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REVIEW

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Red to Red – the Marine Bacterium *Hahella chejuensis* and its Product Prodigiosin for Mitigation of Harmful Algal Blooms

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Harmful algal blooms (HABs), commonly called red tides, are caused by some toxic phytoplanktons, and have made massive economic losses as well as marine environmental disturbances. As an effective and environment-friendly strategy to control HAB outbreaks, biological methods using marine bacteria capable of killing the harmful algae or algicidal extracellular compounds from them have been given attention. A new member of the γ-*Proteobacteria*, *Hahella chejuensis* KCTC 2396, was originally isolated from the Korean seashore for its ability to secrete industrially useful polysaccharides, and was characterized to produce a red pigment. This pigment later was identified as an alkaloid compound, prodigiosin. During the past several decades, prodigiosin has been extensively studied for its medical potential as immunosuppressants and antitumor agents, owing to its antibiotic and cytotoxic activities. The lytic activity of this marvelous molecule against *Cochlodinium polykrikoides* cells at very low concentrations (~1 ppb) was serendipitously detected, making *H. chejuensis* a strong candidate among the biological agents for HAB control. This review provides a brief overview of algicidal marine bacteria and their products, and describes in detail the algicidal characteristics, biosynthetic process, and genetic regulation of prodigiosin as a model among the compounds active against red-tide organisms from the biochemical and genetic viewpoints.

Keywords: Algicide, natural pigment, prodiginine, tripyrrole antibiotic, dinoflagellate

Occasionally, some species of toxic phytoplanktons grow rapidly in seawater leading to a phenomenon called the harmful algal bloom (HAB), which has made a disturbance in the ocean ecosystem by massacring fish, shellfish, and other marine life to result in a massive economic loss in fishery. Thus, the massive economic and environmental costs have provoked research on finding effective strategies to decrease the HAB occurrence and to manage it. For example, treatments of algicidal copper sulfate [10, 65], clay flocculation [58], or UV irradiation [1] have been investigated and applied for the removal of harmful algae. However, some showed toxic side effects to other marine organisms, or resulted in an unexpected environmental disturbance. Alternatively, biological control using cells of an algicidal macro- or microorganism or natural algicidal compounds produced from the organism has been proposed as an environment-friendly strategy.

The abundance of certain marine bacteria increased during the decline of several algal blooms [72]. Interestingly, some of them were able to secrete metabolic compounds [14, 26, 66] that can specifically kill harmful phytoplanktons, suggesting that the extracellular algicidal compounds might be used as a biological control agent in natural seawaters. However, despite the importance of the role of marine algicidal bacteria and their algicidal agents in algal bloom control, systematic information on them is still lacking. This review is intended to provide a summary of the natural algicides and their producer marine bacteria, and to describe the biosynthetic process of prodigiosin, as a model compound among the algicides from the biochemical and genetic points of view. Since the prodigiosin producer *Hahella chejuensis* is the first species among the algicidal bacteria of which the full genome sequence (7.2 Mb) has been determined [31], the genome-based research on the prodigiosin production would provide an academic basis for controlling HABs in the natural marine environment.

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Table 1. Algicidal marine bacteria and their products effective against harmful algae.

