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MINI-REVIEW

Polyunsaturated fatty acids in marine bacteria and strategies to enhance their production

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Abstract

Polyunsaturated fatty acids (PUFAs) play an important role in human diet. Despite the wide-ranging importance and benefits from heart health to brain functions, humans and mammals cannot synthesize PUFAs de novo. The primary sources of PUFA are fish and plants. Due to the increasing concerns associated with food security as well as issues of environmental contaminants in fish oil, there has been considerable interest in the production of polyunsaturated fatty acids from alternative resources which are more sustainable, safer, and economical. For instance, marine bacteria, particularly the genus of *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, *Psychromonas*, *Vibrio*, and *Alteromonas*, are found to be one among the major microbial producers of polyunsaturated fatty acids. Recent developments in the area with a focus on the production of polyunsaturated fatty acids from marine bacteria as well as the metabolic engineering strategies for the improvement of PUFA production are discussed.

Keywords Polyunsaturated fatty acids · Docosahexaenoic acid (DHA) · Eicosapentaenoic acid (EPA) · Marine bacteria · Metabolic engineering

Introduction

Polyunsaturated fatty acids (PUFAs), specifically omega-3 PUFAs comprising of eicosapentaenoic acid (EPA) and its counterpart docosahexaenoic acid (DHA), are considered as essential groups of fatty acid that contribute significantly toward human health. For instance, the Japan EPA lipid intervention study (JELIS) has indicated that EPA consumption minimized the huge coronary events by 19% in patients having a past record of cardiovascular disorder (Yokoyama et al. 2007). The AMR101 study revealed that intake of pure EPA decreased the considerable amount of triglyceride (TG) in patients with serious hypertriglyceridemia (Bays et al. 2011). Moreover, clinical evidence has clearly indicated that EPA and DHA can prevent breast cancer, diabetes, Alzheimer's diseases, allergic diseases, inflammatory bowel disease, and chronic disorder (Jicha and Markesbery 2010; Pishva et al. 2012; van den Elsen et al. 2012; Raatz et al. 2013; Sato et al. 2013; Zulyniak et al. 2013; Kunisawa et al. 2015). EPA and DHA are also regarded as significant components for visual and neurological growth in infants (Voigt et al. 2002; Das and Fams 2003; Ramakrishnan et al. 2010). DHA is a valuable constituent of the membrane phospholipids in the brain, retina, and spermatozoa (Bazan 2009; Rapoport et al. 2011; Mandal et al. 2014). EPA and DHA perform a significant function in the cellular membrane structure which are precursors to many important eicosanoids, e.g., prostacyclins, prostaglandins, and leukotrienes (Funk 2001; Ma et al. 2004). Considering their great health benefits, EPA and DHA have become useful dietary components for human consumption (Siriwardhana et al. 2012; Swanson et al. 2012). Besides their

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use as health additives, EPA and DHA are also crucial in the pharmaceutical, aquaculture, terrestrial animal feed, pet food, and personal care (Xue et al. 2013).

PUFAs cannot be produced by humans; hence, they must to be ingested through diet (Nichols et al. 2010; Schober ME 2016). Sources of omega-3 PUFAs made for consumption are generally from fish and fish-derived products. Fish species such as Scombridae, Clupeidae, and Salmonidae contain the maximum amount of DHA and EPA in the diet (Rubio-Rodríguez et al. 2010). However, fish oils are not sustainable and hazardous due to their poor taste and contamination from toxic metals such as cadmium, lead, and mercury (Domingo et al. 2007). They are also unstable, and the cost of purification is high. Meanwhile, global fish population is dwindling and environmental pollution of marine habitat causes global problems (Domingo et al. 2007; Cao et al. 2012; Gong et al. 2014). Hence, an alternative source that provides sustainable PUFAs and its inexpensive purification process need to be obtained.

In this regard, some microorganisms especially deep-sea bacteria are able to produce PUFAs (Gladyshev et al. 2013). This review provides an outline of the fundamental aspects of marine bacterial PUFA production. It will also explain the biosynthesis pathway of PUFAs in bacteria, genetic alteration of bacteria to enhance the PUFA production, and strategies for attaining higher-quantity formation of PUFA in bacteria. Finally, some future perspectives on PUFA production are discussed with special emphasis on metabolic engineering.

Function of PUFAs in bacteria

Cold adaptation

Most PUFA-producing bacteria inhabit deep ocean environment that is characterized by low temperature and high pressure; hence, they are referred to as psychrophilic (psychrotrophic and/or piezophilic) in nature (Delong and Yayanos 1987; de Carvalho and Caramujo 2012; Bianchi et al. 2014; Yoshida et al. 2016). The existence of such conditions contributes to the adaptation of the bacteria that leads to the production of PUFAs. These bacteria can change the content of hopanoid, protein, carotenoid, and sterol, as well as the composition of fatty acid in the cell membrane (Alain et al. 2002; Kahlke et al. 2012; Cao et al. 2014). Survival of psychrophilic microbes in extremely cold habitats is contributed by the absorption of specific fatty acids into the membrane. This enables the transportation of nutrients and preserves fluidity (de Carvalho and Fernandes 2010; Nogi 2011; Bianchi et al. 2014).

EPA is a prerequisite for the growth of deep-sea bacteria under a psychrophilic and piezophilic environment (Yoshida et al. 2016). For example, *Shewanella livingstonensis* Ac10 is a psychrotrophic bacterium isolated from seawater of the Antarctic sea. It produces a large amount of EPA (Kawamoto et al. 2009). At a low temperature of 4 °C, the EPA composition of the membrane phospholipids of *S. livingstonensis* Ac10 is almost 5% of total fatty acids and, concurrently, the EPA composition became insignificant after the incubation at 18 °C. Meanwhile, the Ac10 EPA-deficient mutants grow along with the wild-type strain at 18 °C. On the other hand, the growth of the mutant was greatly inhibited at 4 °C and this growth retardation was redeemed after the addition of EPA into the culture medium. This justifies that EPA is vital for the survival of *S. livingstonensis* Ac10 at low temperature (Kawamoto et al. 2009). *Shewanella piezotolerans* WP3 was characterized as a psychrotolerant and piezotolerant marine bacterium originated from a sediment sample of the western Pacific Ocean at the depth of 1914 m, and it grew at an optimal temperature and pressure of 15–20 °C and 0.1–20 MPa, respectively (Wang et al. 2004; Usui et al. 2012). *S. piezotolerans* cells contain EPA in the membrane. The depletion of EPA in the membrane of this bacterium led to growth inhibition at low temperature (4 °C, 0.1 MPa) and high pressure (20 °C, 20 MPa), showing that EPA was essential for the growth of this bacterium (Wang et al. 2004; Usui et al. 2012). However, there are exceptions where EPA is not required in some EPA-producing bacteria and the absence of it can be reimbursed by an elevated amount of monounsaturated fatty acids (MUFAs) or branched chain fatty acids. For instance, it was recently proven that the growth inhibition of *Photobacterium profundum* SS9 mutant strain EA10 which was deficient in EPA synthesis was fully remunerated in vivo by a sufficient amount of MUFAs. In comparison, the *P. profundum* SS9 mutant strain EA3, which was lacking MUFA production, appeared sensitive at a low temperature and high pressure and this sensitivity was overcome by the supplementation of exogenic monounsaturated fatty acid instead of EPA (Allen et al. 1999). Additionally, *Shewanella marinintesina* IK-1 was an EPA-producing psychrotrophic bacterium isolated from the intestine of a squid, had a total EPA content of approximately 15% of TFAs at 4 °C, but became lesser when grown at 27 °C. It was still noticed that mutant IK-1Δ8, which was defective in EPA synthesis grew along with the *Shewanella marinintesina* IK-1 cell at 4 °C (Yoshida et al. 2016). Based on this evidence, EPA is not essential for the growth of this particular species at a lower temperature.

