Omega-3 fatty acid obtained from *Nannochloropsis oceanica* cultures grown under low urea protect against Abeta-induced neural damage

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Abstract Amyloid-beta (Abeta) protein is a key factor in the pathogenesis of Alzheimer's disease (AD). Moreover, it has been reported that oxidative stress is involved in the biochemical pathway by which Abeta can lead to neuronal dysfunction. Recently, docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; C20:5n-3) have been reported to protect against AD. However, these omega-3 fatty acids are frequently obtained from fish oil and may contain heavy metals. In this study, we utilized *Nannochloropsis oceanica* to produce omega-3 fatty acid. We observed that when urea levels (nitrogen source) were lowered from 2 to 0.2 g/L in *Nannochloropsis oceanica* cultures, EPA production increased. Moreover, EPA in *Nannochloropsis oceanica* effectively promoted antioxidant activity to counter the Abeta-induced oxidative stress in Neuro-2A cells. These results indicate that *Nannochloropsis oceanica* may be potentially used as a therapeutic agent or as a functional food that promotes protection against AD.

Keywords Amyloid-beta (Abeta) · Oxidative stress · Eicosapentaenoic acid (EPA) · *Nannochloropsis oceanica* · Functional foods

Introduction

Docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; C20:5) are major components of fish oil. These omega-3 unsaturated fatty acids have been reported to protect against Alzheimer's disease (AD) (Sadli et al. 2013; Sahlin et al. 2007), and against oxidative stress in neural cells (Yang

et al. 2012). Recently, omega-6 unsaturated fatty acids such as arachidonic acid and linoleic acid have been found to play a role in the development of inflammation (Al-Taani et al. 2013). However, this pro-inflammatory effect can be suppressed in vivo by omega-3 unsaturated fatty acids (Nobre et al. 2013).

There are many alternative EPA and DHA sources, including bacteria, fungi, plants, and microalgae, that are currently being investigated for commercial use. However, algae are the most abundant primary producers of EPA and DHA, although some can be mixotrophic or heterotrophic. The term *algae* implies a division of lower plants which contain cellular chlorophyll that primarily inhabit aquatic environments, but these organisms are widespread in other environments as well. Microalgal strains are recognized as excellent sources of proteins, carbohydrates, lipids, and vitamins, and utilized for both food and as feed additives (Rocha et al. 2003).

Chlorella sp. (Yongmantichai 1999) and *Nannochloropsis* sp. have been identified as well-known sources of EPA, an important polyunsaturated fatty acid. *Spirulina* sp. has been recognized for its high protein content (Babadzhanov 2004). Microalgae, the primary producers of EPA and DHA in the marine food chain, can produce long chain omega-3 fatty acids, and naturally grow under a variety of autotrophic, mixotrophic, and heterotrophic culture conditions (Rubio-Rodríguez et al. 2010). Autotrophic and mixotrophic microalgae fix atmospheric carbon dioxide during photosynthesis, can grow on non-arable land, and have short harvesting times (Rubio-Rodríguez et al. 2010; Schenk et al. 2008). A comparison of EPA and DHA sources revealed that microalgae are able to produce greater quantities of EPA and DHA than the other common sources.

Oxidative stress is considered the main factor responsible for the ageing process, and in the pathophysiology of various diseases (D'Autreaux and Toledano 2007). Oxidative stress occurs as a result of excessive ROS generation or reduced activity of the antioxidative stress response systems.

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Oxidative stress has been thought to contribute to the general decline in cellular functions, and is associated with a number of human diseases including AD (Cacho-Valadez et al. 2012). AD is one of the most common neurodegenerative diseases of humans. Amyloid-beta (A β) deposits have been found in the brains of patients with AD (Yao et al. 2007). The “amyloid cascade hypothesis,” one of the well-accepted hypotheses of AD suggests that A β and its aggregated forms (including oligomers, protofibrils, and fibrils) may result in toxicity thereby leading to neurodegeneration (Milojevic and Melacini 2011; Shankar et al. 2008). The association between ROS, A β , and neural injury has also been previously reported (Butterfield et al. 2002).

The development of an efficient large-scale culture system for the commercial production of EPA and DHA would address a major global need. Therefore, we investigated the potential use of microalgae as in vitro biofactories for the large-scale production of omega-3 fatty acids against AD.

