Journal of Marine Science and Technology

Volume 30 | Issue 1 Article 6

Polyketides and terpenes in marine microalgae: Ecological roles and mechanisms of biosynthesis

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Recommended Citation

Xie, Yunxuan and Elsheikh, Mohammad (2022) "Polyketides and terpenes in marine microalgae: Ecological roles and mechanisms of biosynthesis," *Journal of Marine Science and Technology*: Vol. 30: Iss. 1, Article 6.
DOI: 10.51400/2709-6998.2566
Available at: https://jmstt.ntou.edu.tw/journal/vol30/iss1/6

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Acknowledgements

This work was supported in partial by the NSFC (81502953). The views expressed herein are those of the authors and do not necessarily reflect the views of the funding agency.

RESEARCH ARTICLE

Polyketides and Terpenes in Marine Microalgae: Ecological Roles and Mechanisms of Biosynthesis

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Abstract

Marine microalgae comprise a large number of polyketides and terpenes identified to date with highly diverse molecular scaffolds and the biological activities (Kalaitzis et al., 2010) [1]. These compounds play significant roles in marine food web and nutraceutical supplementation in marine animals. Compounds of this class are often synthesized by gene clusters with dedicated domains responsible for carbon chain extension and modification. The unique molecular structure of these compounds encourages people to investigate their biosynthetic mechanisms in-depth. In this review, we summarize relevant articles to unravel the shared mechanisms of these compounds to further understand the variety of domain organizations and the subsequent impact on the carbon chain diversity. In addition, the ecological role of these compounds and their modes of distribution in natural sea water is also discussed. We expect this study to bring multifaceted aspects regarding the assemblage and the ecological function of these secondary metabolites and to provide information to those who wish to obtain further understanding in these compounds whose structure and ecological function remain underexplored.

Keywords: Marine microalgae, Polyketides, Terpenes, Biosynthetic mechanisms

1. Introduction

Marine microalgae are ubiquitous marine microbes capable of synthesizing a variety of bioactive secondary metabolites through carbon chain extension. There are more than 20,000 of known species of microalgae that grow in marine environments [2]. The distribution of microalgae in different ecosystems leads to diverse chemical compositions, making them attractive for industrial bioprocessing. Some of the microalgae contain photosynthetic apparatus that allows the fixation of carbon dioxide into metabolically active compounds such as polyketides and terpenes. These compounds are products from complicated intracellular carbon anabolism and display a broad scope of biological activities. Many commercially high-value compounds, including carotenoids, bioactive peptides and polyunsaturated fatty acids (PUFA), compete for intracellular two-carbon units (acetyl-CoA) for their biosynthesis [3,4]. For some photosynthetic microalgae, carbons can be further assembled into more complicated scaffolds such as microalgae toxins [1]. A few microalgae species produce toxins whose chemistry have been intensively studied for decades. Despite of the clarification in their chemical structure, chemoenzymatic catalysis remain poorly understood so far.

Polyketides share a common biosynthetic origin despite of their highly diversified molecular structure. Assemblage of the carbon backbone of all polyketides is catalyzed by polyketide synthase (PKS) in a manner similar to fatty acid biosynthesis [5]. Both classes of compounds are constructed via the sequential decarboxylative Claisen condensation of smaller carboxylic acid units to create carbon backbone. To be specific, the initiation unit (acetyl-CoA) was loaded onto the acyl-carrier protein

Received 13 June 2021; revised 14 September 2021; accepted 7 November 2021.
Available online 15 March 2022.

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https://doi.org/10.514/2709-6998.2566

(ACP), and was then transferred to ketosynthase (KS) domain onto its active site cystein pre-modified by phosphopantetheinylation reaction (Fig. 1). Continued chain extension occurs by using extension unit malonyl-CoA, which is continuously loaded onto the active site serine of the ACP domain pre-modified with phosphopantetheinyl group. Condensation reaction occurs between the initiation unit and extension unit to form diketide under the catalysis by KS domain. For iterative polyketide synthase, same steps are repeated on the same module until products with designated chain lengths are obtained. For non-iterative polyketide synthase, the diketide products are passed onto the ACP domain of the second module to initiate another round of catalysis at KS domain. Presence of additional domains in the module such as ketoreductase (KR) and dehydratase (DH) allows post-condensation modifications to bring over the diversified product scaffolds. Both types of PKS maintain product releasing mechanism by thioesterase domain either as free acid or as cyclized products. Terpenoids, as exemplified by carotenoids, are formed by two common precursors, i.e. isopentenyl-pyrophosphate and dimethylallyl pyrophosphate [6,7] as acetyl-CoA derivatives. The conversion of dimethylallyl pyrophosphate (DMAPP) to geranylgeranyl pyrophosphate (GGPP) is catalyzed by geranylgeranyl pyrophosphate (GGPP) synthase, followed by condensation of two molecules of geranylgeranyl pyrophosphate to phytoene by phytoene synthase and eventually to β-carotene by lycopene β-cyclase [8]. For different polyketides and terpenoids, the core mechanism of carbon chain assemblage remains the same (Figure S1).

