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Bacterial induction and inhibition of a fast necrotic response in *Gracilaria conferta* (Rhodophyta)

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Abstract

Of 45 bacterial isolates from healthy tips of *Gracilaria conferta* (Schousboe ex Montagne) J. et G. Feldmann, 29% were identified as 'conditional inducers' of an apical necrosis. That is, the isolates induced necrotic tips in *G. conferta* within 16 h after elimination of most of the resident microflora from the alga. Several disinfectants and antibiotics were screened for their ability to induce algal susceptibility to the bacteria and to suppress uncontrolled appearance of tip necrosis. Treatment with 100 mg L^{-1} Cefotaxim + 100 mg L^{-1} Vancomycin over three days was the least damaging and most efficient. Tip necrosis was related to isolates of the *Corynebacterium-Arthrobacter*-group and to the *Flavobacterium-Cytophaga*-group. The damaging effect occurred due to the bacterial excretion of active agents and was not correlated with a capability to degrade agar. The damaging influence of four *Cytophaga*-like strains was inhibited by 20 of 40 isolates. This protective effect was caused by very different organisms. In five of six cases examined further, the effect was not cellbound, but due to the excretion of agents. These were not antimicrobially active, but inactivated necrosis-inducing excretions. These results indicate that epiphytic bacterial degradation or inactivation of damaging agents is a protecting factor in *Gracilaria*, which prevents the alga from being harmed by epiphytes.

Introduction

Thallus whitening is a problem in aquaculture of species of *Gracilaria*, *Eucheuma* and *Kappaphycus* (Largo et al., 1995). The symptom can be induced by abiotic stress factors (Friedlander & Gunkel, 1990; Mtolera et al., 1995) and, to a lesser extend, by biotic agents (Weinberger et al., 1994; Jaffray & Coyne, 1996). In Israel, necrotic apical epidermis cells were always observed as the first symptom of the disease in *Gracilaria conferta*, and a bacterium was detected which significantly induced this tip necrosis within seven days after addition to algal cultures (Weinberger et al., 1994). These controlled infection experiments were, however, only poorly reproducible, which indicated possible interference of the resident epiphytic microflora of the alga. Removal of interfering microorganisms was therefore regarded as necessary.

Sterilization of seaweeds is a difficult process and reviews have been presented by McCracken (1989) and Aguirre-Lipperheide and Evans (1991). Most sterilization methods damage not only bacteria, but the alga as well. Further, one treatment is usually not sufficient to achieve sterilization, due to different sensitivities of the various microorganisms, especially against antibiotics. The usual practice is to develop a protocol using mechanical and chemical treatments, which together are lethal for all microorganisms, but do not kill the alga. After successful sterilization, the alga must be kept axenic for a relatively long time in order to recover and regenerate thalli. Only few cases are known in which cultures of red seaweeds were sterilized and

successfully kept free of bacteria (Iwasaki, 1965; Fries, 1974). Most of the successful methods had to identify supplements to the medium needed by the alga and presumably provided by microorganisms under natural conditions (Evans & Trewavas, 1991). Taking the given difficulties of sterilization into consideration, we decided to try a different approach, looking for a simple and fast way to eliminate not all, but most bacteria from relatively large quantities of algal material, so that their interfering effect would be negligible in short-time infection experiments.

The purpose of this study was to develop a method for the examination of damaging and protecting bacterial impacts on *G. conferta* and to verify that epiphytic bacteria contribute to the resistance of this species against bacterial damage.

Materials and methods

Bacteria epiphytic on *G. conferta* were isolated and differentiated. Various antibiotics and desinfectants were screened in a series of bioassays for their ability to control the development of necrotic tips and to increase the algal sensitivity against bacterial necrosis inducers. Finally all bacterial isolates were tested in further bioassays for their capability to damage or protect algae that had previously been freed from interfering epiphytes.

Cultivation, isolation and characterization of bacteria

Bacteria were cultivated at 25 °C or 33 °C in darkness, either in liquid medium on a shaker or on nutrient agar. The liquid medium was autoclaved seawater, containing 5 g L^{-1} bacto-peptone (Difco) and 0.5 g L^{-1} yeast extract (Difco). Nutrient agar was prepared by the addition of 15 g L^{-1} agar (Difco).

