

Chemical Deterrence of a Cyanobacterial Metabolite against Generalized and Specialized Grazers

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Received: 16 May 2006 / Revised: 10 October 2006 / Accepted: 16 October 2006 /
Published online: 25 October 2006
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Abstract Pitipectolide A is the major secondary metabolite in a persistent population of the cyanobacterium *Lyngbya majuscula* from Guam. Because this cyanobacterium is of low preference to many sympatric consumers, we tested the hypothesis that this compound acted as a chemical defense. Pitipectolide A was deterrent to urchins, two species of amphipods, and small herbivorous crabs, whereas it did not deter feeding by the sea hare *Stylocheilus striatus*, which readily consumes cyanobacteria. Although our comparison included various small invertebrates, which are often tolerant to algal chemical defenses, this cyanobacterial compound proved deterrent at natural concentrations to all but one of the consumer species tested. Thus, the production of defensive secondary metabolites is likely related to the common occurrence of *L. majuscula* on this coral reef despite the abundance of diverse grazers.

Keywords Chemical defense · Cyanobacteria · Guam · *Lyngbya majuscula* · Mesograzers · Pitipectolide

Introduction

Marine benthic filamentous cyanobacteria have provided natural products chemists with a wealth of novel compounds. This is particularly true for cyanobacteria of the genus *Lyngbya* (Paul et al., 2001). *In vitro* activities of many of these compounds have been studied as part of screening programs in drug discovery, and a number of potent toxins are known. Yet, out of hundreds of compounds isolated and purified, few studies have addressed the ecological roles (Paul et al., 2001; Cruz-Rivera and Paul, 2002).

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Because these benthic cyanobacteria can form extensive blooms that can have detrimental effects on marine life, fisheries and (sometimes) human health, the study of bottom-up *versus* top-down controls of these blooms is important (e.g., Thacker et al., 2001). Herbivory is a strong top-down ecological force controlling the structure and dynamics of marine macroalgae and has favored the evolution of a variety of chemical defenses in seaweeds (Hay and Steinberg, 1992; Paul et al., 2001). Some evidence suggest that chemical defenses could also mediate the persistence of benthic cyanobacteria in areas of high herbivory such as coral reefs (Paul et al., 2001; Cruz-Rivera and Paul, 2002). However, tests of individual compounds against diverse grazers are scarce. This is important because studies testing crude extracts alone (e.g., Cruz-Rivera and Paul, 2002) cannot discern whether the observed deterrence is the result of individual metabolites, or of additive or synergistic interactions among different chemicals. Also, since intraspecific variation in the production of secondary metabolites is well documented for cyanobacteria (Paul et al., 2001), testing individual compounds might provide information on redundancy of roles for diverse compounds endemic to specific populations of the same cyanobacterial species.

In this study, we test a natural role of the major secondary metabolite from a persistent population of the cyanobacterium *Lyngbya majuscula* as a feeding deterrent to sympatric consumers from Guam. We hypothesize that chemical defenses allow mats of this cyanobacterium to persist in an area of high herbivore density and diversity.

Methods and Materials

Lyngbya majuscula was collected from Piti Bomb Holes in Guam, Mariana Islands (13°30' N, 144°45'E). Large mats of the cyanobacterium were collected by snorkeling, brought to the laboratory, freeze-dried, and extracted in 1:1 ethyl acetate/methanol. After extracting $\times 3$, crude extract was concentrated by using rotary evaporation and partitioned between ethyl acetate and water. The concentrated organic phase was separated by Si Bond-Elut column, and pitipeptolide A eluted with hexane/ethyl acetate (1:3). The mixture was dried and further separated by semipreparative normal-phase HPLC (Econosil Silica) by using an isocratic system of hexanes/ethyl acetate (3:7) (Luesch et al., 2001).

Artificial diets were made following the methods described in Hay et al. (1998), with 4 g of freeze-dried, ground *Ulva* (= *Enteromorpha*) *clathrata*, 0.72 g of agar, and 36 ml deionized water per batch of food. *Ulva* spp. (a number of which were formerly classified under the genus *Enteromorpha*) are routinely used in marine chemical ecology for testing the palatability of compounds because of their lack of chemical or structural defenses that might interact with tested metabolites (Hay et al., 1998). The species used here is readily consumed by a variety of grazers from Guam (Cruz-Rivera and Paul, 2002). Treatment foods were made by coating *U. clathrata* with pitipeptolide A at the natural concentration of 0.8% of dry mass (Pangilinan, 2000). This was accomplished by dissolving the compound in enough ethyl ether to thoroughly soak the powdered alga and then removing the solvent with rotary evaporation. Control foods were made with *U. clathrata* treated with ethyl ether alone. Control or treatment *Ulva* were homogeneously mixed with hot agar and poured individually onto plastic screen mesh to produce food strips. Upon cooling, food-covered screen mesh was cut to desired sizes. For small consumers, the foods were lightly pressed onto strips of screen mesh. For urchins, which consume larger amounts, foods were poured onto molds that were pressed against plastic screen (Hay et al., 1998). Consumption was quantified as the number of squares cleared of food by the grazers.

