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Short review

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INSECTICIDAL ACTIVITY – A NEW BIOACTIVE PROPERTY OF THE CYANOBACTERIUM *FISCHERELLA*

ABSTRACT: Cyanobacterial biofilms serve as food and shelter for benthic invertebrates, such as juvenile insects. Chironomids are often the most widely distributed and abundant insect larvae in freshwater ecosystems. As a consequence of high grazing pressure, effective defence mechanisms can be expected in biofilm-forming organisms. The presence of chemical defence was studied in 12 axenic and monoxenic cyanobacterial species. Flakes of cyanobacterial biofilms were offered to *Chironomus riparius* (Meigen) over a period of 8 days. Mortality and body-length of the surviving animals were used as indicators for the toxicity of the cyanobacteria and their suitability as food. Toxicity and inhibition of larval growth were found for several cyanobacteria tested. *Fischerella* sp. (ATCC 43239) was the most active and caused 100% mortality in *Chironomus* larvae within 24 h. Mortality was also high (87%) for larvae fed with *Aphanothece* sp. Moderate toxicity (40–60% mortality) was found for *Calothrix* sp. (PCC 7507), *C. braunii* Bornet et Flahault, *C. thermalis* (Schwabe) Hansgirg and a cyanobacterium of the LPP group designated JU 5. Mortality of 7–33% was observed for *Calothrix parietina* (Nägeli) Thuret, *Oscillatoria brevis* (Kützing) Gomont, *Cylindrospermum* sp., *Nostoc* sp., *Calothrix anomala* Mitra and a cyanobacterium of the LPP group designated 5 KB. Differences depending on the cyanobacterial food offered were also seen in the lengths of surviving larvae. *Fischerella* sp. (ATCC 43239) was studied in more detail to

chemically characterise the observed insecticidal activity. The insecticidal activity could be extracted with 60% aq. methanol from the fresh biomass and caused 100% mortality in *Chironomus*.

A literature survey was performed on the bioactive compounds so far isolated and characterised from *Fischerella* and related Stigonematales. It is noticeable that no insecticidal activity has been shown for any of these compounds yet. The newly found insecticidal property of *Fischerella* may lead to the identification of bioactive compounds which may be important as chemical defence against insect grazers.

KEY WORDS: cyanobacterial biofilm, chemical defence, *Fischerella*, *Chironomus*, insecticide

1. INTRODUCTION

Juvenile insects are a prominent group of invertebrate grazers in running and standing waters. Their grazing on benthic biofilms has a strong impact on the abundance of algal and cyanobacterial species (Hart 1985, Hill and Knight 1987). The insect communities that develop on stony lake shores are often dominated by caddisfly and chironomid larvae (Harrison and Hildrew 1998). These mainly herbivorous insects influence the productivity of periphyton (Lamberti

and Resh 1983) and the structure of algal assemblages (Peterson *et al.* 1998). Hart (1985) reported that filaments of the cyanobacterium *Microcoleus* sp. were avoided by insect grazers. Though not suggested by the authors, chemicals may be responsible for this discrimination.

Among insect larvae, juvenile chironomids are often the most abundant insects in fresh water. A comprehensive overview on their biology and ecology was given by Armitage *et al.* (1995). Larvae of chironomids use algae, detritus and associated microorganisms, macrophytes, woody debris and invertebrates as food sources. Chironomids can be considered as an important link in aquatic food webs because of their efficient conversion of primary into secondary production (Benke 1998). Secondary consumers, such as fish, waterfowl and predating insects, prey on juvenile chironomid larvae, and adult insects are consumed by birds, bats and amphibians.

Non-selective grazing of chironomids can result in a sharp decline in biomass as shown for epiphytic algae in Lake Kasumigaura, Japan (Takamura *et al.* 1990), where grazing on algae and cyanobacteria was described for chironomids which were living in surficial sediment and for species living in biofilms. Brook (1954) studied chironomid larvae from sand-filter beds of waterworks and observed that the larvae largely ingested filamentous green algae and diatoms. Juvenile chironomids of the genus *Cricotopus* live in the biofilm of the benthic cyanobacterium *Nostoc parmelioides* (Kützing). The interaction is considered to be mutualistic as *Nostoc* provides food and shelter for the insect larvae and the cyanobacterium profits from the larvae, which cement the biofilms to the substratum (Brock 1960) and may also enhance productivity of the cyanobacteria by mineralization of biomass and release of nutrients.

