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AGENCY FOR INTERNATIONAL DEVELOPMENT
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MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C7-131 PR# 2
"The Alga Spirulina as a Source of Linolenic Acid and Other Valuable Chemicals"

Attachment

DUPLICATE

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CDR PROJECT NO. C7-131

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The Alga Spirulina as a Source of Linolenic Acid and Other Valuable Chemicals

Interim Report No. 2

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Collaboration

In the first year there were several visits from both groups. Dr. Boonsom and Ms. Reungjitchachawaly visited the lab at Sede-Boker for an extended period for training and methodology transfer. In the following months,there were 3 visits of the Israeli group in Thailand. Prof. Richmond visited the group at February and delivered several seminars on algal biotechnology as well as assesed the scientific situation of the lab and made several recommendation. His visit was followed by Dr. Vonshak's in March who addressed problems concerning indoor and outdoor algal cultivation. Dr. Cohen visited in April and dealt mainly with experimental design and data analysis and also with problems concerning chemical analysis of fatty acids and pigments and the design of an outline of a separation scheme for the chemicals of interest.

Results

Fatty acid composition of Spirulina strains

Nineteen strains of *Spirulina* were cultivated under the same conditions. All the tested strains except *S. subsalsa* contained the same fatty acids (Table 1). The predominant fatty acids were palmitic acid (16:0), GLA, linoleic acid (18:2) and oleic acid (18:1), as already reported. A great diversity was found in the distribution of fatty acids in the various *Spirulina* strains, which is greater for the unsaturated acids. The proportion of 16:0 was consistent, ranging between 44.6 and 54.1% of the total fatty acids. The percentage of C_{18} acids varied greatly: 18:1 ranged from 1.0% to 15.5%; 18:2 between 10.8% and 30.7% and GLA between 8.0% and 31.7%. The lowest fatty acid content

(% of ash free dry weight) among freshwater strains of Spirulina was found in strain L2 (3.0%); L1 had the highest content (5.6%).

In fourteen strains, high levels of GLA were accompanied by low levels of 18:2 and vice versa (strain 1928, L_1, B_3 and Eth being, however, exceptions to this pattern). For example, in strain Mad the percentage of 18:2 and 18:3 (with respect to total fatty acids) were 10.8% and 31.7%, respectively, while in strain 2340 the percentages of these fatty acids were 30.7% and 8.0%, respectively. Strains SB, Mad and Cat had the highest GLA contents, while strains 2340 and 2342 had the lowest. The tested strains are arranged in Table 1 in decreasing order of GLA content.

A strain which was tentatively identified as S. subsalsa, a marine Spirulina, was the only one to deviate from the above patterns of fatty acid composition and was significantly different from all other tested strains. It had a very low fatty acid content and contained no GLA. However, it was very high in 16:1.

Table 1. Fatty acid content* of *Spirulina* strains†

	Fatty acids							
Strain	16:0	16:1	18:0	18:1	18:2	GLA	Total‡	GLA‡
SB	44.6	4.4	0.5	6.4	17.1	27.0	5.2	1.4
Mad	47.0	0.5	0.7	9.3	10.8	31.7	4.2	1.3
Cat	47.6	2.5	1.0	8.0	15.3	25.6	5.1	1.3
Art. B	46.1	1.0	1.6	10.9	13.6	26.8	4.7	1.3
1928	47.3	2.0	1.0	2.9	18.1	28.7	4.3	1.2
L1	45.0	1.4	1.0	15.5	16.4	20.7	5.6	1.2
AR	49.1	2.2	1.0	6.4	15.7	25.6	4.3	1.1
B4	49.6	2.1	0.7	5.0	16.5	26.1	3.9	1.0
B2	47.3	3.4	0.8	5.8	20.7	20.7	3.8	1.0
G	49.2	2.9	0.9	8.0	15.7	23.3	4.0	0.9
PC	52.5	2.4	0.8	7.2	14.0	23.2	4.0	0.9
B3	52.9	2.2	1.1	7.6	13.7	22.5	4.1	0.9
Art. A	48.5	2.4	1.3	6.0	15.8	26.0	3.4	0.9
Eth	54.1	2.6	1.0	7.7	13.5	21.3	4.1	0.9
L2	50.7	1.1	0.8	7.3	14.3	25.8	3.0	0.8
Minor	46.8	1.2	1.5	12.0	18.4	20.1	3.6	0.7
2342	47.5	1.6	0.5	9.3	21.8	19.3	3.8	0.7
2340	49.3	2.2	1.2	8.6	30.7	8.0	3.2	0.3
Subsalsa	49.2	35.0	1.7	1.0	13.1	—	1.6	—

* Wt percent of total fatty acids.
† Cultures were grown at 35°.
‡ Weight as percentage of biomass (ash-free dry wt-AFDW).