Materials and methods

Chemicals

Fetal bovine serum (FBS) was purchased from Life Technologies (Auckland, New Zealand). Dimethyl sulfoxide (DMSO) was purchased from Wako Pure Chemical Industries (Saitama, Japan). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), sodium dodecyl sulfate (SDS), 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), Triton X-100, trypsin, *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA), *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA), and Abeta25-35 were purchased from Sigma Chemical Co. (St Louis, MO). Dulbecco's modified Eagle's medium (DMEM), penicillin, and streptomycin were purchased from HyClone Laboratories (Logan, UT).

Growth conditions

Nannochloropsis oceanica was purchased from Fisheries Research Institute (COA) in Pintong (Taiwan). Inoculation density of cultures in production were $(1.5 \text{ to } 1.8) \times 10^6$ cell/mL. These cultures were continuously aerated by using an air pump without additional carbon dioxide. The laboratory temperature was kept at 20 ± 2 °C. In Group I, nitrogen source was provided from 2 g/L urea to maintain cell growth, but 0.2 g/L urea was used in Group II to elevate fatty acid synthesis. *Nannochloropsis oceanica* were collected directly from the bioreactor and centrifuged at 4,000 rpm for 4 min. Biomass cakes of samples were washed with 0.5 M NaCl and bidistilled water in order to eliminate non-biological material such as mineral salt precipitates. Then the biomass was freeze-

dried and stored -20 °C. The growth of *Nannochloropsis oceanica* was measured in the absorbance at 682 nm.

Sample extraction

Biomass of *Nannochloropsis oceanica* was extracted by isopropanol with ultrasonication (Ultrasonic Delta DC600H, Tainan, Taiwan) for 40 min. After filtration, the extracts were concentrated and freeze-dried.

Assay for fatty acid

Sample was pre-treated with the method of Christie (1982), and fatty acid was quantified by gas chromatography (GC). Briefly, the analysis of fatty acid in pigeon pea was carried out on an Hitachi gas chromatograph system (TRACE 2000, Japan) equipped with a column: fused silica column Rt-2330 (30 m $\times$ 0.32 mm, ID. 0.2 μ m thickness), a detector (flame ionization detector), and carrier gas (N₂, 1.5 mL/min). The GC measurement was according to our recent study (Dai et al. 2013).

Neuro-2A cell culture

Neuro-2A neuroblastoma cell line was obtained from the Bioresource Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsin Chu, Taiwan). Cells were grown in DMEM medium, supplemented with 10 % heat-inactivated FBS, glutamine (2 mM), and L-glutamine (2 mM) at 37 °C, in a humidified atmosphere of 95 % air and 5 % CO₂.

Cell viability assay

Neuro-2A cell viability was determined with the MTT assays. MTT solution as added to each well for an additional incubation of 4 h at 37 °C. After the addition of DMSO, the absorbance at 570 nm (formation of formazan) was measured (Hsu et al. 2010). Furthermore, cells were treated by Abeta25-35 (1, 10, 25, and 50 μ M) with or without samples for different times, and the protection effects of samples against Abeta-induced Neuro-2A cell damage were investigated in following experiments. Mixed DHA/EPA was used as positive control. The DHA and EPA levels in mixed DHA/EPA group were respectively 1.44 and 8.32 % in Group I, and 2.83 and 26.73 % in Group II.

Antioxidation measurement

For assay of glutathione (GSH), the reduced GSH content of cell homogenate was determined as previously described (Van Dam et al. 1999). Cell homogenate was mixed with TCA (50 mg/mL) mixture and incubated for 5 min, centrifuged at