Some cyanobacteria are a suitable food for *Chironomus* (Ali 1990), but others are reported to be less favourable. Observations indicate that some species may be indigestible and toxic. An example is given by Johnson *et al.* (1989). They observed high concentrations of the cyanobacterium *Microcystis* and the diatom *Melosira* in the gut of *Chironomus*

plumosus (L.) collected from the sediment of the eutrophic Lake Vallentunasjön, Sweden. The total number of *Microcystis* cells (showing strong or weak fluorescence) did not decrease during gut passage but the ratio of weakly fluorescent cells became more abundant. This decrease of fluorescence intensity can be taken as an indication that *Microcystis* is attacked by the digestive enzymes of the gut, but efficient and complete digestion of the cells seems not to be possible, most likely due to the presence of protease inhibitors (Yamaki *et al.* 2005).

In a feeding experiment under laboratory conditions, some cyanobacteria (*Lyngbya* cf. *aeruginosa* (Kützing) Gomont and *Anabaena flos-aquae* (Lynagbye) Brébisson) allowed complete development of the chironomid *Glyptotendipes paripes* Edwards whereas *Microcystis* fed larvae showed significant body mass reduction and death before completion of their development (Frouz *et al.* 2004).

Cyanobacteria, like other biofilm-forming benthic organisms, are under control of invertebrate grazers such as protozoa, worms, crustaceans, snails and insect larvae. As a consequence of this grazing pressure, the presence of chemical defence mechanisms in benthic cyanobacteria might be expected. This topic appears poorly studied, however, compared to pelagic interactions between phytoplankton (including cyanobacteria) and invertebrate grazers. In this study, therefore, we focused our efforts on finding chemical defence of benthic cyanobacteria against insect larvae and on studying the compounds responsible for such reactions.

2. CHEMICAL DEFENCE OF BENTHIC CYANOBACTERIA AGAINST *CHIRONOMUS*

Axenic and monoxenic cyanobacteria that exhibit biofilm growth habit were grown in 300-ml Erlenmeyer flasks. Flakes from these cultures were offered larvae of *Chironomus*. We used natural populations of *Chironomus* that were collected from the stony shore of Lake Zürich. In addition, to minimize variation between the test animals and to achieve independence from the population dynamics of the larvae in the lake, we established a laboratory culture of *C. riparius*

(Meigen). We offered flakes of biofilms suspended in artificial lake water (moderately hard, Weber 1993) to the *Chironomus* larvae. Toxicity tests were performed in 24-well tissue culture plates over a period of 8 days. Results presented here relate to tests with larvae from the laboratory cultures of *C. riparius*.

Twelve cyanobacterial biofilms of different species were tested for toxicity to *C. riparius* (Table 1). The highest acute toxicity was found for *Fischerella* sp. (ATCC 43239). All animals in wells that were supplied with this cyanobacterium were dead within 24 h. As *Chironomus* died very soon after feeding on *Fischerella*, the biofilm of the filamentous, multicellular *Fischerella* was only slightly reduced at termination of the experiment. The biofilms of the other cyanobacteria were continuously grazed by *C. riparius* larvae during the eight days of observation. To avoid starvation of the animals, the surviving larvae were supplied every day with fresh flakes of cyanobacterial biofilms.

Although effects were much less than for *Fischerella* sp. (ATCC 43239), mortality was

also observed in assays with the other eleven biofilm-forming cyanobacteria. Several of these cyanobacteria had already shown toxic effects in tests with *Chironomus* larvae from Lake Zürich. In general, the chironomids collected from the lake were more sensitive than the animals from the laboratory cultures. *Aphanothece* sp. caused mortality in 26 of 30 larvae. This cyanobacterium is a strong producer of mucilage, which may have inhibited ingestion and digestion. Inadequacy of mucilaginous algae and cyanobacteria as potential food organisms has been discussed extensively for planktonic species (Porter 1973, Lampert 1987). Moderate toxicity was observed for biofilms produced by *Calothrix* sp. (PCC 7507), *C. braunii*, *C. thermalis* and a cyanobacterium of the LPP group designated JU 5 isolated from epipellic biofilms of Lake Türlensee, Switzerland. Biofilms of *C. parietina*, *Oscillatoria brevis*, *Cylindrospermum* sp. and a cyanobacterium of the LPP group designated 5 KB isolated from a biofilm in Taiwan caused weaker toxic effects. Mortality was low on *Nostoc* sp. and *C. anomala*.

Table 1. Percentage mortality of larvae from a laboratory culture of *Chironomus riparius* which were fed on different cyanobacterial biofilms. Each biofilm was tested on 30 newly hatched larvae. The body lengths of the surviving larvae were measured at the end of the eight days of incubation (mean \pm standard deviation).

