Fatty acid Compositions of two Microalgae Species used in Mariculture in Bahrain: A New Source of Microalgae for Aquaculture

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ABSTRACT

The microalgal *Chlorella sp.* and *Isochrysis galbana* strains that have been used by the National Mariculture Centre in Bahrain to rear aquatic animals since the 1980s were investigated for fatty acid compositions. The results of the strain identification have confirmed the identity of the strain *Isochrysis galbana* and corrected the identity of the strain *Chlorella sp.* to *Picochlorum sp.* The fatty acids profile has shown that polyunsaturated fatty acids (PUFAs) were the major forms of fatty acids in both species. The most abundant PUFAs were C18:4n3, and 18:2n-6 in *Picochlorum sp.*, and C18:4n3, C22:5n3, and C18:3n6 in *Isochrysis galbana*. Omega 3 fatty acids (38.56±1.76%) were higher in *Isochrysis galbana* in comparison with *Picochlorum sp.* (16.89±3.63%); whereas omega 6 fatty acids were higher in *Picochlorum sp.* (36.53±1.91%) than in *Isochrysis galbana* (22.30±0.86%).The sum of EPA and DHA was 8.26±0.50% and 6.56±0.47% in *Isochrysis galbana* and *Picochlorum sp.* respectively. The n-6/n-3 fatty acids ratio was 0.58 for *Isochrysis galbana* and 2.16 for *Picochlorum sp.* Our results suggest that both strains can be considered as a good food source for commercial production in aquaculture and that the mixing of both species will provide a balanced nutrition for animal growth in aquaculture.

تركيب الاحماض الدهنية في الطحالب المستخدمه في استزراع الأحياء البحرية في مملكة البحرين: مصدر جديد من الطحالب لتربية الأحياء المائية

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الكلمات الدالة

الأحماض الدهنية، Isochrysis galbana ، Picochlorum sp تربية الأحياء البحرية، الطحالب.

المُستلخص

تناول البحث دراسة أنواع الأحماض الدهنية في سلالتين من الطحالب الميكروسكوبية *Chlorella sp.* و *Isochrysis galbana* التي تستخدم في المركز الوطني لتربية الأحياء البحرية في البحرين منذ عام 1980. نتائج الحمض النووي للسلالتين أكدت هوية الطحلب *Isochrysis galbana* وتم تصحيح هوية الطحلب الاخر من *Chlorella sp.* الى *Picochlorum sp.* . عملية تحليل الأحماض الدهنية أظهرت أن الدهون غير المشبعة (PUFAs) هي الاكثر توافرا في كلا النوعين. الأحماض الدهنية الغير مشبعة و التي كانت أكثر تركيزا في الطحالب هي: C18:4n3 و 18:2n-6 في *Picochlorum sp.* وكانت C18:4n3 وC22:5n3 و C18:3n6 في *Isochrysis galbana*. الحمض الدهني اوميغا 3 (38.56±1.76%) موجود بتركيز أعلى في *Isochrysis galbana* مقارنة بالطحلب *Picochlorum sp* (16.89±3.63%). بينما الحمض الدهني اوميغا 6 كانت بتركيز أعلى في *Picochlorum sp.* (36.53±1.91%) مقارنة ب *Isochrysis galbana* (22.30±0.86%) . مجموع كل من حمض *eicosapentaenoic (EPA)* و *docosahexaenoic (DHA)* يساوي8.26±0.50% و 6.56±0.47% في *Isochrysis galbana* و *Picochlorum sp.* بالترتيب. نسبة الأحماض الدهنية n-6/n-3 كانت 0.58 في *Isochrysis galbana* و 2.16 في *Picochlorum sp.* وتشير النتائج ان كلا السلالتين ممكن اعتبارهما مصدر جيد للغذاء للإنتاج التجاري و تربية الأحياء المائية و أن خلط الأنواع سيوفر التغذية المتوازنة لنمو الحيوانات في مراكز تربية الأحياء المائية.

Introduction

The decline of fish stocks as well as catch from wild fisheries in the recent years has lead to a substantial focus on aquaculture and the need for suitable microalgae as an important food source in the commercial rearing of many aquatic animals (Borowitzka, 1997). Microalgae are important diverse group of photoautotrophic organisms that are used as primary producers in mariculture to feed rotifer, copepod, daphnia, brine shrimp etc. which are subsequently used to feed late larval and juvenile fish and crustaceans (Volkman, *et al*., 1989).

The use of cultivated microalgae which compose the phytoplankton as primary food source for the larvae/juvenile stages of bivalves, crustacean and fish in mariculture as well as zooplankton is well documented (Tredici, *et al*., 2009; Conceição, *et al*., 2010; Guedes and Malcata, 2012). Although several hundred microalgae species have been investigated, only few are currently used in aquaculture including *Isochrysis galbana* and *Chlorella sp*. based on several practical considerations such as strain availability, cell physical characteristics, nutritional values, digestibility, and absence of toxins or irritants (Muller-Fuega, *et al*., 2003a; Muller-Fuega, *et al*., 2003b; Muller-Fuega, 2004; Tredici, *et al*., 2009; Anon, 2010; Guedes and Malcata, 2012).

The success of aquaculture relies on several factors among which the average lipid and fatty acid contents of phytoplankton since they are used in the artificial nutrition chain (phytoplankton-zooplankton-fish) in which the next members of this chain are unable to synthesize some fatty acids (Martin-Creuzburg and Von, 2004). Therefore, the total amount and the relative proportion of fatty acids especially those that belong to the omega 3 and omega 6 families which can be affected by nutritional and environmental factors are of special interest (Brown, *et al*., 1997; Tzovenis, *et al*., 2003).

Marine *Chlorella* and *Isochrysis galbana* are widely used as a food source for commercial production in aquaculture to feed aquatic animals such as molluscs, shrimp, and fish (Sukenik and Wahnon, 1991; Elert and Woffrom, 2001; Wacker, *et al*., 2002). They are considered as a major source of essential long-chain and polyunsaturated fatty acids (Sukenik and Wahnon, 1991). The concentration and more importantly the ratios of some highly polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), arachidonic acid (AA), and docosahexaenoic acid (DHA) are of major importance in determining the nutritional value of microalgae (Reitan, *et al*., 1997; Apt and Behrens, 1999).