We summarize studies to demonstrate the charateristics of polyketide synthases available for marine protists to assemble polyketides and their derivatives in marine protists. Our focus is to summarize different types of polyketide synthases, probing their putative functions to illustrate the diversified catalytic mechanisms available in marine protists in making scaffolds of individual polyketides. Amino acid sequences of representative polyketide synthases with well-established catalytic activity are further analyzed with regard to their protein homologues sharing similar modes of catalysis. Candidate strains with plethora polyketide synthases are phylogenetically analyzed to give a clear idea of mining better polyketide producers among unexplored marine protists. We show that marine protists bear a significant potential for discovering new metabolic features allowing combinatorial biosynthesis for the production of novel polyketide products. In addition, marine microalgae thraustochytrids are shown to have unusual carbon incorporation efficiency into the secondary metabolic pathway and could be an ideal host for combinatorial biosynthesis. The presence of noncanonical polyketide synthases also suggests efforts to be worth pursued to elucidate their

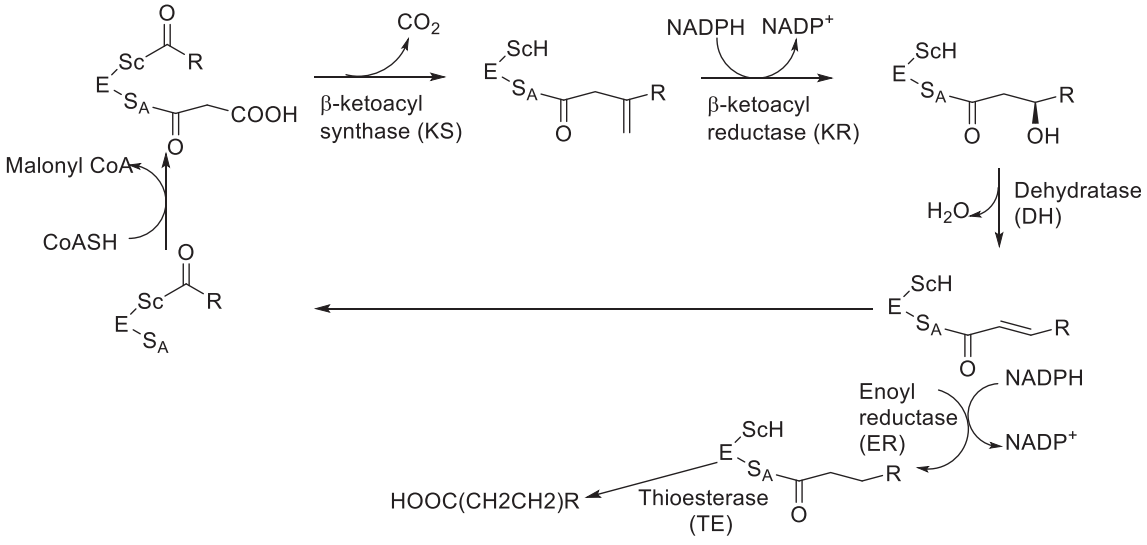


Fig. 1. Assemblage of polyketide scaffolds and its release from phosphopantetheinylated enzyme cluster.

catalytic mechanisms and the subsequent applications. The ecological functions of these compounds are illustrated.

2. Polyketides from marine microalgae

Polyketides from marine microalgae are comprised of two large families, linear polyketides and polyketides with ring structure [1,9]. Linear polyketides include fatty acids of medium or long carbon chain lengths and are good nutrient supplement with multiple bioactivities [10]. Most of these bioactive fatty acids can be found in heterotrophic marine animals such as seafood and fish. They are shown to be transferred via food web from marine microalgae to its higher level predators [11,12]. Generally, multiple carbon−carbon double bonds can be found within these bioactive fatty acids, making them easily to be oxidized. Microalgae produces a vast scope of fatty acids with different chain lengths and degrees of desaturation. Normally those with less than four carbon atoms can be used for producing natural gases. Longer carbon chain fatty acids are of gasoline origin and are used for burning as petroleum. Longer carbon chain fatty acids can be used as biodiesels. Polyunsaturated fatty acids possessing even longer carbon chains (such as DHA and EPA) are regular dietary supplements that provides essential antioxidants to animals and human for normal brain and retinal functions. Phylogenetic analysis suggests that high yield strains for producing long chain polyunsaturated fatty acids are present that include *Nannochloropsis* sp., *Isochrysis* sp. etc. [13−15]. They are particularly good at enriching omega-3 fatty acids and can transfer into different predators via secondary trophic link, entering into marine biological nutrient circulation [15,16] (Fig. 2). Caprellid amphipods, for instance, is an important natural dietary component in a variety of marine fish. They are mostly included in epibiotic communities and live on a variety of natural substrata. Environment with rich organic loading offers better nutrient to ensure fast growth and quick reproductive maturity of caprellid [17]. Feeding caprellid amphipods into finfish ensures a longer body length and more body weight. Marine larval fish that are fed on lipid-encapsulated rotifers after being treated with oil emulsions also possess a more stable body composition of the larvae fish [18]. Copepods (*Acartia grani*, *Centropages hamatus* and *Eurytemora affinis*) are also included in the optimization of feed quality to grow juvenile marine fish. Enrichment in total fatty acids and carotenoids in the dietary menu of copepods increased the feeding quality of marine fish culture

