmed the insecticidal activity of microcystins. Another insecticidal compound that showed toxicity against *Helicoverpa armigera* (Lepidoptera) was a glycine-rich peptide of *Scytonema* sp. (Sathiyamoorthy and Shanmugasundaram, 1996). Unsaturated fatty acids of *Oscillatoria agardhii* were found to be the cause of mortality in larvae of the mosquito *Aedes albopictus* (Harada et al., 2000), and more recently a known cryptophycin-producing strain of *Nostoc* sp. was reported to produce toxins to *Helicoverpa armigera* and was evaluated as a potential source of natural pesticides (Biondi et al., 2004).

Benthic cyanobacteria that are the natural habitat of juvenile insects may have a higher capacity to produce insecticides than do pelagic cyanobacteria. To discover chemically protected cyanobacteria, we tested 10 biofilm-forming cultures for toxicity to larvae of chironomids, which are the most widely distributed and frequently the most abundant insect larvae in freshwater (Cranston, 1995). The screening of 10 biofilm-forming cyanobacteria showed marked differences in the development and survival of the tested larvae. The control, which contained no food, and 7 of 10 biofilms tested showed no negative effect on the insect larvae. *Oscillatoria* 29134, for example, caused no mortality and allowed a normal development of the larvae. Obviously, the *Oscillatoria* 29134 biofilm was used as suitable food. Recently, three epilithic *Oscillatoria* species have been described as high-quality food resources for intertidal grazers in protein, carbohydrate, and calorific values (Nagarkar et al., 2004). However, cyanobacteria are generally considered as poor food because of their nutritional composition, poor ingestibility and digestibility, and the synthesis of toxins (Porter