The present work examined the fatty acid compositions of the microalgae *Isochrysis galbana* and *Chlorella sp*. which were purchased in the 1980s (no record of suppliers names and addresses) from France and Malaysia respectively by the National Mariculture Centre, Ministry of Municipalities and Urban Planning in Bahrain to rear aquatic animals. Although the nutritional quality of microalgae is an important factor for the animal being reared (Brown, *et al*.,1989), the nutritional value of those microalgae had never been studied.

1. Materials and Methods

1.1. Microalgae cultures and sample preparation

The microalgae *Chlorella sp*. and *Isochrysis galabana* cultures were obtained from the National Mariculture Centre, Ministry of Municipalities and Urban Planning. Both cultures were grown aerobically in a sterile Walne's media (Walne, 1970) prepared in seawater of 30‰. They were kept at 18°C under continuous illumination of approximately 100 μmol photon $m^{-2}s^{-1}$ in large controlled Bioreactors (volume exceeding 200L).

Algal cultures were maintained in axenic conditions for long term usage by continuously transferring pure cultures into agar plates kept in controlled incubators. Alternatively, a small volume of pure cultures (250mL) were stored at 4^{o} C for 2 months. A 10mL aliquot was removed from each culture during exponential growth phase for cell count. Cell densities were determined for three replicates of each alga with a Neubauer haemocytometer and an Olympus compound microscope.

1.2. Strain identification of microalgae cultures

The microalgae cultures were sent to CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE, a strain identification service, Roscoff Culture Collection, STATION BIOLOGIQUE, Place Georges Teissier, 29680 ROSCOFF for strain identification by DNA extraction and 18S rRNA sequencing. The following primers were used for amplification of target genes: forward (63F): 5'- ACGCTT-GTC-TCA-AAG-ATT-A- 3' and reverse (1818R): 5'- ACG-GAAACC-TTG-TTA-CGA- 3'. PCR conditions were run using the following parameters: initial denaturation at 95˚C for 5 min, followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 52˚C for 30 sec, and extension at 72˚C for 1 min and 30 sec. A further extension was then completed at 72˚C for 10 min. Subsequently, data analysis were done by comparing sequenced amplicons to GenBank database.

1.3. Chlorophyll determination

Thirty milliliters of each algal culture were collected on 47mm GFF filters and used to analyze chlorophyll *a* and *b* concentrations in *Chlorella sp*.; chlorophyll *a* and *c* concentrations in *Isochrysis galbana*. Chlorophyll contents were extracted using 90% acetone. The extracts were then centrifuged for 10 minutes at 4000x *g*, and the chlorophyll concentrations were measured by spectrophotometer (Perkin Elmer Lambda XLS) using the appropriate equations, following UNESCO protocol (Vohra, 1966).

1.4. Lipid extraction

Lipid extraction was carried out following the procedure of Bligh and Dyer (1959) with some modifications. Lipids were extracted with 15 ml methanol, 7.5 ml chloroform twice, and 10 ml water, with vigorous mixing for 30 seconds after each addition. The mixture was centrifuged for 10 minutes at 2500 rpm and the extract in the chloroform layer was transferred into a pre-weighed test tube. The chloroform layer was evaporated to dryness under nitrogen (N_2) and the test tube was weighed to determine total lipid content. Lipid extracts were stored at -20^{o}C until further analysis.

1.5. Fatty acids methylation and analysis

Fatty acids methylation was performed on the stored samples according to the method of Morrison and Smith (1964). Fatty acid methyl esters (FAMEs) were prepared by drying 5 ml of the chloroform phase under nitrogen in the leak-proof reaction tubes, followed by the addition of 4 ml borontrifloride-methanol (14%) to the residue. The tubes were then flushed with nitrogen before sealing, heated in a water bath for 90 min at 100^{o}C. After cooling, methanol (4 ml) and benzene (3.45 ml) were added, tubes were incubated at 100^{o}C water bath under nitrogen atmosphere for 30 min. The mixture was then transferred into 50 ml Pyrex reaction tubes, extracted by adding 22.9 ml pentane and 11:45 ml water. The upper pentane phase containing FAMEs was then separated, dried under nitrogen, and the residues resuspended in methylene dichloride.

FAMEs analysis was carried out with a Perkin Elmer Autosystem XL Gas Chromatography (GC) FID equipped with metal column 6 ft in length, 1/8 in diameter, CSP 2310 3% and 2300% on 100/120 chrom WAW support (Supelco, Switzerland). Column temperature was 250^{o}C, injector and detector temperature was 300 ^{o}C. The carrier gas was nitrogen at a flow rate of 36.5 ml/min. Identification of FAMEs was based on the comparison of their peak area ratio against authentic standards PUFA No. 1, Marine Source, Cat. No. 47033 and PUFA No. 2, Animal Source, Cat. No. 47015-U supplied by SUPELCO USA.

1.6. Data analysis

The statistical analysis was performed using the statistical package from Excel 97 (Microsoft Corporation). Results are presented as means of 3 replicates ± standard deviation (SD). The mean values of each measured parameter were also statistically compared using ANOVA single factor and student's *t*-test. Differences with p value < 0.05 were considered statistically significant

2. Results

2.1. Strain identification of microalgae cultures

The blast analysis of the obtained sequences from the first strain (initially identified as *Chlorella sp.*) has shown 99.6% similarity with

Nannochloris sp. (KMMCC 357) and 99.5% with *Picochlorum sp.* (UTEX 2491) (Appendix 1). Since the algae is a marine strain, it was therefore re-identified as *Picochlorum sp.* (Chlorophyta, Trebouxiophyceae).

The second strain (initially identified as *Isochrysis galbana*) has shown 99.5% similarity with *Isochrysis sp.* (CCAP 927/14) and 99.5% with *Isochrysis galbana* isolate AL. Blast analysis has confirmed the identification of this species as *Isochrysis galbana* (Hatophyta, Prymnesiophyceae) (Appendix 1). Sequencing results of the 2 strains are shown in Appendix 2.