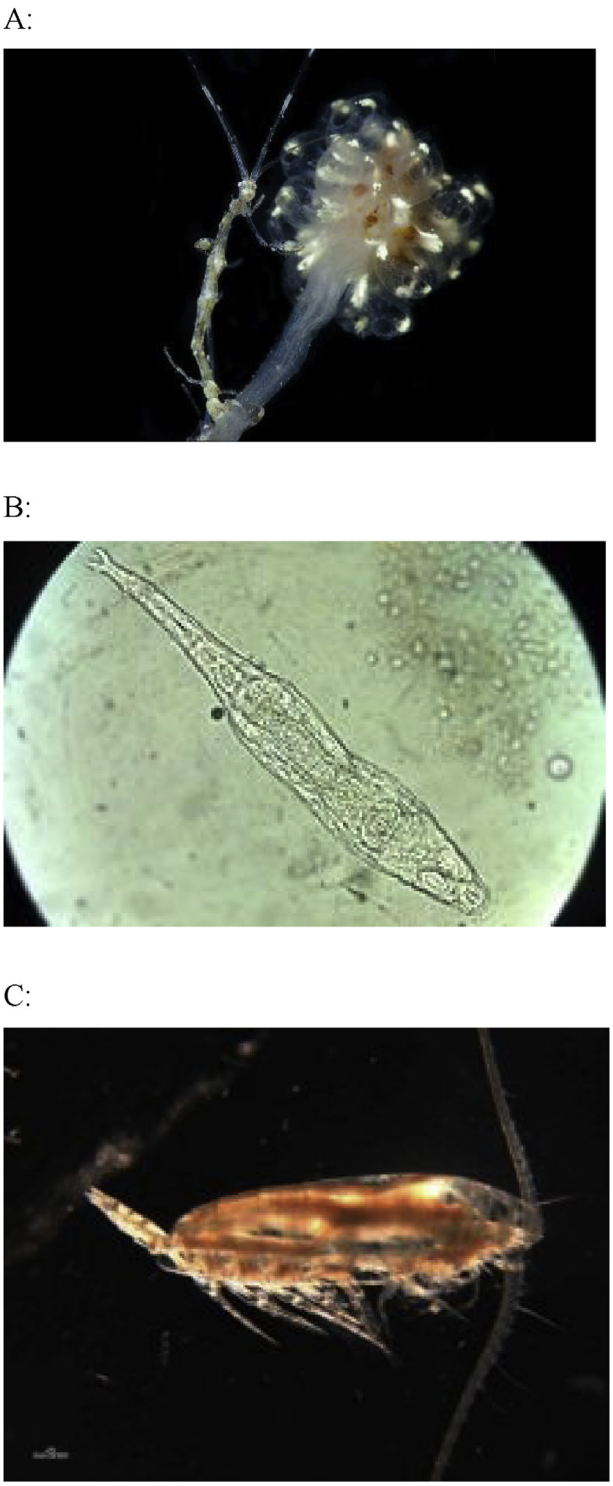


Fig. 2. Major secondary predators for fatty acid circulation in food web (A: Caprellid amphipods, B: Rotifers, C: Copepods).

[19]. These feeding experiments suggest the presence of the potential nutrient transfer pathways from microbes to higher level predators. Photosynthetic algae also synthesize secondary metabolites

with extreme long carbon chain length, most of which are harmful toxins and are posing threat to marine animals [1]. Microalgal toxins penetrate into the body of other seafood during the outbreak of algal bloom, causing massive death of these animals.

3. Linear polyketides

Among most linear polyketides, polyunsaturated fatty acids are the most intensively studied compounds. Fermentative studies address the use of appropriate carbon and nitrogen sources to augment the yield of desired fatty acids and to commercialize microalgae strains particularly with capacity in producing nutraceutically important fatty acids in high yield. In recent years, microalgae has provided a few heterotrophic strains that offer high yield production of bioactive polyunsaturated fatty acids such as docosahexaenoic acid, ecosapentanoic acid, etc. [20−25,27]. Mechanistic studies in microalgal capacity to synthesize polyunsaturated fatty acids at molecular level were facilitated by the conduction of whole genome sequencing of a few microalgae strains, unraveling the presence of large amount of unusual polyketide sequence tags. Among them, a few have been characterized via heterologous expression in model prokaryotic hosts such as *E. coli* [26a,b]. Unlike canonical polyketide synthases, these synthases do not maintain conserved sequence markers that allows the easy identification of the PKS genes from genome sequencing data. Many sequences remain uncharacterized, in part, due to the difficulties to obtain a clear sequence boundary to denote the full gene. Even for genes that were successfully expressed in heterologous host, the substrate specificities and catalytic mechanisms of the corresponding enzymes remain obscure. Total metabolite analysis indicated that these enzymes maintained multiple substrate binding capacities and could increase the yield of more than one fatty acids in the heterologous host. Further inspection into the domain organization and the identification of catalytic center are needed to explain the mechanism of catalysis.

Transcriptomic studies also illustrate the whole cell regulatory mechanism in the biosynthesis of major polyunsaturated fatty acids in microalgae. Preliminary studies (Wang et al., unpublished data) indicated that the change in salinity in culture medium affects the dynamics in metabolite profile. It was reported that lower salinity stimulated the formation of osmotic sensor molecule, the alpha-tocopherol and contributed to the increased lipid oxidation efficiency [28]. Modulation in the carbon and nitrogen source also affects the total biosynthetic efficiencies of major secondary compounds [29−33]. Higher yield in polyunsaturated fatty acids was obtained when both glycerol and glucose were used as carbon sources [34,35]. Analysis in key gene transcription efficiency suggests that rate-limiting enzymes in major fatty acid biosynthetic pathways are transcribed at higher level when appropriate carbon sources are used [35−37]. Key genes that are associated with central carbon metabolism (such as short-chain amino acid catabolism, tricarbolylic acid cycle) also achieve higher transcription level compared to un-optimized culture conditions.

