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FINAL REPORT

May, 1986 - May, 1991

Submitted to the Office of Science Advisor
U.S. Agency for International Development

Strain selection of *Spirulina* algae suitable for mass production - A novel agrotechnology for arid lands

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Project Number: C5-222

Grant Number: 936-5544-G-00-6033-00

A.I.D. Grant Project Officer;

Project Duration: 3+2 years

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Executive Summary

Project Purposes

The main purposes of the project were:

1. Screen for local *Spirulina* strains from different areas in Thailand
2. Evaluate the physiological characteristics of the strains: their productivity, chemical composition and capability to grow outdoors in brackish water.
3. Set up a demonstration pond for cultivation of *Spirulina* in the East-North of Thailand (Khonkan) in order to evaluate and demonstrate the feasibility of locally growing *Spirulina* biomass to be used for aquaculture.

Project Accomplishments

The main accomplishment of the project was the ability to develop and transfer the know-how for outdoor mass cultivation of *Spirulina* to the Thai partner. During the project a few visits of the Thai collaborator to the laboratory in Israel and visits of the Israeli PI to Thailand were used for training and demonstration of practical approaches in the subject of algal biotechnology.

This accomplishment is well reflected in 2 ways:

1. The Thai collaborator is now running an independent research project funded by the Thai government on related topics of mass cultivation of *Spirulina*
2. A demonstration pond is operated in the Fish Research Station in Khonken. Besides being used for demonstration and training, the biomass produced is used for further evaluation of its potential as a source for feed in aquaculture.

Objectives and Problems Addressed

Fresh water is already used to the point of exhaustion in many arid lands, particularly in Israel and many of the less-developed countries (LDC), bearing further development of conventional agriculture. Potentially, however, many such regions may be able to support intensive agriculture production, being endowed with abundant solar irradiance, high temperature throughout the year and saline water. These resources are not suitable for most of the conventional agricultural crops. Algalculture of suitable strains may represents an appropriate agrotechnology for such regions.

This work was aimed to the tactics for enhancing bioproductivity, based on growing algal biomass using local isolate strains and brackish or saline water resources.

The main advantages of microalgal agriculture are:

a. Algal cultivation is an efficient biological system for use of solar energy to produce organic matter. Due to near year-round production, complete area cover, and absence of water limitations, algal cultures are five to ten times more productive than conventional agriculture.
b. Many species of algae can be induced to produce particularly high concentrations of compounds of commercial interest such as proteins, lipids, starch, natural pigments and biopolymers.
c. The life cycle of most algae is completed within several hours, which makes genetic selection and improvements in the species relatively easy and fast. It also allows much more rapid development and demonstration of production processes than for conventional agricultural processes.

d. For many regions of low bioproductivity throughout the world, perhaps the most important aspect of the cultivation of microalgae relates to the ability of many species to thrive in seawater and/or brackish water, resources which are not suitable for conventional agricultural plants.

e. Algal mass cultivation systems can be easily adapted to various levels of skills of operation, from fully automated to labor intensive, depending on the local conditions.

Spirulina, a blue-green filamentous alga (cyanobacteria), is one of the most promising candidates for algal biomass cultivation under outdoor conditions. *Spirulina* biomass is currently produced at several commercial production sites, including Mexico, Thailand, USA and Israel. Almost all the product in the form of powder or pills is marketed as health-food. Further development of this industry is limited due to:

a) High cost of production;

b) *Spirulina* market is still limited to the health-food market;

c) The biological know-how is still limited, facing problems such as culture contamination by other algae, grazers of *Spirulina* overtaking the cultures and loss of cultures due to photooxidation.

The aim of this work was to provide an answer to some of these problems, by obtaining a variety of strains selected for their adaptability to the environmental and growth conditions in Thailand and other tropic areas.

Obtaining such strains may help in turning the somewhat limited technology of *Spirulina* into a novel biotechnology. Producing *Spirulina* biomass as a multipurpose product at a lower price, and using it for feed, food and other chemicals would increase the economic feasibility of such a technology.

Previous work

Spirulina is a cyanobacteria (family Oscillatoriaceae) with helical, multicellular filaments which may be 50-500 m long and 10 m wide. Several *Spirulina* species (e.g. *S. platensis*, *S. maxima*) are found as a single dominating organism in alkaline soda lakes with pH as high as 11.

Zarrouk was the first to provide detailed experimental results on the basic nutritional and temperature requirements of *Spirulina*. Vonshak et al. described various aspects that relate to the optimization of outdoor system for the production of *Spirulina* in Israel. Laboratory studies on the effects of population density, the extent of stirring and the various engineering aspects, on the production of *Spirulina* have been reported. *Spirulina platensis* has been collected from the salty lakes and ponds along the northern shores of Lake Tchad since time immemorial, sun-dried and eaten by the Kanembou people. A similar alga was apparently collected and dried by the Aztec Indians. *Spirulina* filaments may be easily separated from their medium, have high digestibility and a mild flavor, and contain up to 70% protein of excellent quality.

Methods and Results

Collecting *Spirulina* strains from natural habitats: A few field trips were conducted during the first and second years of the project. Table 1 shows some of the water characteristics from which strains were collected. The enclosed map of Thailand is for the orientation of the site locations.

Samples were collected by using phytoplankton collecting procedures. A complete chemical analysis of the water was performed in the laboratory. Single filaments of *Spirulina* strains were isolated, using micromanipulation techniques and used for obtaining monoalgal cultures. Once monoalgal cultures were obtained, the optimal

temperature and the main nutrient requirement were determined, and the general morphological characteristics of each strain was recorded.