2.2. Cell volume and chlorophyll contents

The cell concentration of *Picochlorum sp.* and *Isochrysis galbana* was examined during the exponential growth phase and found to be 11.17×10^7 cell.mL^{-1} (± 1.8 SD) and 4.57×10^7 cell. mL^{-1} (± 0.35SD) respectively.

Chlorophyll analysis revealed that *Picochlorum sp.* has 2.2 mgL^{-1} (±0.4 SD) and 0.57 mgL^{-1} (±0.06 SD) of chlorophyll *a* and *b* respectively. *Isochrysis galbana* was found to contain higher concentration of chlorophyll *a* of 10.8 mgL^{-1} (±0.58 SD) and 9.8 mgL^{-1} (±1.7 SD) of chlorophyll *c* (Figure 1). There was a significant correlation between cell count and chlorophyll *a* concentration in both algal cultures (R^2 is 0.95 in *Picochlorum sp.* and 0.75 in *Isochrysis galban*

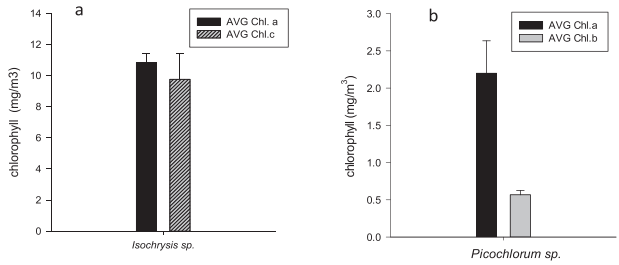

Figure 1. Chlorophyll concentrations in *Isochrysis galabana (a)* and *Picohlorum sp. (b)*

2.3. Fatty acid compositions

The fatty acid contents of the microalgae *Isochrysis galbana* and *Picochlorum sp.* at the exponential phase are shown in Table 1. There was a significant difference ($p \leq 0.05$) in saturated, unsaturated, monounsaturated and polyunsaturated fatty acid values (%) between the two species. The major fatty acids of *Isochrysis galbana* were C18:0, C18:4n3, and C24:1n9; whereas the predominant fatty acids of *Picochlorum sp.* were C16:0, C18:0, C18:2n6, C18:4n3, and C24:1n9.

The major components of the saturated fatty acids in *Picochlorum sp.* were: palmitic (C16:0), and stearic (C18:0); whereas C18:0 was the only saturated fatty acid detected in *Isochrysis galbana*. Among the unsaturated fatty acids, the predominant components were: octadecatetraenoic acid (C18:4n3), and tetracosenoic (C24:1n9) in *Isochrysis galbana*, whereas linoleic acid (18:2n6), 20:1n9, octadecatetraenoic acid (C18:4n3), and tetracosenoic (24:1n-9) were the predominant unsaturated fatty acids in *Picochlorum sp.*

The highest proportion of unsaturated fatty acid was in C24:1n9 (18.11±1.84%) and C18:4n3 (21.07±2.15%), whereas the highest proportion of saturated fatty acids being C18:0 (13.72±0.57%) and C16:0 (16.08±1.12%) in *Isochrysis galbana* and *Picochlorum sp.* respectively. The proportion of EPA (4.41±0.36 and 4.40±0.21%) was higher than those of DHA (3.85±0.14 and 2.16±0.26%) in both *Isochrysis galbana* and *Picochlorum sp.* respectively.

Picochlorum sp. was characterized by lower unsaturated (71.32±6.65%), monounsaturated (16.95±1.11%), polyunsaturated (54.34±5.54%), n3 (16.89±3.63), DHA (2.16±0.26%) %), and higher n6 (36.53±1.91%) fatty acids and when compared with *Isochrysis galbana* unsaturated (86.28±4.80%), monounsaturated (25.42±2.18%), polyunsaturated (60.86±2.62%), n3 (38.56±1.76%) fatty acids, DHA (3.85±0.14%), and n6 (22.30±0.86%) fatty acids ($p = 0.05$). As a consequence, n6/n3 ratio (2.16) was significantly higher ($p = 0.05$) in *Picochlorum sp.* than the mean value obtained in *Isochrysis galbana* (0.58) and the sum of EPA and DHA (6.56±0.47) was significantly lower ($p = 0.05$) in *Picochlorum sp.* than that of *Isochrysis galbana* (8.26±0.50%).

Table 1. Fatty acid compositions of *Isochrysis galbana* and *Picochlorum sp.* (initially identified as *Chlorella sp.*).

Fatty acids	*Isochrysis galbana*	*Picochlorum sp.* (initially identified as *Chlorella sp.*)
C16:0	Nd	16.08±1.12
C16:1 n7	Nd	0.85±0.04
C18:0	13.72±0.57	12.60±0.71
C18:1 n9	5.88±0.30	1.13±0.13
C18:1 n7	Nd	0.96±0.15
C18:2 n6	7.06±0.25	11.90±1.47
C18:3 n6	8.54±0.30	4.99±0.44
C18:3 n3	4.38±0.25	2.09±0.37
C18:4 n3	17.20±0.85	21.07±2.15
C20:1 n9	Nd	Nd
C20:5 n3 EPA	4.41±0.36	4.40±0.21
C22:1 n11	1.43±0.04	2.73±0.17
C22:4 n6	6.70±0.31	Nd
C22:5 n3	8.72±0.16	6.81±0.64
C22:6 n3 DHA	3.85±0.14	2.16±0.26
C24:1 n9	18.11±1.84	12.24±0.62
SFAs	13.72±0.57	28.68±1.83
UFAs	86.28±4.8	71.32±6.65
MUFAs	25.42±2.18	16.95±1.11
PUFAs	60.86±2.62	54.34±5.54
n-3	38.56±1.76	16.89±3.63
n-6	22.30±0.86	36.53±1.91
n6/n3	0.58	2.16
EPA+DHA	8.26±0.50	6.56±0.47

The values are expressed as % of total fatty acid methyl esters; mean ± SD.
SFAs: saturated fatty acids, UFAs: unsaturated fatty acids, PUFAs: polyunsaturated fatty acids, MUFAs: monounsaturated fatty acids, EPA: Eicosapentaenoic acid, DHA: docosahexaenoic acid. Nd: not detected.