4. Unusual polyketides from marine microalgae—structure and biosynthesis

4.1. Mode of carbon scaffold organization–different HAB toxins and their biosynthetic mechanism

Among natural producers of polyketides, bacteria and microalgae make up the majority of all available species (particularly those that are laboratory culturable). Natural bioactive polyketides available in nature maintain dramatically different scaffolds as a result of different assemblage mechanisms [38]. Bacteria developed various strategies in making polyketides by creating variations in polyketide chain lengths and modes of ring structure. Dinoflagellates rivals their bacteria counterparts by offering biosynthetic machinery to assemble scaffolds with unusual carbon chain length and structure complexity. Maitotoxin, which was isolated from the dinoflagellate *Gambierdiscus toxicus*, represents the largest non-peptidic, non-polymeric natural product described to date (Fig. 3A, B, C). Similar molecular scaffolds were also observed for some polyether natural products such as brevetoxin A and ciguatoxin (isolated from dinoflagellates *Gymnodinium breve* and *Gymnodinium toxicus*), the only known natural products possessing five, six, seven, eight and nine membered rings in the same molecule. These polyether compounds are recognized primarily for their notorious toxicity, while their unusual scaffolds inspired research in their assemblage mechanisms remain poorly understood.

Difficulties in studying the biosynthetic mechanisms of these polyketides are, in partial, attributed to the fact that dinoflagellates are recalcitrant to laboratory cultivation, particularly to meet the demand in the production of secondary products [39]. Dinoflagellates mostly grow as autotrophic microorganism but rarely produce toxic polyketides in medium when exogenous substrates are provided. In an *in-vitro* experiment that tests the production of

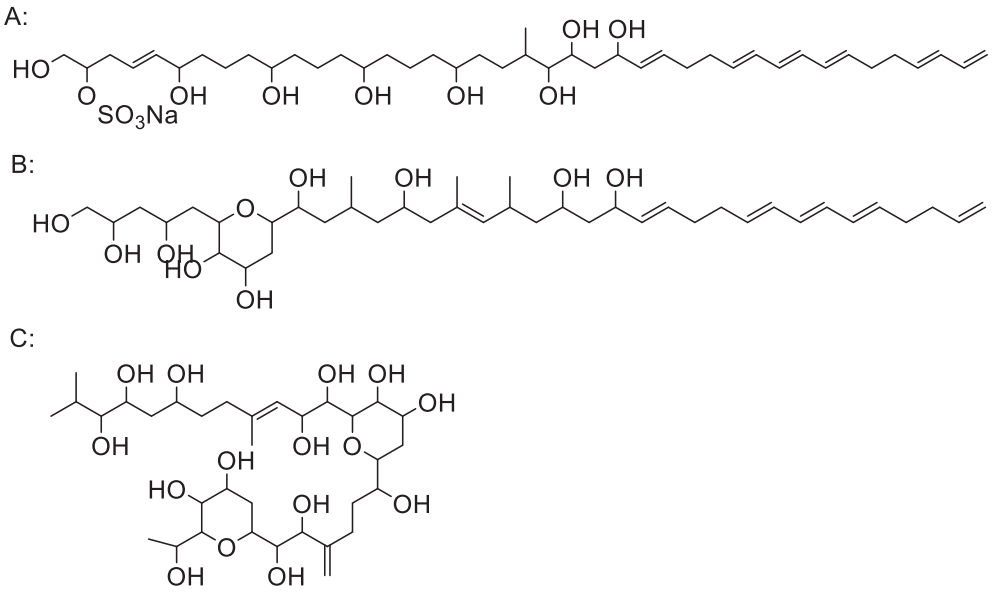


Fig. 3. Structure of maiotoxins (A: AM1, B: AM2, C: AM3).

natural toxic compounds, a stable isotope feeding experiment was performed using 1-^{13}C, 2-^{13}C and 1, 2-^{13}C labelled sodium acetate to evaluate the incorporation patterns of each labelled carbon. In brevetoxin B, three distinctive incorporation patterns were observed, including six c-m-m (m: methyl group in sodium acetate, C: carbonyl group in sodium acetate), one c-m-m-m and one c-m-m-m-m modes. Feeding experiments with single labelled succinate [2, 3-^{14}C] as substrate suggests no visible incorporation of radio-labelled substrate into brevetoxin scaffold [40]. Examination in carbon incorporation pattern of sodium acetate in other compounds (such as okadaic acid, goniodomin and amphidinolide J) suggests the presence of two incorporation patterns that are originated from sodium acetate. For okadaic acid, a Favorski rearrangement occurs to delete C-1 in acetate (Fig. 4A). This explained the labelling pattern in okadaic acid, brevetoxins, amphidinolide J and goniodomin A as well as 1, 2 and 1, 4 oxygenation patterns found in other polyketides from zooxanthellae [41]. Heterologous expression also offers direct evidence to decipher the biosynthetic mechanisms of HAB toxins such as saxitoxins (Fig. 4B-D). Adding intermediate compounds in tricarbolylic acid cycle into culture medium (such as malate, succinate and α-ketoglutarate) clearly enhances saxitoxin production in the heterologous bacterial host. It is therefore reasonable to deduce that bacteria be the major host for saxitoxin production, the presence of dinoflagellates provides essential nutrition for symbiotic bacteria and enhances its productivity.

The complexity in the biosynthetic mechanisms of saxitoxins were further characterized by a series of *in-vitro* enzymatic assays that involves the use of cell-free lysate and substrate feeding experiments. It was demonstrated that two saxitoxin derivatives with 1-NH can be converted to 1-NOH upon incubation in the cell free extract obtained from *Alexandrium tamarense*. At the presence of adenosine 3′-phosphate-5′-phosphosulfate, 21-NH can be modified into 21-NSO_3^- in *Gymnodinium catenatum*. Saxitoxin modification enzyme was also detected in shellfish, suggesting that the complete biosynthetic pathway may comprise enzymes from other microbes. Identification of the accurate open-reading frame coding for individual proteins and purifying enzymes *in vitro* still represent a big technological hurdle that retards the characterization of their catalytic activities precisely. UV-induced mutation in coupling with medium colony screening provides a rapid evolution strategy to obtain highly toxic producing strains [42].

