[1] J. H. Clark, *Green Chem.* **1999**, *1*, 1.
[2] J. O. Metzger, *Angew. Chem.* **1998**, *110*, 3145; *Angew. Chem. Int. Ed.* **1998**, *37*, 2975; M. Eissen, J. O. Metzger, E. Schmidt, U. Schneidewind, *Angew. Chem.* **2002**, *114*, 402; *Angew. Chem. Int. Ed.* **2002**, *41*, 414.
[3] R. Gedey, F. Smith, K. Westaway, H. Ali, L. Baldisera, L. Laberge, J. Roussel, *Tetrahedron Lett.* **1986**, *27*, 279.
[4] A. R. von Hippel, *Dielectric Materials and Applications*, MIT Press, Cambridge, MA, **1954**.
[5] C. Gabriel, S. Gabriel, E. H. Grant, B. S. J. Halstead, D. M. P. Mingos, *Chem. Soc. Rev.* **1998**, *27*, 213.
[6] R. S. Varma, *Green Chem.* **1999**, *1*, 43.
[7] S. Chaddick, *Tetrahedron* **1995**, *51*, 10403.
[8] C. R. Strauss, R. W. Trainor, *Aust. J. Chem.* **1995**, *48*, 1665; C. R. Strauss, *Aust. J. Chem.* **1999**, *52*, 83.
[9] N. Elander, J. R. Jones, S.-Y. Lu, S. Stone-Elander, *Chem. Soc. Rev.* **2000**, *29*, 239.
[10] A. Loupy, A. Petit, J. Hamelin, F. Texier-Boullet, P. Jacquault, D. Mathe, *Synthesis* **1998**, *9*, 1213.
[11] N. Kuhnert, *Nachr. Chem.* **2001**, *49*, 306.
[12] A. Loupy, D. Monteux, A. Petit, J. M. Aizpurua, E. Dominguez, C. Palomo, *Tetrahedron Lett.* **1996**, *37*, 8177.
[13] R. Laurent, A. Laportiere, J. Dubac, J. Berlan, S. Lefeuvre, M. Audhuy, *J. Org. Chem.* **1992**, *57*, 7099.
[14] R. N. Gedey, J. B. Wei, *Can. J. Chem.* **1998**, *76*, 525.
[15] P. Goncalo, C. Roussel, J. M. Milot, J. Vebrel, *J. Chem. Soc. Perkin Trans. 2* **1999**, 2111.
[16] I. Bagnell, M. Bliese, T. Cablewski, C. R. Strauss, J. Tsanaktsidis, *Aust. J. Chem.* **1997**, *50*, 921; J. Y. An, I. Bagnell, T. Cablewski, C. R. Strauss, R. W. Trainor, *J. Org. Chem.* **1997**, *62*, 2505.
[17] N.-F. K. Kaiser, U. Bremberg, M. Larhed, C. Moberg, A. Hallberg, *Angew. Chem.* **2000**, *112*, 3741; *Angew. Chem. Int. Ed.* **2000**, *39*, 3595.
[18] L. Öhberg, J. Westman, *Synlett* **2001**, 1296.
[19] A. Stadler, C. O. Kappe, *J. Chem. Soc. Perkin Trans. 2* **2000**, 1363.
[20] R. Gupta, A. K. Gutpa, S. Paul, P. L. Kachroo, *Ind. J. Chem.* **1995**, *34B*, 151.
[21] A. Dandia, M. Saha, H. Taneja, *J. Fluorine Chem.* **1998**, *90*, 17.
[22] A. Stadler, C. O. Kappe, *Eur. J. Org. Chem.* **2001**, 919.
[23] S. Anto, G. S. Gethvoldsen, J. R. Harding, J. R. Jones, S.-Y. Lu, J. C. Russell, *J. Chem. Soc. Perkin Trans. 2* **2000**, 2208.
[24] W.-M. Yau, K. Gawrisch, *J. Labelled Compd. Radiopharm.* **1999**, *42*, 709.
[25] N. Kuhnert, T. N. Danks, *Green Chem.* **2001**, *3*, 68.

