European Journal of Plant Pathology **101:** 585–599, 1995.

Mini review

The potential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi

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Received 13 September 1994; accepted 22 March 1995

Key words: damping-off fungi, foliar and soilborne plant pathogens, antibiotics, anti-bacterial compounds, anti-fungal compounds, seaweeds

Abstract

Cyanobacteria (blue-green algae) and eukaryote algae occur in freshwater, marine, and terrestrial (soil) habitats. In fact, these microorganisms comprise most of the world's biomass. Although the cyanobacteria are mostly photoautotrophic, some are facultative heterotrophs, capable of growing on certain substrates in darkness. Also, some are non-phototrophic and hence, are obligate heterotrophs. A number of cyanobacteria and eukaryote algae, particularly macroalgae, produce various, biologically active compounds. These include antibiotics which in laboratory tests inhibited bacteria and fungi that incite diseases of humans. In addition, the following fungi which are of interest to plant pathologists, were inhibited *in vitro* by substances produced by various cyanobacteria: The saprophytes *Chaetomium globosum, Cunninghamella blakesleeana*, and *Aspergillus oryzae*, and the plant pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Extracts from seaweeds (macroalgae) sprayed on plants have been reported to reduce the incidence of *Botrytis cinerea* (gray mold) on strawberries, *Erysiphe polygoni* (powdery mildew) on turnips, and damping-off of tomato seedlings. Because many cyanobacteria and algae produce a large number of antibacterial and antifungal materials, are almost never a threat to the environment, and many can be grown in quantity in mass culture, they are suitable candidates for exploitation as biocontrol agents of plant pathogenic bacteria and fungi. Much additional work remains to be done however, to thoroughly evaluate cyanobacteria and algae and their products for this role.

Introduction

Biological control

Biological control of plant pathogens in a broad sense encompasses the utilization of methods which involve the use of organisms other than man [Campbell, 1989]. Although this strategy has existed for a long time, a marked increase in research in this area has occurred recently. One principal impetus for this enhanced activity are constraints on the use of chemical pesticides. These include environmental concerns, high purchase costs, and ever-increasing government restrictions and regulations [Maloy, 1993].

Fungi and bacteria are the chief biological agents that have been studied for the control of plant pathogens, particularly soilborne fungi. In addition, viruses, amoebae, nematodes, and arthropods have been mentioned as possible biocontrol agents [Whipps and McQuilken, 1993]. Although the cyanobacteria (blue-green algae), which 'constitute the largest, most diverse, and most widely distributed group of photosynthetic prokaryotes' [Stanier and Cohen-Bazire, 1977], together with the eukaryote algae, 'make up most of the world's biomass', [Cannell, 1993], they have received little attention as potential biocontrol agents of plant diseases.

Cyanobacteria

Approximately 150 genera representing more than 1,000 species of cyanobacteria have been described

[Rippka *et al.*, 1979]. Originally placed with the algae because of their ability to carry out photosynthesis, they are now recognized as belonging to the subclass of Gram-negative prokaryotes [Stanier and Cohen-Bazire, 1977]. Cyanobacteria are probably best known for the production of toxins by certain species that live in fresh- and saltwater [Lawton *et al.*, 1991]. These toxins are associated with the mass growth ('algal blooms') of these microorganisms and affect fish, birds, and mammals. One cyanobacterium, *Lyngbya majuscula* Gomont, was reported to cause a severe dermatitis in humans who came into contact with it while swimming off Oahu, Hawaii [Grauer, 1959; Banner, 1959]. The active principle was isolated and named lyngbyatoxin A by Cardellina *et al.* [1979]. Other toxins produced by cyanophytes include anatoxins A, B, C, and D from *Anabaena flos-aquae* (Lyng.) Bréb., saxitoxin and related compounds from *Aphanizomenon flos-aquae* (L.) Ralfs, toxic peptides from *Microcystis aeruginosa* Kütz. em. Elenkin, and debromoaplysiatoxin from *Lyngbya gracilis* Rabenh. [Moore, 1977].

Certain cyanobacteria can sometimes cause problems on golf courses and other sports turf areas. These include the production of an unsightly, scum-like growth on the grass surface, abundant mucilaginous 'slime' that may cause players to slip, and the possible interference with water percolation through the turf due to the formation of a so-called 'black layer' [Baldwin and Whitton, 1992].

A substantial number of cyanobacteria are terrestrial. Species of cyanobacteria (Cyanophyceae) representing 38 genera have been reported to be soil inhabitants [Metting, 1981]. The cyanobacteria 'are often the dominant microalgae in soils' [Zimmerman, 1992]. Many cyanobacteria carry out photosynthesis and can grow only in the presence of light (obligate photoautotrophs) but some species can grow on certain substrates in darkness (facultative heterotrophs). Khoja and Whitton [1971] tested 24 species of cyanobacteria (representing 12 genera) in a basal inorganic, liquid medium containing 0.01 M sucrose. The majority of these species were capable of growing in the dark, albeit at a lower rate compared to growth in the light. Rippka *et al.* [1979] grew 178 strains of cyanobacteria (representing 22 genera) on a mineral culture medium supplemented with either fructose, glucose, or sucrose. Seventy-eight of these strains demonstrated unequivocal growth on at least one of these three carbohydrates, and were thus classified as being facultative photoheterotrophs. It is now recognized that some cyanobacteria can synthesize photosynthetic pigments and exhibit active photosystems during heterotrophic growth in the absence of light [Stanier and Cohen-Bazire, 1977].

Some species of cyanobacteria can fix nitrogen and thus add significant amounts of this element to soils in temperate and tropical regions [Metting, 1981]. Research has been carried on for some time in China and India on the use of nitrogen-fixing cyanobacteria, particularly in rice paddies [Li, 1988; Venkataraman, 1986]. Methods for stimulating the growth of cyanobacteria in agricultural soils include proper fertilization and irrigation regimes, and adding live cyanobacteria and their metabolites to soil ('algalization') [Shtina, 1992]. In addition, many cyanobacteria produce a large variety of secondary metabolites, particular antibiotics and biotoxins [Carmichael, 1992].