Pitipeptolide A from *Lyngbya majuscula* (Piti)

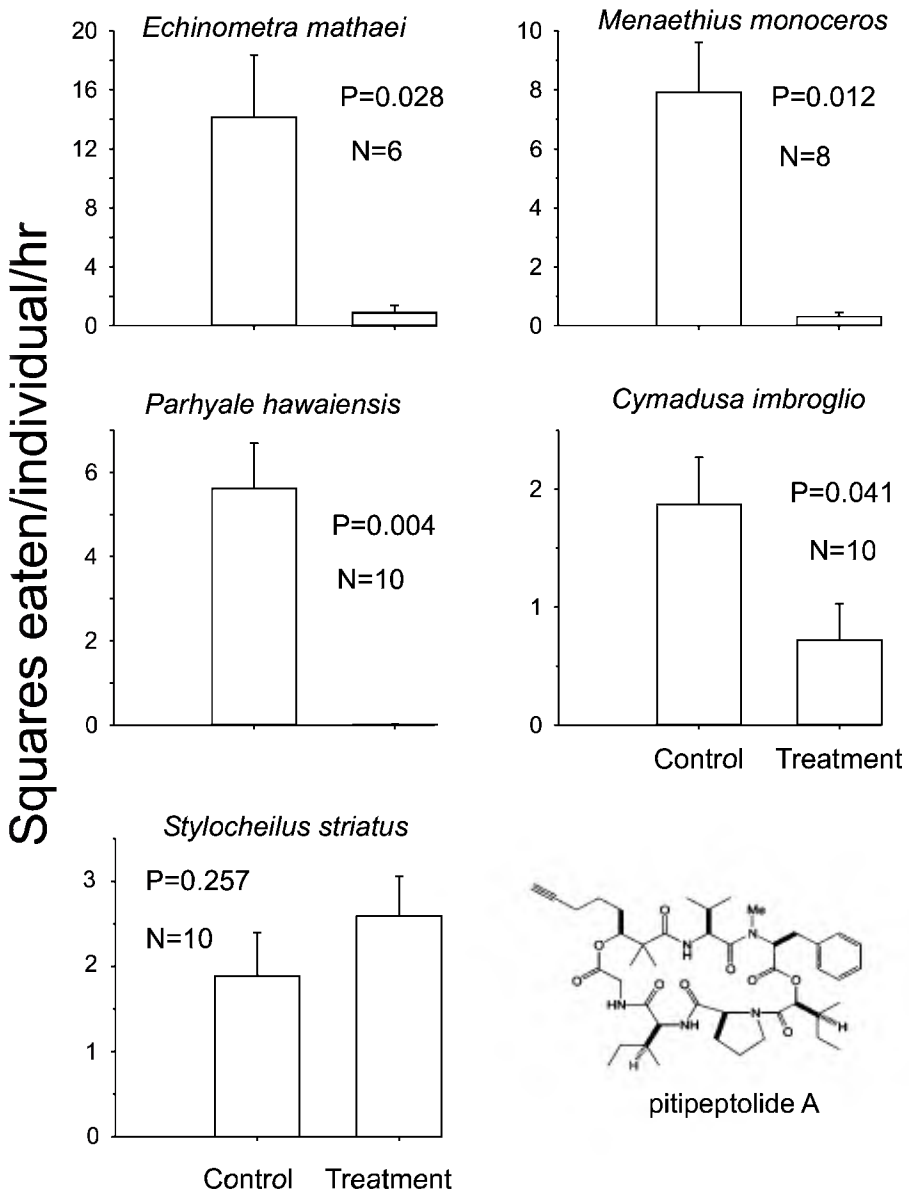


Fig. 1 Consumption of artificial diets containing or lacking pitipeptolide A by five invertebrate consumers from Guam. Bars represent mean (± 1 SE) amount of food eaten by individuals per hour (see [Methods and Materials](#)). Analyses are by paired *t*-tests

Treatment and control foods were simultaneously offered to five invertebrate consumers: the sea urchin *Echinometra mathaei*, the small majid crab *Menaethius monoceros*, the gammaridean amphipods *Parhyale hawaiiensis* and *Cymadusa imbroglia*, and the small sea hare *Stylocheilus striatus* (= *S. longicauda*; see Rudman, 1999). Grazers were obtained from algal and cyanobacterial mats collected by snorkeling at Piti Bomb Holes, Pago Bay, and from flow-through tanks at the University of Guam Marine Laboratory. The tanks receive unfiltered water from the adjacent reef, and organisms continuously recruit from the field.