Cyanobacterial strain assayed	Axenic culture	Mortality (%)	Length (μ m)
<i>Fischerella</i> sp. (ATCC 43239)	+	100(*,**)	-
<i>Nostoc</i> sp. (ATCC 43238)	+	7(*)	3054 \pm 647
<i>Aphanothece</i> sp. (Taiwan)	-	87(*)	1554 \pm 42
<i>Cylindrospermum</i> sp. (ATCC 29412)	+	33(*)	1908 \pm 299
<i>Calothrix anomala</i> (SAG 1410-4)	-	10(*)	3850 \pm 802
<i>Calothrix thermalis</i> (SAG 37.79)	-	40(*)	2482 \pm 640
<i>Calothrix braunii</i> (ASW)	-	50	2414 \pm 494
<i>Calothrix</i> sp. (PCC 7507)	+	60	1780 \pm 347
<i>Calothrix parietina</i> (PCC 6303)	+	20	3008 \pm 533
<i>Oscillatoria brevis</i> (Taiwan)	-	33	3012 \pm 753
JU 5 (Türler See)	-	53	1815 \pm 391
5 KB (Taiwan)	-	33	2244 \pm 641

*Cyanobacterial strain has shown toxic effects in preliminary tests on *Chironomus* larvae from Lake Zürich.

**All larvae showed mortality within 24 hours.

Body-lengths of the surviving larvae of the bioassays were measured after 8 days incubation time and tested by analysis of variance (ANOVA). The low number of variance (ANOVA). The low number of four surviving larvae fed with *Aphanothece* sp. was not included in this analysis. Body lengths were log-transformed to meet the assumptions for ANOVA. Effects of the single treatments were tested by an honest significant difference test (HSD). Differences in body length of the larvae indicated that the cyanobacteria tested significantly affected the growth of the larvae (Table 1 and Fig. 1). Larvae fed with *C. anomala* did not only show low mortality but also larger body length than the other larvae. *Nostoc* sp., *Oscillatoria* sp. and *C. parietina* also allowed body lengths of more than three millimetres. Other larvae, such as those fed with the biofilms designated JU 5 and *Calothrix* sp. (PCC 7507), reached less than two millimetres and were significantly smaller.

We focused our studies on the compounds responsible for the acute toxicity of *Fischerella* sp. (ATCC 43239) with the intention of characterising the bioactive agents responsible for the insecticidal effect

on *Chironomus*. Fresh biomass of *Fischerella* was extracted with 60% aq. methanol. This treatment removed the insecticidal compounds from the cells and when the extract was tested, high mortality to *C. riparius* larvae was observed. The solvent-free residue of an equivalent to 4.1 mg (wet weight) extracted biomass, dissolved in 1 ml of water (containing 1% methanol), caused 100% mortality to the tested larvae within 24 h. This confirmed the presence of insecticidal compounds in *Fischerella* sp. (ATCC 43239). The elucidation of the structures of the compounds in this toxic fraction is under investigation (Becher and Jüttner 2005). Since a large number of bioactive compounds has already been described in *Fischerella* and phylogenetically related species, we present an overview of the current literature on secondary metabolites.

3. SECONDARY METABOLITES ISOLATED FROM STIGONEMATALES (CYANOBACTERIA)

A review of the literature provides a bewildering record of bioactive metabolites isolated from *Fischerella* and other species

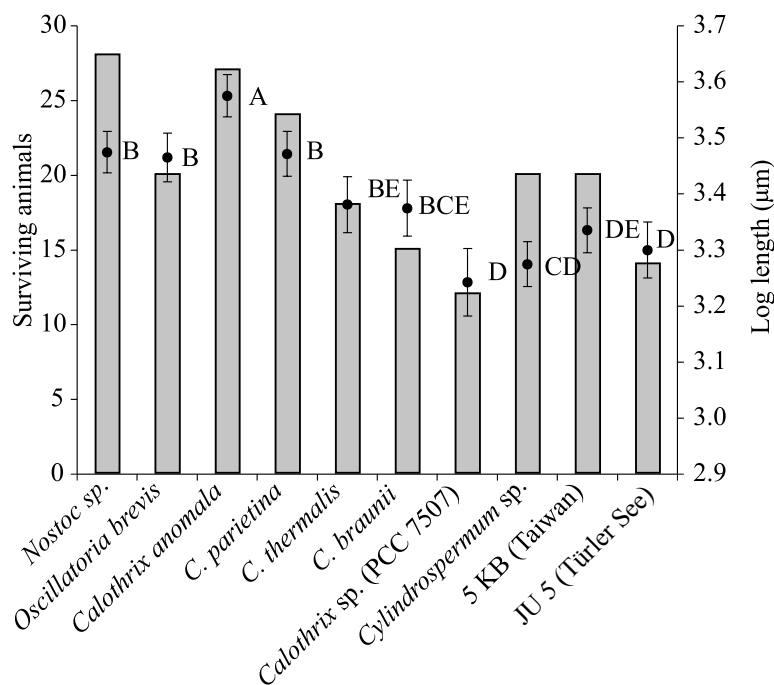


Fig. 1. Numbers (bars) and log of body lengths (points, mean squares with standard errors) of treatments with ≥ 12 surviving *C. riparius* larvae after eight days of incubation on different cyanobacterial biofilms. Points labelled with the same letters are not significantly different (HSD following ANOVA; $F(9, 188) = 25.34, P < 0.05$).

of the order Stigonematales. Table 2 gives an overview of the compiled information about the chemistry of the metabolites, their bioactivities and the cyanobacterial sources. With the exception of fischerellins, parsiguine and ambigols, the compounds listed are indole derivatives.

