

## FAS or PKS, lipid biosynthesis and stable carbon isotope fractionation in deep-sea piezophilic bacteria

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Piezophilic microorganisms are prokaryotes that display optimal growth at pressures greater than 0.1 MPa (megapascal). Most of piezophilic bacterial isolates fall into the gamma-subgroup of the Proteobacteria and are affiliated with one of five genera *Shewanella*, *Photobacterium*, *Moritella*, *Colwellia*, and *Pyschromonas*. This paper reviews the taxonomy, characteristics of fatty acid biomarkers of deep-sea piezophilic bacteria and the relationships between fatty acids and bacterial phylogeny. The biosynthesis of fatty acids and carbon isotope fractionation is also discussed.

**Keywords:** piezophilic bacteria, deep-sea, lipids, carbon isotopes, fatty acid synthases, polyketide synthases

### 1. Taxonomy and phylogeny of deep-sea piezophilic bacteria

Piezophilic microorganisms have been isolated from many regions around the world, displaying pressure optima for growth that span the entire range of pressures existing in the ocean. Isolates of deep-sea piezophilic Archaea from deep-sea hydrothermal vent or Bacteria and Eukarya from cold deep-sea habitats have been obtained. Although only a handful of piezophilic Archaea have been cultured, these isolates span a broad collection of both the Euryarchaeota and the Crenarchaeota kingdoms [1–5]. In contrast, culture based studies of Bacteria (mostly from amphipods, fish, and sediment samples) have thus far resulted in the isolation of a narrow phylogenetic assemblage of gamma-Proteobacteria within the orders Alteromonadales and Vibrionales, including *Colwellia*, *Moritella*, *Photobacterium*, *Pyschromonas*, and *Shewanella* species [6–18]. Exceptions to the genera listed above include two reports of the isolation of a moderately piezophilic sulfate-reducing species, *Desulfovibrio profundus*, obtained from a deep sediment sample in the Japan Sea and from a hydrothermal vent chimney in the East Pacific Rise [19, 20], and a thermophilic member of the Thermotogales isolated from a vent [21]. Additionally, a piezophilic Gram-positive member of genus *Carnobacterium* has been described [22], that is related to a piezotolerant relative isolated from the deep seafloor sediment of the Nankai Trough [23]. It is believed that these isolates represent only a small fraction of the phylogenetic and physiological diversity present in hadal and abyssal environments. All of these “confirmed” inhabitants of the cold deep-sea form distinct clades within phyla of microbes from polar regions suggesting common ancestry and that adaptations to low temperature could be a pre-requisite for the initial acclimation to the deep-sea [22, 24]. However, these isolates probably only represent the copiotrophic opportunists (r-strategists) and new culturing approaches [25–27] will have to be developed in order to isolate other members of the community.

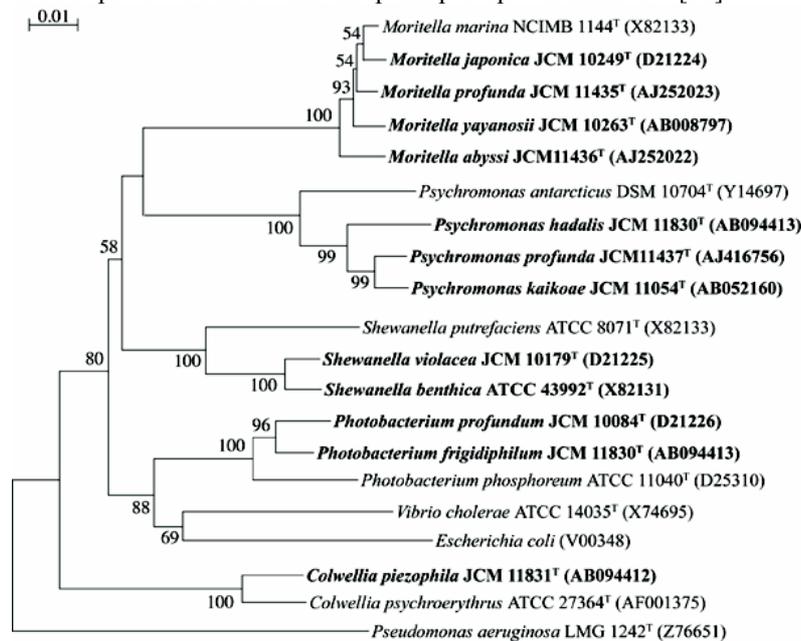
Thus far, most of piezophilic bacterial isolates fall into the gamma-subgroup of the Proteobacteria according to phylogenetic classifications based on 5S and 16S ribosomal RNA gene sequence information [6, 28, 29]. Those cultivated psychrophilic and piezophilic deep-sea bacteria were affiliated with one of five genera within the gamma-Proteobacteria subgroup: *Colwellia*, *Moritella*, *Photobacterium*, *Pyschromonas*, and *Shewanella* (see Fig. 2). Fig. 1 shows the phylogenetic relations between the taxonomically identified piezophilic species (shown in bold) and other bacteria within the

