World J Microbiol Biotechnol (2014) 30:1869–1878
DOI 10.1007/s11274-014-1607-2

ORIGINAL PAPER

Antarctic microorganisms as source of the omega-3 polyunsaturated fatty acids

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Received: 17 July 2013 / Accepted: 17 January 2014 / Published online: 29 January 2014
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Abstract Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are long-chain polyunsaturated fatty acids (PUFAs) that belong to the omega-3 group. They are essential fatty acids found in phospholipid of cell membranes. There is strong evidence that these nutrients may also favorably modulate many diseases. Primary sources of omega-3 PUFAs in the human diet are fish and fish-derived products. The fishing industry worldwide, however, is becoming unable to satisfy the growing demand for these PUFAs. A promising cost-effective alternative source of PUFAs is bacterial production. We identified 40 Antarctic marine bacterial isolates by 16S rRNA gene sequence analysis. Fifteen genera in three phyla were represented in the collection. Isolates were tested for ability to produce EPA using a method in which their ability to reduce 2,3,5-triphenyltetrazolium chloride (TTC) is determined and by gas chromatography coupled to mass spectrometry (GC–MS). All isolates could reduce TTC, and GC–MS analysis showed that four produced EPA and that six produced DHA. We show for the first time that isolates identified as *Cellulophaga, Pibocella* and *Polaribacter* can produce EPA and DHA, only DHA or only EPA, respectively. One isolate, *Shewanella* sp. (strain 8-5), is indicated to be a good candidate for further study to optimize growth and EPA production. In conclusion, a rapid method was tested for identification of new EPA producing strains from marine environments. New EPA and DHA producing strains were found as well as a potentially useful PUFA production strain.

Keywords Antarctic marine bacteria · Psychrophile and psychrotrophic microorganism · Polyunsaturated fatty acids · EPA · DHA

Electronic supplementary material The online version of this article (doi:10.1007/s11274-014-1607-2) contains supplementary material, which is available to authorized users.

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Introduction

Docosahexaenoic acid (DHA) 22:6(n-3) and eicosapentaenoic acid (EPA) 20:5(n-3) are essential polyunsaturated fatty acids (PUFAs) that belong to the omega-3 group. These fatty acids are vital constituents of human metabolism and there is abundant evidence linking their health-beneficial properties and dietary consumption (Bang et al. 1976; Kromann and Green 1980; Burr et al. 1989). PUFAs confer flexibility, fluidity and selective permeability to cell membranes. The brain is particularly rich in arachidonic acid (ARA) and DHA, the latter also being a ligand for a retinoid X receptor (de Urquiza 2000). EPA has a beneficial effect on the cardiovascular system. PUFAs in membrane phospholipids are precursors for biosynthesis of prostaglandins, leukotrienes and thromboxanes. These compounds bind to specific G-protein-coupled receptors signalling diverse cellular responses, e.g., inflammation, vasodilation, blood pressure, pain and fever (Funk 2001). PUFAs, derivatives and analogues are thus important neutraceutical and pharmaceutical targets (Colquhoun 2001).

PUFAs cannot be synthesized by humans, and must be supplied in the diet (Gayathri et al. 2010; Nichols et al.

2010; Nikoopour and Griffiths 2008; Petrie and Singh 2011). Omega-3 PUFAs produced for consumption are mainly from fish and shellfish. Accumulation of omega-3 PUFAs in marine fish tissue is actually due to the intake of PUFAs produced de novo by marine microorganisms. Today, over 10 tons of fish oil is produced every year. Ninety percent of this oil is destined for aquafarming (Petrie and Singh 2011; Sijtsma and de Swaaf 2004; Wijesundera et al. 2011). Demand for omega-3 PUFAs continues to grow as applications for use in food and pharmaceutical industries increases and as fish populations decline throughout the world. Fish oil purification is a very expensive process and the final products have unpleasant taste and smell. Such difficulties have prompted a search for alternative ways to produce omega-3 PUFAs (Amiri-Jami et al. 2006; Gayathri et al. 2010; Lewis et al. 1999; Petrie and Singh 2011; Sijtsma and de Swaaf 2004; Wijesundera et al. 2011; Yokochi et al. 1998).

Bacterial production of long chain PUFAs is a promising alternative approach for large scale production (Shene et al. 2010; Das 2008; Shin et al. 2007). Bacteria are easily cultured and stored, and are also a renewable source of PUFAs (Sijtsma and de Swaaf 2004; Nichols et al. 1993). Production strains can be genetically modified to optimise high level production of specific PUFAs. (Amiri-Jami et al. 2006; Bergé and Barnathan 2005; Hinzpeter et al. 2006; Nichols et al. 1999).