For the isolation of bacteria, apparently healthy material of *Gracilaria conferta* was collected from different tanks. Algal tips were homogenized and plated out on nutrient-agar, using standard methods. Bacterial cells were isolated from visible colonies after 3–7 days of incubation at 33 °C, and transferred to fresh nutrient agar. Transfer to fresh medium was repeated once or twice per week.

All bacterial isolates underwent the following series of standard tests: gram stain, oxidase, OF test and hydrolysis of gelatin. The capability of strains to degrade agar was indicated by liquification of nutrient agar or sinking of colonies into it. Isolates considered to be 'halophilic' showed no or retarded growth on nutrient agar that had been prepared with distilled water instead of seawater. The sensitivity of strains against bacterial culture supernatants or antibiotics in sterile seawater was examined in the following way: nutrient agar was inoculated with 100 μL of a two-day old liquid culture of the strain in question. Autoclaved and dried discs of lens paper (6 mm $\varnothing$) were soaked with 10 μL of test dilution and put on the agar. Inhibition zones could be detected after 3 days of incubation at 33 °C. All strains were identified tentatively according to Bergey's manual of determinative bacteriology (Holt et al., 1994) and to the scheme of Ezura et al. (1988).

General design of bioassay

All experiments were performed with apparently healthy material of *G. conferta*, previously grown under seminatural conditions in different tanks and ponds (Friedlander & Levy, 1995). It was not possible to always use material from the same pond, since algae in all ponds developed necrotic tips from time to time. Branchlets of *G. conferta* with 5–20 healthy tips (approx. 50 mg fresh weight) were introduced into test tubes (18 mm $\varnothing$) with caps containing 9 mL of autoclaved seawater and antibiotics or bacteria were added. All treatments were carried out under sterile conditions. The test tubes were incubated at 25 °C on a shaker (100 strokes min^{-1}) and exposed to 12.5 h of artificial light (cool white, 40 μmol photon s^{-1} m^{-2}) per day.

'Necrotic' or 'bleached' tips were those that showed a distinct region of unpigmented cells in the apical epidermis when observed from the side with a magnification lens (magnification factor: × 20), using high illumination and a black background. Bleached and non-bleached tips were counted. Approximate relative risks of bleaching compared to control treatments were calculated from odd ratios and tested for significance with the χ^2-test (Fisher & van Belle, 1993).

Testing of chemical control agents

Chemicals used were from Sigma, except for Betadine (= Polyvinylpyrrolidone-iodine, Aldrich), Vancomycin (Teva) and Cefotaxim (Roussel). Antibiotic solutions added to each test tube were 200 μL or less. Disinfectants were diluted in autoclaved seawater. Algal branches (0.8–1.2 g) were incubated for defined time periods in 200 mL of these dilutions. They were

Table 1. Characteristics of 45 bacterial strains isolated from *G. conferta*. + = positive, w = weak positive, - = negative reaction. F/O = Type of carbohydrate degradation (F = fermentative and oxidative, Ow = weak oxidative, - = None).

Group	Strains isolated	Gram reaction	Colony colour	(u)nicellular/ (f)ilaments	(r)ods/ (c)occi	(l)ong, (s)traight, (c)urved (i)rregularly shaped	F/O	Oxidase	Motile	Gelatine degrading	Agar degrading	Halo-philic
Coryn.-Arthr. 1	13 (A-M)	+	white	u/f	r	s/c/i	F	+	+	+	-	+
Coryn.-Arthr. 2	1	+	yellow	u/f	r	i	F	+	+	+	-	+
Coryn.-Arthr. 3	1	+	white	f	r	i	F	+	-	-	-	+
Coryn.-Arthr. 4	1	+	white	f	r	i	F	+	-	+	-	-
Coryn.-Arthr. 5	1	+	white	u	r	s	F	+	+	-	-	+
Coryn.-Arthr. 6	3 (A-C)	+	pink	u/(f)	c/(r)	(i)	Ow	-/w	-/w	-	-	-
Micrococcus 1	4 (A-D)	+	white	tetrads	c	-	Ow/-	-	-	-/w	-	-
Cytophaga 1	5 (A-E)	-	orange	u	r	l, s, gliding	F	+	-	-	+	+
Cytophaga 2	2 (A-B)	-	yellow	u	r	l, s, gliding	Ow	-	-	+	+	-
Flav.-Cyt. 3	1	-	yellow	u	r	l, s	Ow	-	-	-	-	+
Flav.-Cyt. 4	2 (A-B)	-	yellow	u	r	l, s	Ow	+	-	-	-	+
Flav.-Cyt. 5	8 (A-H)	-	orange	u	r/(c)	l, s	Ow	-/w	-	-	-	+
Flav.-Cyt. 6	2 (A-B)	-	yellow	u	r	l, s	Ow	+	-	+	-	+
Vibrio 1	1	-	white	u	r	s	F	+	+	-	+	+