5. Brevetoxin

Brevetoxins are a group of molecules that feature a ladder-like, all-*trans*-fused polycyclic ether toxins [43,44] with two carbon backbones assembled individually to make up their carbon scaffolds (Fig. 5A-C). Systematic investigation in brevetoxin synthases was carried out to explain their biosynthetic mechanisms, particularly in regard to the formation of the ether bonds [45]. These ether bonds are not the typical characteristics of polyketide products in

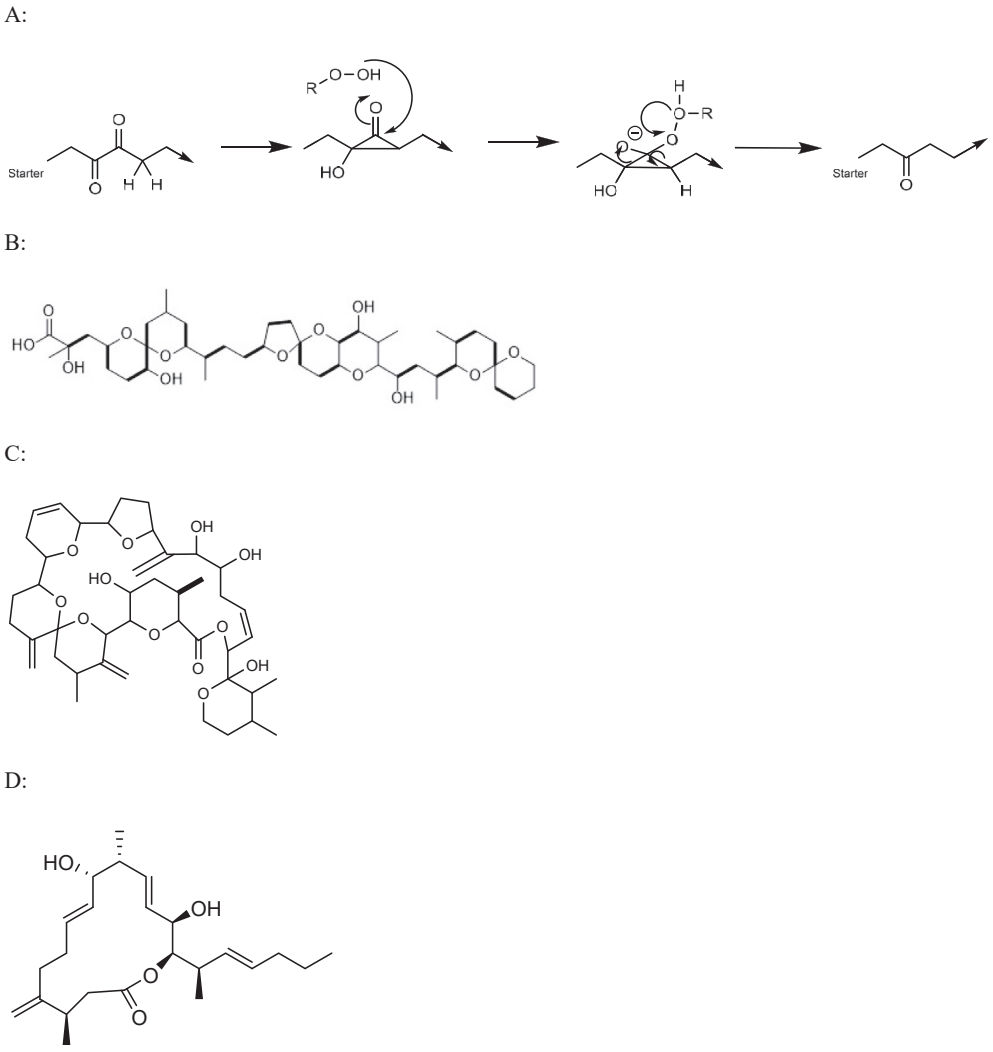
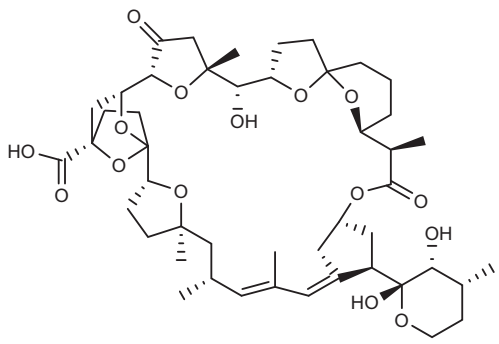


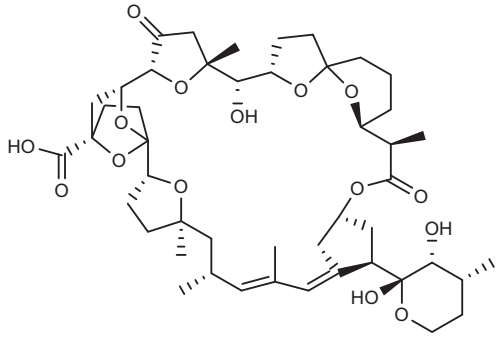
Fig. 4. Schematic illustration of Favorski rearrangement (A) and the formation of okadaic acid (B), goniodomin (C) and amphidinolide J (D).