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gamma-Proteobacteria subgroup. The taxonomic features of the five piezophilic genera are described below.

**Taxonomy of the genus *Shewanella*.** Members of the genus *Shewanella* are Gram-negative, aerobic and facultatively anaerobic gamma-Proteobacteria [30]. The type strain of this genus is *Shewanella putrefaciens*, which is formerly known as *Pseudomonas putrefaciens* [30, 31]. *Shewanella* piezophilic strains, PT-99, DB5501, DB6101, DB6705, and DB6906, DB172F, DB172R, and DB21MT-2 were all identified as members of the same species, *S. benthica* [13, 24]. The psychrophilic and piezophilic *Shewanella* strains, including *S. violacea* and *S. benthica*, produce eicosapentaenoic acid (EPA) and thus the production of such long-chain polyunsaturated fatty acid (PUFA) is a property shared by many deep-sea bacteria to maintain the cell-membrane fluidity under conditions of extreme cold and high hydrostatic pressure environments [32]. *S. violacea* strain DSS12 has been studied extensively, particularly with respect to its molecular mechanisms of adaptation to high-pressure [33–35]. This strain is moderately piezophilic, with a fairly constant doubling time at pressures between 0.1 MPa and 70 MPa, whereas the doubling times of most piezophilic *S. benthica* strains change substantially with increasing pressure. As there are few differences in the growth characteristics of strain DSS12 under different pressure conditions, this strain is a very convenient deep-sea bacterium for use in studies on the mechanisms of adaptation to high-pressure environments. Therefore, the genome analysis on strain DSS12 has been performed as a model deep-sea piezophilic bacterium [36].



**Fig. 1** Phylogenetic tree showing the relationships between isolated deep-sea piezophilic bacteria (in bold) within the gamma-Proteobacteria subgroup determined by comparing 16S rRNA gene sequences using the neighbor-joining method. The scale represents the average number of nucleotide substitutions per site. Bootstrap values (%) are shown for frequencies above the threshold of 50%. Accession numbers were shown in parentheses. This figure is kindly provided by Y. Nogi, JAMSTEC.

**Taxonomy of the genus *Photobacterium*.** The genus *Photobacterium* was one of the earliest known bacterial taxa and was first proposed by Beijerinck in 1889 [37]. Phylogenetic analysis based on 16S rRNA gene sequences has shown that the genus *Photobacterium* falls within the gamma-Proteobacteria and, in particular, is closely related to the genus *Vibrio* [14]. *Photobacterium profundum*, a novel species, was identified through studies of the moderately piezophilic strains DSJ4 and SS9 [14]. *P. profundum* strain SS9 has been extensively studied with regard to the molecular mechanisms of pressure regulation [38] and subsequently genome sequencing and expression analysis [39]. Recently, *P.*

*frigidophilum* was reported to be slightly piezophilic: its optimal pressure for growth is 10 MPa [40]. Thus, *P. profundum* and *P. frigidophilum* are the only species within the genus *Photobacterium* known to display piezophilicity and the only two known to produce PUFA, eicosapentaenoic acid (EPA). No other known species of *Photobacterium* produces EPA [14].

**Taxonomy of the genus *Colwellia*.** Species of the genus *Colwellia* are facultative anaerobic and psychrophilic bacteria [41]. In the genus *Colwellia*, the only deep-sea piezophilic species reported was *C. hadaliensis* strain BNL-1 [41], although no public culture collections maintain this species and/or its 16S rRNA gene sequence information. Bowman et al. [42] reported that *Colwellia* species produce docosahexaenoic acid (DHA). We have recently isolated the obligately piezophilic strain Y223G<sup>T</sup> from sediment at the bottom of the deep-sea fissure of the Japan Trench, which was identified as *C. piezophila* [16]. This strain did not produce EPA or DHA, whereas high levels of unsaturated fatty acids (16:1 fatty acids) were produced.