then rinsed three times for 10 s in fresh sterile seawater before placing into test tubes. The extend of necrotic tips formation or other damage was determined after three days of incubation. To estimate the elimination of bacteria by antibiotics, 100 μL old algal medium were plated out on nutrient agar after shaking the test tube for 10 s on a vortex. The agar was examined for colony-forming units after three days of incubation at 25 °C in darkness.

Testing of bacterial impacts

To free algae from antibiotics, the old medium was carefully decanted and 9 mL fresh autoclaved seawater were then added, decanted again after shaking the test tube on a vortex for a few seconds, and finally replaced by 9 mL fresh autoclaved seawater as new medium. Bacterial isolates were precultivated in liquid medium for two days at 25 °C, when their optical density at 490 nm had reached more than 0.8 (measured against sterile medium). Aliquots of the bacteria were diluted in sterile seawater. Their final dilution factor after addition to algal cultures was 1:2138. Sterile medium was used in control experiments instead of bacterial cultures and diluted in the same manner. Four different isolates of the group '*Cytophaga* 1' (see below) were selected for several experiments as the attacking organisms and inoculated together, such that the final dilution factor of each of the four isolates was 1:8554.

Cells and supernatants of bacterial cultures were tested separately in some experiments. For separation, the cultures were centrifuged at 12 000 × g for 1 h. Cells were washed once and resuspended in the same volume of autoclaved seawater after removal of the supernatant. To verify that the supernatant was free of cells, 100 μL supernatant was plated on nutrient agar and incubated for three days at 33 °C. The bleaching impact of bacterial cultures, cells or supernatants was evaluated after overnight incubation of 16 h.

Results

Isolate characterization

Forty-five bacterial isolates grew under the chosen conditions. They were related to four major groups: *Corynebacterium-Arthrobacter (= Coryn.-Arthr.)*, *Flavobacterium-Cytophaga (= Flav.-Cyt.)*, *Micrococcus* sp., and *Vibrio* sp.). These could be differentiated into subgroups (Table 1). Forty percent of the isolates were related to the genus *Coryn.-Arthr.* and belonged to six subgroups. Four isolates were related to the genus *Micrococcus*, forming tetrads of white cocci. One strain was identified as *Vibrio* sp., having the capability to degrade agar. Seven *Cytophaga*-like isolates were also capable of degrading agar and showed a gliding activity. *Cytophaga* 1 was identical in all observed characteristics with a bacterium that was previously

Table 2. Approximate relative risk of tip bleaching during 16 h of exposure of *G. conferta* to *Cytophaga* 1 after 3 days of algal exposure to different combinations of antibiotics. Bold letters mark treatments that resulted in significant susceptibility to bacterial impacts ($\alpha < 0.05$). Van = Vancomycin, Cef = Cefotaxim n_{treat}, $n_{control}$: sample sizes of groups treated and untreated with *Cytophaga* 1.

Pretreatment: 3 days incubation in	Approximate relative risk	98% confidence interval	$n_{control}/n_{treat}$
no antibiotics	1.4	0.7–2.8	99/99
Van 100 mg L^{-1}	2.5	1.4–4.6	103/106
Van 100 mg L^{-1} + Cef 100 mg L^{-1}	5.4	2.8–10.4	78/92

identified to attack *G. conferta* ('Type ORI-1', Weinberger et al., 1994). The remaining 13 isolates were related to the *Flav.-Cyt.*-group and were incapable of degrading agar and not motile.

Elimination of epiphytic bacteria

The occasional 'spontaneous' development of necrotic tips that is known from semi-natural culture conditions was also observed when *G. conferta* was cultivated *in vitro*. During three days of incubation the necrotic tips reached a level of 42.9% ($n = 309$). Three different disinfectants (Betadine, hydrogen peroxide, sodium hypochlorite) were tested at various concentrations and incubation times. None of the treatments resulted in a significant reduction of bleaching, while all agents caused bleaching at high concentrations.