Shewanella violacea strain DSS12 is a deep-sea bacterium discovered from the Ryukyu Trench at the depth of 5110 m. It survives under a moderate piezophilic condition with optimal growth at 30 MPa and 8 °C, respectively. However, it can still grow at 0.1 MPa. *S. violacea* DSS12 cells contain a large amount of EPA in their membrane of approximately 15% at 8 °C (Nogi et al. 1998a; Usui et al. 2012). Usui et al. (2012) studied the changes in the membrane of *S. violacea* using the

newly modified system that enabled the measurement of fluorescence anisotropy under high pressure. The findings indicated that EPA prevented the membrane from becoming hyperfluid and maintained membrane stability against structural changes in pressure. This finding disagrees with the general concept that high membrane fluidity is the main feature of microorganisms dwelling at a low-temperature and high-pressure environment. The finding further suggests that maintaining a certain level of membrane physical properties under high pressure is more significant than supplying membrane fluidity alone. EPA also played a vital role in *S. violacea* strain DSS12 cell division under high pressure because of the cell structure of EPA-deficient *S. violacea* mutant which undergoes filamentation at 30 MPa (Kawamoto et al. 2011; Usui et al. 2012).

Antioxidant role of PUFAs

It was reported that PUFAs such as EPA, DHA, and arachidonic acid (ARA) could exert antioxidant function in bacterial cells through membrane-shielding effects. Bacterial cells usually resist the attack of H_2O_2 using catalase or other sorts of H_2O_2-scavenging enzymes (Nishida et al. 2006; Okuyama et al. 2008; Nishida et al. 2010; Yoshida et al. 2016). The antioxidant function of PUFAs was initially studied using *Escherichia coli* DHα that had been genetically manipulated to produce EPA (Nishida et al. 2006). The recombinant *E. coli* (denoted as EPA+) become more resistant when treated exogenously with reactive oxygen species (ROS) such as H_2O_2 and *tert*-butyl hydroperoxide (*t*-BHP) compared to the wild type that lacked the EPA production (denoted EPA−). However, to support this hypothesis, catalase-deficient *E. coli* UM2 transformed with EPA biosynthesis gene clusters (*pfaA-E*) could produce EPA of approximately 7–8% of the total fatty acids. This transformant developed more resistance to an externally added H_2O_2 than the normal control strain (Nishida et al. 2006). In these two recombinants of *E. coli*, EPA shielded the cells from the oxidative effects of exogenic H_2O_2, viz. growth retardation, oxidation of cellular proteins, and rupture of the cell structure (Okuyama et al. 2008). Likewise, the antioxidative role of EPA was indicated in the marine bacterium *Shewanella marinitestina* IK-1 that typically contained EPA (approximately 20%) and in its EPA-deficient mutant IK-1Δ8 (Nishida et al. 2007; Okuyama et al. 2008; Nishida et al. 2010). Moreover, EPA-producing and ARA-producing bacteria from seawater source were discovered using the concept of estimating resistance to H_2O_2 (Tilay and Annapure 2012). This research provided the primary evidence that supports the idea of an antioxidative function of PUFAs which includes both omega-3 and omega-6.

The mechanism for the cell membrane-shielding effect of long-chain PUFAs (LCPUFAs) such as EPA, DHA, and ARA has been suggested in bacteria. In this mechanism, the large hydrophobic interface is built between the bilayers of cell membrane phospholipids acylated with LCPUFAs in combination with a medium-chain saturated or monounsaturated fatty acid, and this interface hinders the entry of exogenous hydrophilic compound (e.g., H_2O_2) through the membrane (Nishida et al. 2007; Okuyama et al. 2008; Hori et al. 2011). Moreover, the hydrophobicity allows the entry of hydrophobic compounds (Yoshida et al. 2016).

Production of PUFAs in marine bacteria

Most PUFA-producing bacteria belong to the class of Gammaproteobacteria (Freese et al. 2009) such as *Shewanella*, *Photobacterium*, *Colwellia*, *Vibrio*, *Moritella*, and *Psychromonas* family (see Table 1) (Fang et al. 2004; Nogi et al. 2007). Another group of marine bacteria that produces PUFAs is found within the *Flavobacterium* group, e.g., *Flexibacter* and *Psychroserpens* (Nichols et al. 1993; Bergé and Barnathan 2005; Freese et al. 2009; Nogi 2011).

Shewanella

Shewanella spp. are considered as a vast genus of marine bacteria comprising Gram-negative, facultative anaerobic, motile, straight or curved rod-shaped, and non-fermentative bacteria. The genus consists of psychrophilic and mesophilic species which are the main producers of PUFAs (EPA). Today, numerous *Shewanella* species (both psychrophilic and psychrotolerant) have been discovered and characterized by cold environments (Kato and Nogi 2001). A high level of EPA in *Shewanella* species is primarily associated with the mechanisms of cold adaptation (Russell and Nichols 1999). Examples of *Shewanella* species that produce EPA are presented in Table 1. Most of the species were discovered from deep ocean and found to contain 2–36.6% of EPA of total fatty acids (Deming et al. 1984; Delong and Yayanos 1986; Delong et al. 1997; Nogi et al. 1998a; Miyazaki et al. 2006; Xiao et al. 2007).

Photobacterium

The genus *Photobacterium* was one of the oldest bacterial taxa and was first discovered by Beijerinck in 1889 (Farmer III and Hickman-Brenner 2006). Species belong to this genus are often described as Gram-negative, facultative anaerobic, plump, straight, and rod-shaped bacteria. They are motile (using one to three uncovered flagella) and require a high concentration of sodium ions for growth. This genus is unique due to the ability of some members to produce visible light (Seo et al. 2005; Moi et al. 2017). *Photobacterium* spp. are regarded as a special class of deep-sea bacteria because of their capability to produce PUFAs, particularly EPA (Allen