8,000×g for 10 min under 4 °C. The homogenate was reacted with DTNB for 5 min under 4 °C. The absorbance was measured at 412 nm, and the concentration of GSH was calculated using the absorbance expressed by $\mu\text{mole/mg protein}$. GSH peroxidase (GPx) activity was determined as previously described (Mohandas et al. 1984). Briefly, 0.1 mL of homogenate was mixed with 0.8 mL of 100 mM potassium phosphate buffer (1 mM EDTA, 1 mM NaN_3 , 0.2 mM NADPH, 1 unit/mL glutathione reductase (GR), and 1 mM GSH, pH 7.0) and incubated for 5 min at room temperature. Thereafter, the reaction was initiated after adding of 0.1 mL of 2.5 mM hydrogen peroxide (H_2O_2). GPx activity was calculated by the change of the absorbance at 340 nm for 5 min. In another reaction containing 0.1 M phosphate buffer (1 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 50 mM GSSG, and 0.1 mM NADPH, pH 7.0), 0.1 mL of liver homogenate was added for GR activity determination (Bellomo et al. 1987). The decreased absorbance at 340 nm was measured for 3 min. The catalase (CAT) activity was determined by the method of Aebi (1984). The 50 μL of homogenate mixed with 950 μL 0.02 M H_2O_2 was incubated at room temperature for 2 min. The CAT activity was calculated by the change of the absorbance at 240 nm for 3 min. The glutathione S-transferase (GST) activity was determined as previously described (Habig et al. 1974). 100 μL of homogenate was mixed with 20 μL of 50 mM CDNB (1-chloro-2,4-dinitrobenzene) and 880 μL of 100 mM phosphate buffer (pH 6.5) that contained 1 mM GSH. The GST activity was calculated by the change of the absorbance at 340 nm for 5 min.

Assay for malondialdehyde (MDA) level

The homogenate was centrifuged for 30 min at 2,500×g and under −4 °C. The homogenate was stored at −80 °C for the following experiments. MDA, which is one of the lipid peroxidation products, was determined by the method of Buege and Aus (1978). Briefly, 1 mL of the homogenate was mixed with 1 mL of cold trichloroacetic acid (TCA) (75 mg/mL) to precipitate proteins and then centrifuged at 1,500 rpm. The supernatant was reacted with 1 mL of thiobarbituric acid (8 mg/mL) in boiling water for 45 min. Lipid peroxidation products were estimated by measuring the concentration of thiobarbituric acid reaction substances (TBARS) in fluorescence at 530 nm ex/552 nm.

Statistical analysis

Above data are expressed as means±SD. The software of ANOVA was used to evaluate the difference between multiple groups. If significance was observed between each group, Duncan's multiple range was used to compare the means of two specific groups, and $P < 0.05$ was considered to be significant.

Results and discussion

Effect of nitrogen as a nutrient for cell growth and biomass production

High biomass and commercially acceptable EPA and DHA production can be achieved with microalgae grown in media containing carbon and nitrogen, and controlled pH and temperatures (Griffiths and Harrison 2009; Chen et al. 2013). The growth rate could be regulated by the addition of nutrients such as glucose, nitrogen, sodium, as well as oxygen concentration, temperature, and pH. These conditions resulted in high cell densities and DHA production (Ward and Singh 2005). However, a deficiency in nutrients suppresses the growth of algae (Pramitha and Lipton 2011).

In our pre-test, we had found that limit nitrogen source at 2.5 g/L urea did not inhibit cell growth. In this study, we observed that the addition of 2 g/L urea (Group I) to growth medium significantly enhanced the growth of *Nannochloropsis oceanica* during 14 days of culture at 25 °C in presence of 5 % CO_2 . However, decreasing the urea concentrations to 0.2 g/L inhibited cell growth (Fig. 1a). This result indicates that nitrogen was a necessary factor for cell growth. In addition, the absence of nitrogen at various temperatures markedly affected biomass production (Fig. 1b). The biomass of *Nannochloropsis oceanica* was elevated in Group I (2 g/L urea) as well as Group II (0.2 g/L urea) because of an elevation in temperature from 15 to 30 °C while in culture. We also found a significant difference in biomass productivity between Group I and Group II, when cultured at 30 °C.

Effect of CO_2 on cell growth and biomass production

Microalgae are by far the most abundant primary producers that can be found in aquatic systems. Microalgae photosynthetically convert light energy and carbon dioxide (CO_2) into biomass such as carbohydrates (Park et al. 2011), proteins (Becker 2007), and lipids (including DHA and EPA) (Harwood and Guschina 2009). When we lowered urea levels, we observed enhanced DHA and EPA production in *Nannochloropsis oceanica*.