Of 11 antibiotics that were tested over broad concentration ranges, eight significally inhibited the bleaching of *G. conferta* (Figure 1). The strongest inhibitory effect was detected after application of 100 mg L^{-1} Vancomycin in combination with 100 mg L^{-1} Cefotaxim, which reduced the approximate relative risk of 'spontaneous' bleaching from 1 to 0.1. Neomycin and Chloramphenicol had no effect and relatively high concentrations of Erythromycin (>0.1 mg L^{-1}), Rifampicin (>0.1 mg L^{-1}) and Colistin (>1 mg L^{-1}) had promoting effects on bleaching.

The impact of various treatments on the susceptibility of *G. conferta* to bacterial attacks was tested by adding *Cytophaga* 1 (strains A, C, D and E together) to algal cultures. Addition to algae grown for 3 d without antibiotics did not result in significantly increased risk of tip bleaching within 16 h (Table 2). Similarly, no significant bacterial induction of bleaching was detected after a pretreatment with 0.1% Betadine over 5 min and after 3 d of incubation in various concentrations and combinations of Carbenicillin, Doxycyclin, Neomycin, Trimethoprim, Streptomycin, Cefotaxim, Colistin, Erythromycin and Rifampicin (data not shown). Application of 100 mg L^{-1} Vancomycin, in contrast, increased bleaching (Table 2) and combination with 100 mg L^{-1} Cefotaxim led to the clearest result: the approximate relative risk of tip bleaching within 16 h after addition of *Cytophaga* 1 increased from 1 to 5.1.

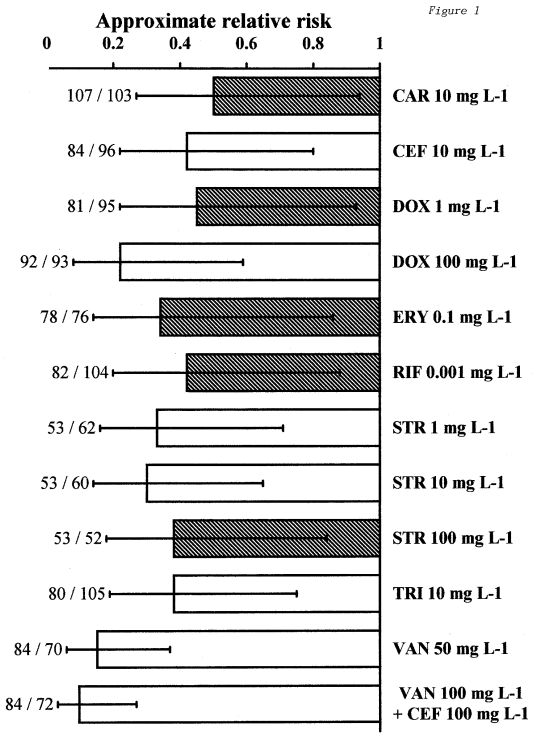

Figure 1. Impact of different antibiotics on bleaching of *G. conferta* during 3 days of incubation. Approximate relative risk ± 95% confidence interval, sample sizes of control and treatment groups are shown respectively. Shaded and white bars indicate $\alpha \leq 0.05$ and 0.01 respectively.

The reduction of 'spontaneous' bleaching and the increased algal susceptibility to bacterial induction of bleaching after addition of 100 mg L^{-1} Vancomycin + 100 mg L^{-1} Cefotaxim were correlated with a reduction in the number of epiphytic bacteria: no colony-forming units were detected in 100 μL medium taken from 28 of 38 test tubes (= 73.7%) in which *G. conferta* was grown with antibiotics for 3 d. The 38 control tubes that lacked antibiotics, always contained bacteria.

Forty percent of necrotic tips developed on algae not treated with 100 mg L^{-1} Vancomycin + 100 mg

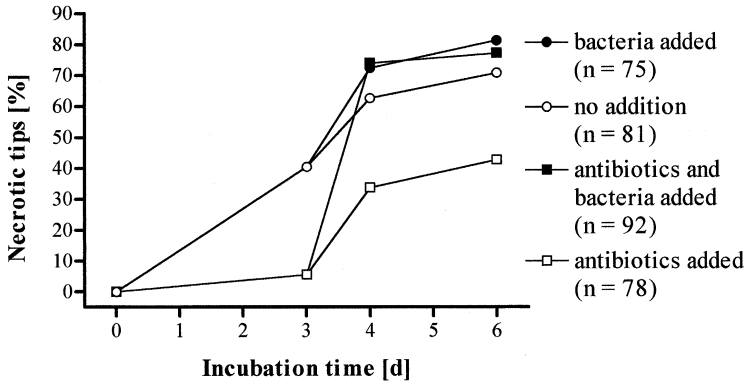

Figure 2. Development of necrotic tips in *G. conferta* during six days, as affected by 3 days of treatment with 100 mg L^{-1} Vancomycin + 100 mg L^{-1} Cefotaxim and addition of *Cytophaga* 1 on day 3.