Hapalindoles, the largest group of cyanobacterial indole alkaloids, have tricyclic and tetracyclic structures and show different biological activities. The large number of possible stereoisomers of hapalindoles complicates the differentiation of the derivatives and makes the analysis rather difficult. The first hapalindoles were isolated from the terrestrial cyanobacterium *Hapalosiphon fontinalis* (Agardh) Bornet by Moore *et al.* (1984). The lipophilic extract of *H. fontinalis* was characterised to be antimycotic and antialgal (in the sense of "antiblue-green algal" = anticyanobacterial). Moore *et al.* (1987a) later described this activity as antibacterial. Antigreenalgal activity of hapalindoles was shown in a subsequent paper of Moore's group (Bonjouklian *et al.* 1988) and in other studies (Doan *et al.* 2000, Etchegaray *et al.* 2004). In the study by Doan *et al.* (2000) toxicity directed to Gram positive and Gram negative bacteria, cyanobacteria, green algae, fungi and mouse myeloma cells was shown. Toxicities associated with hapalindoles were also described for extracts from *Hapalosiphon laingii* Hoffmann, an isolate from the surface of a dead coral debris on Laing Island, Papua New Guinea. A mixture of extracted hapalindoles and hapalindolinones killed the nauplii of *Artemia* sp. (Klein *et al.* 1995). An inhibitory effect to RNA polymerase was described as primary target of 12-epi hapalindole E isonitrile. However, this inhibition site does not seem to be the only target and other effects can be expected (Doan *et al.* 2000, 2001).

Fischerindole L from *Fischerella muscicola* (Thuret) Gomont was the first representative isolated of another class of indole alkaloids, the fischerindoles. This tetracyclic alkaloid was described as antifungal (Park *et al.* 1992). Four more fischerindoles were isolated from the freshwater cyanobacterium *Hapalosiphon welwitschii* W. et G. S. West in an extensive study by Stratmann *et al.*

(1994). The production of fischerindole was also described for an additional strain of *F. muscicola* (Jimenez *et al.* 1999).

Ambiguine isonitriles were first isolated from the terrestrial cyanobacteria *Fischerella ambigua* (Nägeli) Gomont, *Hapalosiphon hibernicus* W. et G. S. West and *Westiellopsis prolifica* Janet. The indole moiety of these alkaloids is modified by an isoprene unit. These compounds are described as fungicidal (Smitka *et al.* 1992).

Biological activities were found for several other indole alkaloids from *Fischerella* and other Stigonematales. Hapalindolinones and welwitindolinones belong to the oxindoles, a subgroup of indole alkaloids which is known from plants and marine organisms (Lin *et al.* 1990, Carletti *et al.* 2000). Hapalindolinone A, one of the indole alkaloids which were isolated from the terrestrial cyanobacterium *Fischerella* sp. (ATCC 53558), was characterised as an inhibitor for the binding of arginine vasopressin in kidney tissue (Schwartz *et al.* 1987). *Hapalosiphon laingii* was described as another producer of hapalindolinones (Klein *et al.* 1995). Welwitindolinones were first isolated from *H. welwitschii* and the edaphic *Westiella intricata* Borzi isolated from a soil sample (Stratmann *et al.* 1994). N-Methylwelwitindolinone C isothiocyanate, the major indole alkaloid in these two cyanobacterial strains, was apparently responsible for the multi-drug resistance reversing activity in tumour cells. This activity was subsequently confirmed for N-methylwelwitindolinone C isothiocyanate and its non-methylated derivative (Smith *et al.* 1995, Zhang and Smith 1996). Insecticidal activity against blowfly larvae of the lipophilic extract of *W. intricata* is mentioned in the work of Stratmann *et al.* (1994). Unfortunately, though the authors associate the toxicity against blowfly larvae with N-methylwelwitindolinone C isothiocyanate, no data are shown for this property in their work or the relating references. Welwitindolinones were also found in *F. muscicola* and *F. major* Gomont (Jimenez *et al.* 1999).

Tjipanazole D, an indolocarbazole present in *F. ambigua*, showed no significant biological activity (Falch *et al.* 1993) although tjipanazoles have previously been described

Table 2. Bioactivities and sources of metabolites characterised in cyanobacteria of the order Stigonematales and of *Tloypothrix* (Noctocales).