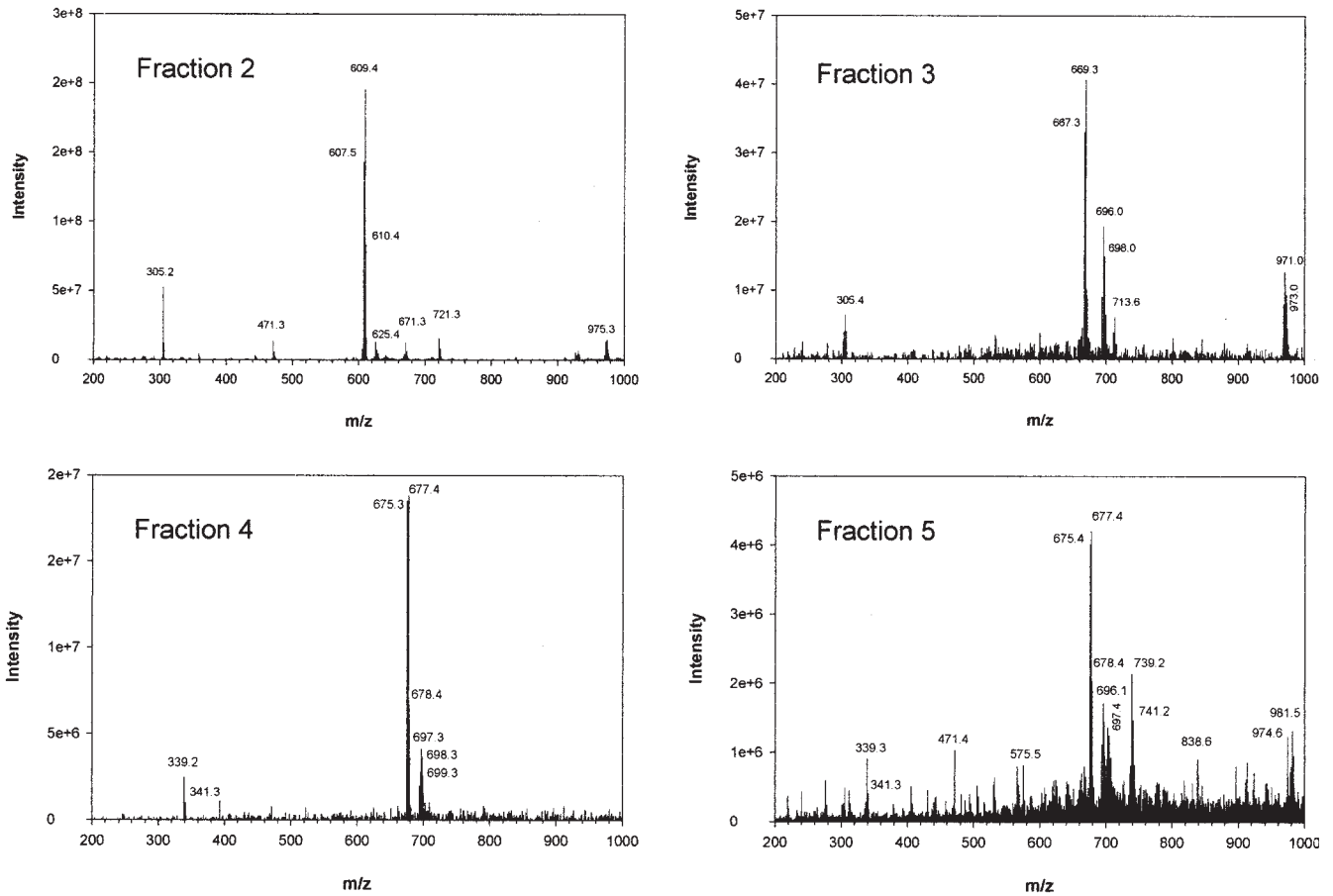


Fig. 5. Mass spectra (ESI) of fractions 2–5.

and Orcutt, 1980; Lampert, 1987; DeMott and Moxter, 1991; Rohrlack et al., 2003). Therefore, an explanation for the successful metamorphosis on poor food would be that endogenous reserves of the larvae were sufficient to complete their development.

Three cyanobacterial biofilms had negative effects on the chironomids. *Calothrix anomala*, *Anabaena* 78-12-A, and *Fischerella* 43239 caused mortality of the incubated insect larvae. In cases in which no mortality was observed by *Anabaena* 78-12-A and *Calothrix anomala*, both cyano-

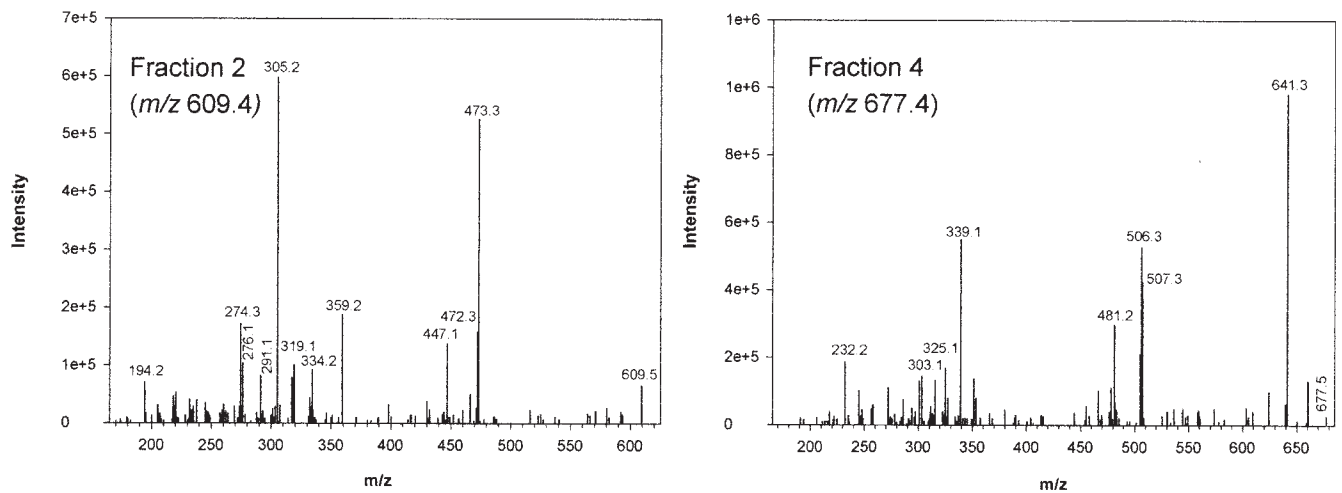


Fig. 6. Tandem mass spectra (MS/MS; ESI) of quasimolecular ions m/z 609.4 (fraction 2) and m/z 677.4 (fraction 4).