Algae

The total number of identified species of algae has been estimated at approximately 40,000 [Raven *et al.*, 1992] and, together with the cyanobacteria, comprise most of the world's biomass [Cannell, 1993]. The majority of eukaryote algae are placed in 18 taxonomic classes [Wainwright *et al.*, 1993]. Although most algae are found in freshwater or marine habitats, species belonging to 147 genera have been reported from soil [Metting, 1981]. These organisms are mainly photosynthetic but a number are facultatively heterotrophic and some nonphotosynthetic species are obligately heterotrophic [Kaplan *et al.*, 1990; Metting, 1981; Parker, 1961; Parker *et al.*, 1961]. The algae are a rich and largely untapped source of a vast assortment of biologically active products [Metting and Pyne, 1986; Cannell, 1993; Radmer and Parker, 1994].

The purpose of this mini-review is to acquaint plant pathologists with the cyanobacteria and the eukaryote algae in the hope that it will encourage them to evaluate these microorganisms and their products for the control of plant pathogenic bacteria and fungi.

Antibiotic activity of cyanobacteria

(NB. In this section, unless otherwise noted, all cyanophytes listed are terrestrial, all bioassays were carried out on agar, and all bacteria are human pathogens. Also, the taxa mentioned in this and the following section, and their products (when known), plus the bacteria and fungi against which they were effective, are summarized in Table 1).

Table 1. Summary of *in vitro* antibiotic activity reported for cyanobacteria and algae towards various bacteria and fungi

A. Bacteria

Taxon	Active compound	Reference
	Bacillus cereus (S)[a]	
Lyngbya majuscula (C)[b]	NI[c]-MA[d]	Moikeha and Chu, 1971
	Bacillus pumilus (S)	
Chlorococcum humicolum (A)[e]	NI	Pande and Gupta, 1977
	Bacillus subtilis	
Acrosiphonia coalita (AM)[f]	Oxylipins	Bernart *et al.*, 1993
Asparagopsis taxiformis (AM)	NI	Ballantine *et al.*, 1987
Chlorococcum humicolum	NI	Pande and Gupta, 1977
Gloiosiphonia verticillaris (AM)	Gloiosiphones A and B	Chen *et al.*, 1993
Gracilaria verrucosa (AM)	NI	Ballantine *et al.*, 1987
Hormothamnion enteromorphoides (C)	Hormothamnin A	Gerwick *et al.*, 1989
Lyngbya majuscula	NI	Welch, 1962
	Bacillus typhosus (HP)[g]	
Lyngbya majuscula	NI	Gupta and Shrivastava, 1965
	Escherichia coli (HP)	
Chlorococcum humicolum	NI	Pande and Gupta, 1977
Gloiosiphonia verticillaris	Gloiosiphones A and B	Chen *et al.*, 1993
Gracilaria bursa-pastoris (A)	NI	Ballantine *et al.*, 1987
Gracilaria corticata (AM)	NI	Sastry and Rao, 1994
Hormothamnion enteromorphoides	Hormothamnin A	Gerwick *et al.*, 1989
Hypnea cervicornis (AM)	NI	Ballantine *et al.*, 1987
Mastigocoleus testarum (C)	NI	Kobbia and Zaki, 1976
Padina tetrastromatica (AM)	NI	Sastry and Rao, 1994
Sargassum wightii (AM)	NI	Sastry and Rao, 1994
	Gaffkya tetragena (HP)	
Lyngbya majuscula	NI-SA[h]	Moikeha and Chu, 1971
	Mycobacterium balnei (HP)	
Lyngbya majuscula	NI	Moikeha and Chu, 1971
	Mycobacterium phlei (HP)	
Lyngbya majuscula	NI	Moikeha and Chu, 1971
Mastigocoleus testarum	NI	Kobbia and Zaki, 1976
	Mycobacterium smegmatis (HP)	
Lyngbya majuscula	NI	Moikeha and Chu, 1976
L. majuscula	Malyngolide	Cardellina *et al.*, 1979
	Proteus vulgaris (S)	
Gracilaria corticata	NI	Sastry and Rao, 1994
Padina tetrastromatica	NI	Sastry and Rao, 1994
Sargassum wightii	NI	Sastry and Rao, 1994