All samples collected were examined microscopically for existence of *Spirulina*. If filaments of *Spirulina* were observed, the collected samples were enriched with nutrients to increase the total concentration of algae. Plate 1 shows some of the enriched cultures. After enrichment, *Spirulina* filaments were picked in order to obtain monoalgal cultures (See Plate 2).

All strains isolated were studied under outdoor conditions as reported in detail in the semi-annual reports. A few of of strains were chosen on the basis of productivity and overall stability for the scale up studies.

A 100 m^2 pond was set up in the National Inland Fisheries Research station in Khonkan (see attached scheme). During this time, a training program was set up in the NIFI laboratory in Bangkok for the personnel to operate the site.

Plate 3 shows the site in Khonkan, including the pond, the harvesting and drying process. The biomass produced is used now to carry out nutritional tests.

The center in NIFI is used as a training center and back-up for supply of know-how and strains.

Further work: Our main recommendation is to ensure further funding and support for 2 most important activities:

1. Operation of the pond in Khonkan as a demonstration and on-site training site
2. Continue the research on further improvement of strains and operational parameters

Attached is a more detailed description of the chemical analysis of the algal strains (Appendix A). Two more papers are currently under preparation.

Table 1: Water Quality of the "found Spirulina" samples, during trip Jan. 14-18, 1987 To NE. Thailand.

le NO.	Place	pH	Alkalinity	Total Hardness	Chlorinity	Note	
						Thai	Israel
4	Dan Kuntot District, Nakhon Ratchasima	7.8	320	224	6.18	-	
11	Ban Kumdin village, Khonkan	8.9	600	285	4.04	✓	
13	Muang District, Khonkan	8.5	420	408	0.79	✓	
15	Ban Non-Sung School, Khonkan	8.2	240	143	1.21	-	
17	Kosumpisai District, Mahasarakham	9.0	600	224	0.79	-	
18	Kosumpisai District, Mahasarakham	9.5	200	428	0.42	-	
19	Muang District, Mahasarakham	9.2	660	163	1.02	-	
20	Muang District, Mahasarakham	9.6	200	326	0.46	-	
21	Muang District, Mahasarakham	8.4	300	302	0.70	-	
22	Fisheries Station, Mahasarakham	9.4	130	458	0.73	-	
23	Fisheries Station, Mahasarakham	10.25	140	469	0.33	-	
30	Muang District, Khonkan	9.0	310	408	0.79	-	
35	Chum Pae Disteict, Khonkan	7.6	300	346	0.56	-	

Alkalinity (mg/l as $CaCO_3$)

Total Hardness (mg/l as $CaCO_3$)

Chlorinity (mg/l)