3. Discussion

It is well established that the fatty acid contents of microalgae produced for aquaculture especially highly unsaturated fatty acids such as EPA (C20:5n3), AA (C20:4n6), and DHA (C22:6n3) are of great importance (Reitan, *et al*., 1997; Apt and Behrens, 1999). Therefore, the evaluation of fatty acids status in microalgae is very important criterion that must be met in order to ensure the use of microalgae having a high nutritional quality in aquaculture. Furthermore, it has been suggested that the mixing of more than one species of the most frequently used microalgae including *Isochrysis galbana* and *Chlorella sp*. will provide more balanced nutrition thus improving animal growth in aquaculture (Borowitzka, 1997; Apt and Behrens, 1999; Muller-Feuga, 2000; Becker, 2004).

Lipid content of microalgae are influenced both qualitatively and quantitatively by several factors including their taxonomic position usually species or strain (Becker, 2003; Muller-Feuga, *et al.*, 2003a), culturing conditions including nutrient

deprivation, light quality, photon flux density (PFD), photoperiod (L/D cycle), and temperature (Converti, *et al*., 2009; Mata, *et al*., 2010). Knowledge on lipids and fatty acids accumulation in *Chlorella* species under different growth modes remains largely unknown (Liu, *et al*., 2011). The microalgal culture *Picochlorum sp.* supplied by the National Mariculture Centre, Ministry of Municipalities and Urban Planning was purchased and always considered as *Chlorella sp*. However, the results of the strain identification have revealed that the microalgae *Chlorella sp.* culture does in fact belong to a different strain (*Picochlorum sp.*). Therefore, the fatty acids profile of the *Picochlorum sp.* used in the present study were compared with those from previous studies of *Chlorella sp*. and *Picochlorum sp.*

The major fatty acids of *Picochlorum sp.* detected in the present study were C16:0 (16.08±1.12%), C18:0(12.60±0.71%), C18:2n6 (11.90±1.47%), C18:4n3 (21.07±2.15%), and C24:1n9 (12.24±0.62%). The results are not consistent with those reported by other investigators regarding *Chlorella sp*. except for the high percentage of C16:0 recorded in all studies and C18:2n6 reported in several studies (Table 2). Furthermore, both EPA (4.4±0.21%) and DHA (2.16±0.26%) were detected in the *Picochlorum sp.* in the present study, whereas both EPA (C20:5n3) and DHA (C22:6 n3) were deficient in most studies of *Chlorella sp*. with the exception of Watanabe, *et al*. (1983) and Shinichi, *et al*. (1983) in which high level of EPA (27.8%), and (16.0-38.4) were reported respectively and trace amount of DHA (0.3%) was reported by Watanabe, *et al*. (1983). On the other hand, the proportion of SFAs (28.68±1.83%), UFAs (71.32±6.65%), PUFAs (54.34±5.54%) reported in this study are similar to those reported by the others: SFAs (15.89-48.4%), UFAs (42-85.9%), PUFAs (21-51%) (Table 2). However, the n3 fatty acids percentage were generally lower (16.89±3.63%) in the present study in comparison to the others (19.7-46.2%).

In addition, this study has reported the presence of the fatty acids C18:1n7, C18:3n6, C18:4n3, C20:5n3, C22:5n3, and C22:6n3, and the first to report the presence of C24:1 n9 in the of *Picochlorum sp.* It has been reported that Chlorophytes (*Dunaliella spp*. and *Chlorella spp*.) are not suitable as a single species diet because of their PUFA deficiency and therefore low nutritional value (Brown, *et al*., 1997). Although, most green algae have low presence of PUFA C20 and 22:6n3; some marine *Chlorella* species can be an exception having up to 30% 20:5n3 (Watanabe, *et al*., 1983). The presence of the PUFA reported here with very long hydrocarbon chains (C20-24) makes them an ideal diet source of lipids for aquaculture. However, the low concentrations of n3 fatty acids detected in the present study are considered a draw back on the quality of their nutritional value.

The comparison data presented in Table 2 shows a great deal of inconsistency regarding the fatty acid contents and proportions of *Chlorella sp.* However, most studies have reported the presence of the following fatty acids but with different proportions C14:0, C16:0, C18:0, C18:1n9, C18:2n6 and C18:3n3 due to different cultivating conditions as explained by the researchers (Petkov and Garcia, 2007). These results are consistent with the present study except for the fatty acids C14:0 that was not recorded.

Table 2. Fatty acid compositions of the microalgal strain *Picochlorum sp.* (initially identified as *Chlorella sp.*) in comparison to marine *Chlorella sp.*

Fatty acids	1	2	3	4	5	6
C14:0	Nd	5.2	1.58	9±0.5	-	2.0
C16:0	16.08±1.12	19.7	13.08	25±1.5	19.4	19.6
C16:1 n9	Nd	-	-	-	10.9	-
C16:1 n7	0.85±0.04	30.5	-	2±0.1	-	6.2
C16:2	-	-	-	10±0.8	-	3.6
C16:3n4	-	-	9.56	9±0.7	-	-
C16:3n3	-	-	-	-	-	12.0
C16:4	-	-	-	-	-	-
C18:0	12.60±0.71	0.70	1.23	0.9±0.2	4.2	3.3
C18:1 n9	1.13±0.13	2.70	6.68	5±0.7	23.1	5.7
C18:1 n7	0.96±0.15	-	1.2	-	-	1.6
C18:2 n6	11.90±1.47	2.4	17.54	20±1.2	11.7	11.8
C18:3 n6	4.99±0.44	-	-	-	1.5	0.3
C18:3 n3	2.09±0.37	0.2	20.02	19±0.9	21.1	22.3
C18:4 n3	21.07±2.15	-	-	-	Nd	0.1
C20:1 n9	Nd	-	0.12	-	Nd	0.1
C20:3 n3 and C 20:4n3	-	3.6	-	-	Nd	0.2
C20:4 n6	-	-	-	-	-	0.5
C20:5 n3 EPA	4.40±0.21	27.8	-	-	Nd	1.3
C22:1n11	2.73±0.17	-	-	-	-	-
C22:4 n6	Nd	-	-	-	-	-
C22:5 n3	6.81±0.64	1.7	-	-	-	-
C22:6 n3 DHA	2.16±0.26	0.3	-	-	Nd	-
C24:1 n9	12.24±0.62	-	-	-	-	-
Others (C10:0, C12:0, C14:0, C14:1)					8.2	6.8
SFA	28.68±1.83	25.6	15.89	34.9±2.2	23.6	24.9
USF	71.32±6.65	69.2	55.13	65.0±4.4	-	25.6
MUFA	16.95±1.11	33.2	8.01	7±0.8	38.2	25.6
PUFA	54.34±5.54	36.0	47.12	51±2.1	34.3	40.1
n3	16.89±3.63	33.6	20.02	19±0.9	21.1	35.8
n6	36.53±1.91	2.4	17.54	20±1.2	13.2	12.1
n6/n3	2.16	0.07	0.88	1.05	0.63	0.34
EPA+ DHA	6.56±0.47	28.1	-	-		