Chemical structures	Bioactivities	Cyanobacteria	Places of isolation	References
<i>Hapalindoles</i>				
Indole alkaloids with tri- or tetracyclic structures; chlorinated or non-chlorinated, isonitrile or isothiocyanate functionalities	Antimycotic, antialgal, antibacterial, anticyanobacterial	<i>Hapalosiphon fontinalis</i> (ATCC 39694); <i>Fischerella muscicola</i> (UTEX 1829); <i>H. welwitschii</i> (UH strain IC-52-3); <i>H. laingii</i> (strain 89-785/4); <i>H. delicatulus</i> (UH isolate IC-13-1); <i>Fischerella</i> sp. (JAVA 94/20); <i>Fischerella</i> sp. (CENA 19)	Soil, Marshall Islands; Terrestrial; freshwater; Freshwater, soil, Australia; Surface of dead coral debris, Laing Island, Papua New Guinea; Terrestrial, edaphic, Australia; Indonesia; Sediment of floodplain Amazon river, Brazil	Moore <i>et al.</i> 1984, 1987a, Bonjouklian <i>et al.</i> 1988, Park <i>et al.</i> 1992, Stratmann <i>et al.</i> 1994, Klein <i>et al.</i> 1995, Huber <i>et al.</i> 1998, Doan <i>et al.</i> 2000, 2001, Etchegaray <i>et al.</i> 2004
<i>Fischerindoles</i>				
Tetracyclic indole alkaloid; chlorinated or non-chlorinated, isonitrile or isothiocyanate functionalities	Antifungal	<i>F. muscicola</i> (UTEX 1829); <i>H. welwitschii</i> (UH strain IC-52-3); <i>F. muscicola</i> (UH strain HG-39-5)	Terrestrial; freshwater; Freshwater, soil, Australia; Terrestrial, epilithic, Nan Madol, Pohnpei, Micronesia	Park <i>et al.</i> 1992, Stratmann <i>et al.</i> 1994, Jimenez <i>et al.</i> 1999
<i>Ambiguines</i>				
Indole alkaloids with isoprene unit; isonitriles and nitriles	Fungicidal	<i>F. ambigua</i> (UTEX 1903); <i>H. hibernicus</i> (UH isolate BZ-3-1); <i>Wesiellopsis prolifica</i> (UH isolate EN-3-1); <i>H. delicatulus</i> (UH isolate IC-13-1)	Terrestrial; Terrestrial; Terrestrial; Terrestrial, edaphic, Australia	Smitka <i>et al.</i> 1992, Huber <i>et al.</i> 1998
<i>Hapalindolinones</i>				
Oxidised indole alkaloids; chlorinated or non-chlorinated	Inhibition of arginine vasopressin binding	<i>Fischerella</i> sp. (ATCC 53558); <i>H. laingii</i> (strain 89-785/4)	Soil, Everglades, Florida, USA; Surface of dead coral debris, Laing Island, Papua New Guinea	Schwartz <i>et al.</i> 1987, Klein <i>et al.</i> 1995
<i>Welwitindolinones</i>				
Oxidised indole alkaloids; chlorinated or non-chlorinated, isonitrile or isothiocyanate functionalities	Multi-drug resistance reversing activity	<i>H. welwitschii</i> (UH strain IC-52-3); <i>Wesiella intricata</i> (UH strain HT-29-1); <i>F. muscicola</i> (UH strain HG-39-5); <i>F. major</i> (UH strain HX-7-4)	Freshwater, Australia; Soil, Moen Island, Truk Atoll, Caroline Island, Micronesia; Terrestrial, epilithic, Nan Madol, Pohnpei, Micronesia; Terrestrial, epipelagic, Tamil Nadu, India	Stratmann <i>et al.</i> 1994, Smith <i>et al.</i> 1995, Zhang and Smith 1996, Jimenez <i>et al.</i> 1999