**Taxonomy of the genus *Moritella*.** The type strain of the genus *Moritella* is *Moritella marina*, previously known as *Vibrio marinus* [43], which is one of the most common psychrophilic organisms isolated from marine environments. However, *V. marinus* has been reclassified as *M. marina* gen. nov. comb. nov. [44]. *M. marina* is closely related to the genus *Shewanella* on the basis of 16S rRNA gene sequence data but is not a piezophilic bacterium. Strain DSK1, a moderately piezophilic bacterium isolated from the Japan Trench, was identified as *Moritella japonica* [12]. This was the first piezophilic species identified in the genus *Moritella*. Production of DHA is a characteristic property of the genus *Moritella*. The extremely piezophilic bacterial strain DB21MT-5 isolated from the Mariana Trench Challenger Deep at a depth of 10,898 m was also identified as a *Moritella* species and designated *M. yayanosii* [11]. The optimal pressure for the growth of *M. yayanosii* strain DB21MT-5 is 80 MPa; this strain is unable to grow at pressures of less than 50 MPa but grows well at pressures as high as 100 MPa (Kato et al., 1998). Approximately 70% of the membrane lipids in *M. yayanosii* are unsaturated fatty acids, which is a finding consistent with its adaptation to very high pressures [11, 45]. Two other species of the genus *Moritella*, *M. abyssi* and *M. profunda*, were isolated from a depth of 2,815 m off the West African coast [17]; they are moderately piezophilic and the growth properties are similar to *M. japonica*.

**Taxonomy of the genus *Psychromonas*.** Deep-sea isolates of the genus *Psychromonas* are psychrophilic and are closely related to the genera *Shewanella* and *Moritella* on the basis of 16S rRNA gene sequence data. The type species of the genus *Psychromonas*, *Psychromonas antarctica*, was isolated as an aerotolerant anaerobic bacterium from a high-salinity pond on the McMurdo ice-shelf in Antarctica [46]. This strain did not display piezophilic properties. *Psychromonas kaikoa*, isolated from sediment collected from the deepest cold-seep environment with chemosynthesis-based animal communities in the Japan Trench at a depth of 7,434 m, is a novel obligatory piezophilic bacterium [15]. The optimal temperature and pressure for growth of *P. kaikoa* are 10°C and 50 MPa, respectively. This strain produces both EPA and DHA. *P. antarctica* does not produce PUFA (either EPA or DHA). In addition, a moderately piezophilic bacterium, *P. profunda* was isolated from Atlantic sediments at a depth of 2,770 m [18]. This strain is similar to the piezo-sensitive strain *P. marina*, which also produces small amounts of DHA.

## 2. Lipid characteristics of piezophilic bacteria

Lipids are important components of all living microorganisms. Lipids are relatively easily extracted, identified and quantified as compared to other major biochemical components, such as proteins and carbohydrates. Fatty acids in particular are useful biomarkers in this regard because they are present in every living cell and display great structural diversity.

The most abundant lipids detected in piezophilic bacteria are n-alkyl, acetogenic lipids (i.e., fatty acids; Table 1). The fatty acid profiles of a number of piezophilic bacteria have been determined [9, 32, 45, 47-51]. Fang et al. [32] reported detailed fatty acid compositions of cells of *Moritella japonica* strain DSK1, *Shewanella violacea* strain DSS12, *S. benthica* strain DB6705, *S. benthica* strain DB21MT-2 and *M. yayanosii* strain DB21MT-5 grown on Marine Broth 2216. Some of the fatty acids biosynthesized by piezophilic bacteria are also commonly found in surface bacteria: C<sub>12-19</sub> saturated, monounsaturated,

terminal methyl-branched,  $\beta$ -hydroxyl, and cyclopropane fatty acids [9, 32, 47, 52]. Characteristics of phospholipid fatty acids of piezophilic bacteria can be summarized as follows (Table 1):

(1) Piezophilic bacteria biosynthesize typical bacterial fatty acids: C<sub>14-19</sub> saturated, monounsaturated, terminal methyl-branched, hydroxyl, and cyclopropane fatty acids.

(2) Piezophilic bacteria produce abundant monounsaturated fatty acids with multiple positions of unsaturation and geometric configuration (*cis* and *trans*). The proportions of monounsaturated fatty acids can be up to 67% of the total fatty acids.