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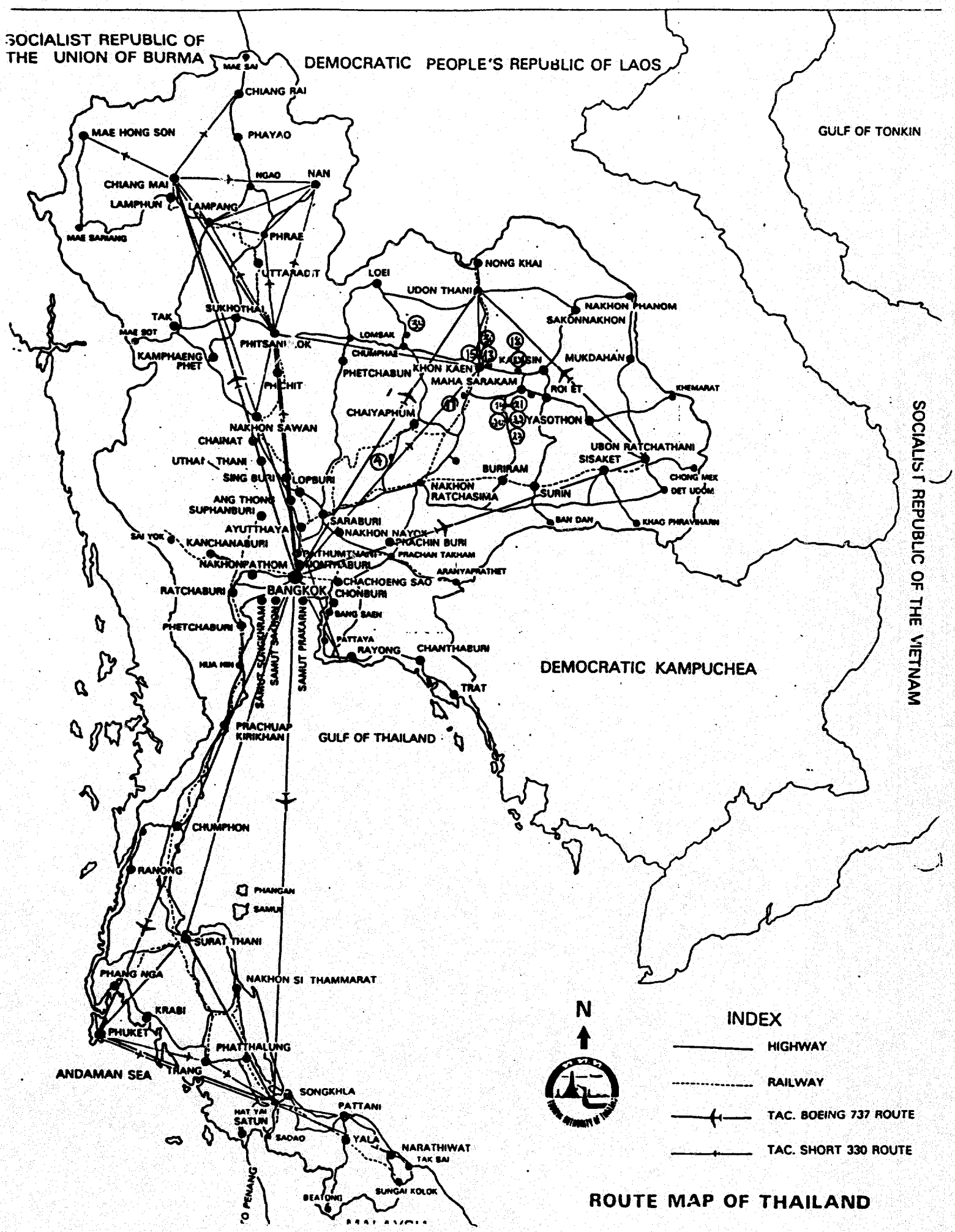
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DEMOCRATIC PEOPLE'S REPUBLIC OF LAOS
GULF OF TONKIN
SOCIALIST REPUBLIC OF THE VIETNAM
DEMOCRATIC KAMPUCHEA
GULF OF THAILAND
ANDAMAN SEA
MAE SAI
CHIANG RAI
MAE HONG SON
PHAYAO
CHIANG MAI
NGAO
NAN
LAMPHUN
LAMPANG
MAE SARIANG
PHRAE
UTTARADIT
LOEI
NONG KHAI
UDON THANI
NAKHON PHANOM
SAKONNAKHON
SUKHOTHAI
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MAE SOT
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CHONG MEK
NAKHON RATCHASIMA
SURIN
DET UDOM
ANG THONG
SUPHANBURI
SARABURI
BAN DAN
KHAO PHRAVIHARN
AYUTTHAYA
NAKHON NAYOK
SAI YOK
KANCHANABURI
PRACHIN BURI
PATHUMTHANI
PRACHAN TAKHAM
NAKHONPATHOM
DONTHABURI
ARANYAPRATHET
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SONGKHLA
PATTANI
HAT YAI
SATUN
SADAO
YALA
NARATHIWAT
TAK BAI
SUNGAI KOLOK
BEATONG
N
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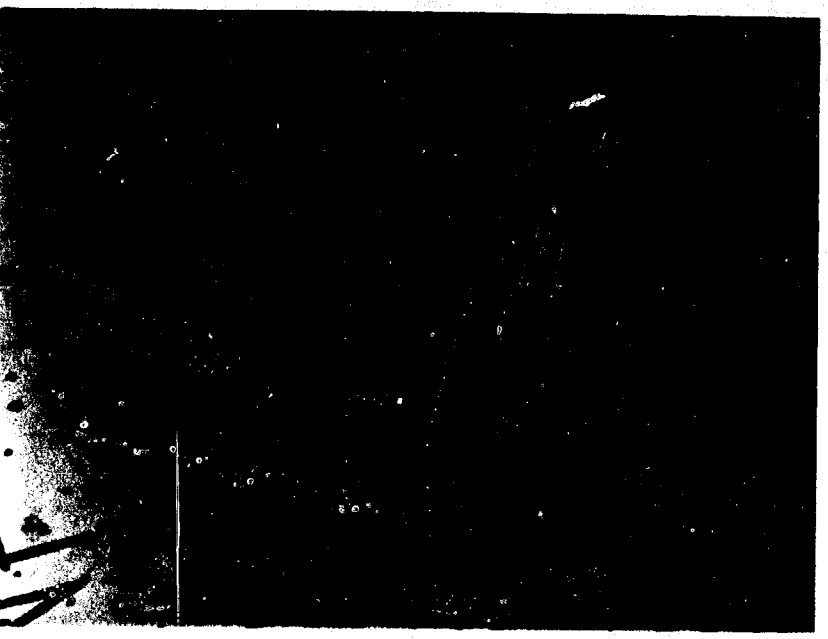
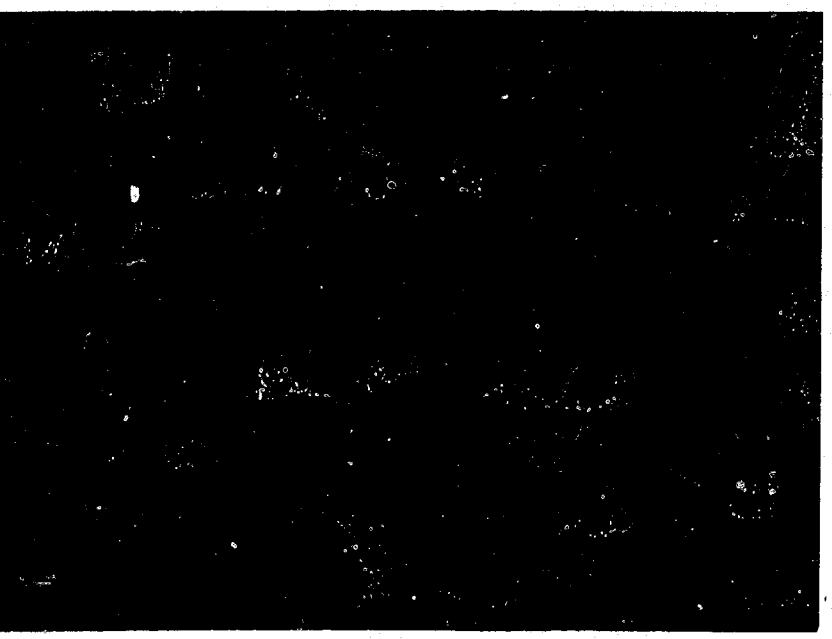
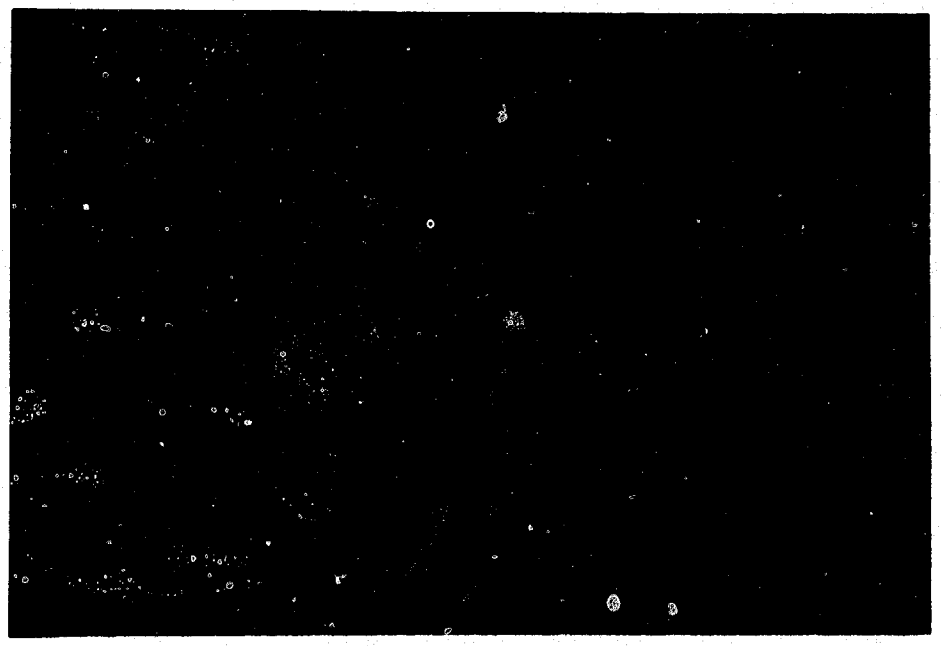
HIGHWAY
RAILWAY
TAC. BOEING 737 ROUTE
TAC. SHORT 330 ROUTE
ROUTE MAP OF THAILAND