Autotrophic microalgae do not require an organic carbon source, and hence circumvent microbial contamination problems faced by heterotrophic cultures. In this study, we investigated the effect of CO_2 concentration (1, 2.5, 5, 10, and 20 %) on biomass production in *Nannochloropsis oceanica* grown at 30 °C. Our results suggested that the biomasses of Group I and Group II increased with increasing CO_2 levels from 1 % CO_2 to 10 % CO_2 . A high quantity of biomass was generated at 10 % CO_2 in both Group I and Group II. However, 20 % CO_2 suppressed biomass production in Group I and resulted in toxicity for Group II (Fig. 2). These

Fig. 1 a The change of *Nannochloropsis oceanica* growth between day 0 and day 14. b The biomass of *Nannochloropsis oceanica* at various temperatures. Group I: 2 g/L urea was provided, Group II: 0.2 g/L urea was provided. *Significantly different between Group I and Group II. Data was shown as mean $\pm$ SD ($n=3$)

data reveal that low nitrogen and high carbon sources induce cell toxicity in *Nannochloropsis oceanica*.

Crude fat, DHA, and EPA generations in *Nannochloropsis oceanica*

Temperature is a key factor that affects fatty acid generation in algae (Borowitzka 1997; Benemann 1992). Therefore, in this study, we investigated the optimal temperature for fatty acid generation. As shown in Fig. 3, *Nannochloropsis oceanica* was cultured in 10 % CO₂ at various temperatures (from 15 to 35 °C) for 14 days at which point crude fat was quantified. Our results demonstrate that lipid synthesis was elevated at 20 °C for Group II (as compared to Group I), but this effect could be suppressed when the temperature was elevated. These data show that lipid synthesis in *Nannochloropsis oceanica* is optimal at lower temperatures.

Additionally, we investigated the quantities of unsaturated fatty acids, including DHA (C22:6), and EPA (C20:5) (Table 1) in microalgae. We found that crude fat was higher in Group II than in Group I [C16:1 (9.34 %), C18:2 (10.31 %), C18:3 (7.53 %), C20:2 (0.84 %), C20:3 (0.42 %), C20:5

Fig. 2 The biomass of *Nannochloropsis oceanica* at various CO₂ concentrations. Group I: 2 g/L urea was provided, Group II: 0.2 g/L urea was provided. *Significantly different between Group I and Group II. Data was shown as mean $\pm$ SD ($n=3$)

26.73 %), and C22:6 (2.83 %)]. In addition, crude fat could not be detected in C20:1, C21:0, C22:0, C22:2, and C22:4. These findings suggest that low nitrogen can promote fatty acid synthesis including DHA and EPA.

Nannochloropsis oceanica protects against Abeta-induced oxidative stress in neuro-2A cells

Alzheimer's disease (AD) is characterized by progressive neurodegeneration leading to loss of cognitive abilities and ultimately death. The characteristic neuropathology of AD is the accumulation of amyloid-beta (Abeta) protein to form plaques and tau phosphorylation that forms tangles in patient brains. Memory deficits and amyloid deposition in the brain have also been observed in rats and mice with several different forms of Abeta mutations (Stephan and Phillips 2005). Specifically, Abeta1-40, Abeta1-42, and Abeta25-35,

Fig. 3 The crude fat of *Nannochloropsis oceanica* at various temperatures. Group I: 2 g/L urea was provided, Group II: 0.2 g/L urea was provided. *Significantly different between Group I and Group II. Data was shown as mean $\pm$ SD ($n=3$)

Table 1 Fatty acid compositions

Fatty acid	Group I %	Group II
C14:0	28.44	29.73
C14:1	0.83	1.27
C16:0	8.43	10.11
C16:1	3.21	9.34
C18:0	1.11	1.98
C18:1	1.25	1.31
C18:2	3.52	10.31
C18:3	2.73	7.53
C20:0	0.14	0.08
C20:1	ND	ND
C20:2	0.14	0.84
C20:3	0.07	0.42
C20:4	0.03	ND
C20:5n-3	8.32	26.73
C21:0	ND	ND
C22:0	ND	ND
C22:1	0.15	0.13
C22:2	ND	ND
C22:4	ND	ND
C22:6	1.44	2.83

ND no determination

Abeta1-56 peptide fragments could influence the learning and memory ability in mice. Abeta25-35 is a toxic Abeta peptide that directly impairs neural function without long-time aggregation (Lesne et al. 2006). Oxidative stress generated from Abeta deposits in the brain of AD patients can result in an increase in the generation of free radicals through NOX pathway (Williams and Gooch 2014) and impair the endogenous antioxidant system (Rammouz et al. 2011; Townsend and Pratico 2005).