Bacterial strain	Isolation site	Algicidal compound (target algae)	Reference
Pseudomonas sp. T827/2B	Unknown	Unknown extracellular protein (*Thallasiosira pseudonanna*)	[3]
Flavobacterium sp. 5N-3	Uranouchi Inlet, Japan	Less than 500 Da, basic compound (*Gymnodinium nagasakiense*)	[14]
Cytophaga sp. J18/M01	The Seto Inland Sea, Japan	ND (*Chattonella antiqua*)	[26, 29, 41]
Alteromonas spp.	The Seto Inland Sea, Japan	Extracellular product (*C. antiqua*)	[27]
Vibrio, *Flavobacterium*, *Acinetobacter*, and *Pseudomonas-Alteromonas* spp.	Tanabe Bay, Japan	ND (*Gymnodinium mikimotoi*)	[71]
Flavobacterium sp. 5N-3	Natural seawater	ND (*G. mikimotoi*)	[13]
Unknown	The Seto Inland Sea, Japan	ND (*C. antiqua* and *Heterosigma akashiwo*)	[28]
Pseudoalteromonas sp. Y	Huon Estuary, Australia	Unknown protein (*Gymnodinium catenatum*, *Chattonella marina*, and *H. akashiwo*)	[46]
The class *γ-Proteobacteria* sp.	Hiroshima Bay, Japan	ND (*H. akashiwo*)	[39, 72]
41-DBG2	Seawater in Gulf of Mexico	Unknown compound (*Gymnodinium breve*)	[9]
Flavobacterium sp. 5N-3	Unknown	ND (*Gymnodinium* sp.)	[41]
Cytophaga sp. AA8-2	Ago Bay, Japan	ND (*Heterocapsa circularisquama*)	[41, 51]
Pseudoalteromonas sp. A28	Ariake Sea, Japan	50 kDa extracellular serine protease (*Skeletonema costatum*)	[44]
EHK-1	The Seto Inland Sea, Japan	ND (*H. circularisquama*)	[40]
Cytophaga sp. 41-DBG2	West Florida shelf waters	ND (*Karenia brevis*, formerly *Gymnodinium breve*)	[49]
Pseudoalteromonas, *Zobellia*, *Planomicrobium* spp., *Cellulophaga lytica*, and *Bacillus cereus*	The Huon Estuary, Tasmania, Australia	ND (*Gymnodinium catenatum*)	[61]
Saprospira sp. SS98-5	Kagoshima Bay, Japan	ND (*Chaetoceros ceratosporum*)	[18]
The class *α-* and *γ-Proteobacteria* and the division *Firmicutes* spp.	The Baltic Sea	ND (*Nodularia spumigena*)	[56]
Bacillus sp. SY-1	Masan Bay of Korea	Bacillamide (*Cochlodinium polykrikoides*)	[32]
Kordia algicida OT-1	Sea water of Masan, Korea	ND (*Skeletonema costatum*, *Thalassiosira* sp., *H. akashiwo*, and *C. polykrikoides*)	[62]
Flavobacterium sp. MA10	Ise Bay, Japan	ND (*G. mikimotoi*)	[30]
Shewanella IRI-160	East coast of the United States	ND (*Pfiesteria piscicida*, *Prorocentrum minimum*, and *Gyrodinium uncatenum*)	[22]
The class *Proteobacteria* and *Cytophaga* spp.	Fjords in Southern Chile	Algal-lytic compounds	[2]
Hahella chejuensis KCTC 2396	Marine sediment from the Marado island, Korea	Prodigiosin (*C. polykrikoides*)	[31]
Alteromonas, Pseudoalteromonas, *Vibrio*, *Cytophaga*, *Cellulophaga*, and *Octadecabacter*, and the family *Rhodobacteraceae* spp.	Coast of Osaka Bay, Japan	ND (*Karenia mikimotoi*, *H. akashiwo*, *Fibrocapsa japonica*, and *C. antiqua*)	[25]
The class *γ-Proteobacteria* sp. MS-02-063	Coast area of Nagasaki, Japan	Pigment PG-L-1 (prodigiosin member) (*H. akashiwo*, *H. circularisquama*, *C. polykrikoides*, *Gyrodinium impudicum*, and *Alexandrium tamarense*)	[53]
Bacillus, *Halomonas*, *Croceibacter atlanticus*, and *Marinobacter* spp.	Great South Bay, New York	ND (*Aureococcus anophagefferens*)	[12]
Pseudoalteromonas SP48	Donghai Sea, China	ND (*A. tamarense*)	[63]

ND, not determined.