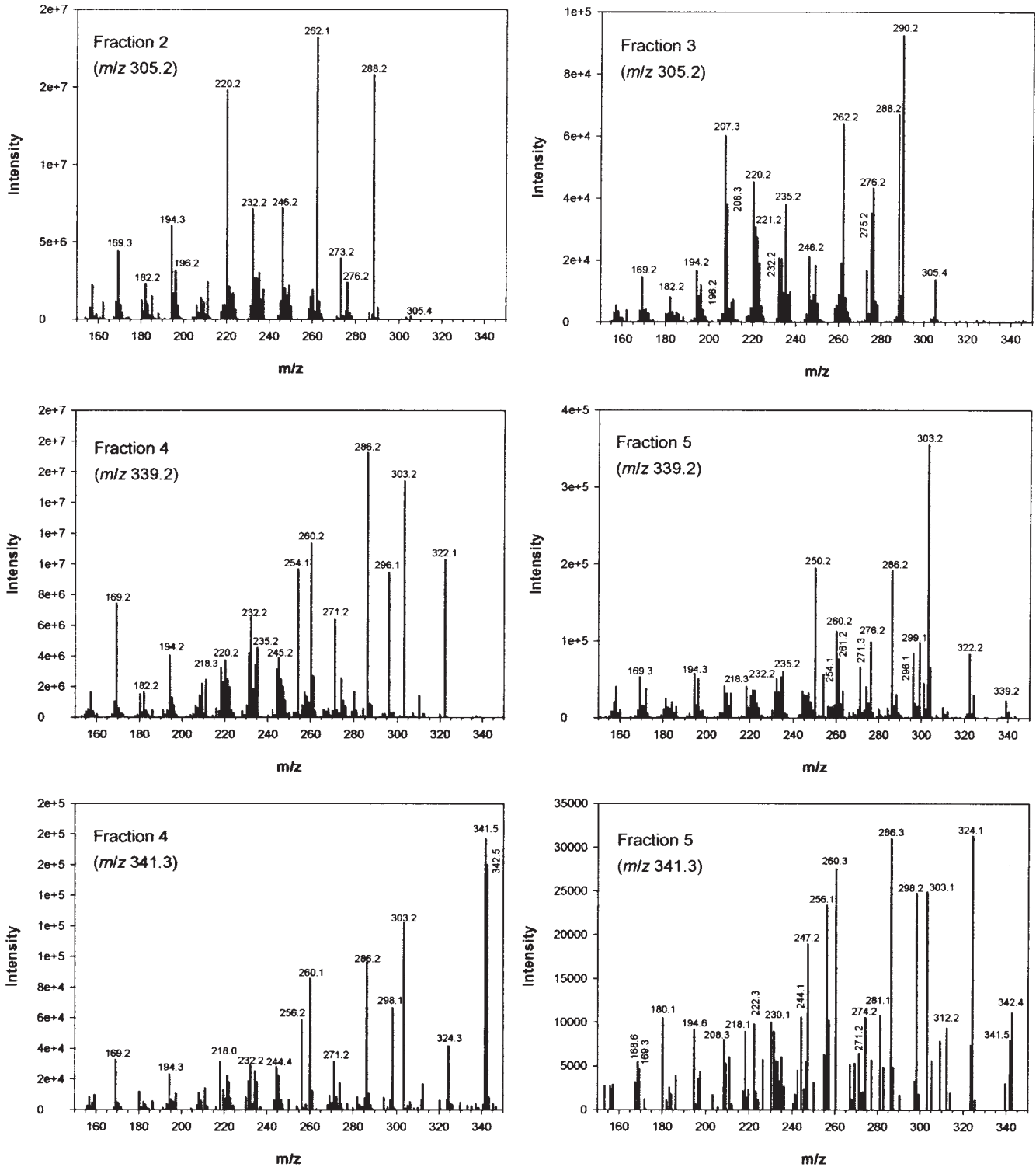


Fig. 7. Tandem mass spectra (MS/MS; ESI) of quasimolecular ions m/z 305.2 (fractions 2 and 3), m/z 339.2 (fractions 4 and 5), and m/z 341.3 (fractions 4 and 5).

bacteria essentially reduced development to the pup stage. These observations mean that the presence of inhibiting or toxic compounds in these two cyanobacterial strains is likely.