1 Present study, the values are expressed as % of total fatty acid methyl esters; mean ± SD in marine *Picochlorum sp.* (initially identified as Chlorella sp.)
2 Watanabe, *et al*. 1983, the values are expressed as % of total fatty acid methyl esters in marine *Chlorella sp*.
3 Pratoomyot, *et al*. 2005, the values are expressed as % of total fatty acid methyl esters in marine *Chlorella sp.*
4 Petkov and Garcia, 2007, the values are expressed as % of total fatty acid methyl esters; mean ± SD in marine *Chlorella sp*.
5 Birkou, *et al*. 2012, the values are expressed as % of total fatty acid methyl esters in marine *Chlorella sp.*
6 Zukova and Aizdaicher, 1995, the values are expressed as % of total fatty acid methyl esters in marine *Chlorella sp.*

The strain identification of the algal culture mistakenly identified as *Chlorella sp.* was clearly identified as *Picochlorum sp.* Although, the strain *Picochlorum sp.* is not considered as one of the commonly used microalgae in aquaculture (Tran, *et al*., 2014), several studies have recommended it as a new candidate for aquaculture and food, and as a potential source of PUFAs, the production of biofuel, and for biotechnological applications (El Abed, *et al*., 2008; de la Vega, *et al*., 2011; Islam, *et al*., 2013; Tran, *et al*., 2014). El Abed, *et al*. (2008) reported a high proportion of C16:00 (18.14±2.87mg/g dry weight), C18:2n6 (19.27±2.45mg/g dry weight), C18:3n3 (28.49±4.34mg/g dry weight), and C18:5n5 (9.22±5.16mg/g dry weight) in *Picochlorum sp.*, whereas Tran, *et al*. (2014) reported high levels of C16:00 (31.49±2.35%), C18:1n9 (37.13±2.09%), and C18:2n6 (20.76±1.93%). Furthermore, Pereira, *et al*. (2013) reported the presence of high levels of the fatty acids C16:00 (26.16±2.03%), C16:1n7 (12.05±1.26%), C18:1n7 (21.96±1.39%), and C18:2n6 (23.76±0.36%) in *Picochlorum sp.* collected from the red sea. High levels of the fatty acids C16:00 (29.48±3.12%), C18:00 (6.00±1.89%), C18:1n7 (9.06±0.28%), C18:2n6 (22.47±2.63%), and C18:3n3 (17.04±0.93%) were also found in *Picochlorum sp.* studied by Yang, *et al*. (2014). Therefore, it can be concluded that the main SFAs of the microalgal strain *Picochlorum sp.* are C16:00 and C18:00; whereas the major UFAs are C18:2n6, and C18:3n3 in most studies including the present work. Furthermore, the fatty acid profile of the strain identified as *Chlorella sp.* prior to this study is more related to the strain *Picochlorum sp.* present in the literature, and hence supports the strain identification results. Therefore, the strain *Picochlorum sp.* which has been used by the National Mariculture Centre since the 1980s can be considered as a good food source for commercial production in aquaculture mainly due to its fatty acid contents including PUFAs, and EPA+DHA.

In general, the fatty acid contents of *Isochrysis galbana* presented in Table 1 agree with those reported in previous studies except for the SFAs C14:0 and C16:0 which were not detected in the present study whereas high concentrations of those fatty acids were reported in most studies (Napolitano, *et al*., 1990; Lin, *et al*., 2007; Yoshioka, *et al*., 2012; Custodio, *et al*., 2014). On the contrary, high levels of the fatty acid C18:0 (13.72±0.57%) were recorded in the present study in comparison to other studies in which C18:0 were either not detected or present in trace amount (Napolitano, *et al*., 1990; Lin, *et al*., 2007; Yoshioka, *et al*., 2012; Custodio, *et al*., 2014) with the exception of the result of Babarro, *et al*. (2001) where high levels of the fatty acid C18:0 (12.79%) were also reported. The important MUFAs were C18:1 n9 and C24:1 n9, while the major PUFAs were C18:2n6, C18:4n3, C18:3n6, C22:4n6, and C22:5n3. It is important to note that UFAs account for 86.28±4.8% of the total in which 60.86±2.62% belong to PUFAs. In addition, the results of the strain identification have revealed that the microalgae *Isochrysis galbana* culture does in fact belong to the strain (*Isochrysis galbana*).

Conclusion

The present study has amended the identity of the microalgal strain *Chlorella sp.* that is being utilized by the National Mariculture Centre, Ministry of Municipalities and Urban Planning in Bahrain to rear aquatic animals since the 1980s to *Picochlorum sp.* Both strains studied (*Picochlorum sp.* and *Isochrysis galbana*) have high levels of PUFAs, and EPA+DHA. The strain *Isochrysis galbana* was characterized by the presence of higher levels of the omega 3 fatty acids whereas high amounts of the omega 6 fatty acids were detected in the strain *Picochlorum sp.* Therefore, the mixing of both species can be considered as suitable feedstock that will provide balanced nutrition for the animal growth in aquaculture.