Biosynthesis of Polyunsaturated Fatty Acids by Polyketide Synthases

Ursula Kaulmann and Christian Hertweck*

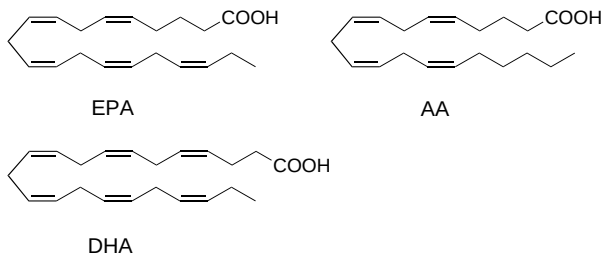
Nature's virtuosity of linking and tailoring simple carboxylic acid monomers to give a huge structural diversity of polyketide and fatty acid metabolites has been a fascinating subject for interdisciplinary research since the early works of Collie at the start of the 20th century.[1] During the past few decades a large body of knowledge has been established by using modern molecular biology methods, from which a deeper insight into the processes employed in fatty acid and polyketide biosynthesis has been gained.[2–4] Both biosynthetic pathways have strong homologies in the chemical mechanisms involved in chain extension and in the common pool of simple precursors (acetyl-CoA, malonyl-CoA),[5, 6] as well as in the character of the enzymes used for chain propagation and processing.[2, 7] In general, polyketides and fatty acids are constructed by repetitive decarboxylative Claisen ester condensations of an acyl-CoA starter unit with (methyl)malonyl-CoA units catalyzed by a β-ketoacyl synthase (KS). This process usually involves a (malonyl)acyl transferase (MAT/AT) and an acyl carrier protein (ACP). In fatty acid biosynthesis, subsequent β-oxo processing by a keto reductase (KR), dehydratase (DH), and an enoyl reductase (ER) generally yields a fully saturated acyl backbone. In contrast to this, the reductive steps in polyketide biosynthesis are partly or fully omitted, thus giving rise to a more complex pattern of functionalization. The elongation/reduction cycles are repeated until a defined chain length is obtained. The thioester-bound substrate is then released from the enzyme complex and may be subjected to further tailoring, such as desaturation (especially fatty acids), oxidation, glycosylation, or methylation (especially polyketides).[8] On the basis of genetic analyses and the architecture of the proteins employed, fatty acid synthases (FASs) and polyketide synthases (PKSs) are both generally classified into two types: type I, in which the active sites are linearly arranged on a large module, and type II, which consists of a dissociable complex of small, discrete monofunctional proteins.[9, 10] Despite remarkable similarities in their setup and functions, PKSs and FASs are clearly distinct in their detailed programming at the amino acid level, and—more importantly—they constitute a branch point between primary and secondary metabolism. It is thus a matter of speculation that both pathways diverged at an early stage during evolution.

In view of this knowledge, a recent discovery that long-chain polyunsaturated fatty acids (PUFAs) can be biosynthesized by bacterial polyketide synthases is ground-breaking news.[11] PUFAs have long been known as essential membrane components in the brain and retina[12, 13] as well as precursors of signaling molecules, such as the prostaglandins, thromboxanes, and leukotrienes,[14, 15] but—until recently—they were believed to be produced exclusively by eukaryotic organisms, not by bacteria. Recently, several research groups have independently identified PUFAs, such as eicosapentaenoic acid (EPA, 20:5n3), docosahexaenoic acid (DHA, 22:6n3), and arachidonic acid (AA, 20:4n6), from various psychro-

[*] Dr. C. Hertweck, Dr. U. Kaulmann
Hans-Knöll-Institut für Naturstoff-Forschung
Beutenbergstrasse 11a, 07745 Jena (Germany)
Fax: (+49) 3641-656-699
E-mail: hertweck@pmail.hki-jena.de