Table 1. Continued

A. Bacteria

Taxon	Active compound	Reference
	Pseudomonas aeruginosa (HP)	
Gracilaria corticata	NI	Sastry and Rao, 1994
Hormothamnion enteromorphoides	Hormothamnion A	Gerwick *et al.*, 1989
Micropeuce mucronata (AM)	NI	Ballantine *et al.*, 1987
Padina tetrastromatica	NI	Sastry and Rao, 1994
Sargassum wightii	NI	Sastry and Rao, 1994
Spyridia filamentosa (AM)	NI	Ballantine *et al.*, 1987
Valonia ventricosa (AM)	NI	Ballantine *et al.*, 1987
	Pseudomonas fluorescens (S)	
Egregia menziesii (AM)	NI	Rosell and Srivastava, 1987
	Salmonella paratyphi A (HP)	
Gracilaria corticata	NI	Sastry and Rao, 1994
Padina tetrastromatica	NI	Sastry and Rao, 1994
Sargassum wightii	NI	Sastry and Rao, 1994
	Salmonella typhi (HP)	
Gracilaria corticata	NI	Sastry and Rao, 1994
Padina tetrastromatica	NI	Sastry and Rao, 1994
	Salmonella typhimurium (HP)	
Gloiosiphonia verticillaris	Gloiosiphones A and B	Chen *et al.*, 1993
Gracilaria corticata	NI	Sastry and Rao, 1994
Hormothamnion enteromorphoides	Hormothamnin A	Gerwick *et al.*, 1989
Padina tetrastromatica	NI	Sastry and Rao, 1994
	Salmonella sp.	
Brachytrichia balani (C)	NI	Kobbia and Zaki, 1976
Mastigocoleus testarum	NI	Kobbia and Zaki, 1976
	Sarcina lutea (S)	
Chlorococcum humicolum	NI	Pande and Gupta, 1977
Desmarestia ligulata (AM)	NI-MA	Rosell and Srivastava, 1987
Eisenia arborea (AM)	NI-MA	Rosell and Srivastava, 1987
Laminaria saccharina (AM)	NI-MA	Rosell and Srivastava, 1987
Lyngbya majuscula	NI-SA	Moikeha and Chu, 1971
Macrocystis integrifolia (AM)	NI-MA	Rosell and Srivastava, 1987
Pleurophycus gardneri (AM)	NI-MA	Rosell and Srivastava, 1987
	Staphylococcus aureus (HP)	
Acrosiphonia coalita	Oxylipins	Bernart *et al.*, 1993
Asparagopsis taxiformis	NI	Ballantine *et al.*, 1987
Brachytrichia balani	NI	Kobbia and Zaki, 1976
Caulerpa vanbosseae (AM)	Caulerpenyne	Schwartz *et al.*, 1990
Chlorococcum humicolum	NI	Pande and Gupta, 1977
Gloiosiphonia verticillaris	Gloiosiphones A and B	Chen *et al.*, 1993

Table 1. Continued

A. Bacteria

Taxon	Active compound	Reference
Gracilaria corticata	NI	Sastry and Rao, 1994
Halimeda opuntia (AM)	NI	Ballantine *et al.*, 1987
Hormothamnion enteromorphoides	Hormothamnin A	Gerwick *et al.*, 1989
Lyngbya majuscula	NI	Welch, 1962
Mastigocladus laminosus (C)	NI	Fish and Codd, 1994
Nostoc muscorum (C)	NI	de Cano *et al.*, 1990
Pandina tetrastromatica	NI	Sastry and Rao, 1994
Phormidium sp. (C)	NI	Fish and Codd, 1994
Sargassum wightii	NI	Sastry and Rao, 1994
	Streptococcus pyrogenes (HP)	
Lyngbya majuscula	Malyngolide	Cardellina *et al.*, 1979

B. Fungi

Taxon	Active compound	Reference
	Aspergillus flavus (PP)[i]	
Tolyphothrix tjipanensis (C)	NI	Bonjouklian *et al.*, 1991
	Aspergillus oryzae (S)	
Anabaena laxa (C)	Laxaphycins	Frankmölle *et al.*, 1992a, b
Calothrix fusca (C)	Calophycin	Moon *et al.*, 1992
Chlorella vulgaris (A)	NI	Matusiak and Krzywicka, 1975
Fisscherella ambigua (C)	Ambiguines	Smitka *et al.*, 1992
Hapalosiphon hibernicus (C)	Ambiguines	Smitka *et al.*, 1992
Westiellopsis prolifica (C)	Ambiguines	Smitka *et al.*, 1992
	Candida albicans (HP)	
Acrosiphonia coalita	Oxylipins	Bernart *et al.*, 1993
Anabaena laxa	Laxaphycins	Frankmölle *et al.*, 1992a, b
Asparagopsis taxiformis	NI	Ballantine *et al.*, 1987
Calothrix fusca	Calophycin	Moon *et al.*, 1992
Fischerella ambigua	Ambiguines	Smitka *et al.*, 1992
Haplosiphon hibernicus	Ambiguines	Smitka *et al.*, 1992
Hormothamnion enteromorphoides	Hormothamnin A	Gerwick *et al.*, 1989
Lyngbya majuscula	NI	Welch, 1962
Mastigocladus laminosus	NI	Fish and Codd, 1994
Nostoc muscorum	NI	de Cano *et al.*, 1990
Phormidium sp.	NI	Fish and Codd, 1994
Tolypothrix tjipanensis	NI	Bonjouklian *et al.*, 1991
Westiellopsis prolifica	Ambiguines	Smitka *et al.*, 1992
	Chaetomium globosum (S)	
Anabaena variabilis (C)	NI	Kellam *et al.*, 1988
	Cryptococcus neoformans (HP)	
Chlorococcum humicolum	NI	Pande and Gupta, 1977
Lyngbya majuscula	NI	Welch, 1962

Table 1. Continued

B. Fungi

Taxon	Active compound	Reference
	Cunninghamella blakesleeana (S)	
Nostoc muscorum	NI	de Mulé *et al.*, 1977
	Penicillium notatum (S)	
Anabaena laxa	Laxaphycins	Frankmölle *et al.*, 1992a, b
Calothrix fusca	Calophycin	Moon *et al.*, 1992
Fischerella ambigua	Ambiguines	Smitka *et al.*, 1992
Haplosiphon hibernicus	Ambiguines	Smitka *et al.*, 1992
Westiellopsis prolifica	Ambiguines	Smitka *et al.*, 1992
	Penicillum sp.	
Lyngbya majuscula	NI	Welch, 1962
	Rhizoctonia solani (PP)	
Nostoc muscorum	NI	de Caire *et al.*, 1990
	Saccharomyces cerevisiae (S)	
Anabaena laxa	Laxaphycins	Frankmölle *et al.*, 1992a, b
Calothrix fusca	Calophycin	Moon *et al.*, 1992
Chlorella vulgaris	NI	Matusiak and Krzywicka, 1975
Fischerella ambigua	Ambiguines	Smitka *et al.*, 1992
Gomphosphaeria aponina (C)	NI	Moon and Martin, 1981
Haplosiphon hibernicus	Ambiguines	Smitka *et al.*, 1992
Westiellopsis prolifica	Ambiguines	Smitka *et al.*, 1992
	Sclerotinia sclerotiorum (PP)	
Nostoc muscorum	NI	de Caire *et al.*, 1987
	Trichophyton mentagrophytes (HP)	
Anabaena laxa	Laxaphycins	Frankmölle *et al.*, 1992a, b
Calothrix fusca	Calophycin	Moon *et al.*, 1992
Fischerella ambigua	Ambiguines	Smitka *et al.*, 1992
Haplosiphon hibernicus	Ambiguines	Smitka *et al.*, 1992
Hormothamnion enteromorphoides	Hormothamnin A	Gerwick *et al.*, 1989
Tolypothrix tjipanensis	NI	Bonjouklian *et al.*, 1991
Westiellopsis prolifica	Ambiguines	Smitka *et al.*, 1992
	Trichophyton cutaneum (HP)	
Chlorella vulgaris	NI	Matusiak and Krzywicka, 1975