1: Cultures of algae in *Spirulina* enriched medium. Those cultures were used for isolation of monoalgal cultures.







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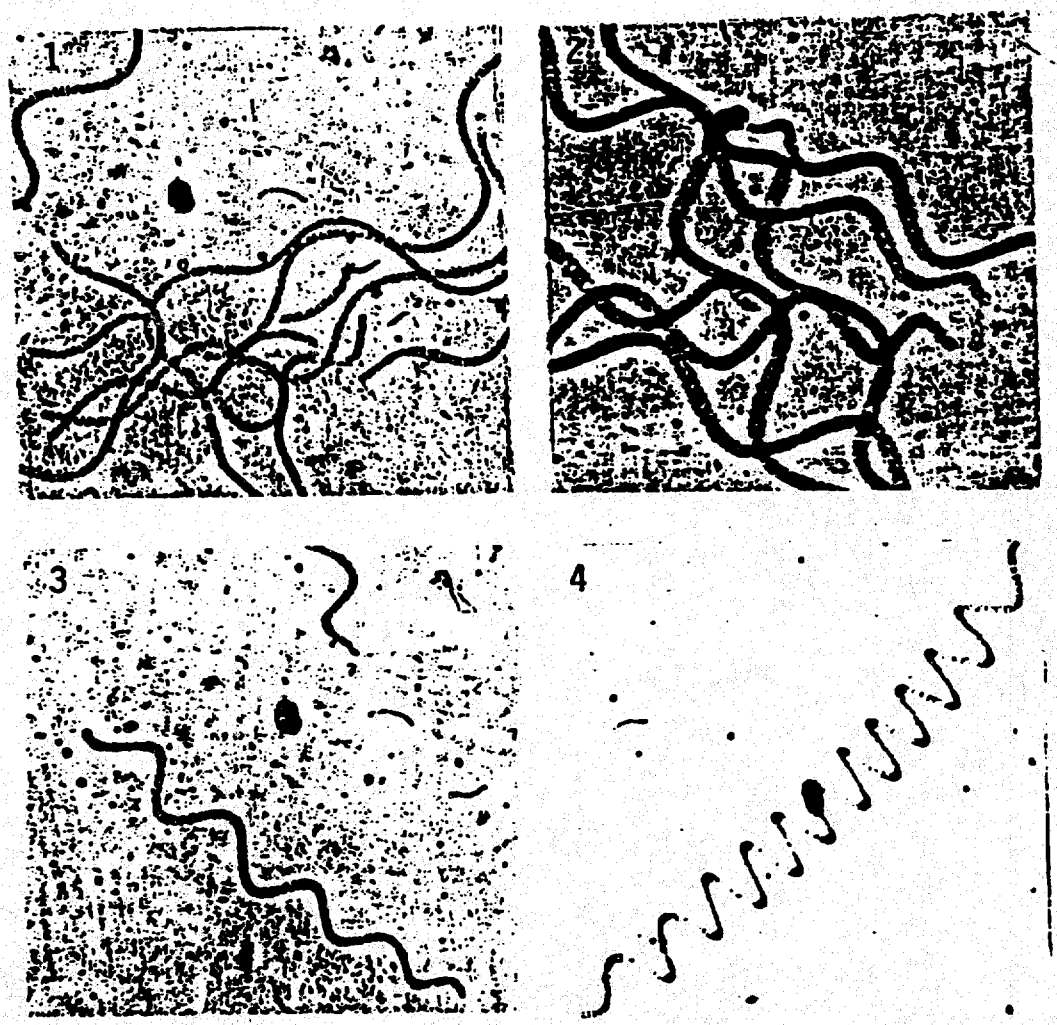