ALGICIDAL MARINE BACTERIA AND THEIR PRODUCTS

Isolation of Algicidal Marine Bacteria

During the past several decades, much research has focused mainly on the isolation and identification of marine algicidal bacteria. Indeed, dozens of marine bacteria, which belong to the phylum *Bacteroidetes* (class *Flavobacteria* and *Sphingobacteria*), *Proteobacteria* (class α- and γ-*Proteobacteria*), or *Firmicutes* (class *Bacilli*), have been isolated from their abilities to kill harmful algae or completely inhibit their growth [48, 61, 62]. It is estimated that γ-*Proteobacteria* and *Flavobacteria* strains account for more than 75% among the total number of marine bacterial isolates that have been described in literature to date (Table 1). Mayali and Azam [48] reported that algicidal bacteria are divided into four major and some less common groups by morphological, biochemical, and molecular methods for taxonomic analyses: the most common groups include members of the genus *Cytophaga* (renamed *Cellulophaga* by Skerratt *et al.* [61]), *Saprospira*, *Pseudoalteromonas*, and *Alteromonas*. Moreover, Sohn and colleagues [62] summarized that the algicidal bacteria isolated from marine and coastal environments have been classified into the genera *Alteromonas*, *Cytophaga*, *Flavobacterium*, *Pseudomonas*, *Pseudoalteromonas*, *Saprospira*, and *Vibrio*. Interestingly, algicidal Gram-positive bacteria (*Planomicrobium* sp. and *Bacillus cereus*) were first reported in 2002 [61]. At this time, a question may well arise about why most of the algicidal marine bacteria belong to the γ-*Proteobacteria* and *Flavobacteria* groups. Since little comprehensive phylogenetic analyses for the reported marine algicidal bacteria have been carried out [41], it might be hard to answer the question at present. However, there is a probability that γ-*Proteobacteria* and *Flavobacteria* strains are present at higher densities in marine environments, and thus the relative frequency for their isolation is higher. Additionally, it could be presumed that the marine bacterial strains in those groups live in symbiosis with algae that are in competition with harmful algae.

Algicidal Compounds from Marine Bacteria

Algicidal bacteria kill algae or inhibit their growth through direct contact with algal cells [18, 47], or indirectly through release of some toxic compounds into the ambient environment [24, 53]. Several algicidal marine bacteria were reported to produce extracellular algicidal compounds: *Pseudomonas* sp. [3], *Flavobacterium* sp. [14], *Alteromonas* spp. [27], *Pseudoalteromonas* spp. [44, 46], *Bacillus* sp. [32], and *H. chejuensis* [31]. However, no further study on the structural identification of algicidal compounds against HAB causers had been carried out until 2000, although a compound responsible for the killing of a dinoflagellate *Gymnodinium nagasakiense*, produced by *Flavobacterium* sp. 5N-3, was described to be a less than 500-Da basic compound [14]. As the first, a 50-kDa extracellular compound from the marine bacterium *Pseudoalteromonas* sp. A28 was reported to have an algicidal effect against diatom *Skeletonema costatum* and characterized as a serine protease [44]. Later, an algicidal small molecule produced from the marine bacterium *Bacillus* sp. SY-1 was identified as bacillamide and characterized to kill *Cochlodinium polykrikoides* (LC_{50} of 3.2 μg/ml after 6 h) [32].

γ-*Proteobacteria* is one of the most prevalent prokaryotic groups in the marine environments [5], showing a broad spectrum of algicidal activity against bloom-forming red-tide phytoplanktons (Table 1). A red pigment showing algicidal activity was isolated from *H. chejuensis* KCTC 2396, a new member of γ-*Proteobacteria*, which has been originally isolated from the coastal marine sediment collected from Marado, Jeju (Cheju) Island, Republic of Korea [43]. The pigment was structurally identified as a well-known bioactive molecule, prodigiosin, which was experimentally demonstrated to have an algicidal activity against *C. polykrikoides* [31], the harmful dinoflagellate causing considerable mortality of aquatic organisms and economic loss in Korean coastal waters [35].

PRODIGIOSIN, AN ALGICIDAL RED PIGMENT

Characterization of Natural Prodigiosin

Naturally occurring prodiginines (6-methoxyprodigiosenes) are a large family of linear tripyrrolyl antibiotics, and are known to be produced by actinomycetes and other eubacterial strains [15]. Since the first isolation in 1929 and the investigation for antibiotic and cytotoxic activities in the 1960s, a red-colored prodiginine, prodigiosin (2-methyl-3-amyl-6-methoxyprodigiosene), produced by *Serratia marcescens*, has been studied for its medical potential as immunosuppressants [16, 18, 21] and antitumor agents [17, 55].