Table 1 Summary of marine bacteria producing PUFAs

Bacteria	Type of PUFA	% of PUFA*	Origin	Reference
Shewanella spp.				
S. gelidimarina ACAM 456	EPA	16.0	Congelation ice, Prydz Bay, Antarctica	Bowman et al. (1997a)
S. benthica ATCC 43992	EPA	16.0	Intestine, holothurians	Bowman et al. (1997b)
S. hanedai ATCC 33224	EPA	22.2	Marine sediment, Arctic Ocean	Bowman et al. (1997b)
S. frigidimarina ACAM 591	EPA	6.8	Congelation ice, Prydz Bay, Antarctica	Bowman et al. (1997b)
S. baltica MAC1	EPA	3.5	Mackerel	Amiri-Jami et al. (2006)
S. violacea DSS12	EPA	1 4	Deep-sea Ryukyu Trench	Nogi et al. (1998a)
S. affinis KMM 3587	EPA	2.1	Sea Bay	Ivanova et al. (2004)
S. halifaxensis HAW-EB4	EPA	7.0	Atlantic Ocean	Zhao et al. (2006)
S. japonica KMM 3299	EPA	8.3	Seawater of Chazhma Bay (Sea of Japan, Pacific Ocean)	Ivanova et al. (2001)
S. olleyana ACEM 9 2	EPA	3.6	Humic-rich river estuary	Skerratt et al. (2002)
S. peizotolerans WP3	EPA	13.4	Pacific deep-sea sediment	Xiao et al. (2007)
S. psychrophila WP2	EPA	7.1	Pacific deep-sea sediment	Xiao et al. (2007)
S. pneumatohori SCRC-2738	EPA	36.6	Intestines of Pacific mackerel	Yazawa (1996)
S. waksmanii KMM 3823	EPA	6.7	Sipuncula (*Phascolosoma japonicum*)	Ivanova et al. (2003)
Photobacterium spp.				
P. frigidiphilum SL1	EPA	6.0	Cold-seep area	Seo et al. (2005)
P. profundum DSJ4	EPA	13.0	Deep-sea sediment	Nogi et al. (1998c)
P. profundum SS9	EPA	7.0	Deep-sea sediment	Allen et al. (1999)
P. profundum SAMA2	EPA	15	Tidal flat sediment at Wadden	Freese et al. (2009)
Colwellia spp.				
C. demingie ACAM 459	DHA	2.2	Antarctic	Bowman et al. (1998)
C. hornerae ACAM 607	DHA	2.1	Antarctic	Bowman et al. (1998)
C. psychrerythraea ACAM 550	DHA	8.0	Antarctic	Bowman et al. (1998)
C. psychrotropica ACAM 179	DHA	0.7	Antarctic	Bowman et al. (1998)
C. rossensis ACAM 608	DHA	6.0	Antarctic	Bowman et al. (1998)
Moritella spp.				
M. japonica JCM 10249	DHA	6	Deep sea	Nogi et al. (1998b)
M. marina MP-1	DHA	12	Deep sea	Nogi et al. (1998b); Kautharapu et al. (2013)
M. yayanosii JCM 10263	DHA	11	Deep ocean	Nogi and Kato (1999)
Psychromonas spp.				
P. kaikoae JT7304	DHA	3	Deep sea	Nogi et al. (2002)
P. marina JCM 10501	DHA	1.6	Coastal area of Okhotsk Sea, Japan	Kawasaki et al. (2002)
Vibrio spp.				
Vibrio sp. strain 5710	DHA	22.7	Deep-sea sediment	Hamamoto et al. (1995)
Vibrio cyclitrophicus	EPA	10	Deep sea	Abd Elrazak et al. (2013)
Vibrio sp. strain A7	EPA	10.7	Intestinal liquor	Ringø et al. (1992a)
Vibrio pelagius	EPA	8.7	Turbot larvae	Ringø et al. (1992b)
Vibrio sp. strain 5705	DHA	21.5	Deep-sea sediment	Hamamoto et al. (1995)
Vibrio sp. strain 5703	DHA	18.6	Deep-sea sediment	Hamamoto et al. (1995)
Vibrio sp. strain 29-1	EPA	19.7	Deep-sea sediment	Hamamoto et al. (1995)
Vibrio sp. strain 814-4	EPA	17.9	Deep-sea sediment	Hamamoto et al. (1995)

*Percentage of PUFAs from the total fatty acids

and Bartlett 2002). These groups of bacteria are characterized as marine psychrophilic and piezophilic (Fang and Kato 2007). Of all the species of *Photobacterium*, only three species are responsible for the production of long-chain PUFAs, which are *P. profundum*, *Photobacterium frigidiphilum*, and *Photobacterium* sp. strain SAMA2 (Table

1). Other members in this genus are involved only in the formation of MUFAs, which usually contain 16 to 18 carbon atoms.

Piezophilic *P. profundum* (strains DSJ4 and SS9) and *P. frigidiphilum* have been found to contain a large amount of long-chain PUFAs, particularly EPA in their membrane (Fang et al. 2002). When *P. profundum* SS9 is grown at either low temperature or high pressure, it produces a high proportion of PUFAs (Allen et al. 1999). *Photobacterium* strain of SAMA2 isolated from tidal flat sediment at the Wadden Sea and North Sea of Germany contained a high quantity of EPA (20:5ω3) at 10 °C. This strain was closely related to *Photobacterium lipolyticum* (Freese et al. 2009). Most members of *Photobacterium* produced terminal-branched (*iso* and *anteiso*) fatty acids (Fang and Kato 2007).

Colwellia

The genus *Colwellia* was initially reported by Deming et al. (1988), describing two facultative anaerobic bacteria: *Colwellia psychrerythraea* and *Colwellia hadaliensis*. Members present in this genus *Colwellia* were described as Gram-negative and motile (except for *Colwellia rossensis*), chemoorganotrophic, having straight or curved rods, psychrophilic, halophilic, consisting of ubiquinone-8 (Q-8) as the prominent isoprenoid quinone, and having a G+C content in the range of 35–42 mol% (Nogi et al. 2004; Zhang et al. 2008; Nogi 2011). Most species produce omega-3 FAs, particularly DHA of which the level increases with the decrease in the optimum growth temperature. This can serve as an adaptation to cold marine habitat (Delong and Yayanos 1986; Delong et al. 1997). Fatty acids with *n*-even chain length, such as *n*-14:0, *n*-14:1ω7*cis*, and *n*-16:ω7, were found dominant in their membrane lipid (Russell and Nichols 1999). Many members of *Colwellia* species previously belonged to the genus *Vibrio*, but analysis of the fatty acid composition could differentiate these two genera by generating an atypical chemotaxonomic grouping (Bowman et al. 1998). Currently, *Colwellia* consists of ten species isolated from marine habitat. Five psychrophilic species such as *Colwellia demingiae*, *C. psychrerythraea*, *Colwellia psychrotropica*, *C. rossensis*, and *Colwellia hornerae* contain DHA (Table 1).

Moritella

DHA production is a common feature of the genus *Moritella*. Members of this genus are described as psychrophilic and/or piezophilic. At present, seven members have been recognized in the *Moritella* genus. They are found in marine environments such as seawater, marine sediments, and abyssal ocean (Nogi and Kato 1999; Benediktsdottir et al. 2000; Urakawa et al. 2000; Xu et al. 2003). Three *Moritella* species which are *M. marina*, *M. yayanosii*, and *M. japonica* (see Table 1) incorporate a large amount of DHA in their membrane. *M. marina* is closely related to the genus *Shewanella*. However, it was grouped as a non-piezophilic bacterium discovered from the North Pacific Ocean (Urakawa et al. 2000). *M. japonica* was described as a moderately piezophilic bacterium which originated from the Japan Trench, while *M. yayanosii* was characterized as an extremely piezophilic bacterium isolated from a Mariana Trench challenger, reaching the depth of 10,898 m (Nogi et al. 1998b; Nogi and Kato 1999). *M. yayanosii* grew optimally at 80 MPa, was incapable of growing at below 50 MPa, and grew well at 100 MPa (Nogi et al. 1998a). Almost 70% of the membrane lipids present in *Moritella yayanosii* strain DB21MT-5 and *Shewanella benthica* strain DB21MT-2 were unsaturated fatty acids (PUFAs) which were the reason why the bacteria can easily adapt to a low-temperature and high-pressure environment (Nogi and Kato 1999; Fang et al. 2000).