(3) Piezophilic bacterial species of the genera of *Shewanella*, *Moritella*, and *Photobacterium* all contain  $\beta$ -hydroxyl fatty acids (note that *P. profundum* SS9 synthesized 3.4% 3OH-12:0 [49], even though the type strain *P. profundum* JCM 10084<sup>T</sup> did not). All piezophilic bacterial isolates examined thus far are Gram-negative and the presence of hydroxyl fatty acids in piezophilic bacteria seems to be consistent with their Gram-negative nature.

(4) Piezophiles biosynthesize high amounts of terminal branched (*iso* and *anteiso*) fatty acids (TBFA) (Table 1). The concentrations of TBFA can be as high as 23% of the total fatty acids. Generally, the *iso*-branched fatty acids are in greater concentrations than *anteiso*-branched fatty acids [32]. It appears that only *Shewanella* and *Photobacterium* species produce TBFA (Table 1). Terminal branched fatty acids are typically found in Gram-positive bacteria (e.g., *Bacillus*) [53]. The presence of these branched fatty acids suggests that they may have a functional role in piezoadaptation.

(5) Piezophilic bacteria contain abundant long-chain polyunsaturated fatty acids, EPA (20:5 $\omega$ 3) and DHA (22:6 $\omega$ 3). Species (*Shewanella* and *Photobacterium*) that produce more monounsaturated fatty acids synthesize less PUFA, whereas those (*Colwellia*, *Moritella*, and *Psychromonas*) that produce more PUFA, branched- and hydroxy fatty acids synthesize less monounsaturated fatty acids.

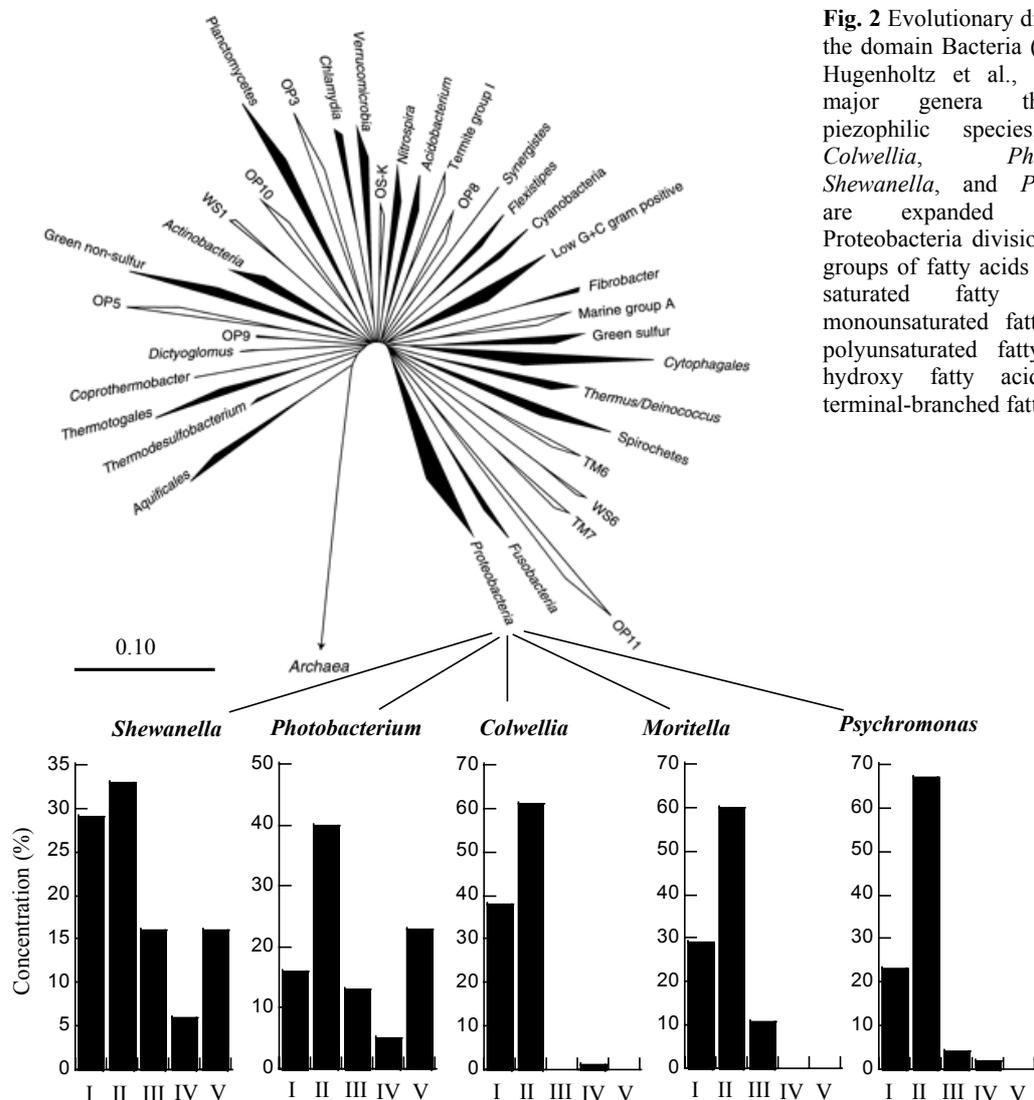
**Table 1** Whole-cell fatty acid composition (%) of the piezophilic isolates (type strains) of the five genera. Sh, *Shewanella benthica* ATCC 43992<sup>T</sup>; Ph, *Photobacterium profundum* JCM 10084<sup>T</sup>; Co, *Colwellia piezophila* Y223G<sup>T</sup>; Mo, *Molitella yayanosii* JCM 10263<sup>T</sup>; Ps, *Psychromonas kaikoe* JCM 11054<sup>T</sup>.