PUFA producing bacteria have frequently been found in very deep ocean habitats with high pressure and very low temperatures. Adaption for producing PUFAs is believed to be part of a strategy to survive such extreme conditions. These bacteria must be able to change protein, sterol, hopanoid and carotenoid content as well as fatty acid composition in the cell membrane. Homeoviscous adaptation is a process that allows cells to change the degree of saturation of these fatty acids to preserve membrane fluidity. This adaptation may involve cis–trans isomerisation, changes in degree of saturation/unsaturation and increase/decrease chain length relationships between fatty acids. Psychrophilic microorganisms survive extremely cold conditions by incorporation of specific fatty acids into the membrane to preserve fluidity and to enable transport of nutrients (de Carvalho and Fernandes 2010; Nogi 2011). EPA and DHA have been used as biomarkers for phylogenetic classification of psychrophilic and piezophilic bacteria because of the limited habitat range in which they are found (Bergé and Barnathan 2005; Freese et al. 2009; Morita et al. 2005; Okuyama et al. 2007; Patnayak and Sree 2005). In addition, marine bacteria having the ability to produce PUFAs have been reported only in some genera in Gammaproteobacteria and Flavobacteria. In the first class, these include *Shewanella, Moritella, Colwellia, Alteromonas* and *Photobacterium.* The second class is represented by *Flexibacter* and *Psychroserpens* (Bergé and Barnathan 2005; Freese et al. 2009; Nichols et al. 1993, 2002; Nogi 2011). Not all species within these genera produce PUFAs (Sani et al. 2010). *Shewanella* is one of the most studied for PUFA production, and species include: *S. hanedai, S. gelidimarina, S. violacea* and *S. béntica* (Nogi 2011); *S. piezotolerans* (Yin and Gao 2011); *S. livingtonensis* (Kawamoto et al. 2009); *S. báltica* (Lee et al. 2009); *S. oneidensis* (Lee et al. 2009; Jeong et al. 2006); *S. marinintestina* (Morita et al. 2005) and *S. japonica* (Ivanova et al. 2001).

Several studies have shown that Antarctic microorganisms produce relatively high proportions of PUFAs including either EPA or DHA, and may be part of an adaptation strategy for extreme cold in this ecosystem (Nichols et al. 1999, 2002; Russell and Nichols 1999; Fogliano et al. 2010). Isolation and characterization of Antarctic marine microorganisms may provide new useful strains for omega-3 PUFA production. In this study, 40 marine Antarctic isolates were identified by16S rRNA gene sequence analysis. These isolates were further characterized for potential ability to produce EPA (Ryan et al. 2010). This was done by testing isolates for ability to reduce "2,3,5-triphenyltetrazolium chloride" (TTC) to triphenyl formazan (TF) and by gas chromatography and mass spectrometry (GC–MS).

Materials and methods

Bacterial strains and culture conditions

Marine bacteria were isolated off the coast of King George Island (KGI) (62°02′S, 58°21′W) December 2008 and 2010. All isolates were grown and purified as single colonies on Marine 2216 Agar solid medium containing per liter 5 g peptone, 1 g yeast extract, 40 g sea salt, 0.008 g Na_2HPO_4, 0.0016 g NH_4NO_3, 0.10 g ammonium ferric citrate and 15 g agar. Plates were incubated at 4 °C for 4–6 days and culture purity was confirmed.

Isolate identification

Forty bacterial isolates were identified by sequence analysis of a defined region of the 16S rRNA gene. Genomic DNA was extracted from cultures grown on Marine 2216 Agar plates for 6 days using the phenol–chloroform method (Alippi and Aguilar 1998). PCR of 16S rRNA gene fragments was done in 40 µL reaction mixtures containing 20 µL of "Fast PCR Master Mix (2X) (Fermentas)", 1.0 µM each of forward primer 27F (5′-AGAGTTTGATC MTGGCTCAG-3′) and reverse primer 1492R (5′-TACG GYTACCTTGTTACGACTT-3′), genomic DNA and water to final volume. PCR reactions were done in a Palm-

Cycler TM (Corbett Research UK Ltd) as follows: initial denaturation at 94 °C 120 s, then 35 cycles of 15 s at 94–96 °C, 30 s at 50 °C, 90 s 70 °C, and a final extension step 300 s at 70 °C. PCR products were analyzed by electrophoresis on 1 % agarose gels. DNA sequencing was done by Macrogen Inc (Korea) using universal primers 27F, 518F and 1492R. DNA sequences obtained from each isolate were aligned by CLUSTALW (Higgins et al. 1994) using MEGA 5.2.2 (Tamura et al. 2011). Assembled DNA sequence data was analyzed by BLASTn (Altschul et al. 1990) and compared with non-redundant nucleotide sequence database (nr/nt) at the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis

Multiple alignments were generated with DNA sequences obtained using CLUSTALW (Higgins et al. 1994). Phylogenetic distance trees were inferred by Maximum-Likehood (ML, Tamura-Nei substitution model) and Neighbour-Joining (NJ, p-distance matrix) analyses, using MEGA 5.2.2 (Tamura et al. 2011). Confidence in topologies was assessed using bootstrapping (1,000 replicates).

Analysis of EPA content using TTC

EPA production by bacteria can be determined by first growing them in Peptone-Yeast extract-Meat extract (PYM) (Yazawa 1996), and then adding TTC to the medium. Bacteria able to produce EPA are also capable of reducing TTC to TF, indicated by the appearance of red color (Ryan et al. 2010). Test tubes with 5 mL PYM medium were inoculated with a given strain, incubated at 4, 15 and 20 °C for 4 days, and then TTC was added 0.1 % (w/v). Cultures were incubated an additional hour at the same temperature, and appearance of red color recorded.