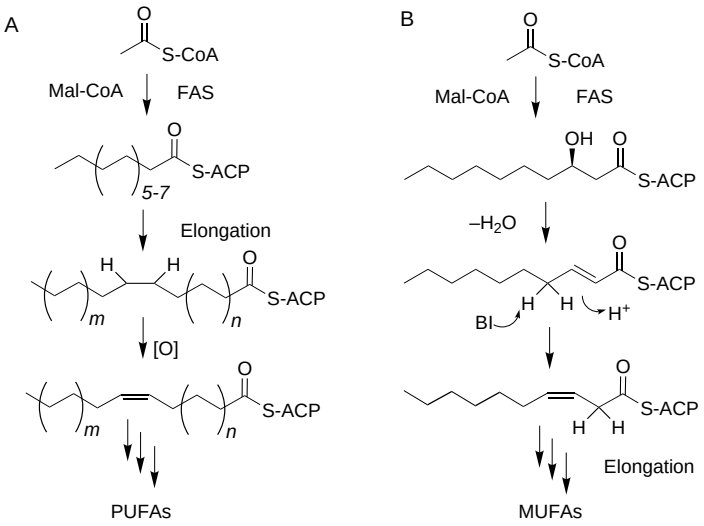
  1433-7851/02/4111-1866 $ 20.00+.50/0

philic bacteria (bacteria thriving at low temperatures), for example, *Shewanella hanedai*, *S. gelidimarina*, and *Colwellia psycherythraea* (Scheme 1).[16–20] As reviewed by Russell and Nichols, a long-existing dogma had thus been refuted, but it remained a mystery as to how these bacterial metabolites can be formed.[16]



Scheme 1. Structures of polyunsaturated fatty acids (PUFAs) found in several psychrophilic marine bacteria and in the protist *Schizochytrium*.

Two major routes are known for the introduction of double bonds into saturated fatty acids. In plants, mammals, and insects, double bonds are inserted by position-specific desaturases using molecular oxygen as a terminal electron acceptor (Scheme 2, route A). Several homoconjugated double



Scheme 2. Biosynthetic pathways (FASs) for unsaturated fatty acids. Route A: aerobic, found in plants and animals. Route B: anaerobic, found in microorganisms.

bonds can be introduced sequentially along this route.[21] In contrast, anaerobic bacteria may form a double bond during an incomplete reductive cycle of the FAS. Subsequently, a specific isomerase activity may catalyze a 2,3-*trans*/*cis* rearrangement (Scheme 2, route B). Unlike the aerobic route, only monounsaturated fatty acids (MUFAs) can be produced by this mechanism.[16] Although anaerobic and aerobic FAS pathways are in fact not mutually exclusive in a single organism,[22] bacterial PUFA biosynthesis remained unclear, since it can function under anaerobic conditions in several marine bacteria. The dilemma was that on the one hand an anaerobic pathway is not capable of introducing more than one double bond per fatty acid, and on the other hand the conventional aerobic pathway had to be excluded.[16] Consequently, it was assumed that another mechanism and/or an electron acceptor other than molecular oxygen had to be present, but it was probably too daring to speculate that methylene-interrupted double-bond patterns could be formed by an entirely different path.

How has this riddle been solved? Clustered genes involved in the synthesis of EPA were isolated for the first time in 1996, thanks to the efforts of Yazawa.[20] He identified five open reading frames (ORFs) from the EPA-producing bacterium *Shewanella* sp. strain SCRC2738, which proved to be necessary and sufficient for EPA production in *Escherichia coli*[23] and in a cyanobacterium (*Synechococcus* sp.).[23, 24] Only recently, the protein products encoded by the EPA cluster have been proposed by researchers of the same research group. Metz et al. identified eleven regions within the five open reading frames as putative enzyme domains.[11] The linear organization of the active site in the ORFs is reminiscent of that in iterative fungal PKSs or type I FASs. Instead of finding genes encoding for fatty acid synthesis, elongation, and aerobic desaturation, as was proposed by others to be involved in bacterial PUFA biosynthesis,[16] it was a great surprise to discover genes that suggest a mixed polyketide/fatty acid biosynthetic pathway exists. In fact, all keto synthases involved in PUFA biosynthesis are homologues of KSs found in bacterial modular type I PKSs, rather than being related to FAS keto synthases.[11] According to Metz et al., ORF5 codes for four PKS-related enzyme domains, which were proposed to function as KS, MAT, ACP (six repeats), and KR. A PKS-related phosphopantetheine transferase and a PKS-related AT are encoded by ORF2 and ORF6, respectively. ORF7 comprises two PKS-related genes (KSs) and two FAS-like genes, which are homologues of the *E. coli* dehydratase FabA. Interestingly, while one KS appears to be fully functional, the second KS lacks a cysteine residue in the active site and is thus assumed to be inactive. This situation is strikingly similar to the iterative type II PKS subunits KS_α and KS_β, the latter also often referred to as the chain length factor (CLF).[25] ORF8 finally codes for a FAS-related putative enoyl reductase.