Neuro-2A cells have been widely used as a model for investigating Abeta toxicity (Calderon et al. 1999). We found that neuro-2A cell viability decreased in a dose-dependent manner upon 48 h treatment with Abeta (Fig. 4a). In addition, this effect was found to be time-dependent in Abeta-treated neuro-2A cells after 6–48 h treatments (Fig. 4b). These findings indicate that Abeta induced cell death in neuro-2A cells. Recently, unsaturated fatty acids including DHA and EPA have been shown to protect against AD and neuronal cell damage (Sadli et al. 2013; Swanson et al. 2012). Our results indicate that the cell viability of Abeta-treated neuro-2A cells could be improved by treatment with extracts of *Nannochloropsis oceanica* from Group II (Fig. 5).

Antioxidant effect of *Nannochloropsis oceanica*

Smith et al. (1996) has proposed that oxidative stress plays a critical role in the development of AD. Lipid peroxidation is

Fig. 4 The cell viability for **a** toxicity of Abeta concentration and **b** induction time of Abeta treatment. Data was shown as mean $\pm$ SD ($n=3$)

one of the major outcomes of free radical-mediated injury, which directly damages membranes and generates a number of secondary products including aldehydes, such as MDA, 4-hydroxy-2-nonenal, and ketones (Slater 1984). Analysis of AD brains reveals an increase in lipid peroxidation products in the amygdala, hippocampus, and parahippocampal gyrus compared with that in age-matched controls (Markesbery and

Fig. 5 Protection of *Nannochloropsis oceanica* for Abeta-induced Neuro-2A cells. *Group I*: 2 g/L urea was provided, *Group II*: 0.2 g/L urea was provided. *Significantly different to Abeta induction group. Data was shown as mean $\pm$ SD ($n=3$)

Fig. 6 **a** MDA generation and **b** GSH level in Abeta-induced Neuro-2A cells. Group I: 2 g/L urea was provided, Group II: 0.2 g/L urea was provided. *Significantly different to Abeta induction group. Data was shown as mean $\pm$ SD ($n=3$)

Lovell 1998). MDA is the most abundant individual aldehyde resulting from lipid peroxidation and thus can be considered a

marker of lipid peroxidation. Abeta-peptide is produced by many different cell types, and is known to circulate in blood and cerebrospinal fluid in a soluble form (Mattson et al. 1997a, b; Maurice et al. 1996). Increased levels of oxidative stress are associated with amyloid plaques and degenerating neurons in the AD brain and peripheral tissues or circulating cells (Smith et al. 1995). Lipid peroxidation and the concentration of reactive oxygen species (ROS) in the cortex, hippocampus, spleen, and astrocytes are significantly elevated in Abeta25-35 injected mice.

The extracts of *Nannochloropsis oceanica* from Group II markedly suppressed MDA production in Abeta-treated Neuro-2A cells than in Group I (Fig. 6a). Moreover, the extracts of *Nannochloropsis oceanica* from Group II resulted in markedly elevated GSH levels in Abeta-treated Neuro-2A cells. These results suggest that *Nannochloropsis oceanica*-induced protection against Abeta-induced neuro-2A cell damage is mediated by the oxidation status.

Elevation of levels of antioxidant enzymes of *Nannochloropsis oceanica*

Oxidative stress is induced by the production of free radicals (Cafe et al. 1996), disruption of calcium homeostasis (Mattson et al. 1997a, b), and apoptosis (Kowall et al. 1991). Histological and biochemical changes, memory deficits, and oxidative stress are observed 1 to 2 weeks after intracerebroventricular administration of the Abeta25-35 peptide into rodent brains (Maurice et al. 1996). Therefore, interventions that protect against Abeta-induced neurotoxicity may have therapeutic value for the treatment of AD (Valko et al. 2007). Our results have shown that *Nannochloropsis oceanica* may have a direct effect on Abeta-induced changes in oxidative stress and the regulation of antioxidant enzymes, while DHA and EPA protect against Abeta toxicity. Removal of ROS by antioxidant enzymes can inhibit lipid peroxidation

Table 2 Activity of antioxidant enzyme in Neuro-2A cell treated by *Nannochloropsis oceanica*