[a] S = Saprophyte
[b] C = Cyanobacterium
[c] NI = Not Identified
[d] MA = Moderate Activity
[e] A = Alga
[f] AM = Alga-Marine
[g] HP = Human Pathogen
[h] SA = Slight Activity
[i] PP = Plant Pathogen

Since 1981, a team of workers at the University of Hawaii, along with colleagues at other institutions in certain instances, have sought to discover new compounds of pharmaceutical interest from cyanobacteria. To this end, they have evaluated for biological activity, hydrophilic and lipophilic extracts from more than 1,500 strains that represented approximately 400 species of cyanobacteria from terrestrial, freshwater, and marine habitats [Patterson *et al.*, 1994]. Antifungal and antiviral activity was observed in a large number of these extracts. The following reports are from the University of Hawaii: Ishibashi *et al.* [1986] isolated from *Scytonema pseudohofmanni* Bharadwaja, several compounds that showed antimycotic activity towards unspecified fungi. These compounds were identified as scytophycins C, D, and E and their structures were elucidated. Eighteen new indole alkaloids with antibacterial and antifungal activity, hapalindoles C-Q and T-V, were isolated from *Hapalosiphon fontinalis* (Ag.) Bornet by Moore *et al.* [1987] and their structures were determined. In a later study, Moore *et al.* [1989] reported the isolation from *H. fontinalis* of six additional compounds, which were identified as minor indole alkaloids and structurally characterized. More than 700 clonal isolates of cyanobacteria from various terrestrial, freshwater, and marine habitats were mass-cultured by Stewart *et al* [1988]. Extracts from about nine percent of these cultures exhibited antifungal activity against one or more of the following test fungi: *Aspergillus oryzae* (Alhb.) Cohn (saprophyte), *Candida albicans* (Robin) Berkh. (human pathogen), *Penicillium notatum* Westling (saprophyte), *Saccharomyces cerevisiae* Meyen (brewing yeast), and *Trichophyton mentagrophytes* (Robin) Blanch. (human pathogen). Patterson *et al.* [1991] obtained extracts from approximately 1,000 strains of cyanobacteria from various habitats and screened them for possible antineoplastic activity. The following cytotoxic antibiotics, produced by 22 strains from seven genera and unique to cyanobacteria, were discovered: acutiphycins, indolecarbazoles, mirabilene isonitriles, paracyclophanes, scytophycins, tantazoles, tolytoxin, toyocamycin, and tubercidin. Bonjouklian *et al.* [1991] found that a lipophilic extract from *Tolypothrix tjipanasensis* De Wild had moderate fungicidal activity against *Candida albicans, Trichophyton mentagrophytes*, and *Aspergillus flavus* Link: Fr. (a producer of mycotoxins). The structures of 15 fungicidal alkaloids from the extract were determined, and were given the name tijpanazoles. Frankmölle *et al.* [1992a] reported that crude ethanolic extracts from *Anabaena laxa* Rabenh. inhibited the growth of the following fungi: *Aspergillus oryzae, Candida albicans, Penicillium notatum, Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*. The fungicidal compounds were isolated and purified and given the name laxaphycins A, B, C, D, and E, and their structures were determined by Frankmölle *et al.* [1992b]. Moon *et al.* [1992] isolated and determined the structure and fungicidal activity of a compound named calophycin, produced by *Calothrix fusca* (Kutzing) Bornet & Flahault. This compound was effective against *Aspergillus oryzae, Candida albicans, Penicillium notatum, Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*. Smitka *et al.* [1992] isolated from *Fischerella ambigua* (Nageli) Gomont, *Hapalosiphon hibernicus* W. & G. S. West, and *Westiellopsis prolifica* Janet, six hapalindole-type alkaloids with fungicidal properties. These compounds were identified as ambiguine isonitriles A-F and their structures were determined. They inhibited the growth of the five test fungi used by Moon *et al.* [1992]. Patterson *et al.* [1993] obtained hydrophilic and lipophilic extracts from about 600 strains (300 species) of cyanobacteria and screened them for antiviral activity. Extracts from approximately 60 strains caused a significant decrease in the cytopathic effect usually associated with viral infection. Reports from laboratories other than at the University of Hawaii (with three exceptions) of cyanobacteria that exhibited antibiotic activity include one by Welch [1962] on *Lyngbya majuscula*, a marine cyanophyte. Filaments of this taxon when ground up and placed on a filter paper disk inhibited the growth of the following fungi: *Candida albicans, Cryptococcus neoformans* (Sanfelice) Vuillemin (human pathogen), and *Penicillium* sp. It also was found to inhibit the growth of the following bacteria: *Staphylococcus aureus* Rosenbach, *Bacillus subtilis* Cohn *emend.* Prazmowski, and *Bacillus typhosus* Zopf [Gupta and Shrivastava, 1965]. The active principle of *Lyngbya majuscula* was found to markedly inhibit the growth of *Mycobacterium balnei* Linell & Nordén, *M. phlei* Lehmann & Neumann, and *M. smegmatis* (Trevisan) Chester, but only slightly or moderately, *Bacillus cereus* Frankland & Frankland (saprophyte), *Gaffkya tetragena* (Gaffky) Trevisan, and *Sarcina lutea* (saprophyte) Schroeter (Moikeha and Chu, University of Hawaii, 1971). *Lyngbya majuscula* was reported to produce an antibiotic, malyngolide, that was effective against *Mycobacterium smegmatis* and *Streptococcus pyogenes* Rosenbach but less effective against *Staphylococcus aureus* and *Bacillus subtilis* (Cardellina *et al.*,