Plate 2: Photomicrographs of four different Spirulina strains
1) Th-11, 2) Th-13, 3) Th-21, 4) Th-30

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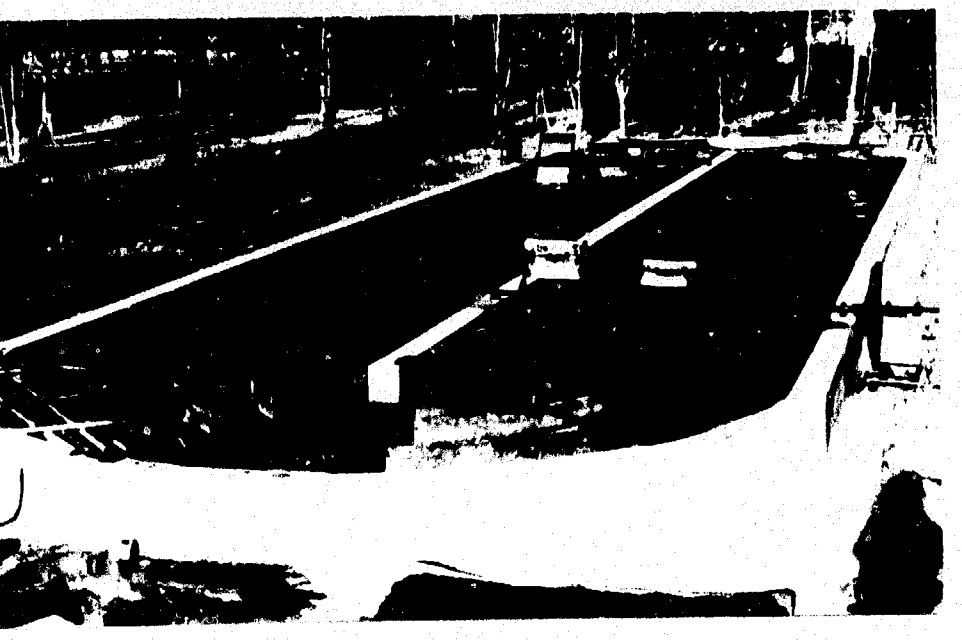

he algal pond including paddle-wheel

b. Harvesting of *Spirulina* biomass using simple nets

rying of the algal biomass

d. The final product

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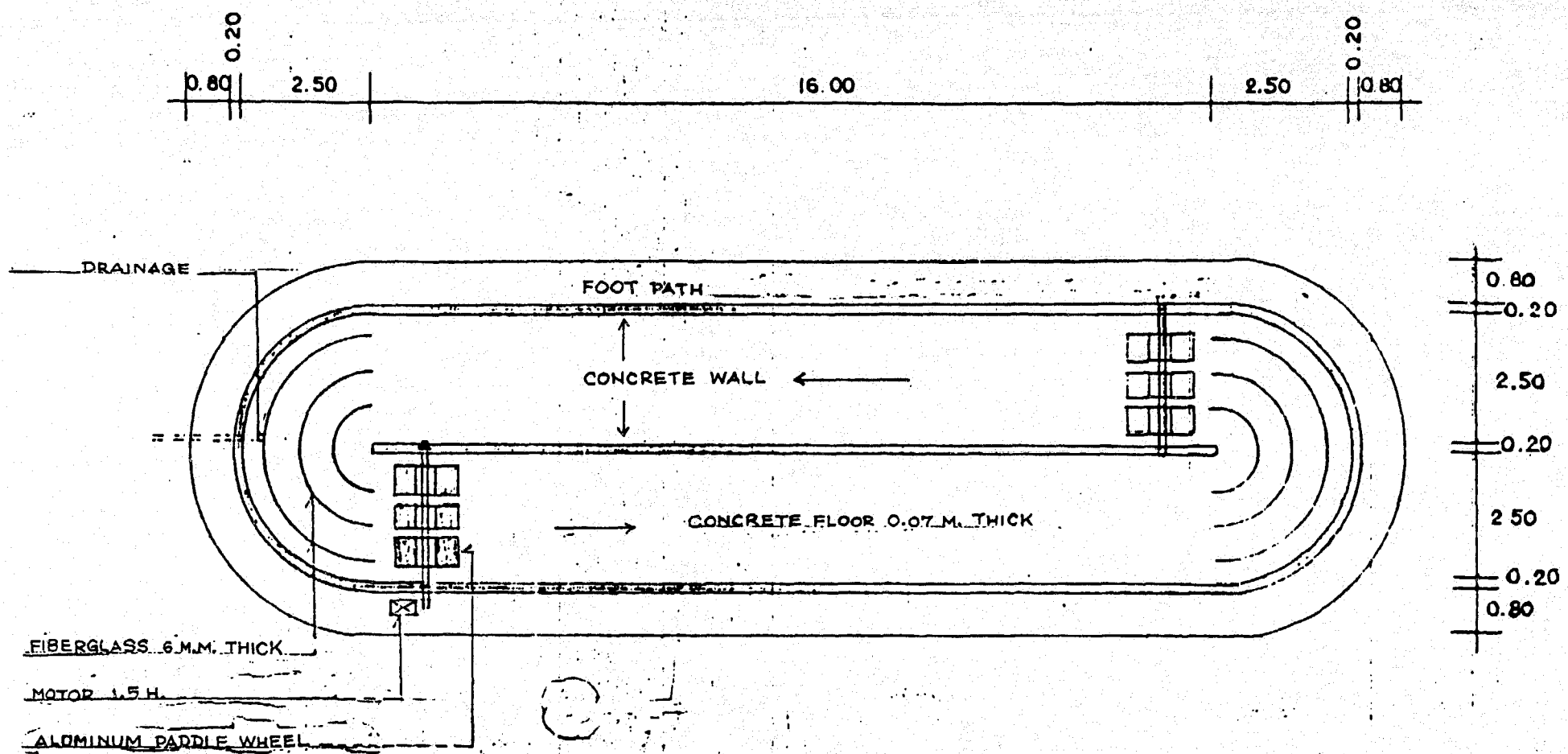