Fatty acid	Sh	Ph	Co	Mo	Ps
12:0	2	2	1	-*	1
14:0	13	3	3	15	6
15:0	-	1	3	1	1
16:0	14	9	31	13	15
18:0	-	1	-	-	-
iso-13:0	5	2	-	-	-
iso-14:0	-	4	-	-	-
iso-15:0	11	2	-	-	-
iso-16:0	-	15	-	-	-
14:1	-	3	9	6	10
15:1	-	2	2	2	2
16:1	31	31	50	53	55
18:1	2	9	1	1	2
3OH-12:0	1	5	1	-	2
3OH-iso-12:0	5	-	-	-	-
3OH-14:0	-	-	-	-	4
EPA (20:5 $\omega$ 3)	16	13	-	-	2
DHA (22:6 $\omega$ 3)	-	-	-	11	2
SAFA	29	16	38	29	23
MUFA	33	40	61	60	67
PUFA	16	13	0	11	4
OHFA	6	5	1	0	2
IBFA	16	23	0	0	0

\* Not detected.

Among the five genera of piezophilic bacteria, species of *Colwellia* and *Moritella* contain DHA, those of *Shewanella* and *Photobacterium* contain EPA, whereas piezophiles in the genus *Psychromonas* synthesize both EPA and DHA [15]. Some newly isolated piezophilic bacterial species of the genera *Colwellia* from the Japan Trench (e.g., *C. piezophila*) produce either no or low levels of EPA and DHA [16]. Clearly, fatty acid composition has provided important information in characterizing piezophilic bacteria in phylogeny and taxonomy [24], piezoadaptation [49, 51], and in biogeochemistry and geomicrobiology [30, 45, 50, 52]. Particularly, EPA and DHA, in combination with other fatty acids, may be used as biomarkers for detecting piezophilic bacteria in deep-sea sediment/water columns.

It has been suggested that a correlation exists between bacterial production of PUFA and the environmental conditions of their habitat [47, 48]. Temperature and pressure combined may have acted as the joint selection pressure for the evolution of bacteria with the ability to produce PUFA [54]. Production of PUFA is a characteristic of piezophilic as well as psychrophilic bacteria [9, 24, 32, 45, 48, 49, 50, 51, 55]. Marine bacteria that biosynthesize PUFA (either EPA or DHA) are Gram-negative bacteria and are distributed in two distinct phylogenetic lineages (Fig. 2). One is the marine genera of the  $\gamma$ -Proteobacteria which includes the genera *Shewanella*, *Colwellia*, *Moritella*, *Psychromonas*, and *Photobacterium*. The other lineage includes the two genera (*Flexibacter* and *Psychroserpens*) of the CFB (*Cytophaga-Flavobacterium-Bacteroides*) group [55]. PUFA-producers are piezophilic and/or psychrophilic. However, not all species of these groups produce PUFA. Bacteria in the genera of *Shewanella*, *Colwellia*, *Moritella*, *Psychromonas*, and *Photobacterium* are true psychrophilic and/or piezophilic [55] and may be the major PUFA-producers in the oceans [50]. Members of the genus *Shewanella* are not unique to marine environments. The deep-sea members of the genera *Shewanella* are different from their surface water counterparts of mesophilic and piezo-sensitive (growth inhibited by increasing pressure) species in that they produce higher amounts of unsaturated fatty acids, particularly PUFA [24]. Thus, the production of PUFA appears to be a unique trait of piezophilic/psychrophilic bacteria. This feature suggests the possible link between fatty acid biosynthesis and environmental conditions. Species in *Flexibacter* and *Psychroserpens* species are psychrophilic and halophilic but not piezophilic and their distributions are limited to the permanent cold areas of the Arctic [57] and Antarctica [42]. Some species of these genera also produce PUFA [42]. Thus, EPA and DHA can be used as an informative (but not exclusive) signature for detecting piezophilic bacteria in deep-sea sediment/water columns [52].