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Appendix 1

Results of BLAST

a. ***Strain 1 (Picochlorum sp.)***

% Pairwise Identity	Accession	Description	Hit start	Hit end
99.60%	JQ315644	Nannochloris sp. KMMCC 357 18S ribosomal RNA gene, partial sequence	1	1564
99.60%	GQ122333	Nannochloris sp. KMMCC C-23 18S ribosomal RNA gene, partial sequence	1	1564
99.50%	AY422077	Picochlorum sp. UTEX 2491 18S small subunit ribosomal RNA gene, partial sequence	1	1611
99.50%	AY422073	Picochlorum oklahomensis 18S small subunit ribosomal RNA gene, partial sequence	1	1611
99.50%	GQ122348	Nannochloris sp. KMMCC C-125 18S ribosomal RNA gene, partial sequence	1	1564
99.50%	GQ122344	Nannochloris sp. KMMCC C-106 18S ribosomal RNA gene, partial sequence	1	1564
99.40%	KF591594	Picochlorum sp. SENEW3 18S ribosomal RNA gene, partial sequence	27	1640
99.30%	KF495093	Picochlorum sp. CTM20019 18S ribosomal RNA gene, partial sequence	47	1658
99.30%	AB058331	Nanochlorum sp. MBIC10208 gene for 18S rRNA, partial sequence	27	1639
99.30%	AY422076	Picochlorum sp. UTEX 2378 18S small subunit ribosomal RNA gene, partial sequence	1	1611
99.30%	AY526738	Picochlorum sp. RCC115 18S ribosomal RNA gene, partial sequence	26	1637
99.20%	JN191236	Picochlorum sp. S1b 18S ribosomal RNA gene, partial sequence	36	1650
99.10%	AB080302	Nannochloris maculata gene for 18S rRNA, partial sequence	46	1658
99.10%	AJ131691	Nannochloris sp. RCC 011 18S rRNA gene	46	1658
98.80%	AB183620	Nannochloris sp. MBIC10596 gene for 18S rRNA, partial sequence, strain: MBIC10596	24	1636
98.80%	AB183584	Prasinoderma sp. MBIC10059 gene for 18S rRNA, partial sequence, strain: MBIC10059	27	1639
98.80%	AB058304	Nanochlorum sp. MBIC10053 gene for 18S rRNA, partial sequence	27	1639
98.70%	AB080303	Nannochloris atomus gene for 18S rRNA, partial sequence, strain:CCAP 251/7	46	1658
98.50%	AB058309	Nanochlorum sp. MBIC10091 gene for 18S rRNA, partial sequence	27	1639
98.30%	X06425	Nanochlorum eucaryotum 18S rDNA	47	1659

Identification:
Domain: Eukaryota
Division: Chlorophyta
Class: Trebouxiophyceae
Order: Chlorellales
Genus: Pichoclorum
Species: sp

B.*Strain 2 (Isochrysis galbana)*

% Pairwise Identity	Accession	Description	Hit start	Hit end
99.50%	DQ079859	Isochrysis sp. CCAP 927/14 18S ribosomal RNA gene, partial sequence	47	1666
99.50%	HM246242	Isochrysis galbana isolate AL 18S ribosomal RNA gene, partial sequence	30	1649
99.50%	KC888124	Haptophyceae sp. 1 EMB-2013 strain PLY562 18S ribosomal RNA gene, partial sequence	30	1649
99.50%	KC888123	Haptophyceae sp. 1 EMB-2013 strain AC620 18S ribosomal RNA gene, partial sequence	30	1649
99.50%	KC888122	Haptophyceae sp. 1 EMB-2013 strain RCC1344 18S ribosomal RNA gene, partial sequence	30	1649
99.50%	KC888121	Haptophyceae sp. 1 EMB-2013 strain RCC1350 18S ribosomal RNA gene, partial sequence	34	1653
99.50%	KC888120	Haptophyceae sp. 1 EMB-2013 strain CCMP463 18S ribosomal RNA gene, partial sequence	34	1653
99.50%	KC888119	Haptophyceae sp. 1 EMB-2013 strain RCC1349 18S ribosomal RNA gene, partial sequence	28	1647
99.50%	KC888118	Haptophyceae sp. 1 EMB-2013 strain AC102 18S ribosomal RNA gene, partial sequence	28	1647
99.40%	DQ075203	Isochrysis sp. zhangjiangensis 18S ribosomal RNA gene, partial sequence	20	1639
99.40%	HM149539	Isochrysis sp. Tun08 18S ribosomal RNA gene, partial sequence	46	1665
99.40%	HM149543	Isochrysis galbana strain Ifremer-Argenton98 18S ribosomal RNA gene, partial sequence	46	1665
99.40%	KC888127	Haptophyceae sp. 1 EMB-2013 strain PLY506C 18S ribosomal RNA gene, partial sequence	37	1656
99.40%	KC888126	Haptophyceae sp. 1 EMB-2013 strain PLY506B 18S ribosomal RNA gene, partial sequence	32	1651
99.40%	KC888125	Haptophyceae sp. 1 EMB-2013 strain PLY506A 18S ribosomal RNA gene, partial sequence	32	1651
99.40%	JN938582	Isochrysis sp. LL-2012 strain IOAC724S 18S ribosomal RNA gene, partial sequence	1	1619
99.40%	GQ118682	Isochrysis galbana strain DB 18S ribosomal RNA gene, partial sequence	17	1637
99.30%	DQ071573	Isochrysis sp. 8701 18S ribosomal RNA gene, partial sequence	17	1625
99.20%	HM149542	Isochrysis galbana strain CCMP1324 18S ribosomal RNA gene, partial sequence	46	1665
99.20%	AJ246266	Isochrysis galbana 18S rRNA gene, strain UIO 102	44	1661

Identification:
Domain: Eukaryota
Division: Haptophyta
Class: Prymnesiophyceae
Order: Isochrysidales
Genus: Isochrysis
Species: galbana