Psychromonas

Members belonging to the genus *Psychromonas* are Gram-negative, curved or straight rod-shaped bacteria, motile or non-motile, chemoorganotrophic, halophilic, aerobic or facultative anaerobic, and psychrophilic. The G+C content present within the DNA molecule is in the range of 38–44 mol%, the dominant isoprenoid quinone is Q8, and the main fatty acids are 16:0 and 16:1ω7. In addition, species in the genus *Psychromonas* possess substantial variations in other physiological characteristics including cell size, the temperature range for growth, piezophily, the presence of gas vacuoles, and carbon source utilization (Mountfort et al. 1998; Nogi et al. 2007; Auman et al. 2010). This genus is closely related to the genera *Shewanella* and *Moritella*. *Psychromonas antarctica* is a prominent member in this genus, and it was originally obtained from a high-salinity pond on the McMurdo Ice Shelf in Antarctica. This bacterium exhibits unique aerotolerant and non-piezophilic anaerobic features (Mountfort et al. 1998). *Psychromonas kaikoae* is considered as a novel obligatory piezophilic bacterium isolated in sediment from the beneath cold-seep area in the Japan Trench at a depth of 7434 m (Nogi et al. 2002). It grows optimally at a temperature and pressure of 10 °C and 50 MPa, respectively. Additionally, this strain can produce both DHA and EPA. In contrast, *P. antarctica* and some recently discovered species such as *Psychromonas agarivorans* and *Psychromonas boydii* did not contain PUFA in their membrane component (Hosoya et al. 2009; Auman et al. 2010). *Psychromonas profunda* has currently been known as a moderately piezophilic bacterium typically isolated from Atlantic sediment at a depth of 2770 m. It was also similar to the piezo-sensitive strain *Psychromonas marina*, which also produced a huge amount of DHA (Xu et al. 2003).

Vibrio

Some members of *Vibrio* sp. produced PUFAs (e.g., EPA and DHA). For example, Ringø et al. (1992a) carried out the EPA screening of 14 bacterial isolates from turbot larvae (*Scophthalmus maximus*). The results revealed that *Vibrio pelagius* was the only bacterial isolate capable of producing a large content of EPA. At a low growth temperature, EPA concentration of the bacterium increased. Moreover, 17 isolates of bacterial strains obtained from the intestinal liquor were also screened for EPA formation. Four isolates identified as *Vibrio* spp. accumulated a large amount of EPA in their total lipid (Ringø et al. 1992b). Similarly, out of 22 psychrophilic and psychotropic *Vibrio* spp. isolated from marine sediment, 12 isolates contained DHA and 5 isolates contained EPA. The optimum growth temperature of DHA producers was below 20 °C, that of EPA was between 20 and 25 °C, but for other bacterial species, it was higher than 25 °C (Hamamoto et al. 1995). Hamamoto et al. (1994) suggested that these PUFAs may play a significant role in the adaptations of marine *Vibrio* species in a low growth temperature and could be used to distinguish between psychrotolerant and psychrophilic deep-sea bacteria. PUFA production decreased as the temperature increased, indicating that the membrane fluidity was adjusted by the composition of PUFAs present in the bacterium (Delong and Yayanos 1986). Some *Vibrio* species that produced EPA and DHA are summarized in Table 1.

Alteromonas

The genus *Alteromonas* was coined by Baumann et al. (1972) and later reexamined by other scientists (Gauthier et al. 1995; Van Trappen et al. 2004). Primarily, the genus comprised a phylogenetically and phenotypically heterogeneous group of Gram-negative, aerobic, heterotrophic, marine, non-fermentative, and motile bacteria using only one polar flagellum (Martinez-Checa et al. 2005; Matsuyama et al. 2015). Many of its species had been systematically reclassified into the following genera such as *Marinomonas*, *Pseudoalteromonas*, and *Shewanella* (Van Landschoot and De ley 1983; Gauthier et al. 1995; Ivanova et al. 2001). At present, *Alteromonas* consists of 11 identified species (Matsuyama et al. 2015; Park et al. 2015). Members of the genus *Alteromonas* were found in the marine habitat, either in the open ocean or at the coast (Matsuyama et al. 2015). *Alteromonas* has a number of distinct characteristics such as moderately halophilic and grows at 4 °C with 7.5–10% (*w*/*v*) sodium chloride (Martinez-Checa et al. 2005). *Alteromonas hispanica* is the representative of the species and was discovered from an inland hypersaline environment. It produces PUFAs at the high growth temperature (32 °C) (Martinez-Checa et al. 2005). This challenges the concept that significant levels of PUFAs are produced only in barophilic and psychrophilic marine species (Nogi et al. 1998c; Russell and Nichols 1999; Martinez-Checa et al. 2005).

Biosynthetic pathway of PUFA production in marine bacteria

Previously, bacteria were thought not to contain PUFAs. It was claimed that only eukaryotes were involved in the production of PUFAs (Fang and Kato 2007). Discovery of PUFAs among marine bacteria had enabled researchers to make an intense investigation on bacterial pathways that synthesized PUFAs (Russell and Nichols 1999). Polyketide synthase (PKS) is a fundamental pathway which catalyzes the biosynthesis of LCPUFAs (Wallis et al. 2002; Fang and Kato 2007). PKS functions independent of fatty acid synthase, elongase, and desaturase (Wallis et al. 2002; Fang and Kato 2007). This was identified in both prokaryotes (bacteria) and eukaryotes (algae) but, more abundantly, in marine bacteria, especially in deep-sea piezophilic bacteria (Metz et al. 2001; Fang and Kato 2007; Gong et al. 2014). The biosynthetic pathway of PUFAs via the PKS route is illustrated in Fig. 1. This pathway uses acetyl-CoA as the putative compound to synthesize EPA or DHA (Cao et al. 2012; Gong et al. 2014).

PUFAs (*n*-3) can also be produced via aerobic desaturase/elongase pathway. This pathway (illustrated in Fig. 2) is present in bacteria, plant, fungi, and microalgae. Specific desaturases and elongases catalyze individual desaturation and elongation steps to produce PUFAs from oleic acid (C18:1ω9) (Sakuradani et al. 2013; Hayashi et al. 2016). Δ-5 Desaturase and Δ-4 desaturase are the important enzymes that play a vital role in the production of EPA and DHA. Any mutant strain that lacks these enzymes cannot completely synthesize ω-3 fatty acid via the desaturation and elongation pathways. All the desaturases and elongases are a membrane-bound acyl-lipid- or acyl-CoA-type enzyme, except for the soluble stearoyl-acyl carrier protein (S-ACP-DES) which initiates the first step. The reactions by the fatty acid desaturation normally occur in the presence of NADPH as the electron donor (see Fig. 2). The existence of NADPH in large number could be a crucial factor for generating the double bond (Cao et al. 2012).

Structure and function of genes responsible for PUFA production

Recent progress in research has identified that only five genes (*pfaA-E*) are required for the biosynthesis of polyunsaturated fatty acids (Fig. 3) (Okuyama et al. 2007). Even though the basic structure of all *pfa* genes necessary for PUFA production shares some similarities, the domain structure of each gene differs slightly (Okuyama et al. 2007). *Shewanella* strain