Fischerella 43239 showed the strongest effects, with acute toxicity within 24 h. This observation was a strong indication of the presence of insecticidal metabolites, and

TABLE II. Selected fragment ions after MS/MS fragmentation of the unchlorinated quasimolecular ion m/z 305 and $^{35}\text{Cl}/^{37}\text{Cl}$ -chlorinated quasimolecular ions m/z 339/341

MS/MS fragment ions of the quasimolecular ion m/z 305 of fractions 2 and 3	MS/MS fragment ions of quasimolecular ion m/z 339 of fractions 4 and 5		MS/MS fragment ions of quasimolecular ion m/z 341 of fractions 4 and 5	
220 $[\text{F}_1\text{H}]^+$	254 $[\text{F}_1^{35}\text{Cl}]^+$	218 $[\text{F}_1^{35}\text{Cl}-\text{H}^{35}\text{Cl}]^+$	256 $[\text{F}_1^{37}\text{Cl}]^+$	218 $[\text{F}_1^{37}\text{Cl}-\text{H}^{37}\text{Cl}]^+$
262 $[\text{F}_2\text{H}]^+$	296 $[\text{F}_2^{35}\text{Cl}]^+$	260 $[\text{F}_2^{35}\text{Cl}-\text{H}^{35}\text{Cl}]^+$	298 $[\text{F}_2^{37}\text{Cl}]^+$	260 $[\text{F}_2^{37}\text{Cl}-\text{H}^{37}\text{Cl}]^+$
288 $[\text{F}_3\text{H}]^+$	322 $[\text{F}_3^{35}\text{Cl}]^+$	286 $[\text{F}_3^{35}\text{Cl}-\text{H}^{35}\text{Cl}]^+$	324 $[\text{F}_3^{37}\text{Cl}]^+$	286 $[\text{F}_3^{37}\text{Cl}-\text{H}^{37}\text{Cl}]^+$
		303 $[\text{339}-\text{H}^{35}\text{Cl}]^+$		303 $[\text{341}-\text{H}^{37}\text{Cl}]^+$

therefore *Fischerella* 43239 was analyzed in more detail. To minimize the phenotypic and genotypic variability of collected animals, a standardized laboratory culture of *Chironomus riparius* was established. To double-check the toxic effects, *Fischerella* 43239 was tested on a larger number of *Chironomus riparius*, and insecticidal activity was confirmed. Insecticidal activity also was found in the methanolic extract of *Fischerella* 43239, and a toxic fraction was obtained by HPLC in a bioassay-guided fractionation. This fraction was applied to electrospray mass spectrometry, where several quasimolecular ions were detected. The ions m/z 339.4/341.4 $[\text{}^{35}\text{ClM}_2+\text{H}]^+ / [\text{}^{37}\text{ClM}_2+\text{H}]^+$ were indicative of chlorine ($^{35}\text{Cl}/^{37}\text{Cl}$)-containing molecules. Furthermore, a quasimolecular ion at m/z 305.4 $[\text{M}_1+\text{H}]^+$ was observed that might represent the corresponding unchlorinated compound. Strong quasimolecular ions at m/z 609.6 $[2\text{M}_1+\text{H}]^+$ and 643.6/645.7 $[\text{M}_1+^{35}\text{ClM}_2+\text{H}]^+ / [\text{M}_1+^{37}\text{ClM}_2+\text{H}]^+$ indicated clustering between two molecules of the mass 304 $[\text{M}_1]$ or clustering of the mass 304 $[\text{M}_1]$ with the mass 338 $[\text{}^{35}\text{ClM}_2]$ or the mass 340 $[\text{}^{37}\text{ClM}_2]$. The quasimolecular ions of m/z 913.5 $[3\text{M}_1+\text{H}]^+$

and 947.5/949.6 $[2\text{M}_1+^{35}\text{ClM}_2+\text{H}]^+ / [2\text{M}_1+^{37}\text{ClM}_2+\text{H}]^+$ could be triple clusters $[3\text{M}]$ between 304 $[\text{M}_1]$ and 304 $[\text{M}_1]$ plus 338 $[\text{}^{35}\text{ClM}_2]$ or 340 $[\text{}^{37}\text{ClM}_2]$.

