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Bacillamide, a novel algicide from the marine bacterium, *Bacillus* sp. SY-1, against the harmful dinoflagellate, *Cochlodinium polykrikoides*

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Abstract—Bacillamide (**1**) was isolated as a new algicide against the harmful dinoflagellate, *Cochlodinium polykrikoides*, from the marine bacterium, *Bacillus* sp. SY-1, and its structure was elucidated by extensive two-dimensional NMR techniques including ^{1}H–^{15}N HMBC analysis and mass analysis. Bacillamide showed algicidal activity against *C. polykrikoides* with LC_{50} of 3.2 μg/ml.

In recent years, harmful algal blooms (HABs) have frequently caused large-scale red tides and mass mortalities of cultured fishes and bivalves in Korean coastal waters. Among the dinoflagellates, *Cochlodinium polykrikoides* is one of the most frequently appearing harmful dinoflagellates responsible for fish kills.[1] Recently, it has been demonstrated that many genera of marine bacteria have algicidal effects and are associated with the termination of algal blooms in natural marine coastal environments.[2,3] The latter findings have raised the possibility of bacterial control of HABs.[4,5] Therefore, algicidal bacteria are considered to be a potentially useful tool to regulate HABs. Considering the bacterial interactions with algal bloom species, in general, indirect attacks are thought to be chemically mediated,[2,6] such as by an extracellular serine protease capable of killing the diatom, *Skeletonema costatum*.[7] However, the identification of algicidal compounds against dinoflagellates has scarcely been accomplished. Here we describe the isolation and structure elucidation of a novel algicide, bacillamide (**1**), produced by a marine bacterium, *Bacillus* sp. SY-1. To our knowledge, this is the first report of an algicide against harmful dinoflagellates such as *C. polykrikoides*.

In the course of our screening program of algicidal bacteria, we isolated a marine bacterium, *Bacillus* sp. SY-1, which had potent algicidal activity against *C. polykrikoides* during the termination of bloom by *C. polykrikoides* in Masan Bay of Korea.[8] An axenic culture of *C. polykrikoides* and *Bacillus* sp. SY-1, and a bioassay method have been described.[9] *Bacillus* sp. SY-1 cells were grown at 25°C with shaking in a 5-L glass flask containing PPES-II medium.[10] After 7 days, the culture broth was centrifuged and then filtrated to obtain a cell-free supernatant, followed by extraction with diethyl ether. The organic layer was concentrated and the residual aqueous suspension was subjected to ODS open column chromatography (YMC-GEL, 5×10 cm) with aqueous MeOH followed by CH_2Cl_2. The 70% MeOH fraction was purified by reversed-phase HPLC (Cosmosil 5C18-MS, 10×250 mm; 60–80% MeOH; flow rate, 2.0 ml min^{-1}; UV detection at 210 nm) to yield bacillamide (**1**, 3.4 mg) as a colorless amorphous powder.

Bacillamide (**1**) showed UV maxima at 279 nm (log ε 3.72) and the positive FAB-MS spectrum of **1** indicated an intense peak at m/z 314. The molecular formula of **1** was established as $C_{16}H_{15}N_3O_2S$ by HRFAB-MS analysis [m/z 314.0969 $(M+H)^+$, (Δ +0.5 mmu)] and ^{13}C NMR spectral data (Table 1). In the ^{1}H NMR spectrum of **1**, the low-field signals peculiar to an indole ring were observed (N(1)H, H-1, 3, 4, 5 and 6). ^{1}H–^{1}H COSY, HMQC and HMBC analyses of **1** easily gave the structure of a tryptamine, and, furthermore, HMBC correlations from H-10 and N(2)H to C-11 revealed that another unit connected to the tryptamine through an amide linkage (Fig. 1). An acetyl unit was assigned

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Table 1. ^{1}H, ^{13}C and ^{15}N NMR data of bacillamide (**1**) in DMSO-d_6