Extraction and analysis of lipids by GC–MS

Strains were cultured in 250 mL Modified Marine Broth containing per liter 5 g peptone, 2 g yeast extract, 20 g sea salt, 0.02 g Na_2HPO_4, 0.05 g $MgSO_4·7H_2O$ and 1 g glucose, at 25 °C and 190 rpm for 48 h. Cells were harvested by centrifugation and lipids were extracted from 1 to 2 g wet biomass according to Burja, et al. (2007). Fatty acid methyl esters (FAME) were prepared and analyzed using AOCS Official Methods Ce 2-66, Ch 1-91 and AOCS Ce 1-62. Samples were analyzed with a gas chromatograph having capillary column Agilent 112-88A7 HP-88 (100 m long, 0.25 mm diam, 0.20 µm film) and Helium as carrier gas (0.5 ml/min). The GC was equipped with a mass spectrometer as detector (Agilent Technologies Mass Selective Detector 5,973 inert). The injection volume used was 5 µl in the scan mode. The oven temperature program was 120 °C for 1 min, increased by 10 °C/min to 175 °C, and then to 240 °C at 3 °C/min. FAME were identified by comparing their retention times with standard mixtures (FAME MIX 47885U, Supelco) and their mass spectrum. Relative fatty acid content (%) was estimated as percentage total peak area obtained by MSDChem. Fatty acid concentrations were calculated by adding a known amount of internal standard (C23:0 methyl ester) and assuming detector response was the same for all fatty acids.

Results and discussion

Forty bacterial isolates were identified and characterized; 37 were Gram-negative rods and 3 were Gram-positive cocci. Sequence analysis of nearly the complete 16S rRNA gene (~1,400 bp) indicated that most were closely related to previously described bacteria (97–100 % similarity except strain 8-5 having 95 % similarity). The affiliation of each isolate, based on BLASTn analysis is shown in Table 1: *Pseudoalteromonas* spp. (1-4, 2-6, 3-3, 3-10, 4-2, 7-1, 7-8, 9-2, 11-5, 2A, 2B, 4B, 5A), *Pibocella* spp. (1-6, 7-9) *Zobellia* spp. (3-7, 10-1), *Granulosicoccus* sp (3-9), *Flavobacterium* spp. (5-1, 14B, 14E), *Colwellia* spp. (7-6, 8-3), *Donghaeana* sp. (7-10), *Cellulophaga* spp. (8-2, 8-6, 9-1, 12-3, 9D, 11B, 15C), *Winogradskyella* sp. (8-2p), *Shewanella* sp. (8-5), *Polaribacter* spp. (5D, 15E), *Psychrobacter* sp. (8A), *Psychromonas* sp. (9A, 3A), *Psychroserpens* sp. (9C) and *Planococcus* sp. (11A). The genera with the greater number of isolates are *Pseudoalteromonas* (13, 32. 5 %) and *Cellulophaga* (7, 17. 5 %).

NJ distance trees shown in Figs. 1 and 2 compares sequences of all isolates in orders Alteromonadales, Pseudomonadales, Flavobacteriales. Also included are sequences of reference strains having highest similarity in the GenBank database at NCBI. Both NJ and ML algorithms gave similar topologies (Figs. 1, 2, 1S, 2S in supplemental material).

Analysis of 16S rRNA gene sequences from isolates and sequences deposited in the GenBank database (Table 1) in general, agree with the close relation inferred from the distribution of sequences in the phylogenetic trees (Figs. 1, 2, 1S, 2S). All isolates were assigned to three phyla (Fig. 3): Bacteroidetes, Firmicutes and Proteobacteria. Proteobacteria are represented by the genera *Psychrobacter, Granulosicoccus, Shewanella, Colwellia, Pseudoalteromonas and Psychromonas.* Within this phylum, Antarctic strains of genera *Pseudoalteromonas, Colwellia* and *Shewanella* have previously been reported to be PUFA producers (Bowman et al. 1997; Gentile et al. 2003; Kawamoto et al. 2009; Russell and Nichols 1999).