Actually, genes with a high homology to the *Shewanella* gene cluster have been found in other PUFA-containing marine bacteria which indicates that the novel PKS pathway may be widely distributed in these organisms. In 1999, Tanaka et al. found four ORFs in the DHA-producing bacterium *Moritella marina* strain MP-1 (formerly *Vibrio marinus* strain MP-1), which had high homology with ORFs 5–8 of the EPA biosynthesis cluster.[26, 27] At that time, Tanaka et al. had already exploited information from the databases and compared the organization of both the EPA and DHA gene clusters. They found that the only difference in the latter was two more regions encoding putative KSs, and five instead of six repetitive copies of ACP. Furthermore, an EPA-deficient strain of the deep-sea bacterium *Photobacterium profundum* SS9 was constructed successfully by gene disruption experiments based on the known EPA ORF3/4 gene sequence of *Shewanella* sp. SCRC2738, which provided another indication of the common distribution of the novel PUFA pathway among psychrophilic marine bacteria.[28] Metz et al. have also

  1433-7851/02/4111-1867 $ 20.00+.50/0

shown for a eukaryote, the marine protist *Schizochytrium*, that DPA and DHA biosynthesis does not necessarily involve membrane-bound desaturases or fatty acid elongation enzymes. Sequences that are located on three ORFs exhibited homology to eight of the eleven domains of *Shewanella* (Figure 1). A horizontal gene transfer is likely to have occurred.[11]

What might a mixed PKS/FAS pathway for bacterial PUFA synthesis look like? In this respect it is useful to recall the function of FabA in *E. coli*.[29] FabA is one of the two β-hydroxyacyl-ACP dehydratases involved in the biosynthesis of fatty acids in *E. coli*. After the acyl chain length has reached ten carbon atoms, FabA is responsible for diverting the flow of intermediates into the unsaturated fatty acid branch of the pathway. Besides catalyzing the dehydration, FabA also isomerizes *trans*-2-decenoyl-ACP to *cis*-3-decenoyl-ACP, which by-passes the following reduction step (ER). The unsaturated acyl-ACP is loaded onto FabB (one of the two β-ketoacyl-ACP synthases) to initiate the first cycle of elongation in the unsaturated fatty acid synthesis. Since the *Shewanella* DHs show high homology to FabA, at least some of the double bonds in EPA from *Shewanella* sp. SCRC2738 are probably introduced by a dehydrase/isomerase mechanism. Furthermore, it has been shown by heterologous expression of the cluster in *E. coli* that EPA biosynthesis occurs anaerobically.[11]