regular cells. It was proposed that carbon chains were truncated after forming the epoxide intermediates, followed by subsequent thioesterification. This allows further chain extension to take place. Deciphering the biosynthetic mechanism at genetic level required complete genome sequencing of *Karenia. brevis* to be done. This attempt, however, was hampered by the presence of permanently condensed chromatin, the lack in the typical transcription activation sequences (such as TATA box) and the presence of many tandem repeats that caused the frame-shift reading of many genes [46]. The large genome size and the presence of non-coding sequences necessitated the screening of cDNA library through reverse transcription assay. A general approach to be used for searching universal polyketide synthases includes high-throughput sequencing, performed with well-defined gene probes to identify individual expression tags within microalgae cells (Table 1). Six different dinoflagellate species were subject to a preliminary assay that was designed to screen the presence of type I polyketide synthases [47]. Two independent KS domains were identified from *Karenia brevis* non-axenic culture samples and seventeen expression sequence tags were considered relating to PKS or FAS. Rapid amplification of cDNA ends (RACE) yielded individual PKS fragments with fragment lengths of approximately 2800bp. Eight sequence tags were identified to contain KS domains (Table 2) by referring to NCBI's Conserved Domain Database (CDD) [48]. Screening the conserved KS domains for other dinoflagellates suggested that two were shared by *Karlodinium veneficum* and *A. tamarense*,

A:



B:



C:

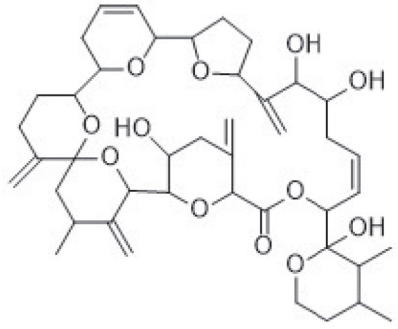


Fig. 5. Chemical structure of toxins in Karenia brevis with trans-fused rings (A: zooxantholatoxins), carbon−carbon bridge six member ring (B: pectenotoxin) and head-to-head ladder fused rings (C: Goniodomin A).

one was conserved in KB1008 and the last one was conserved for *A. tamarense*.

Comparing four PKS fragments unique to *K. brevis* suggested the presence of a "splicer leader" sequence with approximately 100bp upstream to each start codon (Fig. 6) [49]. This splicer leader is considered as a sequence marker of *K. brevis* and can be used to identify novel polyketide synthases isolated from similar strains. To authentuate the classification of PKS in *K. brevis*, amino acid sequence alignment assay using Clustal X was performed with representative sequences for type I PKS (from prokaryotes, fungi, protists and animals) and type II PKS (from prokaryotes). Sequence comparison suggested the complexity of PKSs in different *K. brevis* strains. Among them, *K. brevis* AT2-10L, AT2-15, AT1-6L and Kbrevbac30 contain protist's type I PKS clade while the other Kbrevbac sequences from *K. brevis* culture clearly show bacterial contaminations that are manifested by the presence of bacterial type II PKS clade. It is noteworthy that the splicer leader sequence also play roles in post-transcriptional regulation of PKS synthesis, suggesting that the brevetoxin biosynthesis is under tight cellular regulation. Presence of four PKS genes unique to brevetoxin producing strains suggests that they are directly involved in the biosynthesis of brevetoxins while those conserved genes may participate in the biosynthesis of some common fatty acids or unknown polyketides. The fact that the expression profile of these genes alters according to the change in brevetoxin productivity suggests they are indirectly involved in the biosynthesis of brevetoxin. Although the biosynthesis of polyether brevetoxin is considered to occur primarily through polyketide synthase pathway, the presence of nitrogen containing compounds (such as DTX-5a, DTX-5b [50] and brevisamide [51]) indicated that mixed NRPS-PKS synthases are available to incorporate certain amino acids as a source of nitrogen. RT-PCR analysis demonstrated that both PKS and NRPS were actively expressed within *K. brevis* cultures.

Table 1. PKS transcripts in Karenia brevis strains and other dinoflagellate species + indicates transcripts in Karenia brevis strains and other dinoflagellate species.

	KB1008	KB2006	KB4825	KB5299	KB5361	KB6380	KB6736	KB6842
K. brevis	+	+	+	+	+	+	+	+
K. brevis	+	+	+	+	+	+	+	+
K. brevis	+	+	+	+	+	+	+	+
K. brevis	+	+	+	+	+	+	+	+
Karenia mikimotoi	+	+	+	+				
Karlodinium veneficum							+	+
Amphidinium veneficum	+							
Alexandrium tamarense			+				+	+

Table 2. PKS transcripts identified in K. brevis and proposed functions.

Sequence ID	Base Pairs	Amino Acids	Proposed Function	Sequence Similarity (Protein, origin)	Identity/ Similarity (%)	Accession Number
KB1008	3397	1016	ACP/KS	Beta-ketoacyl synthase (*Anabaena variabilis ATCC* 29413)	32/50	YP_324603
KB2006	2923	944	KS	MxaD (*Stigmatella aurantiaca*)	39/52	AAK57188
KB4825	3288	1023	KS	MmxC (*Cystobacter fuscus*)	29/48	ABA29781
KB5299	1875	515	KR	Protomycinolide IV (*Micromonospora griseorubida*)	43/62	BAC57028
KB5361	3235	857	KS	Type I fatty acid synthase, putative (*Toxoplasma gondii RH*)	39/56	CAJ20333
KB6380	2367	688	KS	Polyketide synthase type I (*Streptomyces aizunesis*)	31/49	AAX98185
KB6736	3361	895	KS	StiG protein (*Stigmatella aurantiaca*)	36/51	CAD19091
KB6842	2413	686	KS	COG3321: Polyketide synthase modules *Nostoc Punctiforme*	30/49	zp_00108,796

Fig. 6. Amino acid alignment of "splicer sequences" in PKS fragments unique to K. brevis (blue region indicating the conserved splicing sequence in the four transcripts).