Table 1 Ability of the antartic marine isolates to reduce the TTC to TF

Clone name/accession number	Database microorganism with highest similarity (% identity)	In solid medium	In liquid medium		
		4 °C	4 °C	15 °C	20 °C
1-4/KF245428	*Pseudoalteromonas* sp. KJF 3-7 (99 %)	+++(GI)	+++	+++	++
1-6/KF365469	*Pibocella* sp. SS9.10 (99 %)	NG	+++	+++	NG
2-6/KF266768	*Pseudoalteromonas* sp. IC2-76 (99 %)	+++	+++	+++	+++
3-3/KF245429	*Pseudoalteromonas* sp. *KJF 3-7* (99 %)	+++(GI)	+++	+++	++
3-7/KF365471	*Zobellia laminariae* KOPRI_22206 (99 %)	+++(GI)	++	++	–
3-9/KF365480	*Granulosicoccus antarcticus* IMCC3135 (99 %)	+++(GI)	+++	+++	+++
3-10/KF228901	*Pseudoalteromonas* sp. AG52 (99 %)	+++	+++	+++	+++
4-2/KF228899	*Pseudoalteromonas* sp. SM495 (99 %)	+++	+++	+++	+++
5-1/KF365464	*Flavobacterium frigidarium* strain NCIMB 13737 (99 %)	NG	+	+++	+++
7-1/KF266769	*Pseudoalteromonas* sp. ArcB853011 (100 %)	+++	+++	+++	++
7-6/KF365475	*Colwellia* sp. JAM-GA24 (98 %)	+++(GI)	++	NG	NG
7-8/KF228900	*Pseudoalteromonas citrea* CIP 105339 (99 %)	+++(GI)	+++	+++	+++
7-9/KF365470	*Pibocella ponti* SS9.10 (99 %)	+++(GI)	+++	+++	+
7-10/KF365477	*Donghaeana dokdonensis* KOPRI_22265 (99 %)	NG	NG	+	NG
8-2/KF266770	*Cellulophaga lytica* strain DSM 7489 (97 %)	+++	+++	+++	+++
8-2p/KF365481	*Winogradskyella* sp. Gap f 141 (99 %)	+++(GI)	++	+	–
8-3/KF365474	*Colwellia* sp. IE7-5 (99 %)	+++	++	+	NG
8-5/KF365473	*Shewanella livingstonensis* KOPRI22225 (95 %)	+++	+++	+++	+++
8-6/KF266771	*Cellulophaga lytica* strain DSM 7489 (97 %)	+++	+++	+++	+++
9-1/KF270631	*Cellulophaga lytica* strain DMS 7489 (97 %)	+++	+++	+++	+++
9-2/KF228902	*Pseudoalteromonas* sp. KJF 2-15 (99 %)	+++	+++	+++	+++
10-1/KF365472	*Zobellia ulginosa* strain S 4-12 (99 %)	++	+++	+++	+++
11-5/KF245430	*Pseudoalteromonas* sp. KJF 3-7 (99 %)	+++(GI)	++	+++	+
12-3/KF270632	*Cellulophaga lytica* strain DSM 7489 (97 %)	+++	+++	+++	+++
2A/KF266767	*Pseudoalteromonas* sp. KJF 8-1 (99 %)	+++	+++	+++	+++
2B/KF228903	*Pseudoalteromonas* sp. KJF 9-4 (99 %)	+++	+++	+++	+++
3A/KF365483	*Psychromonas sp. KJF 14-6* (99 %)	+++(GI)	++	+	–
4B/KF234447	*Pseudoalteromonas* sp. MelAa3 (99 %)	+++	+++	+++	++
5A/KF245427	*Pseudoalteromonas* sp. KFJ 9-4 (99 %)	+++(GI)	+++	+++	+++
5D/KF365468	*Polaribacter* sp. SW 2-4 (99 %)	NG	NG	++	++
8A/KF365479	*Psychrobacter* cryohalolentis K5 (99 %)	+++(GI)	+++	+++	++
9A/KF365482	*Psychromonas arctica KOPRI 24931* (99 %)	NG	+	+	+
9C/KF365476	*Psychroserpen mesophilus* KOPRI22160 (98 %)	+++(GI)	+++	+++	++
9D/KF270634	*Cellulophaga algicola* strain DSM 14237 (99 %)	+++(GI)	++	+++	+++
11A/KF365478	*Planococcus* sp. SS1.3 (99 %)	+++(GI)	+++	++	+
11B/KF270635	*Cellulophaga algicola* strain DSM 14237 (99 %)	+++(GI)	+++	+++	+
14B/KF365465	*Flavobacterium frigidarium* strain NCIMB 13737 (99 %)	NG	+	+++	+
14E/KF365466	*Flavobacterium frigidarium* strain NCIMB 13737 (99 %)	+++(GI)	+	++	+++
15C/KF270633	*Cellulophaga lytica* strain DSM 7489 (97 %)	+++	+++	+++	+++
15E/KF365467	*Polaribacter* sp. SW 2-4 (99 %)	+++(GI)	+++	+++	+++

NG not growing; *GI* growth inhibition, *positive test* + orange, ++ pale red and +++ intense red

Firmicutes is represented by genus *Planococcus*, and Bacteroidetes is represented by the largest number of genera: *Pibocella, Zobellia, Flavobacterium, Donghaeana, Cellulophaga, Winogradskyella, Polaribacter* and *Psychroserpens*. Strains of these genera have also been isolated from Antarctica (Russell and Nichols 1999; Bowman 2006), and PUFA production has been reported only in *Psychroserpen*.

The capacity for isolates to produce EPA was evaluated by the method of Ryan et al. (2010). This is a rapid