Growth phase

Many algal species are known to accumulate lipids during the stationary phase, but no such effect has ever been found in cyanobacteria. Strain Mad cultures were sampled at the mid-exponential phase and at the stationary phase (Table 2). The total fatty acid content was reduced in the stationary phase and the relative amount of PUFA was also decreased. the content of GLA was reduced by ca. 50%.

Table 2. Distribution and content of *Spirulina* (S. mad) fatty acids in different growth phases*

	Fatty acids							
Growth phase	16:0†	16:1†	18:0†	18:1†	18.2†	GLA†	Total‡	GLA‡
Exponential	48.9	2.3	0.8	7.3	12.1	30.5	4.3	1.3
Stationary	52.6	2.7	2.1	12.3	11.1	18.6	3.1	0.6

*At 35°.
† Percentage of total fatty acids.
‡ Percentage of AFDW.

Dilution rate

The dilution rate had a very small effect on the fatty acid composition and content (Table 3). However, as expected, the various pigments are highly affected by the changes in dilution rate, and the phycocyanin content was reduced from 23% of DW at 0.01 d-1 to 10% at 0.6 d-1 (Table 4).

Table 3: Effect of dilution rate on fatty acid composition
Media : Zarrouk's medium, semicontinuous method
Light intensity: 2500 lux, room temperature (29-31oC)
Cultivation vessel - erlenmeyer's flask 1 litre with 500 ml
Working volume - 2 vessels for each dilution rate

Dilution rate (day-1)	Fatty Acids (% of total fatty acids) 16:0	16:1	18:0	18:1	18:2	18:3 (GLA)	GLA (% DW)	TFA (% DW)
0.10	45.0	3.0	1.3	12.0	13.1	20.1	1.0	4.7
0.25	44.7	3.5	1.2	9.6	14.7	23.1	1.0	4.5
0.40	43.7	3.5	1.0	6.0	16.3	24.6	1.1	4.3
0.60	42.1	5.5	1.0	6.2	16.2	23.6	1.0	4.1

Table 4: Effect of dilution rate on pigment content

Dilution rate day-1	Chlorophyll a mg/g DW	% Phycocyanin % DW	DW g/l	Productivity g/l/d
0.01	13.4	22.8	1.35	0.14
0.01	13.4	23.7	1.48	
0.25	12.8	19.8	0.96	0.245
0.25	13.5	18.8	0.99	
0.40	12.4	17.8	0.67	
0.40	11.3	17.6	0.65	0.264
0.60	8.9	10.3	0.44	
0.60	9.4	10.6	0.41	0.255

Effect of salinity

Increasing the medium salinity above 0.75% NaCl resulted in decreased fatty acid content and in a decrease in the percentage of GLA

of total fatty acids. The two effects culminated in a sharp decline in the GLA content (% of dry weight) from 1% at 0.1%-0.5% NaCl to 0.65% GLA at 3.0 NaCl.

The phycocyanin increased slightly at 1.5% NaCl but was drastically reduced at 3% NaCl.

Table 5: Effect of NaCl (0.1, 0.5, 0.75, 1.5, 3.0%) semicontinuous at dilution rate 0.25 day-1 ambient temperature 29-31oC.

NaCl % w/v	D.W. (g/l)	Chlorophyll a (% DW)	Phycocyanin (% DW)	Total FA (% DW)	GLA (18:3) (% DW)
0.10	0.72	1.69	21.7	4.60	1.01
0.10	0.71	1.52	21.8	4.89	1.03
0.50	0.76	1.77	22.8	4.75	1.07
0.75	0.74	1.67	22.8	4.62	1.04
1.50	1.21	1.50	23.9	4.00	0.80
3.00	1.67	1.08	19.0	3.43	0.65

Fatty acid composition

NaCl % w/v	F A (% of total fatty acids)					
	16:0	16:1	18:0	18:1	18:2	18:3
0.10	44.1	4.3	0.9	8.9	14.2	22.4
0.10	42.5	5.7	1.6	10.8	12.5	21.0
0.50	45.0	3.9	1.0	9.9	14.3	22.4
0.75	44.3	4.2	0.9	8.7	15.0	22.4
1.50	43.9	5.4	1.0	11.6	13.5	20.1
3.00	43.0	5.7	1.1	13.3	13.3	19.1