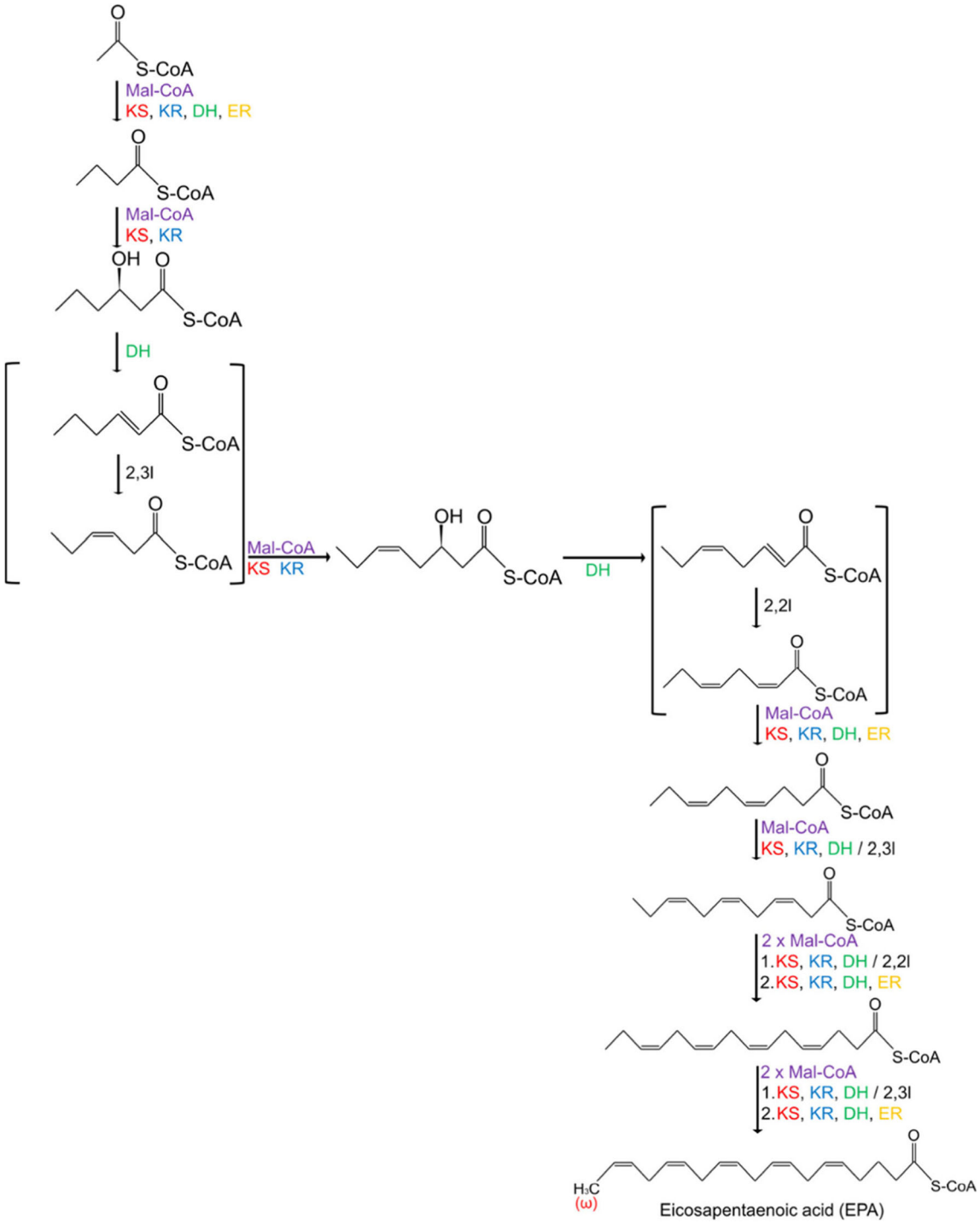

Fig. 1 Proposed polyketide biosynthesis pathway of EPA formation in *Shewanella* sp. SCRC2738 with putative intermediates and key enzymes available as follows: ketoacyl synthase (KS), ketoreductase (KR), dehydratase (DH), enoyl reductase (ER), 2,3I, 2,3-isomerase, and 2,2I,2,3-isomerase. Anaerobic polyketide synthase pathway occurs following a series of reduction, dehydration, and condensation (Gong et al. 2014). It uses acetyl-CoA and malonyl-CoA as the building blocks for the process (Ratledge 2004). Polyunsaturated fatty acid production begins by the condensation of malonyl-CoA together with acetyl-ACP by β-ketoacyl-ACP synthase (KS), yielding the formation of β-ketobutyryl-ACP. β-Ketobutyryl-ACP is further converted by the NADPH-dependent reductase (KR) to β-hydroxybutyryl-ACP. This is accompanied by the dehydration of the β-hydroxy intermediate to form *trans*-2-enoyl-ACP, which is activated by the DH/IS enzyme. The final stage in the process involves NADPH-dependent reduction of the double bond in *trans*-2-enoyl-ACP intermediate by the action of ER. Other reactions in the process are catalyzed by a series of enzyme activities (KS, KR, DH/IS, and ER). This process continuously repeats, resulting in the formation of PUFAs. PKS pathway introduces double bond by the action of a FabA-like dehydratase-isomerase formed during the iterative extension of the fatty acyl chain

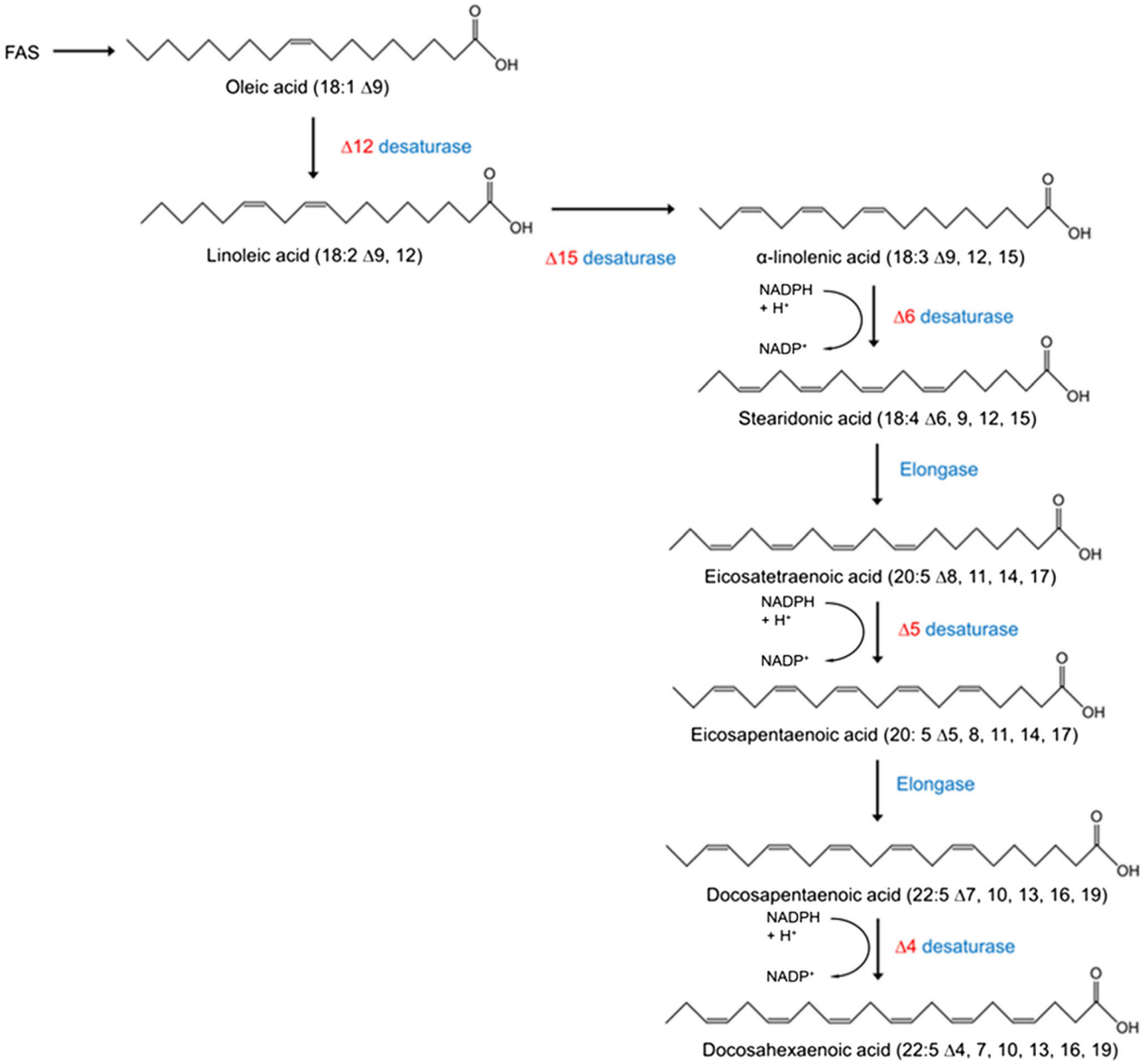

Fig. 2 Schematic diagram of the aerobic desaturation and elongation pathway for ω-3 synthesis. The pathway begins when oleic acid is desaturated by the Δ-12 desaturase, resulting into the formation of linoleic acid. Linoleic acid is the product of a second desaturation step which is catalyzed by Δ-15 desaturase to generate α-linolenic acid. α-Linolenic acid can be converted to stearidonic acid intermediate by Δ-6 desaturase, followed by the elongation of stearidonic acid to produce eicosatetraenoic acid. Further desaturation determined by the activity of Δ-5 desaturase leads to the production of EPA. Eicosapentaenoic acid can be elongated to yield docosapentaenoic acid, which can then be desaturated by Δ-4 desaturase, allowing the formation of DHA