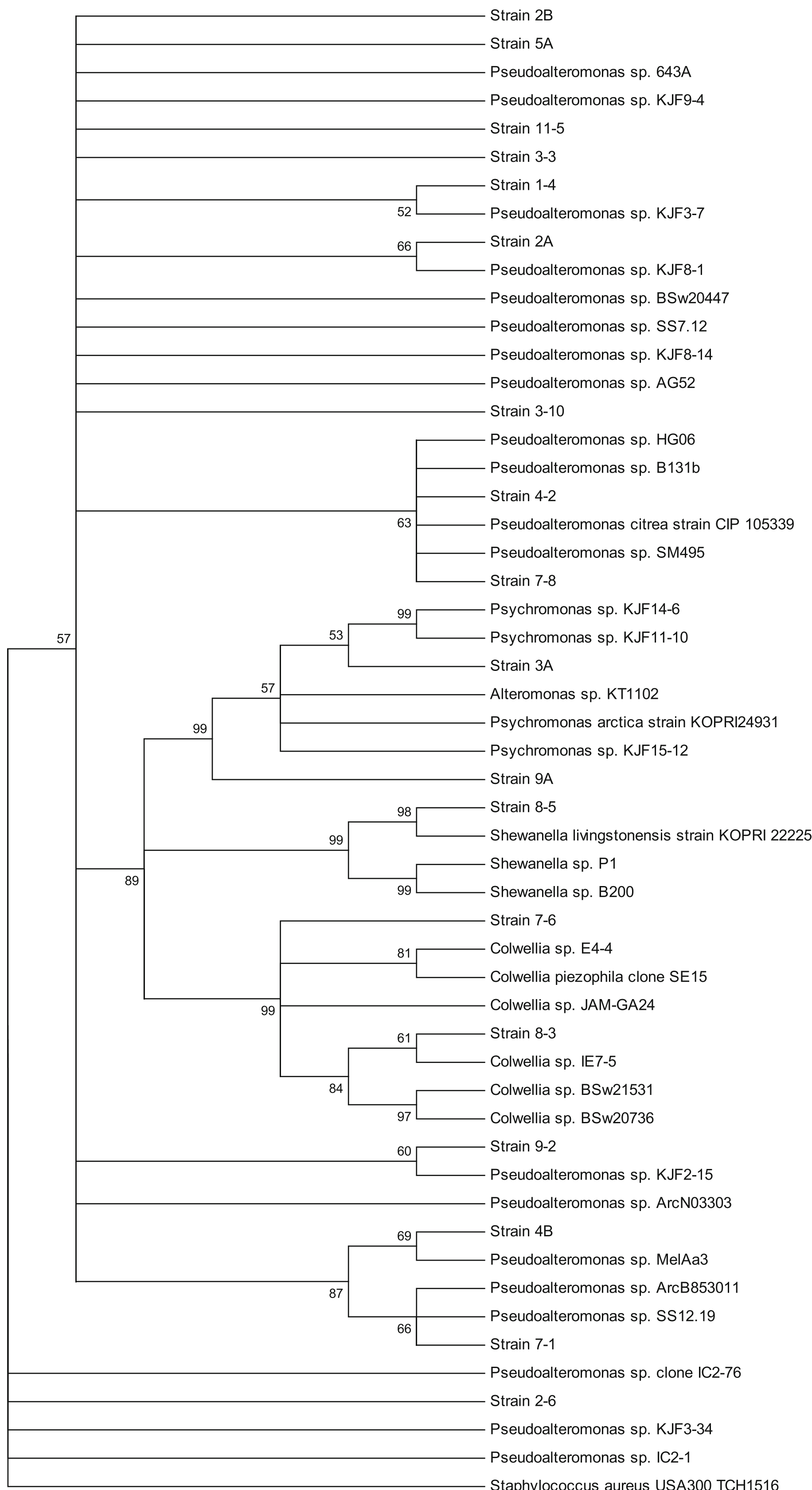


Fig. 1 Rooted phylogenetic tree showing isolates of the order Alteromonadales and Pseudomonadales with highest similarity from the GenBank database of the NCBI and the 18 operational taxonomic units that were used in this analysis. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees (>50 %) in which the associated taxa clustered together in the bootstrap test is shown next to the branches. *Staphylococcus aureus* USA 300 TCH 1516 was used as an outgroup taxon