PLAN

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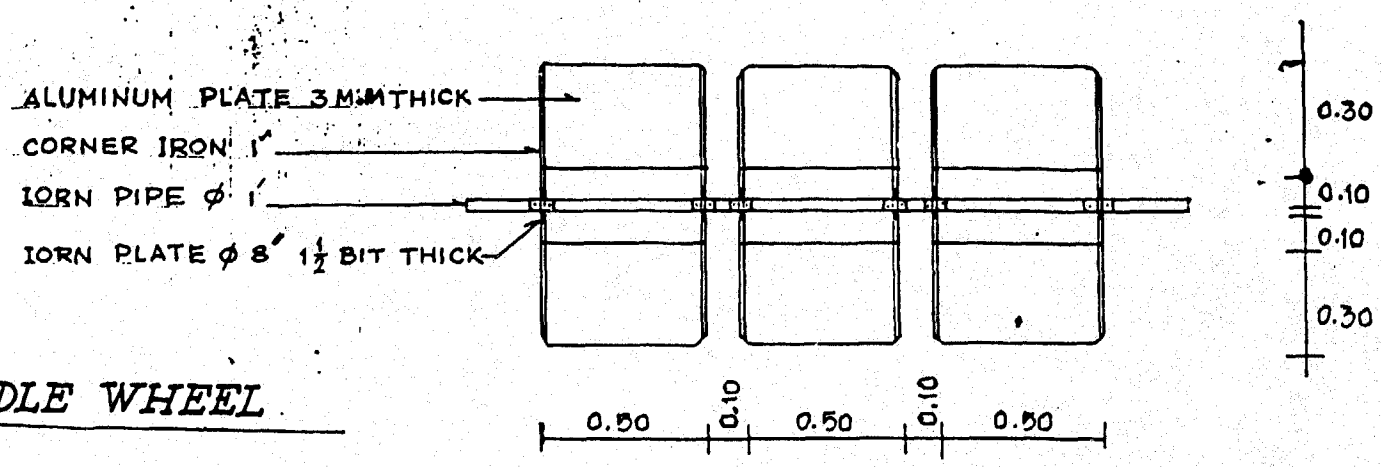

DETAIL OF PADDLE WHEEL

SCALE 1:20

Phytochemistry, Vol. 26, No. 8, pp. 2255-2258, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
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FATTY ACID COMPOSITION OF *SPIRULINA* STRAINS GROWN UNDER VARIOUS ENVIRONMENTAL CONDITIONS

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(Received 1986)

Key Word Index—*Spirulina* species; cyanobacteria; fatty acids; γ-linolenic acid; environment.

Abstract—The fatty acid distribution in 19 strains of *Spirulina* was studied. All but one contained γ-linolenic acid (GLA). No GLA was found in *S. subsalsa*, which had a very high content of palmitoleic acid. The fatty acid content of all but one of the tested strains increased with cultivation temperature and the relative amount of polyunsaturated fatty acid decreased. The highest content of GLA was found at 30-35° for most strains. High light intensities at a high temperature (38°), while not affecting the fatty acid composition, had a drastic effect on the fatty acid content, reducing it by as much as 46%.

INTRODUCTION

The cyanobacterium *Spirulina* is cultivated commercially in several places the world over, mainly as a health food. This alga is protein rich and easily digestable, and it is much larger than unicellular algae, which simplifies harvesting [1].

Spirulina was shown to contain the rare fatty acid, γ-linolenic acid (GLA) 18:3 (6, 9, 12), as early as 1968 [2-5]. This acid was shown to have many therapeutic properties. It is *ca* 170-fold more effective in lowering the plasma cholesterol level than linoleic acid [6], the major constituent of most polyunsaturated oils. In addition, tests on children have shown that GLA is of benefit in treating atopic eczema [7], while in women it appears to reduce the severity of premenstrual syndrome [8]. It was also claimed to have a positive effect in heart diseases, Parkinson's disease, and multiple sclerosis. Direct provision of GLA could thus have an important role in human nutrition.