Subcellular localization of the expressed PKS, NRPS and β-tublin genes within *K. brevis* cells suggested they occurred primarily in nucleus and had no contamination to chloroplasts. Tracing the origin of the PKS-NRPS genes suggests that they are from freshwater cyanobacteria, a microbial strain that produces freshwater toxins. To locate individual PKS genes for brevetoxin biosynthesis, Western blot approach was adopted with probes designed to characterize the keto-reductase domain and keto-synthase domain [52]. Post-transcriptional analysis suggests that in non-toxic cultures, KS abundance is approximately 55−70% less compared to toxic cultures. The discovery that the green fluorescence appears only in the nucleus region indicates that brevetoxin biosynthesis is primarily controlled by chromosomal genes. Screening cDNA libraries for transcripts using type I PKS degenerate probes identified large integral transcripts in *K. brevis*. To further characterize the size of catalytic functional group, an immuno-reactive experiment was performed using degenerate sequences of KS and KR domains to describe the size of the enzymatic products. Anti-KS reaction specified a product of 100 KDa, about the same size as the expected 101 KDa. Anti-KR reaction however, identified a product of 40 KDa, which is slightly different from the theoretical value of 56 KDa. When the immuno-reactive antibody was designed against type I PKS from *Cryptosporidium. parvum*, a product size of 100 KDa was identified. Appearance of the green fluorescence signal indicates PKS resides primarily in chloroplast. Transcriptional analysis of mid-logarithm growth phase of both toxic and non-toxic *K. brevis* suggests KS and KR were associated with brevetoxin biosynthesis. Altered expression level in KS and KR accounts for productivity differences in brevetoxin between toxic and non-toxic *K. brevis* strains. Light induction is very likely to be the reason stimulating brevetoxin biosynthesis and also explains the mechanism in the biosynthesis of other toxic compounds such as okadaic acid in *Prorocentrum lima*. Additional compounds produced by *K. brevis* include brevenal [53], hemibrevetoxin B

[54], brevisamide [51] and phytopigments [55]. There has been no detailed mechanistic study reported for these compounds so far. Tracing the origin of these PKS genes in eukaryotes using phylogenetic analysis strongly indicated the possibility of a horizontal gene transfer within unicellular eukaryotes [56]. A large majority of PKS genes sharing homology with those identified in cyanobacteria suggests the possibility of lateral gene transfer between the two species.

6. Terpenoids from marine microalgae

6.1. Astaxanthin

Marine fish that are fed on microalgal extract generally display better skin colors and skin smoothness compared to those without dietary microalgal supplement. A close inspection into the chemical composition of microalgal extract suggests the presence of terpene that aids in the skin color improvement. A microalgal astaxanthin feeding trial was performed to investigate the effects of natural astaxanthin from *Haematococcus pluvialis* as feed additives on growth, pigmenting efficacy and antioxidant capacity in *Cichlasoma enrinellurn* and *Ciehlasorna sywspiltun*. It is found that supplementation of dietary astaxanthin could effectively enhance growth, skin coloration and the antioxidant capacity of fish [57]. Similar observations were made regarding the muscle and skin color improvement among dietary fish, shrimps and other seafood [58,59].

6.2. Other terpenoids

The supplementation of terpenoids into the feed stock of fish elevated the body pigmentation and antioxidation function while no significant improvement in body weight gain and growth is observed [60]. Besides the improved skin and muscle color, some fish also displayed better immunity against virus infection upon appropriate addition of lipids and terpenes [61−63]. In some other sea animals (such as seahorse and *Moenkhausia sanctaefilomenae*), improved egg and semen quality was also observed [64−66]. Microbial terpene providers include *H. pluvialis*, *Xanthophyllomyces dendrorhous*, *Thraustochytrid* sp. etc. [67−69]. Tracing the delivery of terpenoid compounds from their microbial hosts to higher trophic predators (such as copepods) established the evidence that these nutrients can be transferred, accumulated and metabolized by higher level predators [67,70]. The efficiency of terpene transfer varied upon the nutrient dynamics that are basically conveyed by the type of phytoplankton community available in a particular ecological niche. The enrichment of terpenes from water to fish scales allowed an easy recovery of these nutrients for recycled use [71]. Secondary predators possess diversified conduit efficiencies for the transfer of terpene into higher trophic level [72]. Different sources of terpenoids also had different impacts on skin coloration, suggesting that subtle differences lying within their chemical structure (and therefore their biological activities) affects the absorption efficiency of individual terpenes [73]. Co-supplementation of lipid-solubilized vitamins with terpenes further improved the efficacy of these terpene nutrients [74]. Other compounds that improved skin colour and avoid lipid oxidation also includes small natural compounds with medium polarity (such as alpha-tocopherol), high polarity (such as citric acid) and synthetic pigments [9,17,28,38,42,56,58,75−78]. Despite of different stereochemistry of synthetic pigments, similar enhancing effects were observed in skin coloration and antioxidant capacity.