The production of prodigiosin and its analog is a property found among only a few species of marine bacteria such as genera *Pseudoalteromonas* [57], *Zooshikella* [70], *Serratia* [45], and *Vibrio* [60] included in γ-*Proteobacteria* [54]. In these cases, prodigiosins have been characterized as a by-product and considered as one among the phenotypic features of the marine isolates. Even *H. chejuensis* KCTC 2396 was originally isolated for the ability to produce a large amount of extracellular polysaccharide, which has potential as a biological material in the polymer industry. Although it was able to concomitantly produce a dark red pigment, prodigiosin, the algicidal activity of it had been neither described nor explored as a biological control agent before 2005.

Algicidal Effects of Prodigiosin

Since many natural pigments have been reported to have cytotoxic effects [8, 15, 64] or studied on their usefulness [33, 34, 38], a preliminary test for prodigiosin's algicidal function was carried out. When *C. polykrikoides* were treated

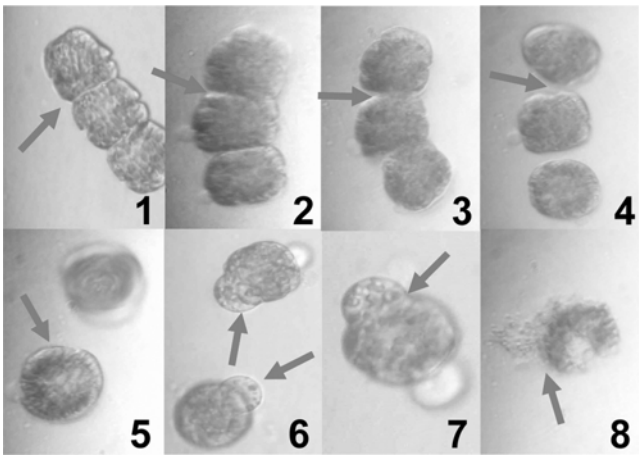

Fig. 1. Algicidal effect of prodigiosin from *H. chejuensis* KCTC 2396 against *C. polykrikoides* BWE0109.
Photomicrographs showing the algal cell-lytic effect were taken 30 min after prodigiosin treatment. 1, Normal vegetative cell; 2–3, rounded shaped cell; 4–5, separated cell; 6–7, swollen cell; 8, burst cell.

with crude red pigment solution containing prodigiosin, the algal cells were rapidly bursted, with the cell number drastically decreasing [31]. When a host range assay was performed to investigate the range of potential target organisms for prodigiosin, it was found that prodigiosin had algicidal activities against a relatively narrow range of dinoflagellates, *C. polykrikoides*, *Gyrodinium impudicum*, and *Heterosigma akashiwo*, and a raphidophyte *Chattonella* sp. (JHY and HKL, unpublished data). Specifically, completely purified prodigiosin showed a significant algicidal activity against *C. polykrikoides* at as low a concentration as 10^{-3} mg/l (1 ppb) within 60 min after co-incubation [31].

Microscopic observation witnessed the rapid lysis of the *C. polykrikoides* cells treated with prodigiosin. More specifically, at a prodigiosin concentration of 10 ppb, the shape of *C. polykrikoides* BWE0109 cells became round, with the boundary between cells being more and more faint. Subsequently, the swollen cells were separated from each other, and cytoplasmic components began to leak out from the walls on the sufficiently swollen cells. Finally, the cells were bursted 30 min after exposure to prodigiosin (Fig. 1). In other cases, phlorotannins from the brown alga *Ecklonia kurome* [52] and rhamnolipid biosurfactant produced by a strain of *Pseudomonas aeruginosa* [66] were able to kill the red-tide algae *Heterosigma akashiwo* and *Karenia mikimotoi*, respectively, through cell lysis.