Groups		GR nmol/min/mg protein	GPx	GST	CAT
Control		132.1 $\pm$ 3.6 ^a	352.1 $\pm$ 1.2 ^a	942.0 $\pm$ 14.2 ^a	21.4 $\pm$ 0.8 ^a
Abeta		46.3 $\pm$ 2.7	89.3 $\pm$ 1.6	614.2 $\pm$ 10.2	4.2 $\pm$ 0.4
Group I	Abeta + Low dosage	51.3 $\pm$ 2.1	101.2 $\pm$ 1.5	657.1 $\pm$ 8.4	4.7 $\pm$ 0.5
	Abeta + Medium dosage	50.7 $\pm$ 1.7	100.7 $\pm$ 2.6	717.4 $\pm$ 11.6 ^a	4.6 $\pm$ 0.4
	Abeta + High dosage	73.3 $\pm$ 1.5 ^a	162.6 $\pm$ 1.9 ^a	704.3 $\pm$ 14.7 ^a	6.8 $\pm$ 0.4
Group II	Abeta + Low dosage	52.4 $\pm$ 2.5	100.4 $\pm$ 2.1	701.7 $\pm$ 20.1 ^a	4.8 $\pm$ 0.8
	Abeta + Medium dosage	67.3 $\pm$ 2.1 ^a	147.3 $\pm$ 1.6 ^a	793.5 $\pm$ 16.3 ^a	5.6 $\pm$ 0.3
	Abeta + High dosage	89.5 $\pm$ 1.7 ^a	203.6 $\pm$ 1.8 ^a	875.6 $\pm$ 14.3 ^a	8.9 $\pm$ 0.2 ^a

^a Significantly different to Abeta-induced group. Data was shown as mean $\pm$ SD ($n=3$). Low dosage: 25 μ g/mL of *Nannochloropsis oceanica* extracts; Medium dosage: 50 μ g/mL of *Nannochloropsis oceanica* extracts; High dosage: 100 μ g/mL of *Nannochloropsis oceanica* extracts. Group I: 10 g/L urea was provided, Group II: 0.2 g/L urea was provided

in cell membranes (Li et al. 1990). Antioxidants such as glutathione peroxidase (GPx), glutathione reductase (GR), glutathione *S*-transferase (GST), and catalase (CAT) clear ROS and protect cells from oxidative damage (Anderson 1996). Our results indicate that the extracts from *Nannochloropsis oceanica* in Group II markedly elevate GR, GPx, GST, and CAT activities in Aβeta-treated neuro-2A cells (Table 2).

Conclusion

This study presents novel findings concerning the effects of *Nannochloropsis oceanica* against Aβeta-mediated bioenergetic alterations in neuro-2A neuroblastoma cells. Our results have shown that *Nannochloropsis oceanica* may directly affect Aβeta-induced oxidative stress and the regulate levels of antioxidant enzymes. Importantly, DHA and EPA protect against Aβeta toxicity. Taken together, the results of our study indicate the potential dietary benefits of DHA and EPA obtained from *Nannochloropsis oceanica* using a culture system with low nitrogen levels, which is a characteristic of neurodegenerative diseases such as Alzheimer's disease.

Disclosure No competing financial interests exist.