Since GLA is quite rare and is not found in foods other than human milk, other sources for this fatty acid were sought. The oil of evening primrose was found to contain 8-12% GLA and is presently its commercial source. Wolf *et al.* [9] showed that GLA exists in some other plants as well, and recently it was reported that GLA can be extracted from the residues of blackcurrant remaining after manufacture of jam and juice products [10].

In this paper, we report on the fatty acid content and composition of various strains of *Spirulina* which can be modified by temperature, light intensity and growth phase. The possibility of selecting high GLA strains of *Spirulina* is currently under investigation.

RESULTS AND DISCUSSION

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 [4]. 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 (7.5%).

In fourteen strains, high levels of GLA were accompanied by low levels of 18:2 and vice versa (strains 1928, L_1, B_3 and Eth being, however, exceptions to this pattern). For example, in strain Mad the percentages 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. Wood [11] questioned the validity of the classification system of the various *Spirulina* strains as Nichols and Wood [2] detected GLA in *Spirulina* while Kenyon *et al.* [12] claimed the existence of two *Spirulina* strains, one containing α-linolenic acid [18:3 (9, 12, 15)] and the other devoid of both acids. Except for the latter, other studies [3-5, 13] reported the occurrence of linolenic acid (in most cases the γ-form) in *Spirulina*. Similarly, the fatty acid composition of *S. subsalsa* makes it doubtful that it is a genuine *Spirulina*.

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

Strain	Fatty acids							
	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	7.9	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).

Effect of temperature

The fatty acid content increases with increasing temperature in many algal species [14], and the composition generally changes so that the proportion of polyunsaturated fatty acids (PUFA) decreases. The latter phenomenon has been explained by the role of PUFA in increasing membrane fluidity at low temperatures [15]. Another explanation is that at lower temperatures more oxygen is dissolved in water and thus more is available for the oxygen-dependent desaturase enzymes [16]. The effects of temperature on the fatty acids of *Spirulina* were studied in eight strains. Several examples are presented in Table 2. Strains Art. B, G and 2340 were studied as they are used in commercial *Spirulina* cultivation at various production sites. *S. minor* was chosen because of the exceptional response of its fatty acids to temperature.

The fatty acid content of the tested strains increased with increasing cultivation temperature, reaching the highest value for most strains at 30–35° and decreasing at higher temperatures. In only one strain (Minor), the maximum content was reached at a lower temperature (25°), and no *Spirulina* strain of those tested exhibited a fatty acid maximum at a temperature higher than 35°. Strains SB, Mad, Cat, and 2342 displayed similar patterns to that of strain Art. B (data not shown).

The fatty acids were less desaturated at higher temperatures; two patterns were observed. In the first, the percentage of GLA decreased while those of 18:2 or 18:1 increased. In the other pattern, an increase of 16:0 at the expense of 16:1 was observed. As a result, in most of the tested strains the percentage of GLA decreased only slightly with increasing temperature (up to 30–35°) while the total fatty acid content increased significantly. The net result was an increase in GLA content with increasing temperature, reaching a maximum for most strains at 30° or 35°, the optimum temperature for growth of most strains being 35°. This is of importance for GLA production from *Spirulina* as higher GLA production rates can be envisaged at this temperature.

Effect of light intensity

The effects of three different light intensities on the fatty acid compositions of two strains of Spirulina cultivated at 32, 35 and 38° were studied (Table 3). The distribution of fatty acids did not change much with light intensity (data not shown), the fatty acid content nevertheless being light-dependent. The content of fatty acids at 300 $\mu E/m^2/sec$ was much lower than at 150 $\mu E/m^2/sec$. This light effect was most pronounced at 38°. A 28–29% decrease in fatty acid content was recorded at 32° and a 22% decrease at 35°. Comparing strains minor and G at these light intensities at 38° showed 46% and 71% decreases, respectively. A slight increase in the percentage of GLA was noted at the high light intensity. However, due to the decreased fatty acid content, the overall effect was a reduction in GLA content which was most strongly demonstrated at 38°. Decreasing the light intensity to 75 $\mu E/m^2/s$ did not cause a further change in the fatty acid content.

The effect of light intensity on the fatty acid content and on the degree of fatty acid unsaturation in algae cannot be generalized, and conflicting data have been reported for different species. *Porphyridium cruentum* grown at 1700 and 8000 lux had fatty acid contents of *ca* 9% and 4%, respectively [17]. A similar effect was observed in *Anacystis nidulans* [18], where the fatty acid content doubled at low light intensity and the degree of unsatur-

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Table 2. Fatty acid content of *Spirulina* strains grown at various temperatures

Strain	Growth Temperature (o)	Fatty acid Composition* 16:0	16:1	18:1	18:2	GLA	Content† FA	GLA
Art. B	25	41.2	7.1	3.6	17.6	28.2	2.7	0.8
	30	44.5	5.1	7.2	14.5	29.7	3.4	1.0
	35	46.1	1.0	10.9	13.6	26.8	4.7	1.3
	38	53.7	2.0	8.2	13.2	21.3	3.9	0.8
G	20	39.5	9.6	1.8	16.4	32.1	2.7	0.9
	25	40.6	8.6	2.2	16.0	31.7	3.2	1.0
	30	44.6	3.1	5.1	17.9	29.0	4.0	1.1
	35	49.2	2.9	8.0	15.7	23.3	4.0	0.9
	38	47.8	0.2	9.3	17.0	25.0	3.3	0.8
Minor	25	43.2	6.9	4.8	20.5	22.2	4.8	1.1
	30	46.2	4.6	9.0	15.0	24.5	3.3	0.8
	35	46.8	1.2	12.0	18.4	20.1	3.6	0.7
	38	47.7	1.1	9.9	22.4	18.2	3.2	0.6
2340	25	43.5	7.9	4.3	21.4	22.9	2.4	0.6
	30	44.4	5.5	4.2	28.6	16.9	4.2	0.7
	35	49.3	2.2	8.6	30.7	8.0	3.2	0.3
	38	50.0	2.0	7.1	32.3	6.0	3.3	0.2

* Percentage of total fatty acids.
† Percentage of AFDW.