**Fig. 2** Evolutionary distance tree of the domain Bacteria (adapted from Hugenholtz et al., 1998). The major genera that contain piezophilic species, *Moritella*, *Colwellia*, *Photobacterium*, *Shewanella*, and *Psychromonas*, are expanded from the Proteobacteria division. The major groups of fatty acids shown are: I, saturated fatty acids; II, monounsaturated fatty acids; III, polyunsaturated fatty acids; IV, hydroxy fatty acids; and V, terminal-branched fatty acids.

### 3. Biosynthesis of fatty acids in piezophilic bacteria

Fatty acids are found in plants, animals, and microorganisms. Given the ubiquity of these important membrane components in biological systems, it is reasonable to assume that the biosynthetic pathway of fatty acids is relatively ancient [58].

Bacteria are known to synthesize fatty acids via the classic fatty acid synthase (FAS) pathway [59] with chain length ranging from C<sub>12</sub> to C<sub>19</sub>. The bacterial FAS pathways are divided into two distinct types called types I and II. In type I pathways which occur in some bacteria, the active sites catalyzing the fatty acid synthesis are found in distinct domains of large polyfunctional proteins. The type I FAS enzymes can have all of the active sites present in a single protein (as in mammals and mycobacteria) or split between two interacting proteins (as in fungi) [60]. The type II systems are found in most bacteria and plants. In the type II systems, each enzymatic activity is found as a discrete protein. These proteins form a dissociable multienzyme complex [61]. Each protein catalyzes an individual reaction in the pathway. The major building block for FAS is acetyl-ACP (acyl-carrier protein). Successive additions of

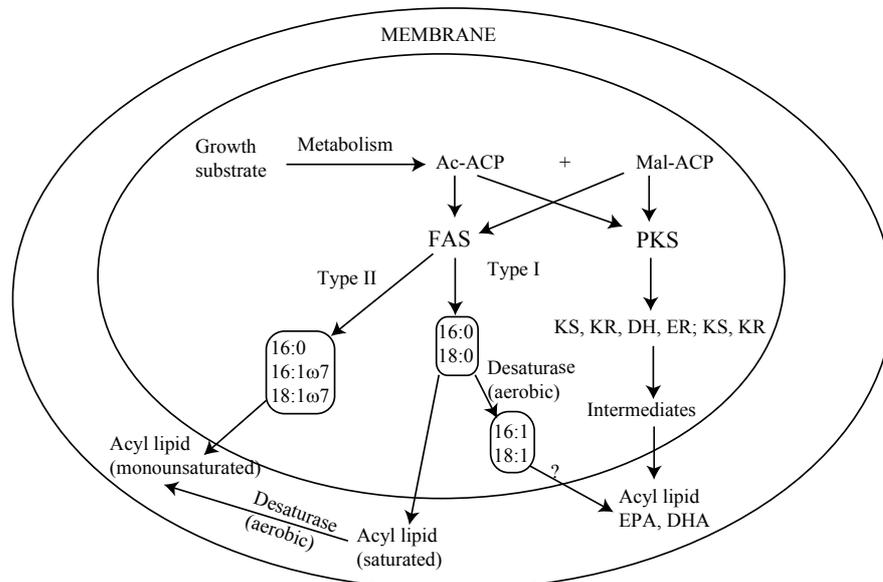
acetyl units produce palmitic acid (saturated C<sub>16</sub>). Monounsaturated fatty acids are synthesized by either the aerobic (by desaturases) or the anaerobic pathway (as a part of the cyclic process of C<sub>2</sub> unit additions after branching at the C<sub>10</sub> or C<sub>12</sub> hydroxy intermediate) [56].

