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FATTY ACID VARIATION AMONG DIFFERENT ISOLATES OF A SINGLE STRAIN OF *ISOCHRYSIS GALBANA*

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Key Word Index—*Isochrysis galbana*; Isochrysidaceae; microalga; fatty acid composition; fatty acid variation between isolates; genetic variation.

Abstract—Fatty acids of 42 isolates of a single strain of *Isochrysis galbana* were analysed. The data showed an evident between-isolates variability in fatty acid content. Underlying genetic variation is suggested as the explanation and biological and practical implications are discussed.

INTRODUCTION

As previously noted [1, 2], published data on the fatty acid composition of *Isochrysis galbana* show wide discrepancies [1–8]. These discrepancies until now have been explained by 'external' factors (i.e. culture conditions, analytical methods, harvest time, etc.), but differences in fatty acid content could also be explained by genetic ('internal') differences between strains of the same species as observed by Volkman *et al.* [1] and suggested by us [2]. Knowledge of the principle cause of fatty acid variation, in this and in other microalgae species, is important both in pure and applied science.

Fatty acid composition is currently studied for chemotaxonomic purposes on algae [9–13] and for the evaluation of microalgae quality as food in aquaculture [14–16]. At the same time, growing attention has been devoted to the *n*-3 fatty acid content of microalgae due to its beneficial role in human health. Several recent studies are focused on comparative screening of some microalgae species as sources of *n*-3 fatty acids [2, 7, 17].

In this paper, evidence is reported of wide variations in fatty acid composition (*n*-3 included) among different isolates of a single culture collection strain of *I. galbana* and some biological and practical implications are discussed.

RESULTS AND DISCUSSION

A new approach has been used to elucidate whether fatty acid content is genetically determined [2]. A single strain was analysed instead of different strains. Fifty-nine isolations were made from a single culture collection strain. One of these isolates (AL41) produced a new set of 42 isolates, analyses on which are presented in this report.

Fatty acids analysed for were 14:0, 16:0, 16:1 (*n*-9), 16:1 (*n*-7), 18:0, 18:1 (*n*-9), 18:1 (*n*-7), 18:2 (*n*-6), 20:0, 18:3, 20:1 (*n*-9), 18:4 (*n*-3), 22:0, 20:4 (*n*-6), 22:1 (*n*-11), 22:1 (*n*-9), 20:5 (*n*-3) (eicosapentaenoic acid), 24:0, 22:5 (*n*-3) and 22:6 (*n*-3) (decosahexaenoic acid). The data shown in Table 1 are the fatty acids for which the average content was greater than 2%.

All isolates were manipulated, cultivated and harvested under identical conditions and all were analysed with the same appartus and method to eliminate 'environmental' variation. All isolates were different in fatty acid composition (Table 1). For example, eicosapentaenoic acid (EPA) content ranged between 16.8 and 29.4% and docosahexaenoic acid (DHA) content between 4.9 and 13.8% of the total fatty acids.

We have evidence of the consistency of our results. During more than six months of continuous culture, one of the isolates (4) was harvested and analysed twice each week. The EPA content during that period ranged between 22.6 and 25.3%; DHA ranged between 9.2 and 11.1%, and the remaining fatty acids showed similar ranges of variation. This shows that this isolate maintains its fatty acid profiles within rather narrow limits over time.

Since culture experiments were conducted under identical conditions, phenotypic (fatty acid) variation among isolates cannot be explained by environmental variation, but solely by genetical differences in the isolates. The most likely explanation is that our parent culture of *I. galbana* was genetically heterogeneous, the fatty acid variation among isolates being an expression of the underlying genetical variation. Following this hypothesis, discrepancies noted in fatty acid composition between different studies on *I. galbana* [1, 2] are easily explained. The question posed by Volkman *et al.* [1] about the hypothetical origin of discrepancies can be answered: taking into account the 'external' factors [7, 8, 18–21], the microalgae genome determines the variation in fatty acid profiles.