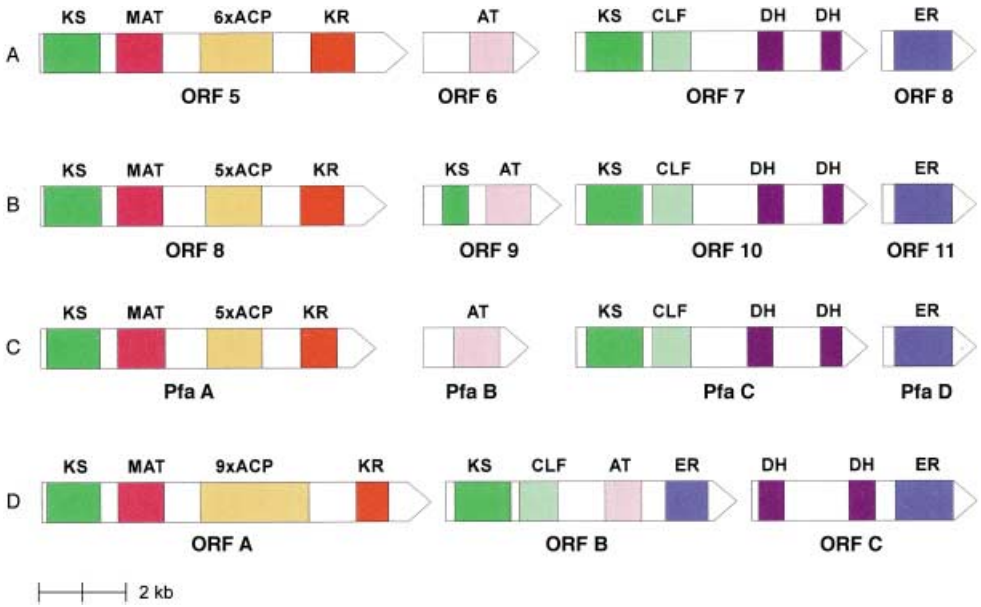
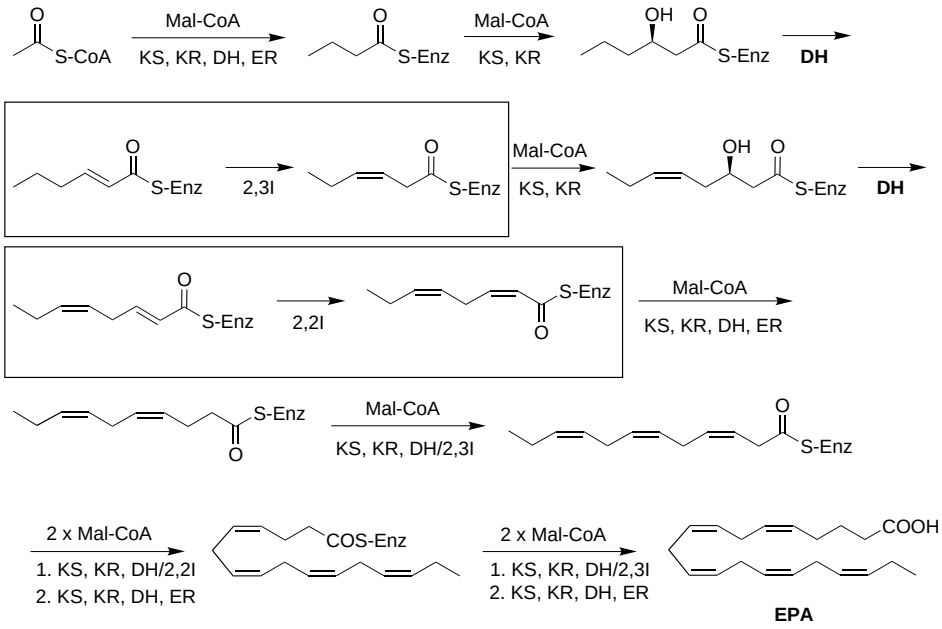


Figure 1. Organization of the core regions of PUFA biosynthetic gene clusters; A: *Shewanella* sp. SCRC-2738 (GenBank accession no.: U73935.1);[11] B: *Moritella marinus* strain MP-1 (GenBank accession no.: AB025342.1);[26, 27] C: *Photobacterium profundum* strain SS9 (GenBank accession no.: AF409100, unpublished data); D: *Schizochytrium* (GenBank accession nos.: AF378327, AF378328, AF378329).[11]



Scheme 3. Proposed biosynthetic pathway of EPA in *Shewanella* sp. with putative intermediates and key catalytic activities employed. Mal-CoA: malonyl-CoA; KS: keto synthase; KR: keto reductase; DH: dehydratase; ER: enoyl reductase; 2,3I: 2,3-isomerase; 2,2I: 2,2-isomerase.