Bacteria are long believed to be unable to produce polyunsaturated fatty acids, which have previously been ascribed exclusively to Eukaryotes [59]. The discovery of polyunsaturated fatty acids in marine bacteria [47, 48, 62, 63] has attracted marine microbiologists and biochemists to revisit the dogma of bacterial biosynthesis of polyunsaturated fatty acids [56]. The recent discovery of bacterial polyketide synthases (PKS) by which polyunsaturated fatty acids are biosynthesized is indeed ground-breaking [64, 65].

Polyketide synthases are classified into two types in a way resembling the classification of FAS [58]. Like type I FAS, type I PKS possesses a multidomain architecture of biosynthesis enzymes and the active sites are linearly arranged on a large module. Type II PKS consists of a dissociable complex of small, discrete monofunctional proteins and carry each catalytic site on a separate protein [58, 66, 67]. Both PKS and FAS use the same core of enzymatic activities and use ACP as a covalent attachment site for the growing carbon chain [64]. It is believed that the FAS and PKS biosynthetic pathways are evolutionary connected but probably diverged at an early stage during evolution [67]. Indeed, both biosynthetic pathways have strong homologies in lipid precursors (acetyl-CoA, malonyl-CoA) and the enzymes for chain propagation and processing [68–71]. Type I possesses a multidomain architecture similar to the type I FAS of fungi and animals; type II PKS have characteristics similar to those of FAS II found in bacteria and plants [58]. However, these two biosynthetic pathways are clearly distinct.

It is clear that the FAS-based biochemical pathway (aerobic or anaerobic) cannot explain the biosynthesis of PUFA by bacteria. Early studies by Nichols and Russell [72], Yazawa [73] and Nichols et al. [74] cleverly pointed to the potential existence of two distinct biosynthetic systems of fatty acids based on the double-bond isomeric pattern of monounsaturated fatty acids (which is characteristic of the anaerobic pathway in bacteria) and the methylene-interrupted PUFA (which are typically found in eukaryotic organisms) detected in bacteria (Russell and Nichols, 1999). Recent ground-breaking research by Metz et al. [64] and Wallis et al. [65] revealed the co-existence of two independent fatty acid biosynthetic systems operating in bacteria: the FAS- and PKS-based pathways. The former is the biosynthetic pathway common to the Bacteria which synthesizes typical bacterial fatty acids. The latter is a fundamentally different pathway which involves polyketide synthases [64] which catalyze the biosynthesis of long-chain polyunsaturated fatty acids (Fig. 3). The PKS pathway apparently act independently of FAS, elongase and desaturase activities to synthesize EPA and DHA without any reliance on fatty acyl intermediate such as 16:0-ACP (acyl carrier protein) [65].

PKS has been found in both prokaryotic and eukaryotic marine microbes [64]. In the Bacteria, strains that are able to produce PKS I include members of all major bacterial groups. In one study, PKS I genes were found in 21% of the 138 bacterial genomes surveyed [58]. Among the major groups of bacteria sequenced, the Proteobacteria contained the most of genomes that possess PKS I genes. In particular, the  $\gamma$ -Proteobacteria contained the most (55%) among the five subgroups of Proteobacteria [58]. It is, therefore, not surprising that the deep-sea piezophilic bacteria, mostly in the  $\gamma$ -Proteobacteria subgroup synthesize polyunsaturated fatty acids such as EPA and DHA. The PKS pathway appear to be widely distributed in marine bacteria [65] as genes with high homology to the *Shewanella* EPA gene cluster (*Shewanella* sp. SCRC-2738) [73] have been found in *Photobacterium profundum* strain SS9 which synthesizes EPA (Allen et al., 1999) and in *Moritella marina* strain MP-1 which contains DHA [75].