L^{-1} Cefotaxim within the first 3 d (Figure 2). Tip bleaching continued after medium change (without addition of bacteria) on the third day, so that 63% of all tips were necrotic on day 4. Addition of *Cytophaga* 1 on day 3 resulted in 72% of necrotic tips after 4 days. The differences between *G. conferta* tips grown with and without bacteria were not significant. Application of antibiotics during the first three days, in contrast, resulted in strong inhibition of tip bleaching (Figure 2). This was followed by increased formation of symptoms after the medium had been replaced by sterile seawater on day 3. The increase was much more distinct when *Cytophaga* 1 was added to the culture after the change of the medium, so that 74% of all tips were bleached on day 4, in contrast to only 34% without addition of bacteria. All treatments resulted in further tip bleaching between day 4 and day 6.

Bacterial induction of symptoms

Treatment with 100 mg L^{-1} Vancomycin + 100 mg L^{-1} Cefotaxim was used as a routine in all the following experiments before the algae were exposed to bacterial impact on the third day. Thirteen out of 45 bacterial isolates significantly increased the risk of tip bleaching ($\alpha \leq 0.05$), compared to control treatments without inoculation of bacteria (Figure 3). They all belonged to *Cytophaga* 1, *Flav.-Cyt.* 3 and *Coryn.-Arthr.* 1. Of the first two groups, all isolates tested significantly influenced algal bleaching. Of *Coryn.-Arthr.* 1, 7 strains were inducers and 6 strains were no inducers of necrotic tips. Cell-free supernatants from liquid cultures of all 13 necrosis-inducing strains (Figure 4) also caused significant damage similar to the combination of cells and supernatants.

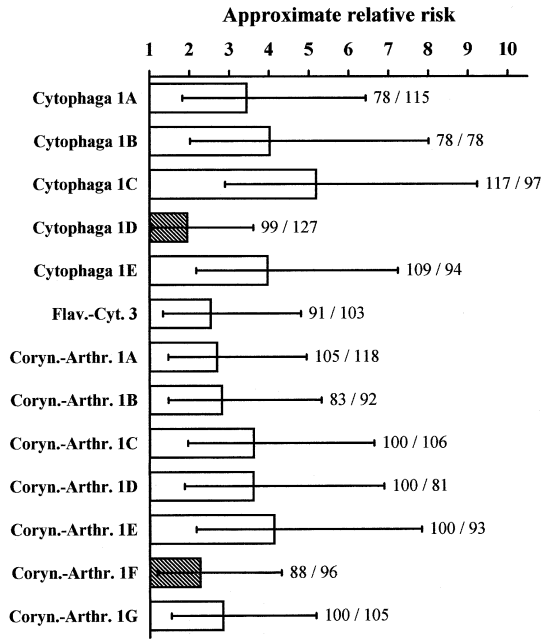

Figure 3. Impact of 13 bacterial isolates on the formation of necrotic tips in *G. conferta* within 16 h, after 3 days of treatment in 100 mg L^{-1} Vancomycin + 100 mg L^{-1} Cefotaxim. Approximate relative risk ± 95% confidence interval, sample sizes of control and treatment groups are shown respectively. Shaded and white bars indicate $\alpha \leq 0.05$ and $\alpha \leq 0.01$ respectively.

Bacterial inhibition of bleaching

Many isolates inhibited the necrosis inducing effect of *Cytophaga* 1 when all bacteria were combined in