SCRC-2738 (presently known as *Shewanella pneumatophori*) isolated from the marine fish intestine in 1996 was believed to be the first step of research into PUFA cluster genes (Yazawa 1996; Okuyama et al. 2007). Yazawa (1996) first amplified a 38-kb DNA fragment of EPA gene cluster from *Shewanella* sp. SCRC-2738 and expressed it in *E. coli*. This resulted in the heterologous formation of EPA (Cao et al. 2012). Homologs of the *Shewanella* PUFA gene cluster were also found in other marine bacteria, e.g., *Photobacterium profundum* (Allen and Bartlett 2002), *Moritella marina* (Tanaka et al. 1999), and *Colwellia psychrerythraea* (Methé et al. 2005).

The *pfaA* gene encodes a multifunction protein that contains the following domains: 3-ketoacyl synthase (KS), malonyl-CoA acyltransferase (MAT), five ACP repeats, and ketoreductase (KR), respectively. *pfaC* encodes a protein containing a domain of two KS repeats, chain-length factor (CLF), and two or three 3-hydroxydecanoyl-ACP dehydratases (DH). *pfaB* and *pfaD* encode for a protein domain of KS, acyltransferase (AT), and enoyl reductase (ER).

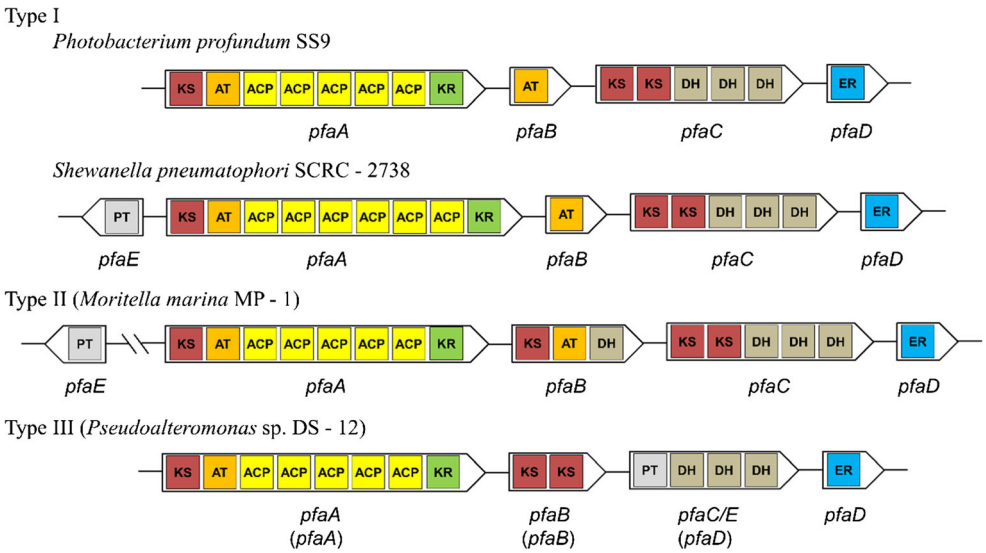

Fig. 3 Organization and arrangement of EPA and DHA gene clusters showing similarities and differences among marine bacteria. The enzyme domain structures are shown in colors

The last *pfaE* gene, which consists of phosphopantetheinyl transferase (PPTase), is situated at the upstream of *pfaA* and is oriented in the opposite direction (Orikasa et al. 2004; Orikasa et al. 2006; Okuyama et al. 2007; Shulse and Allen 2011; Yoshida et al. 2016).

Typically, *pfaA-D* genes were isolated from *P. profundum* SS9 (Allen and Bartlett 2002), but the gene encoding phosphopantetheinyl transferase, corresponding to *pfaE*, was not obtained. However, an EntD-like PPTase gene was unveiled in the *P. profundum* genome. This gene has an open reading frame of 690 bp, composing a protein of about 230 amino acids. Likewise, the EntD-like PPTase gene was cloned from *P. profundum* and expressed in *E. coli* which already carried four genes (*pfaA-D*) from *M. marina* MP-1. The expression of EntD-like PPTase complemented the *pfa* genes in PUFA biosynthesis (Sugihara et al. 2008). Another gene known as orf6 thioesterase was also uncovered upstream of the *pfaA* of *P. profundum*. This gene encodes a multienzyme of the PUFA synthase complex. The closeness of the orf6 gene to the PUFA gene cluster has revealed its participation in PUFA formation (Rodríguez-Guilbe et al. 2013). The arrangement and the organization of the protein domain in *P. profundum* and *Shewanella* sp. SCRC-2738 are highly similar in nature (Cao et al. 2012; Gong et al. 2014). Furthermore, the arrangement of *pfa* gene domains in *M. marina* MP-1 differs from that of *S. pneumatophori* SCRC-2738. Four of the *pfa* genes in DHA-producing bacterium *M. marina* MP-A *pfaA-D* are assembled in the chromosome (Tanaka et al. 1999; Orikasa et al. 2009), whereas *pfaE* is positioned separately (Orikasa et al. 2009). Meanwhile, in EPA-producing bacteria including *S. pneumatophori* SCRC-2738, all the five genes are assembled close to each other (see Fig. 3). Besides that, the *pfaA* gene domains of both *M. marina* MP-1 and *S. pneumatophori* SCRC-2738 contain five and six acyl carrier proteins, respectively. In contrast, the KS domain is absent in *S. pneumatophori* SCRC-2738 but present in *M. marina* MP-1 (Orikasa et al. 2006; Orikasa et al. 2009). Interestingly, the entire three DH domains present in *pfaC* for EPA-producing bacteria were homologous to FabA with a great similarity, whereas in *pfaC* for DHA-producing bacteria, only two DH domains were found similar to that of FabA, and the other domain located in the center was similar to either FabZ or FabA. Furthermore, the domain structure of *pfa* genes in *Pseudoalteromonas* sp. strain DS-12 showed some differences from other strains (see Fig. 3). PfaB contains two KS domains, while PfaC has one PPTase domain and two DH domains. In addition, the downstream end of the pfaC/E gene is composed of a 483-bp fragment of DNA, which was considered the incomplete sequence similar to that of the ER gene (Okuyama et al. 2007).

Strategies for increasing PUFA production in marine bacteria

Researchers have identified several ways to synthesize PUFA in high quantity, which depend on a detailed understanding of the complex pathways and networks in PUFA biosynthesis and major metabolic processes. Some of the major strategies are explained in the section below.