The existence of genetic variation in the parent culture (AL41) merits discussion. In fact, it was surprising as single-cell isolates had been made and reproduction was asexual, thus clonal cultures were expected [2]. The results neccesitate a change of view; micromanipulation may not have been so careful as to exclude the establishment of cultures of more than one cell and, at the same time, there is sexual reproduction in *I. galbana*, as suggested many years ago [22]. So, perhaps isolates were founded with several cells that intercrossed, originating a

Table 1. Fatty acid composition (percentage of total fatty acids) in 42 isolates from a single strain of *Isochrysis galbana*

	Fatty acid								
iso-	14:0	16:0	16:1	18:1	20:0	18:4	20:5	22:6	PUFAs
1	9.9	13.3	13.8	1.9	3.0	7.3	25.2	8.7	41.8
2	9.3	11.1	11.9	2.1	3.0	8.2	26.8	9.8	46.8
3	9.2	11.9	12.0	2.4	2.8	7.5	29.4	9.6	47.2
4	10.7	11.0	12.2	2.4	2.8	7.4	25.9	9.4	43.4
5	9.3	12.2	12.7	2.2	3.2	7.4	26.2	9.2	44.2
6	10.1	12.0	12.7	1.8	3.4	7.7	26.0	10.1	44.4
7	10.8	11.0	12.9	2.3	2.8	7.7	27.2	9.3	44.8
8	9.7	11.2	11.9	1.9	3.5	6.2	24.5	8.9	40.8
9	11.0	13.1	12.6	2.1	3.6	6.2	26.4	8.7	41.9
10	10.5	12.5	12.2	3.2	3.2	8.2	24.7	9.4	43.2
11	9.8	12.2	11.5	5.4	2.2	8.5	24.5	9.4	43.4
12	11.2	10.8	11.5	2.2	2.2	5.7	25.7	8.5	40.9
13	10.5	12.4	12.8	1.7	3.0	7.4	25.7	9.3	43.1
14	10.4	14.0	13.7	1.7	2.6	6.1	21.7	9.3	37.8
15	10.2	16.0	14.5	2.2	3.1	5.5	24.0	9.4	39.9
16	6.8	12.7	12.8	5.7	3.3	8.2	26.5	10.2	45.6
17	9.9	13.3	12.7	4.4	3.4	5.6	22.8	8.2	37.4
18	9.8	19.2	15.3	2.1	2.1	5.5	22.8	8.6	38.2
19	9.0	12.8	12.1	2.8	3.2	5.4	27.3	8.8	42.3
20	10.4	15.6	14.7	3.0	2.8	5.5	23.9	9.2	39.7
21	9.5	16.8	14.4	4.2	2.2	5.6	23.3	9.3	39.3
22	12.8	19.2	19.8	3.6	0.8	4.8	16.8	5.2	28.0
23	14.0	14.0	14.0	2.1	2.8	5.7	22.5	8.0	37.1
24	11.3	15.8	15.3	4.0	2.7	5.7	21.1	8.0	35.4
25	10.8	14.9	13.4	2.8	2.8	6.4	23.5	10.7	41.2
26	9.9	17.3	15.4	2.1	2.9	6.3	23.8	8.5	39.4
27	10.5	14.0	13.7	5.0	3.7	5.7	23.5	8.4	38.9
28	9.6	18.2	15.6	1.8	3.1	5.7	22.9	8.7	38.2
29	10.5	12.0	13.4	3.0	2.2	6.4	24.0	9.8	40.8
30	10.0	18.2	16.1	5.0	2.1	5.6	21.5	8.6	36.5
31	10.2	15.0	15.0	3.3	2.8	6.5	23.9	9.2	40.5
32	11.7	13.6	14.3	2.6	2.8	6.3	24.1	9.5	40.6
33	9.8	16.5	15.2	5.8	2.3	5.9	20.8	8.9	37.0
34	10.5	15.0	12.3	2.0	1.9	4.1	23.9	11.8	40.5
35	6.0	12.7	13.5	2.7	2.6	9.4	28.0	4.9	47.7
36	10.2	13.2	13.6	2.0	3.3	6.8	25.4	10.9	44.4
37	10.9	15.5	13.8	3.0	1.4	4.7	26.3	12.0	44.5
38	6.4	13.2	11.5	3.0	3.3	7.5	27.2	13.8	49.2
39	6.1	11.8	10.2	3.4	2.9	5.6	28.2	8.0	44.8
40	10.6	12.5	12.9	2.3	3.4	7.2	25.9	9.2	45.0
41	9.6	16.1	12.5	3.0	1.7	4.4	24.9	11.4	42.2
42	10.6	13.1	13.1	2.8	3.2	6.4	26.2	8.9	42.1
$\bar{x}$	10.0	14.0	13.5	2.9	2.8	6.4	24.6	9.2	41.4
Std	1.51	2.27	1.65	1.12	0.59	1.16	2.31	1.48	3.80
Max	14.0	19.2	19.8	5.8	3.7	9.4	29.4	13.8	49.2
Min	6.0	10.8	10.2	1.7	0.8	4.1	16.8	4.9	28.0

$\bar{x}$ = overall average.

Std = standard deviation.