Biosynthesis of Prodigiosin in *H. chejuensis*

Production of Several Prodiginines by *H. chejuensis*

H. chejuensis KCTC 2396 produces red pigments showing antibacterial and algicidal activities [31]. The main red-colored metabolite of the pigments was identified as the antibiotic prodigiosin [31]. Through LC-MS/MS analysis, three constituents in the red pigments besides prodigiosin were confirmed as the well-known dipyrrolyldipyrromethene prodigiosin (0.5%), norprodigiosin (4.0%), and undecylprodiginine (1.0%), although their relative peak areas were very small compared with that of prodigiosin (100.0%) [36]. Interestingly, the biosynthetic pathways of prodigiosin and undecylprodiginine are slightly different from each other, and co-production of these molecules in a single microorganism has not been reported up to now. Dipyrrolyldipyrromethene prodigiosin and norprodigiosin were produced *via* the condensation of intermediates and accumulated in the prodigiosin-synthetic mutants of *S. marcescens* [20]. Additionally, four new prodigiosin analogs, each of which was distinguished from prodigiosin (C_5) according to differences in alkyl chain length (C_3–C_7), were detected in small quantities by LC-MS/MS spectroscopy: 2-methyl-3-propyl-prodiginine (1.6%), 2-methyl-3-butyl-prodiginine (2.0%), 2-methyl-3-hexyl-prodiginine (1.3%), and 2-methyl-3-heptyl-prodiginine (17.0%) [36]. Some studies for structure-activity relationships have established that the 4-methoxy group on prodigiosin is important for cytotoxic potency [7]. Since all of the prodiginines produced from KCTC 2396 possess a common linear tripyrrolyl structure and the cytotoxic methoxy group, it is expected that all the new prodigiosin analogs are also bioactive. However, biological activity tests for each of them have not been carried out, owing to their small quantities and the difficulty to purely isolate them from cell culture.

Analysis of the Prodigiosin-Biosynthetic Genes in *H. chejuensis*

During the genome sequencing of *H. chejuensis* KCTC 2396, three fosmid clones of *E. coli* EPI300 possibly producing a red pigment have been selected and fully sequenced. Consequently, the clones were confirmed to contain a complete set of the prodigiosin-biosynthetic gene cluster from the observation that they indeed produce the pigment by successfully expressing and functioning in the heterologous host [31]. Sequence analysis of the genetic element (~35.7 kb) revealed 14 ORFs (Table 2) showing high similarities to the *red* (undecylprodiginine biosynthesis) and *pig* (prodigiosin biosynthesis) genes from *Streptomyces coelicolor* A3(2) and *Serratia* spp., respectively [37]. These ORFs were named *hap* for *Hahella* prodigiosin and assigned as *hapA* – *hapN*. Previously, the *red* and *pig* gene clusters have been known to be dissimilar in gene organization and operon structure, although some genes are assigned to almost identical functions. Specifically, the *red* cluster (~36.1 kb) consisting of 23 genes is separated into four transcriptional units [4], whereas the 14 (~24.8 kb) or 15 (~25.0 kb) *pig* genes are present in a single operon [69]. The organization of the *hap* genes was, as expected, highly

Table 2. Bioinformatic analysis of the amino acid sequences deduced from *hap* genes.