One of the plausible schemes that can be derived from what we know so far was proposed by Metz et al. (Scheme 3): The first elongation step from a putative acetyl-CoA starter molecule and malonyl-CoA as well as a complete cycle of reduction is catalyzed by KS, KR, DH, and ER. The following elongation step occurs by a keto reduction to the β-hydroxy-
ester. The FabA-like bifunctional dehydratase/isomerase would then catalyze dehydration as well as the *trans*-2,3-*cis* rearrangement of the acyl intermediate. In analogy to the requirement of a special β-ketoacyl-ACP synthase in *E. coli* for the elongation of the 3-*cis*-acyl-ACP intermediate, a designated KS might be responsible for chain propagation. After subsequent reduction and dehydration steps, a methylene-interrupted double-bond pattern would require that a *trans*-2,2-*cis* isomerization occurs. A similar reaction is known for the regeneration of 11-*cis*-retinol. For EPA biosynthesis, the same set of reactions is then repeated twice, with the last 2,3-isomerization being followed by an elongation in which a full reductive cycle is employed (Scheme 3). In analogy, the formation of DHA and AA may be explained with only minor modifications. However, the proposed pathway needs to be

  1433-7851/02/4111-1868 $ 20.00+.50/0

verified and studied in more detail by further biochemical analyses. As for fungal iterative type I PKSs, it is completely unresolved how chain length and degree of reduction is controlled at specific steps. Also, the function of multiple ACPs (5–9 copies!) for PUFA biosynthesis is enigmatic.

What benefit will the discovery of PUFA gene clusters in marine psychrophilic bacteria have for us? The finding and functional analysis of these novel gene clusters is relevant for several reasons: It is advantageous to the biotechnological production of PUFAs from marine bacteria. Since there is medical evidence for the relevance of PUFAs to prevent, for example, artheriosclerosis and heart disease, there is a great demand on such drugs.[16, 30] Psychrophilic bacteria could be used as a primary PUFA-rich feedstock in artificial food chains in the aquaculture industry, thereby protecting natural fish stocks. Gene transfer to industrially relevant producer organisms will be a further solution to the supply of PUFAs.[24] Apart from the commercial aspect, PUFA genes will help in bacterial taxonomy to redefine old classifications, to create new groupings, and to trace-back evolution.[17, 31] New strains with active and silent copies of the PUFA genes can be tracked by using molecular biology tools, and more might be learned about the world-wide distribution of strains with PUFA-biosynthesis gene clusters and their ecological importance.[18, 32] Last, but not least, the genes for PUFA biosynthesis are distinct from previously recognized PKSs in both structure and mechanism as well as the novel putative dehydrases/isomerases, and may thus provide new tools for combinatorial biosynthesis of polyketide antibiotics.[33–35]

