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SCREENING OF CULTIVATION MEDIA FOR MICROALGAL SOURCES OF EICOSAPENTAENOIC ACID (EPA)

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INTRODUCTION

Polyunsaturated fatty acids (PUFA) of the ω-3 type are essential to human nutrition because they are building blocks in membrane lipid biogenesis and play important roles in the structure and function of biological membranes. Inclusion of such PUFA in normal human diets has also been advocated in view of claims of lowering plasma cholesterol levels and prevention of coronary heart diseases. A direct intake of those fatty acids is thus generally recommended (Carvalho, 1996). Although the most common source is fish oil, organoleptic and economic queries have provided an impetus for alternative sources, e.g. microscopic algae. Variations in the fatty acid profile of intracellular fats within a species can be caused by differences in growth conditions, namely differences in nutrients availability. In order to determine the medium composition that potentiates EPA production in two microalgae species, four different media (covering a qualitative and quantitative spectrum of nutrients) were tested.

MATERIALS AND METHODS

ORGANISMS: *Nannochloropsis* sp. (s/NANNO-2) from Solar Energy Research Institute (SERI), Golden, CO, USA and *Pavlova lutheri* from Instituto Português de Investigação Marítima (IPIMAR), Lisboa, Portugal.

CULTURE CONDITIONS: Cultures were grown in 100 ml Erlenmeyer flasks, with a working volume of 50 ml. The media used to cultivate each algae were prepared based on information provided by original investigators: (i) GPM, according to F. Haxo Scripps Institution of Oceanography (SERI, 1986), without soil extract, (ii) MN (Borowitzka, 1988), (iii) f/2 Seawater (Guillard and Ryther, 1962), (iv) ASW (Darley and Volcani, 1969) without glycylglycine (SERI, 1986). In i, ii and iii, seawater was substituted by deonized water with 3-3.5% salt. Cultures were incubated in an orbital incubator at 20±2°C, with ca. 40 μmol/m^{2}s, in a continuous light cycle. Cells were harvested after 13 days (late exponential phase) and were analysed for cell mass and fatty acid composition.

CELL MASS: Determinations of cell number, were done with a Neubauer haemocytometer, and ash free dry weight (AFDW), were done with pre-conditioned Whatman GF/C glass fibre filters, dried at 80°C to constant weight and heated at 550°C for 1 hr.

FATTY ACID COMPOSITION: Fatty acid methyl esters (FAME) were obtained by direct transesterification of freeze-dried samples, according to the acidic method described by Lepage and Roy (1984), with modifications introduced by Cohen *et al* . (1988), and using heptadecanoic acid as internal standard. The analysis of FAMEs was carried out by gas chromatography in a 30 m-capillary column of fused silica (CP-Sil 88, Chrompack, The Netherlands) using helium as the carrier gas and a flame ionization detector (FID). Pure standards (SIGMA) were used for calibrations, and fatty acid identifications were based on comparison of sample and standard peak retention times.

RESULTS AND DISCUSSION

The selected media where chosen from media claimed by other investigators as adequate to the algae in question. All experiments were run in duplicate. The mean values for AFDW and cell counts for both algae are tabulated in table 1.

TABLE 1: Results from biomass assays

	P. lutheri		*N.* sp.	
MEDIUM	MEAN AFDW (g/L)	MEAN CELL NR. (cell/ml)	MEAN AFDW (g/L)	MEAN CELL NR. (cell/ml)
GPM	0.610	7.3E6	0.425	34.9E6
MN	0.460	6.5E6	0.450	28.6E6
f/2	0.520	6.0E6	0.535	36.8E6
ASW	0.535	8.6E6	0.550	45.8E6

The results from biomass assays were statistically analyzed by an ANOVA table, with a Fisher's PLSD test to the medium effect, at a 5% significance level. At this level, all media revealed statistically equal performances in terms of biomass results.
Freeze-dried biomass from each flask was transesterified in duplicate, and each duplicate was injected twice in the chromatograph. The average amounts of biomass and fatty acids for the screened standards are presented in table 2.

TABLE 2: Biomass and fatty acid (in mg/litre of culture) in the media tested

	Medium	Biomass (mg/L)	16:0	16:1ω7	18:0	18:1	18:2ω6	18:3ω6	18:3ω3	20:4ω6	20:5ω3	22:6ω3
P. lut.	ASW	1450	1.866	0.332	1.235	0.633	0.179	0	0	0	0.281	0.108
	f/2	1230	1.517	0.265	0.906	0.522	0.085	0	0	0	0.214	0.088
	GPM	1665	2.419	0.387	1.872	0.633	0.209	0	0.067	0	0.346	0.114
	MN	1320	2.117	0.285	1.371	0.890	0.126	0	0.043	0	0.214	0.109
N. sp.	ASW	1615	1.656	0	1.093	0.587	0.203	0	0	0	0	0
	f/2	1465	1.418	0	0.875	0.644	0.160	0	0	0	0	0
	GPM	1280	1.352	0	0.783	0.635	0.178	0	0	0	0	0
	MN	1445	1.052	0	0.592	0.550	0.151	0	0	0	0	0

The major fatty acids (FA) in *P. lutheri* are palmitic (16:0), palmitoleic (16:1ω7), stearic (18:0), oleic (18:1) and eicosapentaenoic (20:5ω3); the proportion of docosahexaenoic acid (22:6ω3) is low, which has important advantages in terms of EPA recovery. *Nannochloropsis* sp. exhibited a profile of FA dominated by 16:0, 18:0 and 18:1, but without EPA.
An unpaired t-test at the 5% significance level was performed with these results in order to determine whether the different amounts observed were significantly different. From the results, it could be concluded that for *P. lutheri*, statistically significant differences in fatty acid amounts can be observed for linoleic acid (18:2) between both ASW and GPM against f/2; since the two first media provide higher amounts of this fatty acid, one of them should be chosen instead of f/2. α-linoleic acid (18:3ω3) is not produced in f/2 and ASW, and their amounts in GPM and MN are not significantly different. With eicosapentaenoic acid, the results and conclusions are the same as for linoleic acid, and for docosahexaenoic acid no statistically significant differences are observed. There is no statistically significant difference at the 5% level for any of the FA amounts detected in *Nannochloropsis* sp. between the different media.
From these results it can be concluded that *Nannochloropsis* sp. is not a suitable source of EPA, while *P. lutheri* is; in terms of biomass production, there is no significant difference between the media compositions tested, but with respect to EPA production, ASW or GPM media should be preferentially chosen instead of MN or f/2. Further studies should be performed, with other physico-chemical variations, in order to further enhance EPA production.

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