Cerulenin treatment

Cerulenin is an antifungal compound and also a special inhibitor of condensing enzyme. Cerulenin was found to inhibit MUFA synthesis but not PUFA synthesis in *M. marina* (Morita et al. 2005). The addition of cerulenin in the culture media had increased (about 1.5- to 5-fold) the composition of DHA in several bacteria such as *Moritella marina* (Morita et al. 2005); *Shewanella violacea* DSS12, *S. benthica* DB21MT-

2, and *M. yayanosii* DB21MT-5 (Fang et al. 2004); *Colwellia psychrerythraea* strain 34H (Wan et al. 2016) as well as recombinant *E. coli* DH5α carrying *pfaE* from *C. psychrerythraea* (Peng et al. 2016); and *Photobacterium profundum* SS9 strain DB110 (Allen et al. 1999).

A similar phenomenon was encountered in the EPA-producing bacterium, *Shewanella marinintestina* strain Ik-1; when cultured in a medium composed of cerulenin, the EPA content increased (Morita et al. 2005). The concentration of cerulenin added varies from 0.5 to 12 μg/ml as well as the number of fold increase (Table 2). Cerulenin treatment causes the downregulation of numerous genes associated with the fatty acid degradation. It also inhibits FabB protein which may lead to the aggregation of the substrate for DHA formation which is malonyl-CoA (Wan et al. 2016).

Nitrogen supplementation and limiting the carbon source in growth media

Supplemental nitrogen such as tryptone and yeast extract increased the EPA and DHA production in *Shewanella putrefaciens* MAC1 and *M. marina* MP-1, respectively (Nikoopour and Griffiths 2008; Kautharapu et al. 2013). *S. putrefaciens* MAC1 produced a higher quantity of EPA (34.7 mg/g dry cell weight (DCW)) when grown in a culture medium composed of 1% of tryptone and 0.5% of yeast extract. Similarly, about fivefold increase in DHA titer was observed when *M. marina* MP-1 was grown in a medium consisting of tryptone and yeast extracts at an equal concentration (10 g/l). The addition of nitrogen greatly increased the overall DHA titer through an increment in the biomass production (Nikoopour and Griffiths 2008; Kautharapu et al. 2013).

The carbon concentration in the growth medium is important in determining the production of PUFAs. For instance, when *S. putrefaciens* MAC1 was cultured in a medium composing an extra 2% of glucose, the quantity of EPA decreased from 15.6 to 11.3 mg/g dry cell weight as compared to the medium without having an extra glucose (Nikoopour and Griffiths 2008) (Table 2). The addition of carbon source enhances the biomass but decreases the DHA or EPA content in the bacterial cells (Kautharapu et al. 2013). Therefore, these studies suggest that decreasing carbon source in the growing medium increases PUFA production by the bacteria.

Decreasing the cultivation temperature

Growth temperature is an important factor affecting PUFA productivities. Most deep-sea bacteria contain the huge amount of PUFAs at low temperature (Allen et al. 1999; Allen and Bartlett 2002; Yang et al. 2007). *P. profundum* SS9 was found to generate a high amount of PUFAs when cultivated at a low temperature. At 4 °C, the EPA content in *Shewanella* sp. KMG427 was higher as compared to 25 °C (Lee et al. 2008b). Similar results were found in *P. profundum* SS9 strain EA2 and *P. profundum* SS9 strain EA3 (Allen et al.

Table 2 Strategies for increasing PUFA production in marine bacteria

Strategies	Strain	Condition	PUFA	Fold increase	Final titer	Reference
Cerulenin treatment	*M. marina* MP-1	0.5 μg/ml cerulenin	DHA	3.4-fold	13.7 mg/l	Morita et al. (2005)
	M. marina MP-1	1.0 μg/ml cerulenin	DHA	3.1-fold	5.0 μg/g DCW	Morita et al. (2000)
	S. marinintestina Ik-1	7.5 μg/ml cerulenin	EPA	5-fold	8 mg/l	Morita et al. (2005)
	M. yayanosii DB21MT-5	12 μg/ml cerulenin	DHA	2.5-fold	19.5%	Fang et al. (2004)
	Recombinant *E. coli* DH5α	1 mg/l cerulenin	DHA	1.3-fold	2.4 mg/l	Peng et al. (2016)
	C. psychrerythraea	12 μg/ml cerulenin	DHA	1.4-fold	120 μg/ml	Wan et al. (2016)
	P. profundum DB110	12 mg/ml cerulenin	EPA	1.5-fold	13.0%	Allen et al. (1999)
Nitrogen supplementation	*M. marina* MP-1	Addition of 10 g/l tryptone and yeast extract	DHA	5-fold	55 mg/l	Kautharapu et al. (2013)
	S. putrefaciens MAC1	Addition of 10 g/l tryptone and 0.5% yeast extract	EPA	1.5-fold	34.7 mg/g DCW	Nikoopour and Griffiths (2008)
Limiting carbon source	*S. putrefaciens* MAC1	No addition of extra glucose	EPA	1.4-fold	15.57 mg/g DCW	Nikoopour and Griffiths (2008)
	Recombinant *E. coli* DH5α	No addition of extra glucose	EPA	5-fold	10%	Lee et al. (2008a)
Decreasing cultivation temperature	*P. profundum* SS9EA2	15 vs 4 °C	EPA	1.1-fold	29.5%	Allen et al. (1999)
	P. profundum SS9EA3	15 vs 4 °C	EPA	5.2-fold	8.3%	Allen et al. (1999)
	Shewanella sp. KMG427	25 vs 4 °C	EPA	1.9-fold	11.5%	Lee et al. (2008b)
	Shewanella olleyana sp. nov.	24 vs 4 °C	EPA	2.3-fold	23.6%	Skerratt et al. (2002)
	Vibrio pelagius	23 vs 4 °C	EPA	1.9-fold	8.7%	Abd Elrazak et al. (2013)
	S. piezotolerans $WP3^{LIV}$	20 vs 4 °C	EPA	1.8-fold	13.6%	Wang et al. (2009)
	Recombinant *E. coli* DH5α	20 vs 10 °C	DHA	7-fold	1.4 mg/l	Peng et al. (2016)

DCW dry cell weight

1999), *Shewanella olleyana* sp. nov. (Skerratt et al. 2002), *V. pelagius* (Abd Elrazak et al. 2013), *S. piezotolerans* WP3 (Wang et al. 2009), and recombinant *E. coli* DH5α (Peng et al. 2016). Changing to lower temperature could enhance the content of PUFAs up to 29.5% (Table 2).

Metabolic engineering in bacteria to enhance the EPA and DHA production

Although a few marine bacteria are able to produce PUFAs (Table 3), the yields are relatively low. The discovery of genetic transformation system in marine bacteria has provided essential ways to enhance the production of omega-3 PUFA through metabolic engineering. One of the metabolic engineering strategies involved is the disruption of competing pathway in PUFA biosynthesis. For example, disruption of the *torA* gene by transposon Tn5 mutagenesis in *Shewanella baltica* MAC1 deactivated the TorA enzyme in the trimethylamine-*N*-oxide (TMAO) reductase respiratory pathway (Amiri-Jami et al. 2006). Hence, the level of TMAO reductase might have been escalated in bacterial cells, and as a result, the anaerobic pathway of bacterial fatty acid biosynthesis elevated. Three *S. baltica* MAC1 mutants produced three to five times more EPA compared to the wild type (Amiri-Jami et al. 2006). Another strategy for metabolic engineering of PUFA production is by transferring the PUFA biosynthesis gene cluster (*pfaA-E*) from PUFA-producing marine bacteria into a few types of host, i.e., *E. coli*, *Synechococcus* sp., *Lactococcus lactis*, and *Pseudomonas putida* (Table 3). Heterologous expression of EPA gene cluster in *E. coli* has increased the level of EPA slightly as compared to the native host just because of the presence of multicopies of the biosynthesis genes. However, the production level was still low (Lee et al. 2006a). To increase the expression level, strong *lacZ* promoter was engineered in front of the *pfaA-D* genes (Lee et al. 2008b). Using different plasmids, the amount of EPA or DHA in engineered *E. coli* strains carrying the *Pfa* gene clusters from *Shewanella* spp. ranges from 0.2 to 22% and from 2.2 to 31.4 mg/g DCW, respectively (Takeyama et al. 1997; Yu et al. 2000; Orikasa et al. 2004; Lee et al. 2006; Orikasa et al. 2007; Amiri-Jami and Griffiths 2010; Amiri-Jami et al. 2015).