Max and min are the highest and lowest values; only data for the most abundant fatty acids are shown (data are shown according to biometrical standards).

genetically heterogeneous culture. Thus, the AL41 'clone' was likely to be not clonal *sensu stricto* (i.e. genetically homogeneous). If this hypothesis is true, it could have wide implications, because many other microalgae cultures reported as clonal might then be false clones, since sexual reproduction may exist in many microalgae.

Our hypothesis is not esoteric from a population genetics view but, in general, genetics has not been taken into account in most studies on microalgae. Moreover, *I. galbana* is surely not an exception, but the illustration of a rule and, probably, many other microalgae species strains are also biochemically and genetically heterogen-

eous. In fact, even bacterial populations are genetically heterogeneous being composed of several clones [23]. Some papers have recently reported variation in reproduction rates between clones of microalgae and suggest genetic inter-clonal variation [24–26]. One reports fatty acid variation between clones of *Skeletonema costatum* which is explained by an identical hypothesis [27].

Simultaneous with the variation observed among our isolates of *I. galbana*, a 'qualitative' pattern can also be observed. The same fatty acids are the most abundant in all isolates (Table 1). Thus, 14:0, 16:0, 16:1 (*n*-7), 18:4 (*n*-3), 20:5 (*n*-3) and 22:6 (*n*-3) were always present in greatest quantities. We could say that these fatty acids are 'typical' or 'characteristic' of our cultured strain, but only of this strain, because each *I. galbana* strain shows a different 'qualitative' fatty acid composition [1–6].

The ignorance of the existence of this basic variation could lead to erroneous assumptions. For example, *I. galbana* is currently reported as a species poor in EPA [1, 3, 5, 23], when this may be true for the T-ISO strain, but not for all *I. galbana* strains [2, 4, 7]. Consequently, *I. galbana* may not have been taken into account in the screening of microalgae species as alternative sources of EPA [17], while some of our isolates had a promising EPA content of nearly 30% (Table 1), and selection might further improve the fatty acid content.

In the same way, the very hypothesis underlying other works (e.g. on chemotaxonomy or food quality evaluation) based on fatty acid content could suffer if the fatty acid variation between and within strains is not considered. For instance, Cohen and Vonshak [9] proposed excluding from the genus *Spirulina* some cyanobacteria morphologically indistinguishable from it on the basis of their fatty acid profiles. This suggestion could be rejected following the results with *I. galbana*. On the other hand, it is conceivable that knowledge of the fatty acid variation could be employed to formulate foods for molluscs by careful selection of strains of various algal species (J. D. Castell, personal communication). At the same time, genetic variation may be used to improve strains for the production of fine chemicals with the usual microbiological methods [29, 30] as is being done in our laboratory.

EXPERIMENTAL

The original culture collection of *I. galbana* Parke was supplied by Dr J. Fábregas (Departamento de Microbiología, Universidad de Santiago de Compostela, Spain). Cells were isolated with a micropipette under microscope from a previous isolate labelled AL41. Each isolate (one or a few cells each) was transferred to a test tube for cultivation, 42 isolates were successful. All isolates were cultivated under identical conditions: medium Algal 1 (from Nutrición Avanzada S. A.' Spain), continuous light intensity of 55 W m^{-2}, temp. 20°, pH 8 and specific air supply rate of 2 v v^{-1} min^{-1}.

Filtered seawater (from the Almería coast, Mediterranean Sea) was sterilised in an autoclave at 120° for 30 min and the complete culture medium was sterilized by filtration through 0.2 μm pore membranes. All cultures were harvested during the stationary phase of growth for subsequent analysis. Biomass was washed with a dil saline soln (9:1000).

Lipids were extd by the method of ref. [31]. Fatty acid methylation was done by direct transterification with AcCl–MeOH methanol (1:20) following the method of ref. [32]. Analyses of Me esters were carried out by GC using a 30 m capillary column of fused silica (SP 2330) i.d. 0.25 mm, 0.2 mm film thickness, split 100:1, an injn vol. of 1.1 μl and a FID. The carrier gas (N_2) flow rate was 0.8 ml min^{-1}, init. temp. 150° during 8 min followed by a 3° min^{-1} to up 190°, staying constant during 25 min. Inj. and detector temps were 220°. Sigma Lipid Standard 189-15, Supelco rapeseed oil mixture and Supelco PUFAs-I patterns were used for the determination.

Each isolate was analysed two or more times in order to eliminate technical errors in the determination of its fatty acid content. Data shown in Table 1 are the averages of two analyses. Pigment content was substracted from total lipids to calculate fatty acid content of total fatty acids. Data are shown with the number of digits according to biometrical rules [33].

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