University of Hawaii, 1979). Gerwick of Oregon State University and colleagues have isolated and identified antibiotic compounds from a number of marine cyanophytes. One of these was a novel compound from *Lyngbya majuscula* which they named curacin A [Gerwick *et al.*, 1994]. This compound exhibited antimitotic and antiproliferative properties and was extremely toxic to brine shrimp. Ramamurthy [1970] obtained indirect evidence that *Trichodesmium erythraeum* Ehrenb. (a marine cyanophyte) produced an antibiotic that was effective against Gram-positive and negative bacteria. Kobbia and Zaki [1976] found that the filtrate from *Brachytrichia balani* (Lloyd) Born. & Flah. inhibited the growth of *Staphylococcus aureus* and *Salmonella* sp., and that from *Mastigocoleus testarum* Lagerheim greatly inhibited *Escherichia coli* (Migula) Castellani & Chalmers, *Salmonella* sp., and *Mycobacterium phlei*. Moon and Martin (University of Hawaii, 1981) reported that a crude extract of *Gomphosphaeria aponina* Kützing (a marine cyanophyte) inhibited the yeast *Saccharomyces cerevisiae*. An extract from *Anabaena variabilis* Kütz, a freshwater cyanobacterium, severely inhibited the growth of the cellulolytic fungus *Chaetomium globosum* Kunze ex Fries [Kellam *et al.*, 1988]. Gerwick *et al.* [1989] isolated a complex mixture of toxic and antimicrobial peptides from the marine cyanophte *Hormothamnion enteromorphoides* Grunow. The major peptide, named hormothamnin A, was found to be active against *Bacillus subtillis, Escherichia coli, Pseudomonas aeruginosa* (Schroeter) Migula, *Salmonella typhimurium* (Loeffler) Castellani & Chalmers, and *Staphylococcus aureus*. In addition, this compound was active against *Candida albicans* and *Trichophyton mentagrophytes*. Gerwick *et al.* [1992] elucidated the total structure of hormothamnin A, which was determined to be a cyclic undecapeptide. Schwartz *et al.* [1990] evaluated cyanobacteria isolated from soil for antifungal properties. This was part of a program including macro- and microalgae, the goal of which was to discover new pharmaceuticals. The culture filtrate from one unnamed cyanobacterium proved active against unnamed filamentous fungi and particularly active against a *Cryptococcus* species. They isolated and characterized the structure of the active compound, which they named cryptophycin. de Cano *et al.* [1990] found that phenolic compounds in extracts from cells of *N. muscorum* significantly inhibited the growth of *Candida albicans* and *Staphylococcus aureus*. The culture filtrate from *Mastigocladus laminosus* Cohn and from a species of *Phormidium*, both from hot-springs, inhibited the growth of *Staphylococcus aureus* and *Candida albicans* [Fish and Codd, 1994].

Perhaps the earliest published report of activity of a cyanobacterium of interest to plant pathologists was made by de Caire *et al.* [1976] of the Centro de Investigación de Biología Marina (CIBIMA) in Argentina, using a culture of *Nostoc muscorum* Ag. isolated from a rice (*Oryza sativa* L.) paddy in Argentina. They studied the effect of dilute, cell-free extracts from cells of this nitrogen-fixing cyanobacterium on the growth of millet seedlings (*Panicum miliaceum* L.) in pots of soil. They noted that this extract checked an outbreak of damping-off in their millet seedlings. However, the identity of the pathogenic fungus that incited the damping-off was not mentioned. In a subsequent study, de Mulé *et al.* [1977] of CIBIMA found that culture extracts of *N. muscorum* inhibited the mycelial development of the saprophyte *Cunninghamella blakesleeana* Lendner in liquid cutlure. In a third study, de Caire *et al.* [1987] found that the growth of the plant pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary was inhibited by aqueous or ethereal extracts from cells of *N. muscorum* or by extracellular products of this cyanobacterium. In a continuation of their work with *N. muscorum*, de Caire *et al.* [1990] evaluated extracellular products and extracts from cells of this cyanobacterium on the growth of an anastomosis group 4 isolate of the plant pathogen *Rhizoctonia solani* Kühn on potato dextrose agar in Petri dishes. Colony diameters were measured three days after seeding the agar with *R. solani* and highly significant results were obtained. Average growth of *R. solani* on the agar which contained extracellular products was inhibited 77% compared to the control. The cell extract inhibited growth by 14%.

Antibiotic activity of algae

(N.B. In the following section, unless otherwise noted, all bioassays were carried out on agar and all bacteria are human pathogens.)