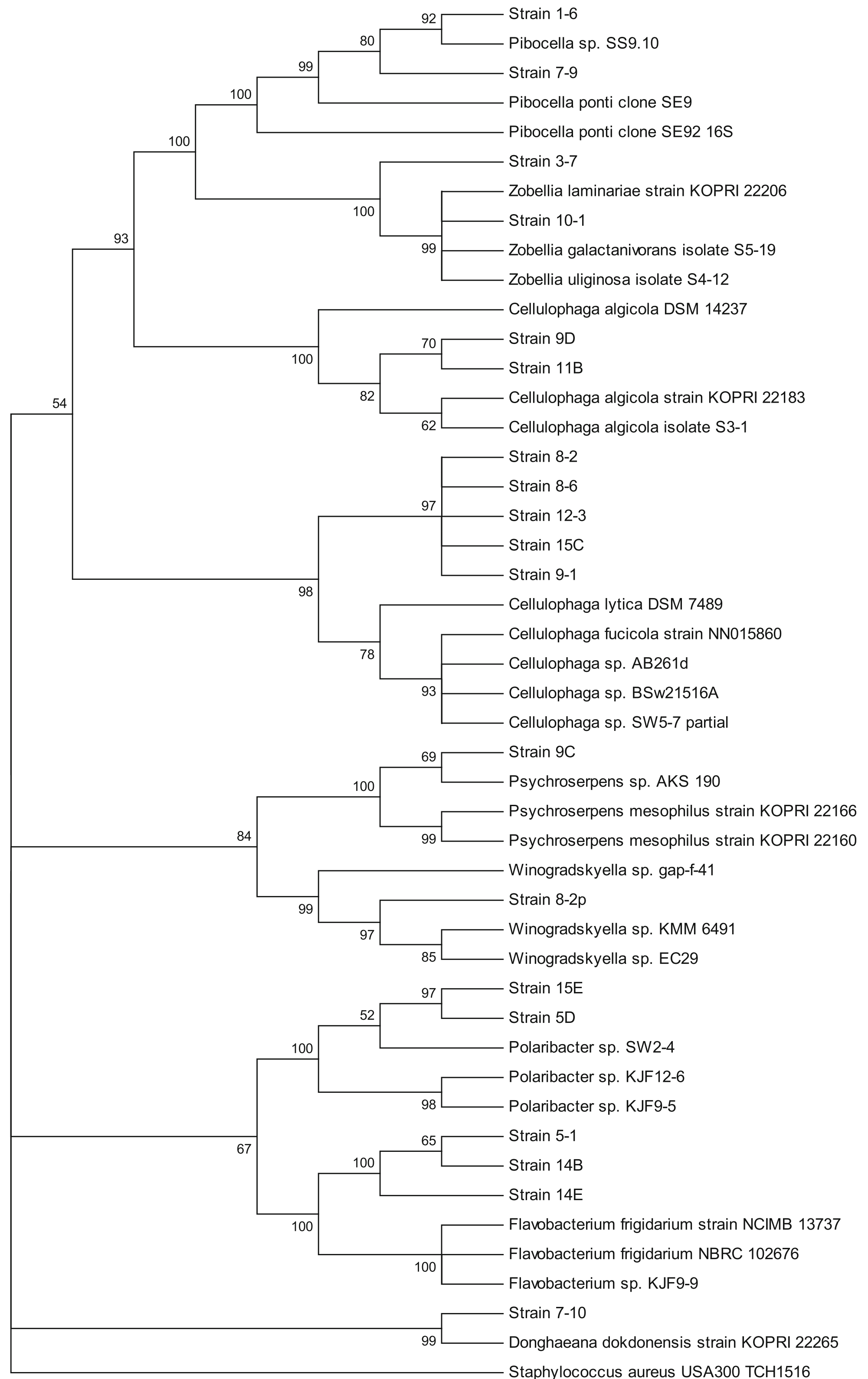
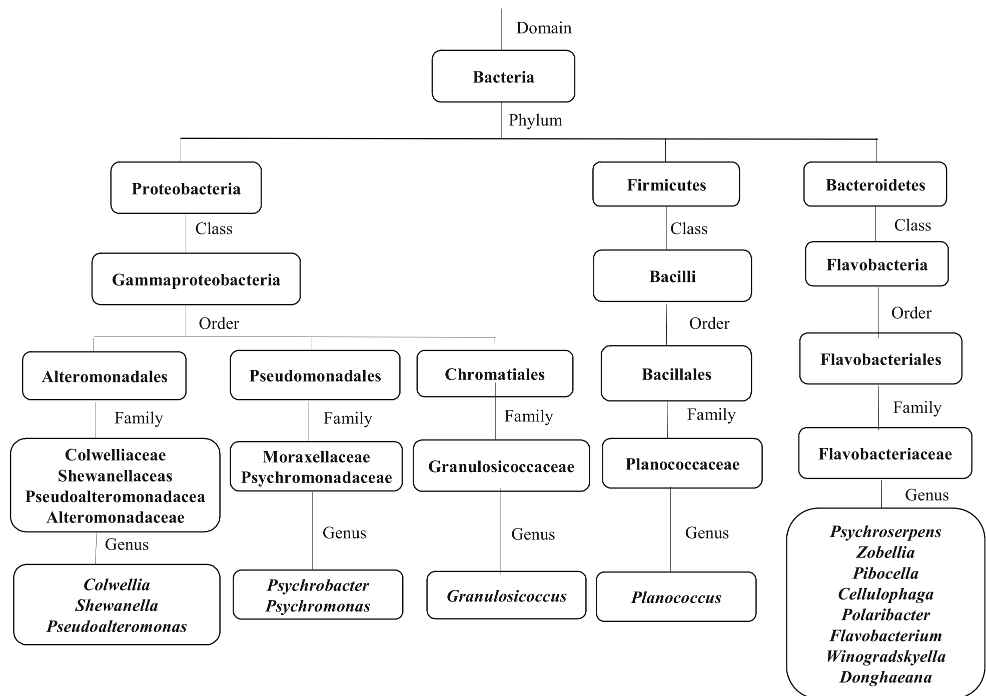


Fig. 2 Rooted phylogenetic tree showing isolates of the order Flavobacteriales with highest similarity from the GenBank database of the NCBI and the 19 operational taxonomic units that were used in this analysis. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees (>50 %) in which the associated taxa clustered together in the bootstrap test is shown next to the branches. *Staphylococcus aureus* USA 300 TCH 1516 was used as an outgroup taxon

screening method that detects enzymatic activity that can be associated with EPA biosynthesis. The method has been successfully used to screen for EPA-producing marine bacteria. The ability to reduce TTC to TF was shown to be directly associated with EPA production in marine Gram-negative bacteria in solid or liquid medium. Bacterial