References

- Aebi H (1984) Catalase in vitro. *Meth Enzymol* 105:121–126
- Al-Taani O, Stephenson JA, Spencer L, Pollard C, West AL, Calder PC, Metcalfe M, Dennison AR (2013) Changes in plasma and erythrocyte omega-6 and omega-3 fatty acids in response to intravenous supply of omega-3 fatty acids in patients with hepatic colorectal metastases. *Lipids Health Dis* 12:64
- Anderson D (1996) Antioxidant defences against reactive oxygen species causing genetic and other damage. *Mutat Res* 350:103–108
- Babadzhanov AS (2004) Chemical composition of *Spirulina plantensis* cultivated in Uzbekistan. *Chem Nat Comp* 40:276–279
- Becker EW (2007) Micro-algae as a source of protein. *Biotechnol Adv* 25:207–210
- Bellomo G, Mirabelli F, DiMonte D, Richelmi P, Thor H, Orrenius C, Orrenius S (1987) Formation and reduction of glutathione-protein mixed disulfides during oxidative stress. A study with isolated hepatocytes and menadione (2-methyl-1,4-naphthoquinone). *Biochem Pharmacol* 36:1313–1320
- Benemann J (1992) Microalgae aquaculture feeds. *J Appl Phycol* 4:233–245
- Borowitzka M (1997) Microalgae for aquaculture: opportunities and constraints. *J Appl Phycol* 9:393–401
- Buege AJ, Aus SD (1978) Microsomal lipid peroxidation. *Meth Enzymol* 52:302–310
- Butterfield DA, Griffin S, Munch G, Pasinetti GM (2002) Amyloid beta-peptide and amyloid pathology are central to the oxidative stress and inflammatory cascades under which Alzheimer's disease brain exists. *J Alzheimers Dis* 4:193–201
- Cacho-Valadez B, Munoz-Lobato F, Pedrajas JR, Cabello J, Fierro-Gonzalez JC, Navas P, Swoboda P, Link CD, Miranda-Vizueté A (2012) The characterization of the *Caenorhabditis elegans* mitochondrial thioredoxin system uncovers an unexpected protective role of thioredoxin reductase 2 in β-amyloid peptide toxicity. *Antioxid Redox Signal* 16:1384–1400
- Café C, Torri C, Bertorelli L, Angeretti N, Lucca E, Forloni G, Marzatico F (1996) Oxidative stress after acute and chronic application of beta-amyloid fragment 25–35 in cortical cultures. *Neurosci Lett* 203:61–65
- Calderon FH, Bonnefont A, Munoz FJ, Fernandez V, Videla LA, Inestrosa NC (1999) PC12 and Neuro 2A cells have different susceptibilities to acetylcholinesterase-amyloid complexes, amyloid25-35 fragment, glutamate, and hydrogen peroxide. *J Neurosci Res* 56:620–631
- Chen CY, Chen YC, Huang HC, Huang CC, Lee WL, Chang JS (2013) Engineering strategies for enhancing the production of eicosapentagenoi acid (EPA) from an isolated microalga *Nannochloropsis oceanica* CY2. *Bioresour Technol* 147:160–167
- Christie WW (1982) A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J Lipid Res* 23:1072–1075
- Dai FJ, Hsu WH, Huang JJ, Wu SC (2013) Effect of pigeon pea (*Cajanus cajan* L.) on high-fat diet-induced hypercholesterolemia in hamsters. *Food Chem Toxicol* 53:384–391
- D'Autreaux B, Toledano MB (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 8:813–824
- Griffiths MJ, Harrison STL (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J Appl Phycol* 21:493–507
- Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 249:7130–7139
- Harwood JL, Guschina IA (2009) The versatility of algae and their lipid metabolism. *Biochimie* 91:679–684
- Hsu WH, Lee BH, Pan TM (2010) Red mold dioscorea-induced G2/M arrest and apoptosis in human oral cancer cells. *J Sci Food Agric* 90:2709–2715
- Kowall NW, Beal MF, Busciglio J, Duffy LK, Yanker BA (1991) An in vivo model for the neurodegenerative effects of beta-amyloid and protection by substance. *Proc Natl Acad Sci U S A* 88:7247–7251
- Lesne S, Koh MT, Kotilinek L, Kaye R, Glabe CG (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 440:352–357
- Li XJ, Zhao BL, Liu GT, Xin WJ (1990) Scavenging effects on active oxygen radicals by schizandrin with different structures and configurations. *Free Radic Biol Med* 9:99–104
- Markesbery WR, Lovell MA (1998) Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol Aging* 9:33–36
- Mattson MP, Begley JG, Mark RJ, Furukawa KA (1997a) Aβeta25–35 induces rapid lysis of red blood cells: contrast with Aβeta1–42 and examination of underlying mechanisms. *Brain Res* 771:147–153
- Mattson MP, Furukawa K, Bruce AJ, Mark RJ, Blanc EM (1997b) Calcium homeostasis and free radical metabolism as convergence points in the pathophysiology of dementia. In: Wasco W, Tanzi RE (eds) *Molecular mechanisms of dementia*. Humana Press, Clifton, pp 103–143
- Maurice T, Lockhart BP, Privat A (1996) Amnesia induced in mice by centrally administered beta-amyloid peptides involves cholinergic dysfunction. *Brain Res* 706:181–193
- Milojevic J, Melacini G (2011) Stoichiometry and affinity of the human serum albumin-Alzheimer's Aβeta peptide interactions. *Biophys J* 100:183–192

- Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ (1984) Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. *Cancer Res* 44: 5086–5091
- Nobre ME, Correia AO, Borges Mde B, Sampaio TM, Chakraborty SA, Goncalves Dde O, Brito GA, Leal LK, Felipe CF, Lucetti DL, Arida RM, Viana GS (2013) Eicosapentaenoic acid and docosahexaenoic acid exert anti-inflammatory and antinociceptive effects in rodents at low doses. *Nutr Res* 33:422–433
- Park JH, Yoon JJ, Park HD, Kim YJ, Lim DJ, Kim SH (2011) Feasibility of biohydrogen production from *Gelidium amansii*. *Int J Hydrogen Energy* 36:13997–14003
- Pramitha VS, Lipton AP (2011) Growth responses of microalgae, *Chlorella salina* and *Isochrysis galbana* exposed to extracts of the macroalga, *Hypnea musciformis*. *Indian J Fish* 58:95–99
- Rammouz G, Lecanu L, Aisen P, Papadopoulos V (2011) A lead study on oxidative stress-mediated dehydroepiandrosterone formation in serum: the biochemical basis for a diagnosis of Alzheimer's disease. *J Alzheimers Dis* 24:5–16
- Rocha MS, Garcia EC, Henriques HF (2003) Growth aspects of the marine microalga *Nannochloropsis gaditana*. *Biomol Eng* 20:237–242
- Rubio-Rodríguez N, Beltrán S, Jaime I, de Diego SM, Sanz MT, Carballido JR (2010) Production of omega-3 polyunsaturated fatty acid concentrates: a review. *Innov Food Sci Emerg Tech* 11:1–12
- Sadli N, Barrow CJ, McGee S, Suphioglu C (2013) Effect of DHA and coenzyme Q10 against Aβeta- and zinc-induced mitochondrial dysfunction in human neuronal cells. *Cell Physiol Biochem* 32:243–252
- Sahlin C, Pettersson FE, Nilsson LN, Lannfelt L, Johansson AS (2007) Docosahexaenoic acid stimulates non-amyloidogenic APP processing resulting in reduced Aβeta levels in cellular models of Alzheimer's disease. *Eur J Neurosci* 26:882–889
- Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussnug JH, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy Res* 1:20–43
- Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14:837–842
- Slater TF (1984) Overview of the methods used for detecting lipid peroxidation. *Meth Enzymol* 105:283–293
- Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF, Kowall N (1996) Oxidative damage in Alzheimer's. *Nature* 382: 120–121
- Smith MA, Sayre LM, Monnier VM, Perry G (1995) Radical ageing in Alzheimer's disease. *Trends Neurosci* 18:172–176
- Stephan A, Phillips AG (2005) A case for a non-transgenic animal model of Alzheimer's disease. *Genes Brain Behav* 4:157–172
- Swanson D, Block R, Mousa SA (2012) Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Nutrition* 3:1–7
- Townsend KP, Pratico D (2005) Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs. *FASEB J* 19:1592–1601
- Valko M, Leibfriz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 39:44–84
- Van Dam PS, Van Asbeck BS, Bravenboer B, Van Oirschot JF, Marx JJ, Gispen WH (1999) Nerve conduction and antioxidant levels in experimentally diabetic rats: effects of streptozotocin dose and diabetes duration. *Metab Clin Exp* 48:442–447
- Ward OP, Singh A (2005) Omega-3/6 fatty acids: alternative sources of production. *Process Biochem* 40:3627–3652
- Williams CR, Gooch JL (2014) Calcineurin Aβeta regulates NADPH oxidase (NOX) expression and activity via nuclear factor of activated T cells (NFAT) in response to high glucose. *J Biol Chem* 289: 4896–4905
- Yang YC, Lii CK, Wei YL, Li CC, Lu CY, Liu KL, Chen HW (2012) Docosahexaenoic acid inhibition of inflammation is partially via cross-talk between Nrf2/heme oxygenase 1 and IKK/NF-kappaB pathways. *J Nutr Chem* 24:204–212
- Yao J, Taylor M, Davey F, Ren Y, Aiton J, Coote P, Fang F, Chen JX, Yan SD, Gunn-Moore FJ (2007) Interaction of amyloid binding alcohol dehydrogenase/Aβ mediates up-regulation of peroxiredoxin II in the brains of Alzheimer's disease patients and a transgenic Alzheimer's disease mouse model. *Mol Cell Neurosci* 35:377–382
- Yongmantichai OP (1999) Growth of and omega-3 fatty acid production by *Phaeodactylum tricomutum* under different culture conditions. *Appl Environ Microbiol* 57:419–425