Most of the studies aimed at discovering biologically active compounds in algae have centered on the marine algae (macroalgae), commonly called seaweeds. These plants have long been used in agriculture and horticulture to feed livestock, for soil fertilization and conditioning and, more recently, in the form of extracts to promote plant growth [Verkleij, 1992]. However, it is only in recent times that seaweed extracts have been screened for compounds that could be of use in the

pharmaceutical industry. An early study reported that extracts from several species inhibited the growth of one or more of the following: *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* [Pratt *et al.*, 1951]. Burkholder *et al.* [1960] reported that 66 out of 131 identified species showed some degree of inhibitory activity towards one or more of the following: *Staphylococcus aureus, Escherichia coli*, and *Mycobacterium smegmatis*, and to *Candida albicans*. In a similar study, Welch [1962] tested homogenized preparations of 35 marine algae for activity against six fungi. Four of the preparations severely inhibited the growth of one or more of the following taxa: *Rhizopus oryzae* Went & Geerligs (plant pathogen), *Mucor racemosus* Fres. (saprophyte), *Aspergillus niger* van Tiegh. (saprophyte), *Penicillium* sp., *Candida albicans*, and *Cryptococcus neoforman.* Khaleafa *et al.* [1975] reported that ethereal extracts from three out of 18 marine algae had a pronounced inhibitory effect on unidentified 'leather moulds'. Accorinti [1983] listed 35 marine and 10 freshwater algae that produced substances that inhibited the growth of *Candida albicans* and several other fungi. Acetone extracts from nine marine algae were assayed against nine species of bacteria by Rosell and Srivastava [1987]. All extracts were inhibitory to at least four of the test microorganisms. The highest level of inhibition was observed in filtrates from *Desmarestia ligulata* (Lightf.) Lamour., *Eisenia arborea* Aresch., *Laminaria saccharina* (L.) Lamour., and *Macrocystis integrifolia* Bory towards *Sarcina lutea*, and from *Egregia menziesii* (Turn.) Aresch. towards *Pseudomonas fluorescens* Migula. Lipid-soluble extracts from 102 species of Caribbean marine algae were assayed for antibiotic activity by Ballantine *et al.* [1987]. Inhibition zones ranged in size from 0.5 to 6.0 mm. Sixty-five (64%) of the extracts demonstrated at least minimal activity against one or more of four bacteria and one fungus. Sixty-one percent of the extracts only showed activity against *Bacillus subtilus* (Bs) and/or *Staphylococcus aureus* (Sa). Only nineteen percent were active against *Candida albicans* (Ca) and 15% against *Pseudomonas aeruginosa* (Pa) and/or *Escherichia coli* (Ec). Extracts from the following algae produced inhibition zones of 3.0 mm or greater against one or more of the assay microorganisms: *Asparagopsis taxiformis* (Del.) Trev. (Bs, Sa, Ca), *Gracilaria bursa-pastoris* (Gmel.) Silva (Ec), *Gracilaria verrucosa* (Huds.) Papenf. (Bs), *Halimeda opuntia* (L.) Lamour. (Sa), *Hypnea cervicornis* J. Ag. (Ec.), *Micropeuce mucronata* (Harv.) Kylin (Pa), *Spyridia filamentosa* (Wulf.) Harv. in Hook. (Pa), and *Valonia ventricosa* J. Ag. (Pa). Ballantine *et al* [1987] mention that a number of species in their study showed temporal variability in their microbial activity. This included inactivity at one sampling time, activity against one set of bioassay microorganisms at a second sampling, and activity against a different set when sampled for the third time. They cite the following factors that may influence the microbial activity of algal extracts: reproductive state, sampling locale, and seasonality. Culture filtrates and organic solvent extracts of 132 marine and 400 freshwater algal cultures were evaluated by Kellam *et al.* [1988] for inhibitory properties against six fungi. Their results indicated that marine algae are a more promising source of antifungal agents than freshwater algae. An extract from the marine green alga *Caulerpa vanbosseae* Setch. et Gard. was found by Schwartz *et al.* [1990] to exhibit 'good activity against a strain of methicillin-resistant *Staphyloccus aureus*'. The active compound was isolated and identified as caulerpenyne. Extracts of four marine red algae were reported by Tariq [1991] to inhibit the lateral growth of colonies of the human pathogenic fungi *Microsporum canis* Bodin and *Trichophyton verrucosum* Bodin. Bernart *et al.* [1993] isolated a novel fatty acid derivative from the marine alga *Acrosiphonia coalita* (Rupr.) Scagel, Garbary, Holden, & Hawkes that inhibited the growth of *Bacillus subtilus, Candida albicans*, and *Staphylococcus aureus*. Chen *et al.* [1993] determined the structure of two antibacterial compounds, gloiosiphones A and B, isolated from the marine alga *Gloiosiphonia verticillaris* (Farlow) Smith. Crude extracts of these two compounds were active against *Bacillus subtilis, Escherichia coli, Salmonella typhimurium*, and *Staphylococcus aureus*. Ballesteros *et al.* [1992] tested extracts from 71 species of marine macrophtes and found that 70% of them demonstrated antifungal activity, 21% antiviral activity, and 6% antibacterial activity. The highest level of antibiotic activity came from extracts from members of the Chlorophyta. Gerwick *et al.* [1994] over a three-year period evaluated more than 500 extracts from marine micro- and macroalgae for anticancer activity. Most of the active compounds (67%) were obtained from the latter. Chloroform extracts from the marine algae *Gracilaria corticata* J. Ag. and *Padina tetrastromatica* Hauck inhibited the growth of *Escherichia coli, Proteus vulgaris* Hauser (saprophyte), *Pseudomonas aeruginosa, Salmonella paratyphi* A (Kayser) Castellani & Chalmers, *S. typhi* Warren & Scott, *S. typhimurium* (Loeffler) Castellani & Chalmers, and *Staphylococcus aureus. Sargassum wightii* (Grev.) J. Ag.

inhibited all of the above except *S. typhi* and *S. typhimurium* (Sastry and Rao, 1994].

Six-hundred and sixty-nine species of marine algae are described and illustrated in the book by Abbott and Hollenberg [1976], *Marine Algae of California*. Descriptions of a large number of marine algae and 563 figures are contained in the book by Schneider and Searles [1991], *Seaweeds of the Southeastern United States*.

The terrestrial chlorophyte *Chlorococcum humicolum* (Naeg) Rabenh. produced a broad spectrum antibiotic which was quite inhibitory to *Crypotococcus neoformans, Bacillus subtilis, Escherichia coli*, and *Staphylococcus aureus*, and to the saprophytic bacteria *Bacillus pumilus* Gottheil and *Sarcina lutea* [Pande and Gupta, 1977]. Matusiak and Krzywicka [1975] reported that an extract from *Chlorella vulgaris* Beij., a terrestrial chlorophyte, inhibited the growth of *Aspergillus oryzae* (43%), *Saccharomyces cerevisiae* Hansen var. *ellipsodeus* (Hansen) Deckker (67%), and the human pathogen *Trichosporon cutaneum* (de Beurm., Gougerot & Vaucher) Ota (21%).