Chemical structures	Bioactivities	Cyanobacteria	Places of isolation	References
<i>Anhydrohapaloxindoles</i>				
Oxidised indole alkaloids; chlorinated or non-chlorinated, isonitrile or isothiocyanate functionalities		<i>H. fontinalis</i> (ATCC 39694)	Soil, Marshall Islands	Moore <i>et al.</i> 1987b, 1989
<i>Fontonamide, Hapalonamides</i>				
Oxidised indole alkaloids; chlorinated or non-chlorinated		<i>H. fontinalis</i> (ATCC 39694)	Soil, Marshall Islands	Moore <i>et al.</i> 1987b, 1989
<i>Tijpanazoles</i>				
Indolocarbazoles	Antifungal	<i>Tolypothrix tijpanasensis</i> (UH strain DB-1-1); <i>F. ambigua</i> (EAWAG strain 108b)	Soil, Vero Beach, Florida, USA; Shallow hollow, Mellingen, Switzerland	Bonjouklian <i>et al.</i> 1991, Falch <i>et al.</i> 1993
<i>Fischerellins</i>				
Pyrrolidines	Inhibition of photosynthetic organisms; Fischerellin A: inhibition of photosystem II, antifungal	<i>F. muscicola</i> (UTEX 1829); <i>F. ambigua</i> (UTEX 1903); <i>F. tisserantii</i> (Treboñ);	Freshwater; terrestrial; Terrestrial; Tree bark of <i>Rhizophora mangle</i> , Las Villas, Cuba	Gross <i>et al.</i> 1991, Hagmann and Jüttner 1996, Papke <i>et al.</i> 1997, Srivastava <i>et al.</i> 1998 and others
<i>Parsiguiine</i>				
Not determined	Antibacterial and antifungal activity	<i>F. ambigua</i> (PTCC 1635)	Paddy-field, Iran	Ghasemi <i>et al.</i> 2004
<i>Ambigols</i>				
Highly chlorinated aromatic compounds	Cyclooxygenase and HIV reverse transcriptase inhibition; antibacterial, antifungal, molluscicidal, cytotoxic, antitrypanosomal	<i>F. ambigua</i> (EAWAG strain 108b)	Shallow hollow, Mellingen, Switzerland	Falch <i>et al.</i> 1993, Wright <i>et al.</i> 2005

as antifungal agents (Bonjouklian *et al.* 1991).

Though the listed bioactivities are rather diverse, no insecticidal activity has yet been shown. This is surprising, as from an ecological point of view, defence against grazing insects might be one of the most important requirements for benthic primary producers, such as cyanobacteria. In a previous publication (Becher and Jüttner 2005), we described the separation of four compounds from *Fischerella* sp. (ATCC 43239) by HPLC. Characteristic UV-absorption spectra and heavily clustered quasi-molecular ions of the electrospray ionisation mass spectra were indicative of compounds also belonging to the group of indole derivatives. Extensive sampling and purification of the compounds is now required, to obtain adequate amounts for structural determinations. Knowledge of the chemical structure is essential to link the insecticidal activity of *Fischerella* sp. (ATCC 43239) to the causative agents. The chemical characterisation of the insecticidal compounds would also allow clarification of their distribution in *Fischerella* and other genera of the order Stigonematales and further evaluation of their ecological significance as chemicals of defence particularly directed against grazing insects.

ABBREVIATIONS AND SUPPORTING INFORMATION: ATCC: American Type Culture Collection, Rockville, USA; SAG: Sammlung von Algenkulturen, Göttingen, Germany; UTEX: Culture collection, University of Texas, Austin, USA; Třeboň: Culture collection, Czech Academy of Sciences, Třeboň, Czech Republic; ASW: Algenkultursammlung Wien, Austria; JU 5 (isolated from Lake Türlensee, Switzerland); biofilms designated as Taiwan (Jüttner and Wu 2000) were collected by F. Jüttner. *Fischerella* sp. (ATCC 43239) was originally isolated from a sample taken in the Philippines (personal communication by C.P. Wolk (Michigan State University, East Lansing, USA)) and Milagrosa Martinez-Goss (University of the Philippines, Los Baños). The culture of *Nostoc* sp. (ATCC 43238) is identical to *Anabaena* 78-12A (renamed *Nostoc* 78-12A), and was obtained from C.P. Wolk. Detailed information on abbreviated culture collections mentioned in the review part of this paper can be

found in the literature cited. Information about culture conditions of cyanobacteria used in our toxicity assays and about culturing of *C. riparius* are available (Becher and Jüttner 2005).

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4. REFERENCES

- Ali A. 1990 – Seasonal changes of larval food and feeding of *Chironomus crassicaudatus* (Diptera: Chironomidae) in a subtropical lake – J. Am. Mosq. Control. Assoc. 6: 84–88.
- Armitage P., Cranston P.S., Pinder L.C.V. 1995 – The *Chironomidae*. The biology and ecology of non-biting midges – Chapman and Hall, London.
- Becher P.G., Jüttner F. 2005 – Insecticidal compounds of the biofilm-forming cyanobacterium *Fischerella* sp. (ATCC 43239) – Environ. Toxicol. 20: 363–372.
- Benke A.C. 1998 – Production dynamics of riverine chironomids: Extremely high biomass turnover rates of primary consumers – Ecology, 79: 899–910.
- Bonjouklian R., Moore R.E., Patterson G.M.L. 1988 – Acid-catalyzed reactions of hapalindoles – J. Org. Chem. 53: 5866–5870.
- Bonjouklian R., Smitka T.A., Doolin L.E., Molloy R.M., Debono M., Shaffer S.A., Moore R.E., Stewart J.B., Patterson G.M.L. 1991 – Tjipanazoles, new antifungal agents from the blue-green alga *Tolypothrix tjipanasensis* – Tetrahedron, 47: 7739–7750.
- Brock E.M. 1960 – Mutualism between the midge *Cricotopus* and the alga *Nostoc* – Ecology, 41: 474–483.
- Brook A.J. 1954 – The bottom-living algal flora of slow sand filter beds of waterworks – Hydrobiologia, 6: 333–351.
- Carletti I., Banaigs B., Amade P. 2000 – Matemone, a new bioactive bromine-containing oxindole alkaloid from the Indian Ocean sponge *Lotrochota purpurea* – J. Nat. Prod. 63: 981–983.
- Doan N.T., Rickards R.W., Rothschild J.M., Smith G.D. 2000 – Allelopathic actions of the alkaloid 12-epi-hapalindole E isonitrile and calothrixin A from cyanobacteria of the genera *Fischerella* and *Calothrix* – J. Appl. Phycol. 12: 409–416.