Fig. 3 Taxonomic categories to which the isolates were assigned



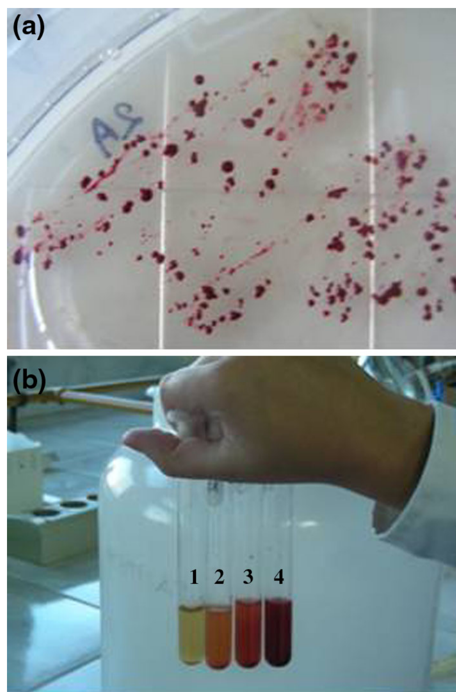


Fig. 4 Reduction of TTC to TF **a** *solid* medium AM 2216, positive (+++) strain 2 A; **b** *liquid* medium PYM, *1*—negative, *2*—positive (+), *3*—positive (++) y *4*—positive (+++)

Table 2 GC–MS detection of EPA and DHA in the ten strains selected

Clon name	mg/g EPA ([a])		mg/g DHA ([a])	
Shewanella sp. 8-5	1.80	1.58	8.60×10^{-3}	8.89×10^{-3}
Colwellia sp. 8-3	0	0	0.01	9.93×10^{-3}
Cellulophaga sp. 9-1	0	0	0	3.68×10^{-3}
Cellulophaga sp. 9D	0.89	0.48	0	0.19
Pseudoalteromonas sp. 9-2	–	–	0	0
Pseudoalteromonas sp. 7-1	0.32	0.07	0.09	0.07
Psychroserpen sp. 9C	0	0	–	–
Flavobacterium sp. 5-1	0	0	0	0
Polaribacter sp. 15E	0	0.31	0	0
Pibocella sp. 7-9	0	0	0	0.54

Assays performed in duplicate; (–) Duplicates not obtained

[a] mg of EPA or DHA/g of cell dry weight

colonies that grow on an agar medium containing TTC becomes red when they able to reduce this compound to TF. Testing isolates previously grown in broth medium was preferable in cases where growth was inhibited by TTC. In this case, TTC is added after growth in the broth culture and red color may develop within an hour. *S japonica* DSM15915 was used as positive control in these experiments (Ivanova et al. 2001). Single colonies of strain DSM15915 grown on agar media containing TTC develop an intense red color. All our isolates that were able to grow on solid medium with TTC also produced colonies with an intense red color (Fig. 4a).

TTC reduction activity in isolates was also tested in liquid medium. The broth cultures were inoculated and incubated 3 days at different temperatures, 4, 15 and 20 °C, since it is known that EPA production may vary