Gene	Representative homolog (gene)	Identity (%)[a]	Organism	NCBI accession number
hapA	L-Prolyl-PCP dehydrogenase (*pigA*)	50	*Serratia* sp. ATCC 39006	CAH55629
hapB	H_2MAP oxidase/dehydrogenase (*pigB*)	41	*Serratia* sp. ATCC 39006	CAH55630
hapC	Condensation enzyme (*pigC*)	53	*Serratia* sp. ATCC 39006	CAH55631
hapD	3-Acetyloctanal synthase (*pigD*)	51	*Serratia* sp. ATCC 39006	CAH55632
hapE	3-Acetyloctanal aminotransferase (*pigE*)	58	*Serratia* sp. ATCC 39006	CAH55633
hapF	HBC *O*-methyl transferase (*pigF*)	58	*Serratia* sp. ATCC 39006	CAH55634
hapG	Peptidyl carrier protein (*pigG*)	46	*Serratia* sp. ATCC 39006	CAH55635
hapH	HBM synthase/aminotransferase (*pigH*)	56	*Serratia* sp. ATCC 39006	CAH55636
hapI	L-Prolyl-AMP ligase (*pigI*)	42	*Serratia* sp. ATCC 39006	CAH55637
hapJ	Pyrrolyl-β-ketoacyl ACP synthase (*pigJ*)	37	*Serratia* sp. ATCC 39006	CAH55638
hapK	Hypothetical protein (*pigK*)	46	*Serratia* sp. ATCC 39006	CAH55639
hapL	4'-Phosphopantetheinyl transferase (*redU*)	29	*Streptomyces coelicolor* A3(2)	NP_630004
hapM	HBM oxidase/dehydrogenase (*pigM*)	31	*Serratia* sp. ATCC 39006	CAH55641
hapN	Oxidoreductase (*pigN*)	58	*Serratia marcescens* ATCC 274	CAH55659

[a]Percentage of sequence identity was obtained by aligning the deduced amino acid sequences using Blastp.

similar to that of the *pig* genes and was determined to be transcribed as one operon by RT-PCR experiments against total RNA from KCTC 2396. Several mutants derived from an *E. coli* clone containing *hap* genes through transposon insertions in *hapA*, *B*, *C*, *D*, *E*, *H*, and *J*, respectively, could not produce prodigiosin at all, proving that those *hap* genes are functionally involved in prodigiosin production in the wild-type KCTC 2396. Based on homology analysis using BLAST searches, the *hap* genes could be divided into three apparent categories: eleven structural genes for biosynthesis of the intermediates MBC and MAP, and one gene for the condensation of MBC and MAP [37] (Fig. 2A).

Proposed Prodigiosin-Biosynthetic Pathway in *H. chejuensis*

It is thought that *H. chejuensis* KCTC 2396 can possess at least two pathways for biosynthesis of prodigiosin and undecylprodiginine. On the other hand, there is also a possibility that this bacterium has key enzymes with reduced substrate specificities. Owing to the characteristics of these enzymes, the bacterium thus would be able to simultaneously