Appendix 2

Sequencing Results
a. ***Strain 1 (Picochlorum sp.):***

>L1 18S rRNA - Asembly Hi Sensitivity P=0.04

AGCCATGCATGTCTAAGTATAAGTTGCTTTATACTGTGAAACTGC-
GAATGGCTCATTAAATCAGTTATAGTTTATTTGATGGTACCTACT-
TACTCGGATACCCGTAGTAATTCTAGAGYTAATACGTGCGTACATCCC-
GACTTCTGGAAGGGACGTATTTATTAGATAAAAGGCCGACCGGGCTT-
GCCCGACTCGCGGTGACTCATGATAACTTCMCGAATCGCATGGMCTC-
GCGCCGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTTTT-
GATGGTAGGATAGAGGCCTACCATGGTGGTAACGGGTGACGGAGA-
ATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATC-
CAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAG-
GTAGRGACAATAAATAACAATACCGGGCCTTTGGTCTGGTAATKGGAAT-
GAGTACAACCTAAACACCTTAACGAGGATCAATTGGAGGGCAAGTCTG-
GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTA-

AGTTGCTGCAGTTAAAAAGCTCGTACTTGGATTTCGGGTGGGGCCT-
GCCGGTCCGCCGTTTCGGTGTGCACTGGCCGGGCCCACCTTGTTGC-
CGGGGACGAGCTCCTGGGCTTTATTGTCCGGGACTCGGAGTCKGC-
GAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAGGCCACCGCTCT-
GAATACATTAGCATGGAATAACACGATAGGACTCTGGCCTATCTTGTTG-
GTCTGTAGGACCGGAGTAATGATTNAGAGGGACAGTCGGGGGCATTC-
GTATTTCATTGTCAGAGGKGAAATTCTTGGATTTATGAAAGACGAAC-
TACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAAC-
GAAAGTTGGGGGCTCGAAGACGATTAGATACCGTCCTAGTCTCAACCATA-
AACGATGCCGACTAGGGATCGGCGGGTGTTTTTTTTGATGACCCCGCCG-
GCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTC-
GCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTG-
GAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTTACCAGGTCCAGA-
CATAGTGAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTG-
GTGGTGCATGGCCGTTCTTAGTTGGTGGGTTGCCTTGTCAGGTTGATTCC-
GGTAACGAACGAGACCTCAGCCTGCTAACTAGTCACGCGTGCTCCGGCAC-
GCGGCGGACTTCTTAGAGGGACTATTGGCGACTAGCCAATGGAAGCAT-
GAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGC-
GCTACACTGATGCATTCAACGAGCCTCTCCTTGGCCGACAGGTCCGGG-
TAATCTTTGAATCTGCATCGTGACGGGGATAGATTATTGCAATTATTA-
ATCTTCAACGAGGAATGCCTAGTAGGCGCAAGTCATCAGCTTGCGTCGAT-
TACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGGGTG

b. *Strain 2 (Isochrysis galbana):*

>L2 18S rRNA - Asembly Hi Sensitivity P=0.04 sequence
AGCCATGCATGTCTAAGTATAAGCGAGTATACTGTGAAACTGCGAATG-
GCTCATTAAATCAGTTATGGTTTATTNTGATGGTACCTTGCTACTTG-
GATAACCGTAGTAATTCTAGAGCTAATACATGCAGGAGTTCCCGACTTCG-
GAAGGGATGTATTTATTAGATAAGAAACCAAACCGGTCTCCGGTTGCGT-
GCTGAGTCATACTAACTGCTCGAATCGCACGGCTTTACGCTGGCGATG-
GTTCATTCAAATTTCTGCCCTATCAGCTTTCGATGGTAGGATAGAG-
GCCTACCATGGCGTTAACGGGTAACGGAGAATTAGGGTTCGATTCCG-
GAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGAAGGCAGCAGGC-
GCGTAAATTGCCCGAATCCTGACACAGGGAGGTAGTGACAAGAAATAA-
CAATACAGGGCTCTTCGAGTCTTGTAATTGGAATGAGTACAATTTA-
CATCTCTTCACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGC-
CGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTA-
AAACGCTCGTAGTCGGATTTCGGGGCGGGCCCGCCGGTCTGCCGATGGG-
TACGCACTGGCGGGCGCGTCCTTCCTCCCGGAGACGGCCGCTACTCTTA-
ACTGAGCGGTGGTCGGAGACGGGATGTTTACTTTGNAAAAATCAGAGT-
GTTTCAAGCAGGCAGTCGCTCTTGCATGGATTAGCATGGGATAAT-
GAAATAGGACTCTGGTGCTATTTTGTTGGTTTCGAGCACCGGAGTAAT-
GATTAACAGGGACAGTCAGGGGCACTCGTATTCCGCCGAGAGAGGT-
GAAATTCTCAGACCAGCGGAAGACGAACGACTGCGAAAGCATTTGC-
CAGGGATGTTTTCACTGATCAAGAACGAAAGTTAGGGGATCGAAGACGAT-
CAGATACCGTCGTAGTCTTAACCATAAACCATGCCGACTAGGGATTG-
GAGGATGTTCCGTTTGTGACTCCTTCAGCACCTTTCGGGAAACTA-
AAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTA-
AAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTATTT-
GACTCACACGGGGAAACTTACCAGGTCCGACATTGTGAGGATTGACA-
GATTGAGAGCTCTTTCTTGATTCGATGGGTGGTGGTGCATGGCCGTTCT-
TAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGAC-
CGCAGCCTGCTAAATAGTGTCCCCAACCCCNTGTTGGGGCTCGCTTCT-
TAGAGGGACAACTTGTCTTCAACAAGTGGAAGTTCGCGGCAATAACAG-
GTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGAT-
GCATTCAGCGAGTCGTCTCCCTTGACCGAGAGGTCCGGGTAATCTTGT-
GAACTTGCATCGTGATGGGGATAGATTATTGCAACTATTAATCTTCAAC-
GAGGAATTCCTAGTAAGCGTGTGTCATCAGCGCACGTTGATTAC-
GTCCCTGCCCTTTGTCAAAGCGCCCGTCGCTCCTACCGATTGAATGATCCC
AGCCATGCATGTCTAAGTATAAGTTGCTTTATACTGTGAAACTGC-
GAATGGCTCATTAAATCAGTTATAGTTTATTTGATGGTACCTACT-
TACTCGGATACCCGTAGTAATTCTAGAGYTAATACGTGCGTA-
CATCCCGACTTCTGGAAGGGACGTATTTATTAGATAAAAGGCC-
GACCGGGCTTGCCCGACTCGCGGTGACTCATGATAACTTCMC-
GAATCGCATGGMCTCGCGCCGGCGATGTTTCATTCAAATTTCT-
GCCCTATCAACTTTTGATGGTAGGATAGAGGCCTACCATGGTGGTA-
ACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCT-
GAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCG-
CAAATTACCCAATCCTGACACAGGGAGGTAGRGACAATAAATAA-
CAATACCGGGCCTTTGGTCTGGTAATKGGAATGAGTACAACCTA-
AACACCTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAG-
CAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGCT-
GCAGTTAAAAAGCTCGTACTTGGATTTCGGGTGGGGCCTGCCG-
GTCCGCCGTTTCGGTGTGCACTGGCCGGGCCCACCTTGTTGC-
CGGGGACGAGCTCCTGGGCTTTATTGTCCGGGACTCGGAGTCK-
GCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAGGCCAC-
CGCTCTGAATACATTAGCATGGAATAACACGATAGGACTCTG-
GCCTATCTTGTTGGTCTGTAGGACCGGAGTAATGATTNAGAGGGA-
CAGTCGGGGGCATTCGTATTTCATTGTCAGAGGKGAAATTCTTG-
GATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAGGAT-
GTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATTA-
GATACCGTCCTAGTCTCAACCATAAACGATGCCGACTAGGGATC-
GGCGGGTGTTTTTTTTGATGACCCCGCCGGCACCTTATGAGA-
AATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCT-
GAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTG-
GAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTTACCAG-
GTCCAGACATAGTGAGGATTGACAGATTGAGAGCTCTTTCTT-
GATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGGTT-
GCCTTGTCAGGTTGATTCCGGTAACGAACGAGACCTCAGCCT-
GCTAACTAGTCACGCGTGCTCCGGCACGCGGCGGACTTCT-
TAGAGGGACTATTGGCGACTAGCCAATGGAAGCATGAGGCAATA-
ACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGC-
TACACTGATGCATTCAACGAGCCTCTCCTTGGCCGACAGGTC-
CGGGTAATCTTTGAATCTGCATCGTGACGGGGATAGATTATTG-
CAATTATTAATCTTCAACGAGGAATGCCTAGTAGGCGCAAGTCAT-
CAGCTTGCGTCGATTACGTCCCTGCCCTTTGTACACACCGCCCGTC-
GCTCCTACCGATTGGGTG