Published reports on biologically active products from algae deal mainly with human pathogenic bacteria and fungi and to a small extent with food spoilage microorganisms. Only a few papers concern studies on the effects of algal extracts on plant pathogenic fungi and apparently none on plant pathogenic bacteria. However, unlike the situation in the cyanobacteria, these algal studies were conducted in *vivo*, not *in vitro*. In one part of a study, turnips (*Brassica rapa* L. var. *rapa*) were grown under conditions that favored the development of *Erysiphe polygoni* DC, the incitant of powdery mildew [Stephenson, 1966]. The study was terminated when the pathogen had covered the entire leaf surface of the most affected plant. In plants receiving overhead watering weekly with a solution of a commercial seaweed extract (one part extract in 120 parts water; taxon not specified), only 15% of the total leaf area was covered by the fungus. In the control plants, 85% of the total foliage was affected. In the second part of a study by Stephenson [1966], overhead watering of strawberry plants (*Fragaria* × *ananassa* Duchesne) with seaweed extract reduced the number of fruit affected by gray mold incited by *Botrytis cinerea* Pers. in three experiments to 4.6%, 4.8%, and 1.7% respectively, compared to 22.5%, 15.4%, and 12.9% respectively, in the control plants. The increase in fruit yield in the treated plants over the controls in the three experiments was 14.8%, 31.9%, and 28% respectively. In the third part of the study, Stephenson [1966] evaluated a seaweed extract for its effect on damping-off incited by an unidentified complex of fungi. Pots of nonsterilized loam planted with tomato [*Lycopersicon lycopersicum* (L.) Karsten var. *lycopersicum*] seeds were watered with seaweed extract each week. The average number of tomato seedlings that reached the fourth leaf stage was 90% in the pots treated with seaweed extract compared to 45% in the controls. Lettuce plants (*Lactuca sativa* L.) sprayed three times during the growing season with an extract from the macrophytic alga *Ascophyllum nodosum* (L.) LeJol. exhibited an incidence of less than 12% of an unspecified disease, compared to 18% in unsprayed plants [Abetz and Young, 1983]. The use of foliar sprays consisting of extracts from cyanobacteria or algae could affect the growth of the treated plants. Kugrens [1980] obtained extracts from two chlorophytes, *Hydrodictyon reticulatum* (L.) Lagerh. and *Scenedesmus quadricauda* (Turp.) Bréb., two terrestrial cyanophytes, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*, and the diatom *Nitzschia palea* (Kütz.) W. Smith, by boiling five to 20 grams of each taxon in 80% ethanol for ten minutes. The radish cotyledon method was employed to bioassay these extracts, using changes in fresh weight to indicate the response of the cotyledons to the algal extracts. All five extracts caused statistically significant reductions in cotyledon fresh weights. Kugrens noted that sometimes potent inhibition occurred when the extracts from *M. aeruginosa* and *A. flos-aquae* were mixed together but when taken individually, cotyledon fresh weight actually increased. Promotion of the growth of higher plants by extracts from cyanophytes and algae has been reported by other investigators, for example, Bentley-Mowat and Reid [1968], Gupta and Shukla [1968], Gupta and Gupta [1973], and Russo and Berlyn [1992].

The chemistry and physiology of toxins produced by cyanobacteria and algae are discussed in a review paper by Ikawa and Sasner [1990].

Sources of pure cultures of cyanobacteria and algae

Pure cultures of many species of cyanobacteria and microalgae are maintained in the Culture Collection of the University of Texas at Austin (UTEX). 'All cultures of the Collection are available without any restrictions as to use by all interested individuals and organizations, both academic and commercial' [Starr and Zeikus, 1993]. A catalog is available [Starr and

Zeikus, 1993] which lists the taxa in the collection, provides descriptions of the various media used for maintaining these taxa, and an extensive bibliography. There is a modest charge for cultures. Inquiries should be directed to: Culture Collection of Algae, Department of Botany, The University of Texas at Austin, Austin, Texas 78713-7640. Other culture collections include that of the American Type Culture Collection, Rockville, Maryland; the Center for the Culture of Marine Phytoplankton, West Boothbay Harbor, Maine; the Culture Collection, Botany Department, University of Toronto, Toronto, Ontario, Canada; Culture Collection of Algae and Protozoa, Institute of Terrestrial Ecology, Cambridge, United Kingdom; the Culture Collection of Algae and Microorganisms of the Institute of Applied Microbiology, University of Tokyo; the Pasteur Culture Collection, Paris; Culture Collection of MicroAlgae, Indian Agricultural Research Institute, New Delhi; Laboratory of Hydrobotanics, Botanical Institute, Czech Academy of Sciences, Trebon, Czech Republic; and the Collection of Algal Cultures, University of Göttingen, Germany.

The World Data Center on Microorganisms has compiled the *World Catalog of Algae* which lists most of the algal collections extant [Zeikus, personal communication, 1994]. Its address is: 2-1 Wako, Saitama 351–01, Japan.

Laboratory and mass cultivation of cyanobacteria and algae

The main advantages of growing microalgae (including the cyanobacteria) for the production of chemicals discussed by Cohen (1986) are, in my opinion, applicable to these taxa with regard to the production of antibiotics:

1. 'Algal cultivation is an efficient biological system for the use of solar energy to produce organic matter ...'
2. 'Algae can be grown well in hot desert climates utilizing sea and/or brackish water ...'
3. 'The life cycle of most algae is completed within several hours ...'
4. 'Many species of algae can be induced to produce particularly high concentrations of compounds of commercial interest ...'