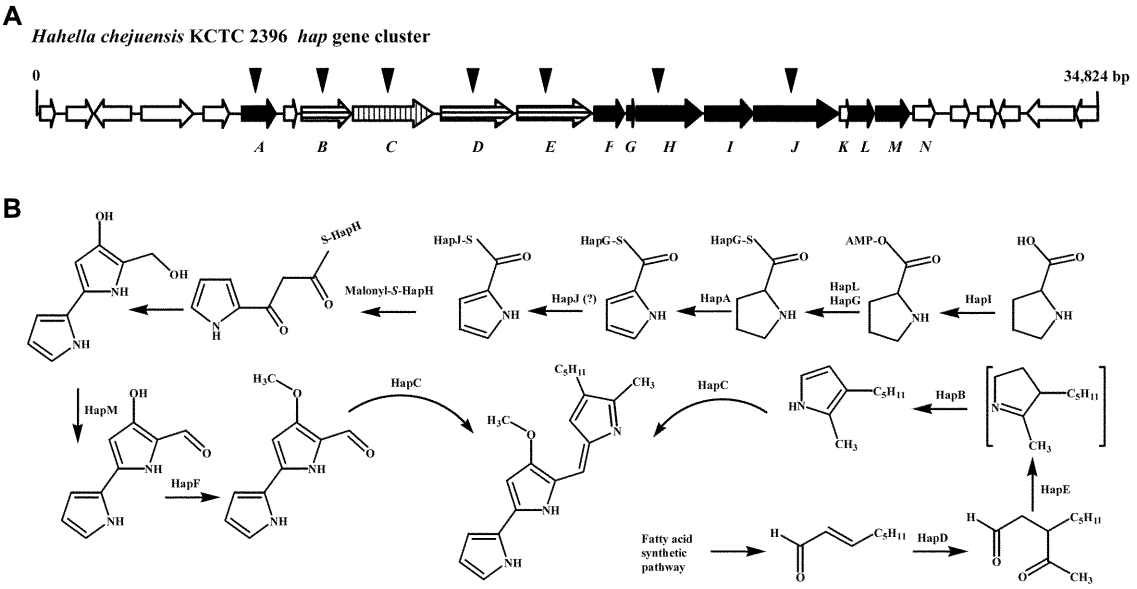

Fig. 2. Organization of the *hap* structural genes (**A**) and proposed bifurcated pathway (**B**) for prodigiosin biosynthesis by *H. chejuensis* KCTC 2396.

The genes for MBC (shade black)/ MAP (horizontal lines) biosynthesis and the single gene for condensation (vertical lines) of the two intermediates are shown. The positions of the transposon insertions is marked with inverted triangles.

produce prodigiosin and undecylprodiginine. Based on the results from the mutagenesis experiments and the comprehensive comparative analysis of *hap* genes with *pig* and *red* genes, a prodigiosin-biosynthetic pathway in KCTC 2396 was proposed as shown in Fig. 2B [37].

Biosynthesis of 4-Methoxy-2,2'-Bipyrrole-5-Carboxyaldehyde (MBC)

The biosynthetic pathways for prodigiosin and undecylprodiginine were proposed with bipyrrole MBC as a common building block. Although MBC was generally supposed to share a similar biosynthetic route, some catalytic reactions and corresponding enzymes have been proposed and described differently between *Serratia* sp. ATCC 39006 [19, 23, 69] and *S. coelicolor* A3(2) [4]. Based on the organization and homology similar with those of prodigiosin-biosynthetic *Serratia pig* genes, the MBC pathway in KCTC 2396 has been proposed. HapI would activate the carboxyl group of L-proline, resulting in L-prolyl-AMP, and then transfer the L-prolyl group to HapG. The prolyl ring of the resultant L-prolyl-*S*-HapG is then successively oxidized to a pyrrole ring by HapA, forming pyrrolyl-2-carboxyl-*S*-HapG, which would be converted into the corresponding β-ketopyrrolyl thioester [19, 69]. The next step is to add an additional pyrrolyl ring, called pyrrole ring B, to the pyrrole ring A derived from L-proline to constitute the bipyrrole moiety of MBC. HapH is expected to be involved in the serine transfer and ketoacyl thioester formation, which would subsequently produce the dihydroxybipyrrole (4-hydroxy-2,2'-bipyrrole-5-methylalcohol, HBM). In order to produce the final product MBC in this bifurcated pathway, the hydroxymethyl group of HBM should be further oxidized into an aldehyde group producing 4-hydroxy-2,2'-bipyrrole-5-carboxaldehyde (HBC), the hydroxyl group of which later would be *O*-methylated. In *Serratia* sp. ATCC 39006, PigM and PigF were proposed for the hydroxymethyl group oxidation and the hydroxyl group methylation, respectively [69]. As in other *hap* genes homologous to *pig* genes, the function of *hapM* and *hapF* is thought to be to catalyze the above two successive reactions.

Biosynthesis of 2-Methyl-3-Amylpyrrole (MAP)

Although MBC was supposed to share similar biosynthetic steps among the microorganisms, two monopyrroles, MAP and 2-undecylpyrrole (a monopyrrolyl counterpart for MAP), were reported to be synthesized through steps different from each other. In accordance with a general concept that the monopyrrolyl biosynthetic-pathway appears to follow the fatty acid biosynthesis, the initial steps for MAP biosynthesis in KCTC 2396 would be similar to that in *Serratia* sp. ATCC 39006. Initially, 2-octenal is expected to be produced as a starting material, through the fatty acid biosynthesis system in KCTC 2396, which would be condensed with pyruvate by HapD, finally producing 3-acetyloctanal. The resulting intermediate would receive a nitrogen atom through a transamination step by HapE, and be spontaneously cyclized to provide the bifurcated monopyrrolyl pathway with MAP. The resulting intermediate pyrroline compound, H_2MAP, would be liable to an oxidation into MAP by HapB. However, the proposed synthetic pathway is still unable to explain the steps for acyl chain initiation, elongation, and termination. In particular, the pathways do not give details for a mechanism for the determination on the acyl chain length. Recently, Mo and coworkers [50] reported that ketoacyl ACP synthases (KASs) with different substrate specificities participate in the initial reaction, and, through the initial steps, the length or type of acyl chains is determined.