- Doan N.T., Stewart P.R., Smith G.D. 2001 – Inhibition of bacterial RNA polymerase by the cyanobacterial metabolites 12-*epi*-hapalindole E isonitrile and calothrixin A – FEMS Microbiol. Lett. 196: 135–139.
- Etchegaray A., Rabello E., Dieckmann R., Moon D.H., Fiore M.F., von Döhren H., Tsai S.M., Neilan B.A. 2004 – Algicide production by the filamentous cyanobacterium *Fischerella* sp. CENA 19 – J. Appl. Phycol. 16: 237–243.
- Falch B.S., König G.M., Wright A.D., Sticher O., Rügger H., Bernardinelli G. 1993 – Ambigol A and B: New biologically active polychlorinated aromatic compounds from the terrestrial blue-green alga *Fischerella ambigua* – J. Org. Chem. 58: 6570–6575.
- Frouz J., Ali A., Lobinske R.J. 2004 – Laboratory evaluation of six algal species for larval nutritional suitability of the pestiferous midge *Glyptotendipes paripes* (Diptera: Chironomidae) – J. Econom. Entomol. 97: 1884–1890.
- Ghasemi Y., Yazdi M.T., Shafiee A., Amini M., Shokravi S., Zarrini G. 2004 – Parsiguine, a novel antimicrobial substance from *Fischerella ambigua* – Pharma. Biol. 42: 318–322.
- Gross E.M., Wolk C.P., Jüttner F. 1991 – Fischerellin, a new allelochemical from the freshwater cyanobacterium *Fischerella muscicola* – J. Phycol. 27: 686–692.
- Hagmann L., Jüttner F. 1996 – Fischerellin A, a novel photosystem-II-inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity – Tetrahedron Lett. 37: 6539–6542.
- Harrison S.S.C., Hildrew A.G. 1998 – Patterns in the epilithic community of a lake littoral – Freshwat. Biol. 39: 477–492.
- Hart D.D. 1985 – Grazing insects mediate algal interactions in a stream benthic community – Oikos, 44: 40–46.
- Hill W.R., Knight A.W. 1987 – Experimental analysis of the grazing interaction between a mayfly and stream algae – Ecology, 68: 1955–1965.
- Huber U., Moore R.E., Patterson G.M.L. 1998 – Isolation of a nitrile-containing indole alkaloid from the terrestrial blue-green alga *Hapalosiphon delicatulus* – J. Nat. Prod. 61: 1304–1306.
- Jimenez J.I., Huber U., Moore R.E., Patterson G.M.L. 1999 – Oxidized welwitindolinones from terrestrial *Fischerella* spp. – J. Nat. Prod. 62: 569–572.
- Johnson R.K., Boström B., van de Bund W. 1989 – Interactions between *Chironomus plumosus* (L.) and the microbial community in surficial sediments of a shallow eutrophic lake – Limnol. Oceanogr. 34: 992–1003.
- Jüttner F., Wu J.-T. 2000 – Evidence of allelochemical activity in subtropical cyanobacterial biofilms of Taiwan – Arch. Hydrobiol. 147: 505–517.
- Klein D., Daloze D., Braekman J.C., Hoffmann L., Demoulin V. 1995 – New hapalindoles from the cyanophyte *Hapalosiphon laingii* – J. Nat. Prod. 58: 1781–1785.
- Lamberti G.A., Resh V.H. 1983 – Stream periphyton and insect herbivores: An experimental study of grazing by a caddisfly population – Ecology, 64: 1124–1135.
- Lampert W. 1987 – Laboratory studies on zooplankton-cyanobacteria interactions – N.Z.J. Mar. Freshwat. Res. 21: 483–490.
- Lin L.-Z., Cordell G.A., Ni C.-Z., Clardy J. 1990 – Two oxindole alkaloids from *Gelsemium elegans* – Phytochemistry, 29: 3013–3017.
- Moore R.E., Cheuk C., Patterson G.M.L. 1984 – Hapalindoles: new alkaloids from the blue-green alga *Hapalosiphon fontinalis* – J. Am. Chem. Soc. 106: 6456–6457.
- Moore R.E., Cheuk C., Yang X.-Q.G., Patterson G.M.L., Bonjouklian R., Smitka T.A., Mynderse J.S., Foster R.S., Jones N.D., Swartzendruber J.K., Deeter J.B. 1987a – Hapalindoles, antibacterial and antimycotic alkaloids from the cyanophyte *Hapalosiphon fontinalis* – J. Org. Chem. 52: 1036–1043.
- Moore R.E., Yang X.-Q.G., Patterson G.M.L. 1987b – Fontonamide and anhydrohapaloxindole A, two new alkaloids from the blue-green alga *Hapalosiphon fontinalis* – J. Org. Chem. 52: 3773–3777.
- Moore R.E., Yang X.-Q.G., Patterson G.M.L., Bonjouklian R., Smitka T.A. 1989 – Hapalonamides and other oxidized hapalindoles from *Hapalosiphon fontinalis* – Phytochemistry, 28: 1565–1567.
- Papke U., Gross E.M., Francke W. 1997 – Isolation, identification and determination of the absolute configuration of fischerellin B. A new algicide from the freshwater cyanobacterium *Fischerella muscicola* (Thuret) – Tetrahedron Lett. 38: 379–382.
- Park A., Moore R.E., Patterson G.M.L. 1992 – Fischerindole L, a new isonitrile from the terrestrial blue-green alga *Fischerella muscicola* – Tetrahedron Lett. 33: 3257–3260.
- Peterson C.G., Vormittag K.A., Valett H.M. 1998 – Ingestion and digestion of epilithic algae by larval insects in a heavily grazed montane stream – Freshwat. Biol. 40: 607–623.