Methods for the isolation and purification of cyanobacteria and microalgae are presented by Acreman [1994] and in the Handbook of Phycological Methods [Stein, 1973]. Cyanobacteria and most microalgae lend themselves to laboratory production in quantity in containers ranging in size from small flasks to large carboys. Methods for both small- and large-scale production of cyanobacteria, as well as laboratory methods applicable to these microorganisms, are presented by Kaushik [1987]. Detailed information on the laboratory cultivation of microalgae (including cyanobacteria) is contained in a chapter by Vonshak [1986]. Discussions of microalgal (including cyanobacterial) biotechnology and agricultural applications can be found in articles by Vonshak [1990], Radmer and Parker [1994], and Zimmerman [1992]. Other recent papers dealing with mass cultivation of algae and cyanobacteria include reports by Tredici *et al.* [1991] on the use of a vertical alveolar panel for the outdoor mass cultivation of microalgae and cyanobacteria, the effects of mixing upon productivity [Bosca *et al.*, 1991], and Wang *et al.* [1991] on large-scale, mixed, mass cultivation of nitrogen-fixing cyanobacteria. Detailed information on the engineering aspects of mass cultivation of cyanobacteria and microalgae, on harvesting microalgal biomass, and methods and economics of industrial production, is contained in chapters by Oswald [1988], Mohn [1988], and Borowitzka and Borowitzka [1989], respectively. Factors which can limit the growth of cyanobacteria and algae are discussed in a chapter by Raven [1988]. These limiting factors include the culture temperature, supply of available nutrients, and light quality and quantity.

In addition, the reader is referred to the following volumes which contain a wealth of valuable information on cyanobacteria and algae: *Algal and Cyanobacterial Biotechnology* [Cresswell *et al.*, 1989], *Handbook of Microalgal Mass Culture* [Richmond, 1986], *Introduction to the Algae* [Round, 1984], *Marine Algae in Pharmaceutical Science* [Hoppe *et al.*, 1979], *Marine Biotechnology*, volume 1, *Pharmaceutical and Bioactive Natural Products* [Attaway and Zaborsky, 1993], *Micro-algal Biotechnology* [Borowitzka and Borowitzka, 1988], *The Ecology of Algae* [Round, 1984], and to a paper by Gerwick [1987], *Drugs from the sea-The search continues.*

Conclusions

For a number of reasons, cyanobacteria and algae are suitable candidates for exploitation as biocontrol agents of plant pathogenic bacteria and fungi: Cyanobacteria and algae produce a large number of antibacterial and antifungal products, many can be

grown in quantity in mass culture, and they are not a threat to the environment (except for the production of toxic blooms in freshwater and marine habitats and slimy areas on turf by a relatively small number of cyanobacteria). Nevertheless, there are two important considerations that have to be taken into account: 1. Although it has been reported in many studies that cyanobacteria and algae are capable of producing substances *in vitro* that can inhibit the growth of bacteria and fungi, it still remains to be proven that these substances are produced in nature. Prokaryotes such as the actinomycetes (particularly *Streptomyces* species), are well known for the vast array of metabolites that they produce *in vitro*, and also have been shown to produce compounds in nature that are antagonistic towards certain plant pathogenic fungi [Whipps and McQuilken, 1993]. However, even if it is shown that cyanobacteria and/or algae do not produce antibacterial and antifungal substances *in vivo*, the substances produced by them *in vitro* may prove useful in controlling bacterial and fungal plant pathogens. 2. Since the majority of species of cyanobacteria and algae are obligate photoautotrophs, they will not be able to grow below the surface of the soil in the vicinity of germinating seeds or plant roots. In addition, many cyanobacteria and algae are not terrestrial but are found in freshwater and marine habitats.

Formulations of intact cyanobacteria and microalgae could be applied to the above-ground portions of plants and it is possible that they might offer protection against pathogenic bacteria and fungi. However, it is my opinion that a greater chance of success might be attained with formulations of culture filtrates or cell extracts from cyanobacteria and algae applied to seeds as protectants against damping-off fungi such as *Fusarium spp.*, *Pythium spp.*, and *Rhizoctonia solani*, or sprayed on leaves to protect them from pathogenic bacteria and fungi. Although pelleting of vegetable seeds to incorporate beneficial materials has been practiced for a number of years, it has not been economically feasible to pellet high volume seeds such as corn (*Zea mays* L. subsp. *mays*) and wheat (*Triticum aestivum* L.). However, technology now exists that will permit seed companies to apply thin films (polymers) containing a chemical pesticide to high volume seeds and remain competitive (Burris, 1994). With additional research, it should be possible to develop thin film formulations of bactericidal and fungicidal cyanobacterial and algal products that would confer protection against soilborne pathogens that attack seeds and seedlings. Given the current awareness of the harmful effects of chemical pesticides on the environment, this may provide sufficient reason for substituting cyanobacterial and algal products for conventional pesticides.

In view of the information presented in this minireview, I hope that plant pathologists will be encouraged to begin evaluating cyanobacteria and algae for use in the biological control of plant pathogenic bacteria and fungi. As a first step, cultures of cyanobacteria and algae could be obtained from various collections, grown in liquid culture, and their cell extracts and filtrates evaluated for *in vitro* activity towards bacteria and fungi that incite economically important plant diseases. I suggest that some of the taxa of cyanobacteria and algae listed in Table 1 would be a good starting point for a screening program of this type.

Acknowledgments

I am greatly obliged to Prof. B. Parker, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, for his thorough review of the manuscript and many helpful suggestions for its improvement. In addition, I would like to thank Dr. S. D. O. Ketchum, College of Pharmacy, Oregon State University, Dr. P. Wolk, Plant Research Laboratory, Michigan State University, and Dr. J. A. Zeikus, Department of Botany, University of Texas, for providing me with helpful information on cyanobacteria and algae.

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