Table 3. The effect of light intensity on fatty acid content in *Spirulina*

Strain	Light intensity uE/m/s	Fatty acid content* 32° FA†	18:3	35° FA	18:3	38° FA	18:3
G	75	3.26	1.02	-	-	3.28	0.86
	150	3.43	0.90	3.82	0.95	3.29	0.79
	300	2.45	0.70	2.99	0.90	0.95	0.26
Minor	75	3.50	0.94	-	-	3.32	0.51
	150	3.27	0.80	3.63	0.73	3.27	0.60
	300	2.36	0.65	2.89	0.70	1.78	0.28

* Percentage of AFDW.
†Fa, Fatty acids.

ation increased. In *Chlorella minutissima* [19], low light intensity had the opposite effect, both on fatty acid content (15% reduction) and on percent PUFA (10% reduction). Similar results were reported for *Nitzschia closterium* [20].

The reasons for the inhibitory effect of high light intensities on fatty acid content described herein are as yet unknown.

Growth Phase

Many algal species are known to accumulate lipids during the stationary phase [21], 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 4). 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%.

The data presented here suggests that *Spirulina* could be utilized as a source for the valuable fatty acid GLA. Since the maximal GLA content was found at the optimal growth temperature, a relatively high GLA production rate may be expected. Also, in many strains GLA is a major fatty acid comprising more than 25% of total fatty acids. The introduction of GLA as a drug will necessitate its purification, the costs of which will be lower, the higher the initial GLA concentration in the fatty acid mixture.

EXPERIMENTAL

Organisms. A culture collection of *Spirulina* strains was established in the Algal Biotechnology Laboratory of the Desert Research Institute at Sede-Boqer. Strains No. 1928, 2340 and 2342 were obtained from the UTEX culture collection (Austin, Texas, USA), strain Art A from Carolina Biological Supplies (Burlington, North Carolina, USA), strain G from Prof. C. Soeder (F.R.G.) (originally isolated from Lake Chad) and strains

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Table 4. Distribution and content of *Spirulina* (S. mad) fatty acids in different growth phases*

Growth phase	Fatty acids							
	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.

Mad and Cat from Dr. Venkataraman (India). Strains PC, B_2, B_3 and B_4 were isolated by the authors from a flood-irrigated field in Thailand. Strains SB, L_1, L_2, Art. B and AR were also isolated by the authors from water reservoirs in Israel. Strain Eth was isolated from a lake in Ethiopia, and *S. subsalsa* was obtained from Dr. E. Tel-Or (Hebrew University of Jerusalem). Stock cultures were held in flasks under constant illumination with frequent dilutions. Isolation procedures and inocula prepn were performed according to ref. [22].

Culture conditions. Cultures (500 ml) were grown on Zarrouk's medium [23] in flat-bottomed 1l flasks placed in a transparent water bath illuminated from below with four cool-white fluorescent lamps providing 150 $\mu E/m^2/sec$ at the surface of the bath, unless otherwise stated. Mixing was achieved with an air CO_2 mixture (99:1) bubbled through a sintered glass tube placed in the bottom of the culture flask. The temp was maintained constant within $\pm 1°$. The marine strain *S. subsalsa* was cultivated similarly in Abeliovich's medium [24]. Cultures (mid log phase unless otherwise stated) were harvested by filtration.

Cultures were grown exponentially (with proper diln) under the experimental conditions for at least six days (depending on the individual culture's growth rate). Although cultures used were not bacteria free, they were cultivated on sterile medium (bacterial counts not exceeding 100 colonies/ml). Ash and chlorophyll content were determined according to ref. [22].

Lipid extraction and transmethylation. Freeze-dried samples of *Spirulina* (100 mg) were treated with 2 ml of MeOH–AcCl (19:1) according to ref. [25]. 17:0 (its absence in the sample was previously checked) was added as an int. standard and the mixt sealed in a vial under an Ar atmosphere and heated to 80° for 1 hr. The vial was then cooled, its contents dil. with 1 ml H_2O and the mixt. extd with 1 ml hexane. The hexane layer was dried (Na_2SO_4), evapd to dryness and redissolved in hexane.

Fatty acid analysis. GC analysis was performed on a SP-2330 fused silica capillary column (30 m, 0.2 mm) at 200° (FID, injector and FID detector temps 230°, split ratio 1:100). Fatty acid Me esters were identified by cochromatography with authentic standards (Sigma Co.) and by GC/MS using a Carbowax capillary column (30 m). CI spectra were obtained at 250 eV with *isobutane* as reactant gas. Fatty acid contents were determined by comparing their integrated peak areas with that of the int. standard. The data shown are mean values of at least two independent samples, each analysed in duplicate.

Acknowledgements—The authors wish to express their gratitude to Mr Gershon Schwartz for helping with the algal cultivation, to Ms Shoshana Didi for algal cultivation and chemical analyses and to Dr Marjorie A. Tiefert for help with the manuscript. This work was supported in part by AID-CDR grants C7-131 and C5-222.

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