Experiments with urchins were conducted in flow-through 20-l tanks ($N=10$). For all other smaller consumers, 16 cm (diam) \times 6 cm (height) dishes were used ($N=10$). Replicates contained single individuals of each consumer species except for the amphipods. Because *P. hawaiiensis* would invariably form precopulatory pairs, and mate guarding could influence feeding behavior, we used four males or four females per replicate. For *C. imbroglia*, the smallest of the consumers tested, five individuals were placed in each replicate. Experiments were monitored over 24 hr, and replicates were scored when more than 50% of either treatment or control foods were eaten. Paired *t*-tests were used to analyze data (squares eaten/individual/hr). Replicates in which no food was consumed after 24 hr were eliminated from the analyses as they offered no information of feeding preference.

Results

Pitipeptolide A at natural concentrations deterred feeding in all but one of the invertebrates grazers tested. The urchin *E. mathaei*, the small algal-dwelling crab *M. monoceros*, and the amphipods *P. hawaiiensis* and *C. imbroglia* all consumed significantly more of the control algal food (without pitipeptolide A) than of the treatment food ($P=0.004\text{--}0.41$, Fig. 1). The exception to this pattern was the small sea hare, *S. striatus*, which was not deterred by natural concentrations of the compound ($P=0.257$, Fig. 1).

Discussion

While hundreds of compounds have been isolated from marine benthic cyanobacteria, and *L. majuscula* in particular, only a handful of studies have addressed the ecological roles of cyanobacterial secondary metabolites (reviewed in Paul et al., 2001). In this work we demonstrate a defensive role for the main secondary metabolite produced by a specific *L. majuscula* population from Guam. Pitipeptolide A was deterrent at natural concentrations against urchins as well as three smaller algal/cyanobacterial-dwelling grazers. Previous studies have shown that *L. majuscula* from this population is also a low preference food for sympatric fishes, suggesting that its chemical defenses might act against a broad array of consumers (Cruz-Rivera and Paul, 2002).

The exception to this pattern of deterrence was the small gastropod *S. striatus*. This sea hare has been collected from at least six cyanobacterial genera and will readily consume cyanobacteria (Cruz-Rivera and Paul, 2002, 2006). It will also accumulate compounds from *L. majuscula*, and their role as potential sequestered chemical defenses has been discussed (Paul et al., 2001; Capper et al., 2005). While it could be expected that this grazer would not be deterred by *Lyngbya* extracts or compounds, studies have shown that different *L. majuscula* compounds and their varying concentrations may stimulate or deter feeding by *S. striatus*, which does not appear to be completely adapted to *L. majuscula* secondary

metabolites (Paul et al., 2001; Cruz-Rivera and Paul, 2002). In this study, however, the natural concentration of pitipeptolide A did not deter feeding by this sea hare.

Other consumers tested here are more generalized than *S. striatus* and will eat macroalgae, sometimes as much as cyanobacteria (Cruz-Rivera and Paul, 2002, 2006). Mesograzers such as the amphipods and crabs used here are often better adapted to secondary metabolites of macroalgae. Living on chemically defended hosts can reduce predation from fishes, although closely related mesograzers can vary greatly in their ability to use a chemically defended host (Hay and Steinberg, 1992; Paul et al., 2001). It is possible that similar advantages are experienced by small consumers preferring, or specializing on, chemically defended cyanobacteria as *S. striatus* does (Cruz-Rivera and Paul, 2002, 2006). If so, then tropical benthic cyanobacteria could fill similar niches as chemically defended macroalgae (as food and shelter) for small marine consumers that have adapted to live, and feed, on benthic cyanobacterial mats (Cruz-Rivera and Paul, 2002).

Acknowledgments Funding for this study was provided by an NIH Postdoctoral Supplement (to E. Cruz-Rivera) to NIH grant CA 53001 (J. Horwitz, P.I.). We thank the reviewers whose comments improved this manuscript. This is contribution # 589 of the University of Guam Marine Laboratory and contribution # 670 from the Smithsonian Marine Station at Fort Pierce.

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