Electrospray mass spectrometry indicated that several compounds were present in this fraction. After varying the solvents and the columns, a cyanopropyl column finally was found that allowed the separation of four peaks in the active fraction. MS analyses of these peaks (fractions 2–5) showed separation of the compounds with the masses 304 and 338/340. Tandem mass spectrometry of the strong quasimolecular ions m/z 609 $[2\text{M}_1+\text{H}]^+$ and 677 $[2^{35}\text{ClM}_2+\text{H}]^+$ confirmed the clustering of the molecules 304 $[\text{M}_1]$ and 338 $[\text{}^{35}\text{ClM}_2]$.

All the compounds exhibited absorption spectra that were typical for indole derivatives. The presence of indole moieties also would explain the strong clustering as described by Yamamoto and Wakisaka (1997). They interpreted clustering of indole and isoquinoline through intermolecular hydrogen-bonding interactions.

MS/MS fragmentation of the quasimolecular ions m/z 305, 339, and 341 showed a similar pattern. Furthermore, the fragmentation of m/z 339 and 341 showed cleavage of hydrochloric acid (H^{35}Cl and H^{37}Cl). These data confirmed the assumption that the compounds with masses of 338 and 340 were chlorinated derivatives of compounds with a mass of 304.

Sixteen cyanobacterial compounds with masses of 304 and 338/340 belonging to the hapalindole and fischerindole alkaloids have so far been described as present in cyanobacteria (Moore et al., 1984, 1987; Schwartz et al., 1987; Park et al., 1992; Stratmann et al., 1994; Klein et al., 1995). Most were isolated by Moore and coworkers (1987) and were characterized as antimycotic, antialgal, and antibacterial compounds. Insecticidal activity has yet to be described for any of these compounds.

Indole alkaloids are common in microorganisms, animals, and especially plants (Cordell et al., 2001; Thomas and Spaggiari, 2002), and they often possess pharmacological and toxicological properties (Mukhopadhyay et al., 1981; Kong et al., 1986). Insecticidal activity was documented for indole alkaloids isolated from *Murraya koenigii* (*Rutaceae*) (Ramsewak et al., 1999) and a *Penicillium* species (Li et al., 2002).

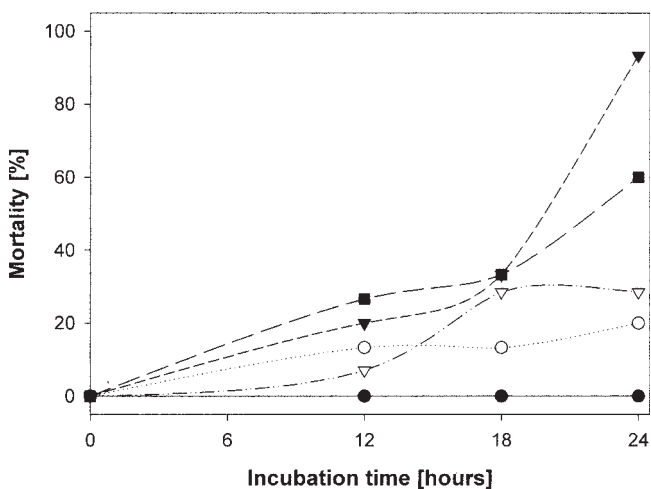


Fig. 8. Mortality of *C. riparius* larvae tested for toxicity of the five HPLC fractions: ● fraction 1 (control), ○ fraction 2, ▼ fraction 3, ▽ fraction 4, ■ fraction 5. The amounts of fractions 2–5 were normalized to the same absorption at 281 nm.

On the basis of our present knowledge, *Fischerella* 43239 could be of potential use as a new source of natural insecticides, but when occurring in reasonable amounts, *Fischerella* also may have an impact on water quality, as already documented for many other secondary metabolites of cyanobacteria (Codd, 1995; Kuiper-Goodman et al., 1999).

The authors gratefully acknowledge the support of the Hydrobiologie-Limnologie Stiftung, Zürich.

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