**Fig. 3** Summary of possible biosynthetic pathways of fatty acyl chains in piezophilic bacterial membrane lipids (modified from [56] and [65] with permission from the authors and the publishers). The saturated and monounsaturated fatty acids are synthesized by the FAS (fatty acid synthase) pathway common to members of the domain Bacteria which include the aerobic (Type I) and anaerobic (Type II) branches. The polyunsaturated fatty acids found in piezophilic bacteria are probably synthesized via the PKS (polyketide synthase) pathway which appears to be unique to marine bacteria. Biosynthesis of PUFA by an aerobic mechanism through sequential elongation and desaturation reactions appears less likely to occur in piezophilic bacteria. Ac-ACP, acetyl-acyl carrier protein; Mal-ACP, malonyl-acyl carrier protein; DH, dehydrase; ER, enoyl reductase; KR, 3-ketoacyl reductase; KS, 3-ketoacylsynthase [52].

#### 4. Carbon isotope fractionation in biosynthesis of fatty acids of marine bacteria

The  $\delta^{13}\text{C}$  of fatty acids can provide insight into lipid biosynthetic pathways. Fang et al. [50] determined carbon isotopic composition of fatty acids isolated from the hyperpiezophilic bacteria *Shewanella benthica* strain DB21MT-2 and *Moritella yanosii* strain DB21MT-5 grown on Marine Broth 2216. The variations of the  $\delta^{13}\text{C}$  values between fatty acids were nearly 8‰ and 14‰ for each strain, respectively. Despite the fact that the two strains were grown on the same medium and under the same temperature/pressure, DB21MT-2 showed a systematic enrichment of  $^{13}\text{C}$  in fatty acids compared to DB21MT-5 on a molecule-to-molecule basis. Polyunsaturated fatty acids (EPA and DHA) exhibited the most depleted  $\delta^{13}\text{C}$  values in both strains. All fatty acids except the odd-carbon-numbered ones from DB21MT-2 were depleted in  $^{13}\text{C}$  relative to bacterial growth substrate (Marine Broth 2216).

In a recent study, Fang et al. [76] examined carbon isotope fractionation in fatty acid biosynthesis in cells of *Moritella japonica* strain DSK1 grown on glucose. Two important findings include (1) carbon isotope fractionation in fatty acid biosynthesis is pressure-dependent; the higher the pressure for growth, the more the fractionation; and (2) PUFA had much more negative  $\delta^{13}\text{C}$  values than other short-chain saturated and monounsaturated fatty acids. It appears that the pressure-dependent carbon isotope fractionation is the result of the effects of high hydrostatic pressure on the kinetics of enzymatic reactions. Fatty acids are biosynthesized from the basic  $\text{C}_2$  unit acetyl-CoA (see discussion above). The magnitude of fractionation is determined by a kinetic isotopic effect ( $\epsilon_{\text{PDH}}$ ) [77]:

$$\epsilon_{\text{FA-substrate}} = (1 - f) \epsilon_{\text{PDH}}$$

where  $\epsilon_{\text{FA-substrate}}$  is carbon isotope fractionation between the substrate and fatty acids and  $f$  is the fraction of pyruvate flowing to acetyl-CoA [78]. This equation is invalid to biosynthesis of fatty acids by piezophilic bacteria because  $f < 0$  if the observed  $\delta^{13}\text{C}$  values of glucose and fatty acids are inserted into the equation. This suggests that the kinetic carbon isotope effect in biosynthesis of fatty acids of piezophilic bacteria,  $\epsilon_{\text{PDH}}$ , is greater than 23‰, a value commonly observed at atmospheric pressure of non-piezophilic bacteria [78]. The  $f$  value was as low as 0.1 and the corresponding  $\epsilon_{\text{PDH}}$  were 31, 35, and 40‰ at 10, 20, and 50 MPa for fatty acids. Therefore, carbon isotopic fractionation in the biosynthesis of fatty acids is pressure dependent. The observed more  $^{13}\text{C}$ -depleted  $\delta^{13}\text{C}$  values of PUFA can be attributed to the operation of the PKS-based pathways in piezophilic bacteria.

## 5. Conclusions

Certain deep-sea piezophilic bacteria synthesize long-chain polyunsaturated fatty acids, EPA and DHA. It appears that biosynthesis of PUFA can be attributed to an independently operating biosynthetic pathway in piezophilic bacteria – the PKS-based pathway. Carbon isotope fractionation in biosynthesis of fatty acids is pressure-dependent, possibly reflecting the effects of pressure on the kinetics of enzymes involved in the biosynthetic processes. The more depleted  $\delta^{13}\text{C}$  values of PUFA found in piezophilic bacteria may be a result of the PKS pathway. Fatty acid biomarkers and stable carbon isotope ratios of these compounds can aid in the characterization of piezophilic bacteria.

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