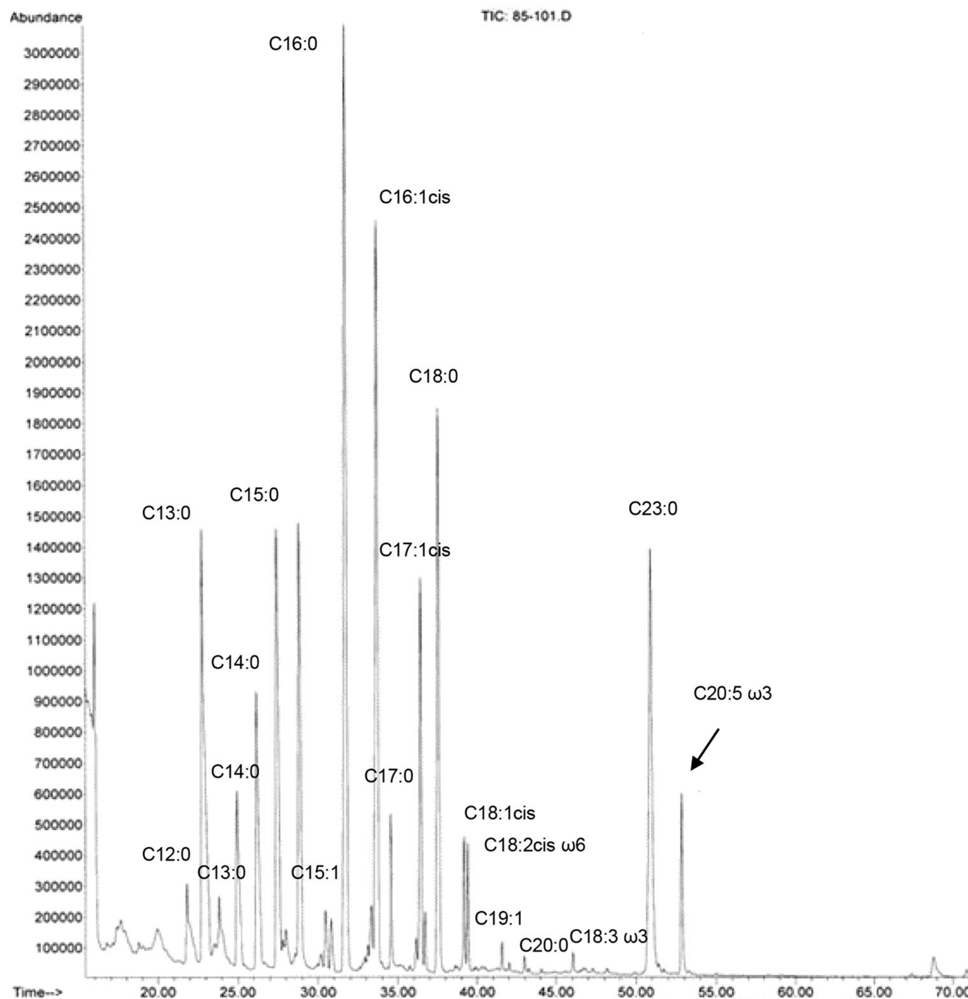


Fig. 5 GC–MS profile of fatty acids methyl esters from strain 8-5. The arrow indicates the peak of EPA

with growth temperature (Freese et al. 2009; Ivanova et al. 2001). Results in Table 1 show that red color intensity varied between isolates and for different temperatures measured. The color intensity is reported as: (+) orange, (++) pale red and (+++) intense red (Fig. 4b). The highest levels of biomass produced and TTC reduction were obtained at 15 °C (30 isolates had a score of +++). We found that all 37 Gram-negative isolates were able to reduce the TTC to TF. If reduction of TTC to TF in marine Gram-negative bacilli is related to EPA production, as predicted by Ryan and co-workers, all of our Gram-negative isolates should be able to produce EPA. These isolates belong to 13 genera, most of which have not been reported to produce EPA. Reduction of TTC to TF has also been used as an indicator of metabolic activity in soil, among other things (Dinamarca et al. 2007; Casida et al. 1964).

To test the prediction of Ryan et al., the ten isolates having highest TTC to TF reduction activity were analyzed to determine EPA production levels. Total fatty acids were prepared from each isolate, and the profiles were obtained by GC–MS. We show that only four of the ten isolates analyzed were confirmed to be capable of EPA production (Table 2). Predominant fatty acids found in the ten isolates were straight-chain saturated fatty acids. The most abundant was palmitic acid (C16:0), except for strain 9-2, followed by monounsaturated fatty acids, next dienoic fatty acids and finally polyunsaturated fatty acids. Strains 7-1 and 8-5 produced eight and five different PUFAs, whereas strains 9C and 5-1 did not contain PUFAs. Strains 8-5, 9D and 7-1 produced both EPA and DHA. Strain 15E produced only EPA, strains 8-3, 9-1 and 7-9 produced only DHA, and strain 9-2 was unable to produce EPA or DHA.

The best EPA production was in *Shewanella* sp. (8-5), with an average EPA content of 1.69 mg/g cell dry weight, and 2 % of total fatty acids (Fig. 5). Results agree with those reported for another strain of *Shewanella* sp. (Freese et al. 2009; Gentile et al. 2003; Kawamoto et al. 2009), where EPA content was 5 and 0.7 % of total fatty acids when cells were grown at 4 and 18 °C, respectively. Strain 8-5 is predicted to be a good production strain in that it

produced four times the amount of EPA produced by the positive control strain *S. japonica* DSM15915 (1.69 mg/g versus 0.40 mg/g).

The best DHA producer was *Pseudoalteromonas* sp. 7-1, with an average content of 0.08 mg/g. This level of DHA production was much smaller compared with that obtained using strain DSM15915 (0.08 vs. 0.72 mg/g). This level of DHA production is also very small compared with production in some strains of *Thraustochytrium* sp., 30–277 mg/g DHA (Bergé and Barnathan 2005). *Colwellia* sp. (8-3) also produced low amounts of DHA (0.01 mg/g), in contrast with production levels by other strains of this genus reported to be producers (Bowman et al. 1997; Nogi 2011).

Conclusions

Forty marine bacterial isolates from the coast of King George Island were identified by 16S rRNA gene analysis. Thirty-seven were Gram-negative representatives of Bacteroidetes and Proteobacteria and all of these could reduce TTC to TF. GC–MS analysis of fatty acids prepared from ten isolates showed that one produced only EPA, three produced only DHA, three produced both EPA and DHA and three were unable to produce EPA or DHA. We found that the TTC reduction to TF screening method was not specific for EPA producer.

Cellulophaga sp. 9D and *Polaribacter* sp. 15E could produce EPA, which has not been reported previously for these genera. Likewise, three of the six isolates that produced DHA, *Cellulophaga* sp. 9-1, *Cellulophaga* sp. 9D and *Pibocella* sp. 7-9, are genera for which DHA production has not been reported. *Colwellia* sp. 8.3 produced relatively low levels of DHA compared with levels produced by other strains in this genus.

Highest amounts of EPA were produced by *Shewanella* sp. 8-5, and accounted for 2 % of the total fatty acids. This is in agreement with studies on EPA production using *Shewanella livingtonensis*. Strain 8-5 holds promise for development of a production strain with growth optimized to produce biomass and EPA.

Acknowledgments We thank Dr. Paul R. Gill for English corrections and valuable comments on this manuscript. This study was supported by a grant from the Instituto Antártico Uruguayo.

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