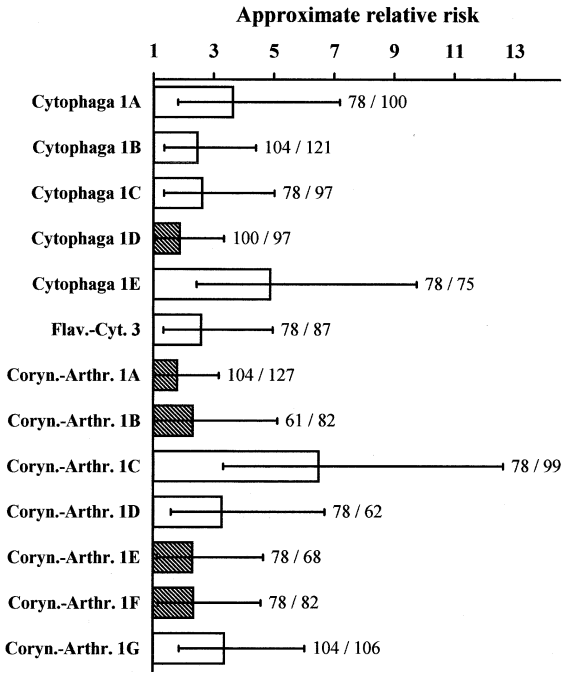

Figure 4. Impact of cell-free culture fluid of 13 bacterial isolates on the formation of necrotic tips in *G. conferta* within 16 h, after 3 days of treatment in 100 mg L^{-1} Vancomycin + 100 mg L^{-1} Cefotaxim. Approximate relative risk $\pm$ 95% confidence interval, sample sizes of control and treatment groups are shown respectively. Shaded and white bars indicate $\alpha \leq 0.05$ and $\alpha \leq 0.01$ respectively.

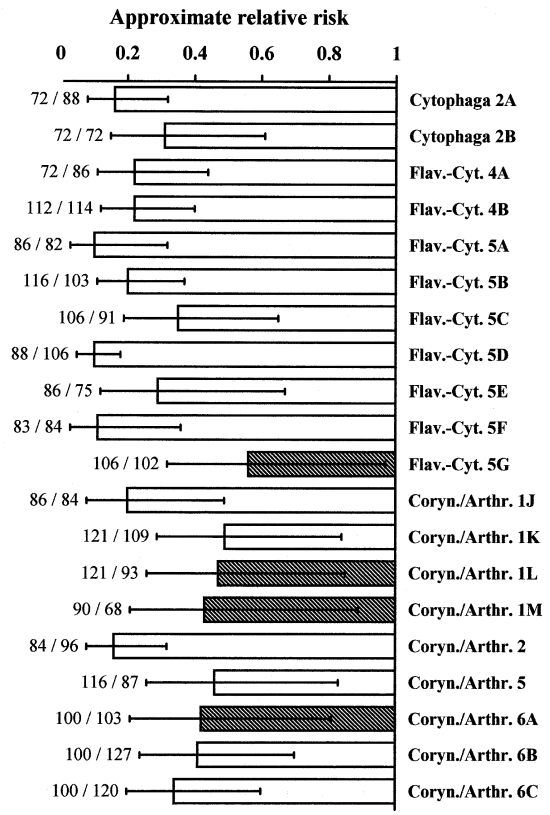

Figure 5. Impact of 20 bacterial isolates on induction of necrotic tips by *Cytophaga* 1 within 16 h, after 3 days of treatment in 100 mg L^{-1} Vancomycin + 100 mg L^{-1} Cefotaxim. Approximate relative risk $\pm$ 95% confidence interval, sample sizes of control and treatment groups are shown respectively. Shaded and white bars indicate $\alpha \leq 0.05$ and $\alpha \leq 0.01$ respectively.

test tubes with *G. conferta* (Figure 5). Of 40 tested strains, 20 had such an antagonistic effect. The groups *Flav.-Cyt.* 6, *Coryn.-Arthr.* 3 and 4, *Micrococcus* 1 and *Vibrio* 1, as well as strains *Coryn.-Arthr.* 5H and 5I, had neither significant inducing nor inhibitory impact on algal bleaching. Some isolates of *Coryn.-Arthr.* 1 damaged, while others protected *G. conferta* branch tips from necrosis.

The supernatant of one isolate, *Coryn.-Arthr.* 1M, did not inhibit the activity of *Cytophaga* 1 (Figure 6), thus only the cells showed an inhibiting effect. Supernatants of five other isolates tested (*Coryn.-Arthr.* 1K and 1L, *Flav.-Cyt.* 5B, 5C and 5D) always showed antagonistic activity, suggesting that inhibiting agents were excreted. The inhibiting supernatants, however, did not show any bactericidal or bacteriostatic effects against *Cytophaga* 1 in agar diffusion tests (Table 3) and inhibition was only detected in control experiments with Vancomycin and Cefotaxim.