Condensation of MBC and MAP

Finally, synthesized MBC and MAP intermediates should be condensed to generate the final product prodigiosin. Previously, an interesting chemical reaction for production of prodigiosin and a prodigiosin-like compound was carried out; chemically synthesized monopyrroles (MAP and ethyl-*meta*-cyclononylpyrrole) were combined with MBC by a general HCl-catalyzed condensation reaction at room temperature, producing prodigiosin and metacycloprodigiosin (ethyl-*meta*-cyclononylprodiginine), respectively, *in vitro* [67]. It indicates that a spontaneous condensation may be completed without a catalytic enzyme in the whole cell. In the case of prodigiosin biosynthesis, however, the activities of the final coupling enzyme were present in both *S. marcescens* wild type and mutants deficient in prodigiosin biosynthesis, and exhibited a maximum during the late log or early stationary phase of cell growth [6]. Most recently, the *pigC* gene encoding the prodigiosin condensing enzyme was detected in the gene cluster of *Serratia* sp. ATCC 39006, responsible for biosynthesis of prodigiosin, and functionally confirmed to be a novel class of pyrrole-condensing enzymes [23, 69]. Since MBC and MAP should undoubtedly be condensed to generate the final product prodigiosin also in KCTC 2396, *hapC* analogous to *pigC* was assigned to the condensing enzyme.

Regulation of Prodigiosin Biosynthesis in *H. chejuensis*

The structural genes (*hapA-N*) encoding the prodigiosin biosynthetic pathway in KCTC 2396 should need regulatory elements for prodigiosin production. In the cases of *Serratia* and *Streptomyces,* complex environmental cues and genetic factors affecting the *pig* and *red* genes for prodigiosin and undecylprodiginine biosynthesis respectively have been verified, [68]. Some decisive regulatory factors for prodigiosin were selected from a genomic library of KCTC 2396 using a heterologous expression system. Among the screened factors, a putative histidine kinase gene and a cognate response regulator of the two-component signal transduction system were confirmed as positive regulators

of prodigiosin biosynthesis *via* molecular deletion and biochemical analysis [42]. The use of a two-component signal transduction system is a common regulatory theme of red-pigment production in *Serratia* and *Streptomyces* [11, 59]. Inversely, a noncoding region located at one end of the *hap* cluster was proved to function as a repressor for red-pigment production. The regulatory determinant present in this noncoding region and the molecular nature of this phenomenon are yet to be explored [42].

As an effort to inhibit or minimize the HAB outbreak, biological control using algicidal marine bacteria has been considered as an alternative to the other practices such as algicidal chemical treatment and clay spraying. Some extracellular compounds produced from the bacterial strains were known to specifically kill harmful phytoplanktons with higher and faster algicidal efficacies. As a representative example, the well-known natural bioactive prodigiosin from *H. chejuensis* KCTC 2396 showed a high algicidal activity against *C. polykrikoides* and other harmful algal cells causing considerable mortality of aquatic organisms in world-wide seawater.

Thus, through this article, we tried to cover potential algicidal agents and their effects for the practical usage in the future, and the biosynthetic routes and regulation of prodigiosin from *H. chejuensis* for the possibility of mass production. To investigate algicidal properties within natural marine environments, *in situ* experiments in a coastal area of the south sea in Korea were carried out with crude red-pigmented extracts containing prodigiosin, and the results appear promising (JHY and HKL, unpublished data). Now, to develop *H. chejuensis* or prodigiosin as a biological or biochemical control agent, the study on the mass production of prodigiosin through optimization of the culture medium and conditions is in progress.

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