- Porter K.G. 1973 – Selective grazing and differential digestion of algae by zooplankton – *Nature*, 244: 179–180.
- Schwartz R.E., Hirsch C.F., Springer J.P., Pettibone D.J., Zink D.L. 1987 – Unusual cyclopropane-containing hapalindolinones from a cultured cyanobacterium – *J. Org. Chem.* 52: 3704–3706.
- Smith C.D., Zilfou J.T., Stratmann K., Patterson G.M.L., Moore R.E. 1995 – Welwitindolinone analogues that reverse P-glycoprotein-mediated multiple drug resistance – *Mol. Pharmacol.* 47: 241–247.
- Smitka T.A., Bonjouklian R., Doolin L., Jones N.D., Deeter J.B., Yoshida W.Y., Prinsep M.R., Moore R.E., Patterson G.M.L. 1992 – Ambiguine isonitriles, fungicidal hapalindole-type alkaloids from three genera of blue-green algae belonging to the Stigonemataceae – *J. Org. Chem.* 57: 857–861.
- Srivastava A., Jüttner F., Strasser R.J. 1998 – Action of the allelochemical, fischerellin A, on photosystem II - *Biochim. Biophys. Acta*, 1364: 326–336.
- Stratmann K., Moore R.E., Bonjouklian R., Deeter J.B., Patterson G.M.L., Shaffer S., Smith C.D., Smitka T.A. 1994 – Welwitindolinones, unusual alkaloids from the blue-green algae *Hapalosiphon welwitschii* and *Westiella intricata*. Relationship to fischerindoles and hapalindoles – *J. Am. Chem. Soc.* 116: 9935–9942.
- Takamura N., Iwakuma T., Aizaki M., Yasuno M. 1990 – Primary production of epiphytic algae and phytoplankton in the littoral zone of Lake Kasumigaura Japan – *Mar. Microb. Food Webs*, 4: 239–255.
- Weber C.I. 1993 – Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027F. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency; Cincinnati, Ohio, (<http://www.epa.gov/waterscience/wet/atx.pdf>).
- Wright A.D., Papendorf O., König G.M. 2005 – Ambigol C and 2,4-dichlorobenzoic acid, natural products produced by the terrestrial cyanobacterium *Fischerella ambigua* – *J. Nat. Prod.* 68: 459–461.
- Yamaki H., Sitachitta N., Sano T., Kaya K. 2005 – Two new chymotrypsin inhibitors isolated from the cyanobacterium *Microcystis aeruginosa* NIES-88 – *J. Nat. Prod.* 68: 14–18.
- Zhang X., Smith C.D. 1996 – Microtubule effects of welwistatin, a cyanobacterial indolinone that circumvents multiple drug resistance – *Mol. Pharmacol.* 49: 288–294.

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