Discussion

Antibiotics and disinfectants may have three different effects on the algal component in a system of macroalgae in relation to protecting and damaging epiphytic bacteria. First, they may directly damage the alga (Aguirre-Lipperheide & Evans, 1991; Kooistra et al., 1991; McCracken, 1989). Second, they may protect the alga through elimination or inactivation of potentially damaging bacteria (Demain, 1995; Sande et al., 1990). Third, bactericidal and bacteriostatic agents may indirectly damage the alga by elimination of protecting bacteria. This would allow potentially damaging components to become virulent.

Hydrogen peroxide and sodium hypochloride, which were tested in the present survey, directly damaged *G. conferta*, causing depigmentation and algal death. High concentrations of Rifampicin, Ery-

Table 3. Inhibitory effects of cell-free supernatants of liquid cultures of five strains of epiphytic bacteria and of Cefotaxim and Vancomycin on four bacterial isolates in agar diffusion tests. $n = 5$. The supernatants added were I: *Flav.-Cyt.* 5B, II: *Flav.-Cyt.* 5C, III: *Flav.-Cyt.* 5D, IV: *Coryn.-Arthr.* 1K, V: *Coryn.-Arthr.* 1L, n.i.: no inhibition zones observed.

Substance tested	Diameter of inhibition zones [mm] on agar inoculated with			
	Cytophaga 1A	*Cytophaga* 1C	*Cytophaga* 1D	*Cytophaga* 1E
Supernatants I, II, III, IV, V	n.i.	n.i.	n.i.	n.i.
Vancomycin 1000 mg L^{-1}	34.4 ± 4.8	33.8 ± 7.0	31.2 ± 2.4	30.0 ± 3.8
Cefotaxim 100 mg L^{-1}	27.1 ± 1.8	28.4 ± 1.6	30.1 ± 2.0	28.5 ± 2.6

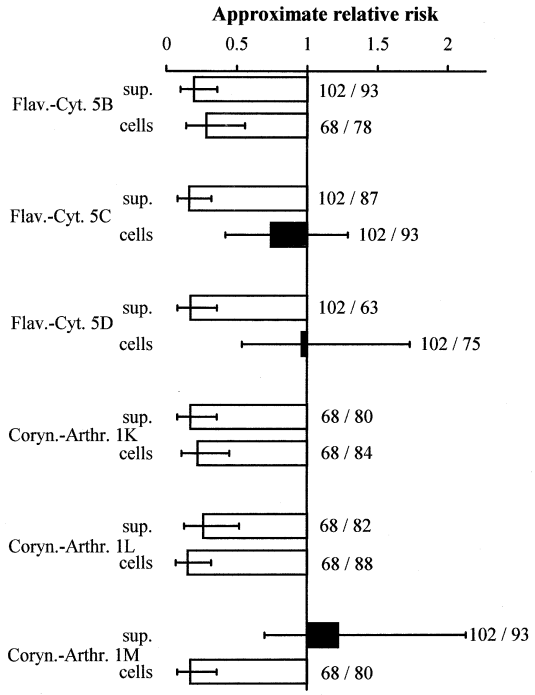

Figure 6. Impact of cells and supernatants of 6 bacterial isolates on induction of necrotic tips by *Cytophaga* 1 within 16 h, after 3 days of treatment with 100 mg L^{-1} Vancomycin + 100 mg L^{-1} Cefotaxim. Approximate relative risk $\pm$ 95% confidence interval, sample sizes of control and treatment groups are shown respectively. Black and white bars indicate $\alpha > 0.05$ and $\alpha \leq 0.01$ respectively.

thromycin, Colistin and Betadine increased tip bleaching and it is difficult to know, whether these sublethal effects were direct or indirect damages.

Several antibiotics reduced tip bleaching and thus protected *G. conferta* to some degree. Vancomycin (50 mg L^{-1}) and combined application of Vancomycin and Cefotaxim (100 mg L^{-1} each) reduced the risk of 'spontaneous' bleaching during three days to less than 0.15. However, significant effects were only detected at specific concentrations. The lack of a correlation between concentration and effect may be due to a combination of 'symptom inducing' and 'symptom preventing' bacteria in the epiphyton of *G. conferta*. Both groups contain a range of species, that may be expected to have different sensitivities against antibiotics (McCracken, 1989; Sande et al., 1990). Varied concentrations may influence the ratio between inducers and inhibitors differently and cause a reduction of bleaching, when inhibiting strains dominate.