c. *Strain 2 (Isochrysis galbana):*

>L2 18S rRNA - Asembly Hi Sensitivity P=0.04 sequence
AGCCATGCATGTCTAAGTATAAGCGAGTATACTGTGAAACTGC-
GAATGGCTCATTAAATCAGTTATGGTTTATTNTGATGGTACCTT-
GCTACTTGGATAACCGTAGTAATTCTAGAGCTAATACATGCAG-
GAGTTCCCGACTTCGGAAGGGATGTATTTATTAGATAAGAAAC-
CAAACCGGTCTCCGGTTGCGTGCTGAGTCATACTAACTGCTC-
GAATCGCACGGCTTTACGCTGGCGATGGTTCATTCAAATTTCT-
GCCCTATCAGCTTTCGATGGTAGGATAGAGGCCTACCATGGCGT-
TAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCT-
GAGAAATGGCTACCACATCCAAGGAAGGCAGCAGGCGCGTA-
AATTGCCCGAATCCTGACACAGGGAGGTAGTGACAAGAAATAA-
CAATACAGGGCTCTTCGAGTCTTGTAATTGGAATGAGTACAATT-
TACATCTCTTCACGAGGATCAATTGGAGGGCAAGTCTGGTGC-
CAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTT-
GTTGCAGTTAAAACGCTCGTAGTCGGATTTCGGGGCGGGCCCGC-
CGGTCTGCCGATGGGTACGCACTGGCGGGCGCGTCCTTCCTCCC-
GGAGACGGCCGCTACTCTTAACTGAGCGGTGGTCGGAGAC-
GGGATGTTTACTTTGNAAAAATCAGAGTGTTTCAAGCAGGCAGTC-
GCTCTTGCATGGATTAGCATGGGATAATGAAATAGGACTCTGGT-
GCTATTTTGTTGGTTTCGAGCACCGGAGTAATGATTAACAGGGA-
CAGTCAGGGGCACTCGTATTCCGCCGAGAGAGGTGAAATTCTCA-
GACCAGCGGAAGACGAACGACTGCGAAAGCATTTGCCAGGGAT-
GTTTTCACTGATCAAGAACGAAAGTTAGGGGATCGAAGACGATCA-
GATACCGTCGTAGTCTTAACCATAAACCATGCCGACTAGGGATTG-
GAGGATGTTCCGTTTGTGACTCCTTCAGCACCTTTCGGGAAAC-
TAAAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCT-
GAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTG-
GAGCCTGCGGCTTATTTGACTCACACGGGGAAACTTACCAGGTC-
CGACATTGTGAGGATTGACAGATTGAGAGCTCTTTCTTGATTC-
GATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTT-
GTCTGGTTAATTCCGTTAACGAACGAGACCGCAGCCTGCTA-
AATAGTGTCCCCAACCCCNTGTTGGGGCTCGCTTCTTAGAGGGA-
CAACTTGTCTTCAACAAGTGGAAGTTCGCGGCAATAACAGGTCT-
GTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGAT-
GCATTCAGCGAGTCGTCTCCCTTGACCGAGAGGTCCGGGTA-
ATCTTGTGAACTTGCATCGTGATGGGGATAGATTATTGCAACTAT-
TAATCTTCAACGAGGAATTCCTAGTAAGCGTGTGTCATCAGCGCAC-
GTTGATTACGTCCCTGCCCTTTGTCAAAGCGCCCGTCGCTCCTACC-
GATTGAATGATCCC