7. Conclusion

Marine microorganisms can synthesize a wide range of bioactive compounds with ecological and nutraceutical significance. These natural compounds include primarily polyketides (such as fatty acids and microalgae toxins) and terpenoids (such as carotenoids). Biosynthesis of polyketides and terpenoids are intercalated. Most of the linear polyketides can be transferred from one biological species to another via food web. Some of these compounds (such as polyunsaturated fatty acids) can even enter into marine mammals at the highest trophic hierarchy. How these molecules are assembled remains not totally unclear till now. Preliminary studies indicated the presence of polyketide synthases and fatty acid synthases that assemble these linear polyketides (mostly from fourteen to twenty-two carbon atoms). However, in photosynthetic organisms, most of the synthases remain uncharacterized due to the lack of precise genome annotation information and subsequent biochemical studies. In limited studies, similar biosynthetic mechanism is found to be shared for linear carbon chain assemblage among the long-

carbon chain natural products in marine microalgae. For compounds with extremely long carbon scaffolds (mostly microalgal toxins with up to sixty carbon atoms), additional synthases are also present to allow the continued incorporation of carbon chain building blocks. Similarities in amino acid sequence and modular organization of the gene clusters for these microalgal toxins suggest that the lateral gene transfer occurs between phylogenetically distant protist strains. Clarifying the process of lateral gene transfer will explain how different marine protists evolve similar mechanisms for the production of these secondary metabolites. Elucidation in the module organization manner will help predict the abundance of these polyketides as natural resources in different marine environments. Terpenoids are another type of marine microalgal nutrients that are present in marine protists and marine mammals. Compared to the studies in polyketides, mechanisms in terpenoids biosynthesis remain further underexplored. What is still needed is the discovery of sequence markers that address the hidden unusual polyketide (terpenoid) synthases with catalytic functions remaining to be identified. Purification and characterization of these unusual synthases will further aid in the exploration and utilization of marine microbial resources.

Conflicts of interest

The author states no conflict of interest.

Acknowledgements

This work was supported in partial by the NSFC (81502953). The views expressed herein are those of the authors and do not necessarily reflect the views of the funding agency.

Appendix

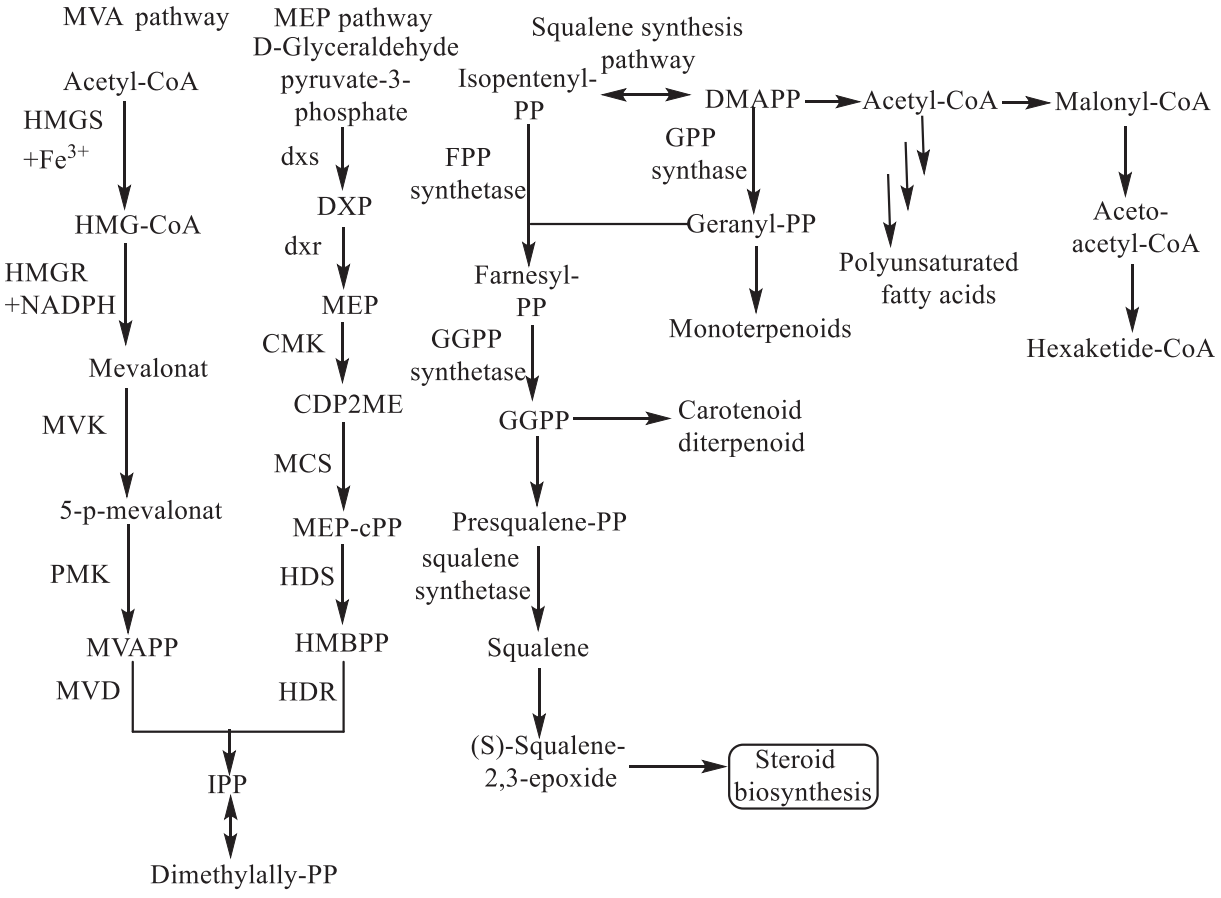


Figure S1. Biosynthesis of terpenes and polyunsaturated fatty acids via intercalated pathway.

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