No.	^{1}H	J (Hz)	^{13}C (mult.)	^{15}N	^{15}N HMBC (^{1}H)
(N1)	10.82	(brs)		130.0	H-1, N(1)H
1	7.19	(brs)	122.6 (d)		
2			111.6 (s)		
3	7.61	(d, 7.7)	118.3 (d)		
4	6.97	(t, 7.7)	118.2 (d)		
5	7.06	(t, 7.7)	120.9 (d)		
6	7.33	(d, 7.7)	111.4 (d)		
7			136.2 (s)		
8			127.2 (s)		
9	2.95	(t, 7.5)	25.2 (t)		
10	3.58	(dt, 7.5, 7.0)	39.6 (t)		
(N2)	8.63	(t, 7.0)		115.0	H-9, H-10, N(2)H
11			159.9 (s)		
12			151.5 (s)		
13	8.64	(s)	130.5 (d)		
(N3)				326.7	H-13
14			166.2 (s)		
15			191.4 (s)		
16	2.69	(s)	25.7 (q)		

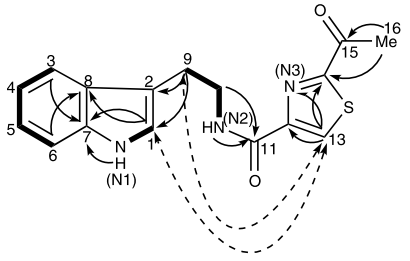

Figure 1. Selected ^{1}H–^{1}H COSY (bold lines) and ^{1}H–^{13}C HMBC (arrows), ^{1}H–^{15}N HMBC (half arrow) and NOESY (dashed arrows) correlations of bacillamide (**1**).

by HMBC cross peak from a singlet methyl signal of H-16 to the ketone carbon signal of C-15. A remaining singlet proton signal of H-13 (δ_H 8.64) showed the HMBC correlations to two unassigned quaternary carbons, C-12 (δ_C 151.5) and C-14 (δ_C 166.2). This fact and the molecular formula of **1** indicated the presence of a thiazole ring, which was also supported by ^{1}H–^{15}N HMBC experiment (Fig. 1 and Table 1). The acetyl unit was connected to C-14 of the thiazole by the HMBC cross peaks from H-16 to C-14. Moreover, the chemical resonances agreed well with those for 2-alkylthiazole-4-carboxamides.[11] Although the correlation from H-13 to C-11 could not be observed by HMBC experiment, NOESY correlations from H-13 to H-1 and H-9 allowed us to assign the connection between the tryptamine unit and the 2-acetylthiazole-4-carboxylic acid unit (Fig. 1). Thus, the gross structure of **1** was elucidated to be 2-acetylthiazole-4-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide.

Bacillamide (**1**) showed algicidal activity against *C. polykrikoides* (LC_{50} after 6 h: 3.2 μg/ml). Furthermore, antialgal, antifungal and antimicrobial activities of **1** were also measured according to the methods previously reported.[9,12] It was found that **1** had significant algicidal activities against a wide range of dinoflagellates and raphidophytes.[13] However, **1** showed neither algicidal activity against microalgae of other phyla such as diatom, green algae, and cyanobacteria,[14] nor growth inhibition against bacteria, fungi and yeast.[15] It is very interesting that **1** possesses strong algicidal activity against dinoflagellates and raphidophytes selectively. Therefore, **1** might become a useful algicidal agent for regulating the blooms of harmful dinoflagellate species such as *C. polykrikoides*. Further studies on the algicidal mechanism of **1** are in progress.

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13. Dinophyceae: *Alexandrium catenella* (LC_{50} after 6 h: 9.4 μg/ml), *Gyrodinium impudicum* (2.3), *Prorocentrum micans* (4.4), *Scrippsiella trochoidea* (50.2); Raphidophyceae: *Chattonella* sp. (3.7), *Heterosigma akashiwo* (1.6).
14. Bacillamide did not show antialgal activity at the concentration of 100 μg/ml against the following species; Baclilariophyceae (*Chaetoceros affinis*, *Skeletonema costatum*), Chlorophyceae (*Chlorella ellipsoidea*, *C. vulgaris*), Cyanophyceae (*Anabaena variabilis*, *Microcystis aeruginosa*).
15. Bacillamide did not show growth inhibition at the concentration of 50 μg/disc against the following species; Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), Fungi (*Mortierella ramanniana*, *Penicillium chrysogenum*), Yeast (*Candida albicans*, *Saccharomyces cerevisiae*).