[1] R. Bentley, J. W. Bennett, *Annu. Rev. Microbiol.* **1999**, *53*, 411.
[2] J. Staunton, K. J. Weissman, *Nat. Prod. Rev.* **2001**, *18*, 380.
[3] D. O'Hagan, *The Polyketide Metabolites*, Ellis Horwood, Chichester, **1991**.
[4] J. W. Campbell, J. E. J. Cronan, *Annu. Rev. Microbiol.* **2001**, *55*, 305.
[5] B. J. Rawlings, *Nat. Prod. Rep.* **1998**, *15*, 275.
[6] B. S. Moore, C. Hertweck, *Nat. Prod. Rep.* **2002**, *19*, 70.
[7] M. P. Crump, J. Crosby, C. E. Dempsey, J. A. Parkinson, M. Murray, D. A. Hopwood, T. J. Simpson, *Biochemistry* **1997**, *36*, 6000.
[8] C. Khosla, R. S. Gokhale, J. R. Jacobsen, D. E. Cane, *Annu. Rev. Biochem.* **1999**, *68*, 219.
[9] D. A. Hopwood, *Chem. Rev.* **1997**, *97*, 2465.
[10] J. L. Harwood, N. J. Russell, *Lipids in Plants and Microbes*, George Allen and Unwin, London, **1984**.
[11] J. G. Metz, P. Roessler, D. Facciotti, C. Levering, F. Dittrich, M. Lassner, R. Valentine, K. Lardizabal, F. Domergue, A. Yamada, K. Yazawa, V. Knauf, J. Browse, *Science* **2001**, *293*, 290.
[12] L. Lauritzen, H. S. Hansen, M. H. Jorgensen, K. F. Michaelsen, *Prog. Lipid Res.* **2001**, *40*, 1.
[13] M. McConn, J. Browse, *Plant J.* **1998**, *15*, 521.
[14] A. Heller, T. Koch, J. Schmeck, K. van Ackern, *Drugs* **1998**, *55*, 487.
[15] R. A. Creelman, J. E. Mullet, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 355.
[16] N. J. Russell, D. S. Nichols, *Microbiology* **1999**, *145*, 767.
[17] D. Nichols, J. Bowman, K. Sanderson, C. Mancuso Nichols, T. Lewis, T. McMeekin, P. D. Nichols, *Curr. Opin. Biotechnol.* **1999**, *10*, 240.
[18] F. DeLong, A. A. Yayanos, *Appl. Environ. Microbiol.* **1986**, *51*, 730.
[19] K. Watanabe, C. Ishikawa, I. Ohtsuka, M. Kamata, M. Tomita, K. Yazawa, H. Muramatsu, *Lipids* **1997**, *32*, 975.
[20] K. Yazawa, *Lipids* **1996**, *31*, S297.
[21] H. Sprecher, Q. Chen, *Prostaglandins Leukotrienes Essent. Fatty Acids* **1999**, *60*, 317.
[22] M. Wada, N. Fukunaga, S. Sasaki, *J. Gen. Appl. Microbiol.* **1991**, *37*, 355.
[23] R. Yu, A. Yamada, K. Watanabe, K. Yazawa, H. Takeyama, T. Matsunaga, R. Kurane, *Lipids* **2000**, *35*, 1061.
[24] H. Takeyama, D. Takeda, K. Yazawa, A. Yamada, T. Matsunaga, *Microbiology* **1997**, *143*, 2725.
[25] B. J. Rawlings, *Nat. Prod. Rep.* **1999**, *16*, 425.
[26] M. Tanaka, A. Ueno, K. Kawasaki, I. Yumoto, S. Ohgiya, T. Hoshino, K. Ishizaki, H. Okuyama, N. Morita, *Biotechnol. Lett.* **1999**, *21*, 939.
[27] N. Morita, M. Tanaka, H. Okuyama, *Biochem. Soc. Trans.* **2000**, *28*, 943.
[28] E. E. Allen, D. Facciotti, D. H. Bartlett, *Appl. Environ. Microbiol.* **1999**, *65*, 1710.
[29] R. J. Heath, C. O. Rock, *J. Biol. Chem.* **1996**, *271*, 27795.
[30] R. S. Lees, *Food Sci. Technol.* **1990**, *37*, 1.
[31] D. S. Nichols, P. D. Nichols, N. J. Russell, N. W. Davies, T. A. McMeekin, *Biochim. Biophys. Acta* **1997**, *1347*, 164.
[32] D. S. Nichols, P. Hart, P. D. Nichols, T. A. McMeekin, *Aquaculture* **1996**, *147*, 115.
[33] L. Rohlin, M. K. Oh, J. C. Liao, *Curr. Opin. Microbiol.* **2001**, *4*, 330.
[34] P. F. Leadlay, *Curr. Opin. Chem. Biol.* **1997**, *1*.
[35] M. Chartrain, P. M. Salmon, D. K. Robinson, B. C. Buckland, *Curr. Opin. Biotechnol.* **2000**, *11*, 209.

  1433-7851/02/4111-1869 $ 20.00+.50/0