Vancomycin (100 mg L^{-1}) in combination with 100 mg L^{-1} Cefotaxim was the only treatment that significantly caused *G. conferta* to be susceptible to bacterial attacks, but could also control tip bleaching. It eliminated all detectable bacteria from the algal medium in 74% of the cases and use of one or both of the agents for up to 14 days did not cause subsequent abnormal development. Both Vancomycin and the beta-lactam Cefotaxim act through blockage of the bacterial cell wall synthesis. This metabolic pathway is only present in prokaryotes and its inhibition is considered to be relatively harmless to eukaryotes (Sande et al., 1990; Kooistra et al., 1991).

Out of 45 bacterial strains isolated by choice from healthy tips of *G. conferta*, 13 were capable of inducing tip bleaching in the same alga. Twenty nine % of all strains could result in thallus whitening and this included taxonomically distinct organisms such as *Flav.-Cyt.* 1 and 3 and *Coryn.-Arthr.* 1A to 1G. Further, necrosis-induction was also possible using the supernatant from all thirteen strains. Both *Cytophaga* 1 and the inducing strains of *Coryn.Arthr.* 1 apparently excrete agents which damage *G. conferta*.

The release of necrotizing agents is an integral part in the pathogenesis of many bacterially induced plant diseases. They may be cell wall degrading enzymes, toxins, or agents that elicit the synthesis of host cell toxic compounds (Šutic & Sinclair, 1991). Jaffray and Coyne (1996) observed that of 10 bacterial isolates that were injected into *Gracilaria gracilis* only agar-

degrading ones induced necrotization. Such a correlation was not observed in the present study. Some vigorous agar degrading strains (*Vibrio* 1, *Cytophaga* 2) did not induce necrotic tips and several necrosis inducing bacteria (*Flav.-Cyt.* 3, *Coryn.-Arthr.* 1) could not degrade agar. The release of cell wall degrading enzymes may nontheless be important for the development of the disease after the algal cuticle and outer cell wall have been penetrated by the microorganism, supporting an increase in agar degrading microorganisms (Lavilla-Pitogo, 1992; Weinberger et al., 1994).

In contrast to enzymes, bacterial toxins and release of autotoxic agents by the alga will cause little changes in the cell wall structure. They typically induce chlorotic lesions of the plant tissue that are limited to a few cells and appearing within 12–24 h (Bailey & O'Connell, 1989, Šutic & Sinclair, 1991). This corresponds with our observations of local symptom formation within 16 h. Some algae are known to be sensitive to bacterial toxins (Berland et al., 1972). Thallus whitening of *Euchuma denticulatum* reportedly results from stress-induced autodestructive release of toxic agents such as hydrogen peroxide and halogenated compounds by the alga (Collén & Pedersén, 1995; Mtolera et al., 1995) and there are indications for similar mechanisms in *Gracilaria* (Pedersén et al., 1995).

The increased algal sensitivity against *Cytophaga* 1 after treatment with antibiotics could be reverted by addition of certain components of the algal epiphytic flora, suggesting that its cause was the elimination of protecting bacterial epiphytes. Of 40 bacterial isolates 50% could inhibit the damaging effect of *Cytophaga* 1 and belonged to a wide spectrum of seven different subgroups of *Flav.-Cyt.* and *Coryn.-Arthr.* Thus, the protective effect may be related to characteristics that are generally widespread among bacteria. Protection was in five out of six cases due to the excretion of protecting agents. Bacterial isolates from seaweeds and marine environments often synthesize antibiotics (Lemos et al., 1985; Fenical, 1993) and the release of such agents by epiphytic and commensalic microorganisms is known to help protect spermatophytes and marine animals from pathogens (Blakeman & Brodie, 1976; Andrews, 1985; Demain, 1995). The inhibiting supernatants, however, did not show any bactericidal or bacteriostatic effects against *Cytophaga* 1 in agar diffusion tests, suggesting that the protective effect must be due to molecular interactions between protecting and necrosis-inducing agents. In conclusion, bacterial epiphytes appear to play a major protecting role for *G. conferta*, similar to spermatophytes (Blakeman & Brodie, 1976; Andrews, 1985) and as has been predicted for seaweeds (Tait, 1991).

Also, the successful establishment of a relatively bacteria-free thallus of *G. conferta* created the conditions to reveal that a major part (73%) of *G. conferta*'s microflora potentially can protect or damage the alga and that the antagonistic effects are from bacterial excretions.

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