In the PKS pathway, the addition of precursors such as malonyl-CoA or acetyl-CoA in engineered microbial cells increased the release of free fatty acid *E. coli* strain (Gong et al.

Table 3 Summary of the metabolic engineering strategies of heterologous production of EPA and DHA in bacteria

Strain	Gene source	Product	Amount	Growth temperature (°C)	Time (h)	Reference
E. coli S17-1	*S. putrefaciens* SCRC-2738	EPA	3.3 mg/g DCW	20	ND	Takeyama et al. (1997)
E. coli JM109	*S. putrefaciens* SCRC-2738	EPA	5.7%	20	ND	Yu et al. (2000)
E. coli JM109	*S. pneumatophori* SCRC-2738	EPA	22%	15	ND	Orikasa et al. (2004)
E. coli XL1-Blue	*Shewanella oneidensis* MR-1	EPA	0.7%	20	34	Lee et al. (2006)
E. coli	*S. oneidensis* MR-1	EPA	7.5%	20	36	Lee et al. (2008a)
E. coli DH5α	*Moritella marina* MP-1	DHA	5.2%	15	96	Orikasa et al. (2006)
E. coli DH5α	*Shewanella* sp. strain SCRC-2738	EPA	12%	20	40	Orikasa et al. (2007)
E. coli DH5α	*M. marina* MP-1 and *S. pneumatophori* SCRC-2738	DHA	0.2%	15	96	Orikasa et al. (2009)
E. coli DH5α	*M. marina* strain MP-1 and *S. pneumatophori* SCRC-2738	EPA	9.2%	15	96	Orikasa et al. (2009)
E. coli EPI300T1	*S. baltica* MAC1	EPA	14%	15	120	Amiri-Jami and Griffiths (2010)
E. coli EPI300T1	*S. baltica* MAC1	DHA	0.4%	15	120	Amiri-Jami and Griffiths (2010)
E. coli Nissle1917	*S. baltica* MAC1	EPA	31.4 mg/g DCW	15	ND	Amiri-Jami et al. (2015)
E. coli DH5α	*C. psychrerythraea* 34H and *S. baltica* MAC1	DHA	2.2 mg/g DCW	15	ND	Peng et al. (2016)
Synechococcus sp.	*S. putrefaciens* SCRC-2738	EPA	0.6 mg/g DCW	17	24	Takeyama et al. (1997)
Synechococcus sp.	*S. putrefaciens* SCRC-2738	EPA	7.5 mg/g DCW	18	22	Yu et al. (2000)
Lactococcus lactis subsp. *cremoris* MG1363	*S. oneidensis* MR-1	DHA	1.35 mg/g DCW	15	24	Amiri-Jami et al. (2014)
Lactococcus lactis subsp. *cremoris* MG1363	*S. oneidensis* MR-1	EPA	0.12 mg/g DCW	15	24	Amiri-Jami et al. (2014)
Pseudomonas putida	*Aetherobacter fasciculatus* SBSr002	DHA	1.4 mg/g DCW	16	24	Gemperlein et al. (2016)

DCW dry cell weight, *ND* not determined

2014). Over-expressing acetyl-CoA carboxylase showed intracellular accumulation of malonyl-CoA, which led to a six-fold increase in the overall fatty acid formation (Davis et al. 2000). However, the addition of 2 mg/l desaturase cofactors NADPH and FAD increased the EPA production to 21 mg/g DCW (Nikoopour and Griffiths 2008).

Metabolic engineering of *Yarrowia lipolytica* to produce EPA

In Table 1, it is shown that marine bacteria engaged in the production of PUFAs but the yields were still low. Some metabolic engineering work in *E. coli*, *Synechococcus* sp., *Lactococcus lactis*, and *Pseudomonas putida* (Table 3) toward enhancing the production of PUFAs still the rate, the titer, and the yield from these organisms could not meet the requirement for commercial purposes are also presented in Table 3. However, *Yarrowia lipolytica* is the only metabolically engineered yeast today that could produce a sufficient amount of EPA as commercial products. It is also the best example of yeast products that substituted a fish-derived product (Xue et al. 2013; Xie et al. 2015). Among oleaginous microorganisms, *Yarrowia lipolytica* is the best investigated species due to both its fascinating features in biotechnology and its ability to be manipulated in the laboratory (Ledesma-Amaro and Nicaud 2016). Many studies suggested that *Yarrowia lipolytica* is a good organism not only for scientific investigation but also for industrial applications. There is a broad knowledge gathered on its genetics, molecular biology, and physiology, respectively (Xie et al. 2015). *Yarrowia lipolytica* has been categorized as "generally recognized as safe (GRAS)" for various industrial applications (Xie et al. 2015; Zhu and Jackson 2015).

Yarrowia lipolytica, the original strain with ATCC no. 20362, does not produce any omega-3 fatty acids. Its fatty acid profile demonstrates that it can synthesize linoleic acid (C18:2$n-6$). However, the wild-type strain was genetically engineered by DuPont to produce a high level of EPA for commercialization. The metabolic strategy used was overexpressing a mixture of enzymes (Δ-9 elongase, Δ-8 desaturase, Δ-5 desaturase, and Δ-17 desaturase) that are required for the synthesis of EPA through Δ9/Δ6 pathway. These genes were obtained from a different group of LCPUFA-producing microorganisms, viz. algae, euglenoids, and fungi (Xue et al. 2013). In addition, the interruption of peroxisome biogenesis contributed significantly to the formation of EPA and metabolism of storage lipid and the elimination of huge by-products (Xie et al. 2015). In DuPont research, three engineered strains with the capacity to produce a large amount of EPA were identified; the engineered Gen I strain Y4305 (Xue et al. 2013) produced EPA exceeding 15% of its DCW, the Gen II strain Z1978 (Hong et al. 2011) produced EPA exceeding 20% of its DCW, and lastly, the Gen III HP strain Z5567 (Hong et al. 2011) accumulated EPA exceeding 25% of its DCW. Two major commercial products were generated using these strains of yeast, namely New Harvest EPA-rich oil for a human nutritional supplement and Verlasso, a sustainably farmed salmon (Xue et al. 2013; Xie et al. 2015).

Concluding remarks and future perspective

EPA and DHA from marine bacteria offer more viable sources for PUFA production. In this review, production of PUFAs in marine bacteria, as well as strategies to achieve large quantity production of PUFAs, was described. In the postgenomic era, advances of omics technology have allowed for the development of novel strains through metabolic engineering, improving the quantity of desired products. However, to maximize the product yields for commercialization, this process cannot work in isolation; it requires the combination of other fields such as synthetic biology, system biology, genetic engineering, and computational modeling and simulation. Synthetic biology techniques may simplify gene modification and speed up metabolic engineering approaches, hence saving both time and resources. Systems biology can also assist to find novel, apparent targets of manipulation to boost the PUFA production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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