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Strain selection of *Spirulina* suitable for mass production

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For the past ten years, we have been involved in an ongoing project aimed at developing the biotechnology for the mass production of *Spirulina*. Our work has pointed out a basic requirement for proper development of this new agrotechnology, *i.e.*, establishing, maintaining and testing a collection encompassing a wide variety of algal species and strains that can be grown under various environmental conditions. Such a collection will also facilitate screening and selection of algae species for high productivity.

A large number of *Spirulina* species throughout the world have been described, but their descriptions concern mainly taxonomic characterization (Dangeard, 1940), and almost no information exists on their potential for production of biomass. We have therefore started a collection of *Spirulina* species and strains available throughout the world. Each species will be characterized from the standpoint of its growth physiology and its performance under outdoor conditions, to evaluate its potential as source of biomass for food and feed.

Some of the *Spirulina* strains already isolated and maintained in a unialgal form in our laboratory are shown in Fig. 1. All strains shown were grown under the same nutritional conditions as well as constant light intensity and temperature. As noted, these strains can be easily distinguished morphologically. In order to develop a more detailed classification protocol, two chemical procedures were adapted: one involves isolation of the photosynthetic membrane fraction of some strains and comparing the protein pattern of each strain on a polyacrylamide gradient gel (Fig. 2). As is evident, few distinguishing differences can be observed. We are now trying to develop this approach further by using another fraction of the cell proteins. The second approach is to undertake a detailed analysis of the fatty acid content and composition of *Spirulina* strains. Eighteen strains of *Spirulina* were cultivated under the same nutritional and environmental conditions. Although all tested strains contained palmitic acid (16:0), linoleic acid (18:2) and oleic acid (18:1), great diversity was found in the distribution of fatty acids in the various strains (Table 1). A basic requirement for using this procedure is growing the different strains under the same conditions, because the fatty acid content of *Spirulina* may vary significantly as a function of the environmental conditions (Cohen *et al.*, 1987).

After twelve years of research we have determined five different physiological criteria that we consider to be of great importance for evaluation of strains capable of high production rates under outdoor conditions. Based on those criteria we have started a screening and evaluation procedure as follows:

1 – Photoinhibition: even in dense cultures, the upper layer of an outdoor algal culture is exposed to high solar radiation (Vonshak *et al.*, 1982). From laboratory studies it can be demonstrated that photosynthesis is saturated at light intensities much less than that of full sunlight. In most algal species studied, photosynthesis is saturated at 1/3 the intensity of full solar radiation and in most cases some photoinhibition is observed at of 60–70% of full sunlight. In some algae, prolonged exposure to high light intensity may cause photooxidative

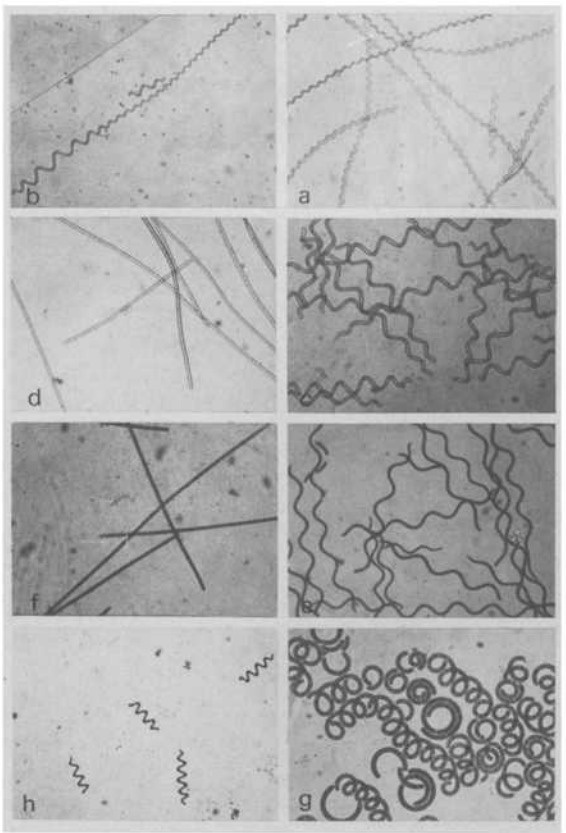

Fig. 1. Different *Spirulina* strains from the culture collection of the Algal Biotechnology Laboratory.

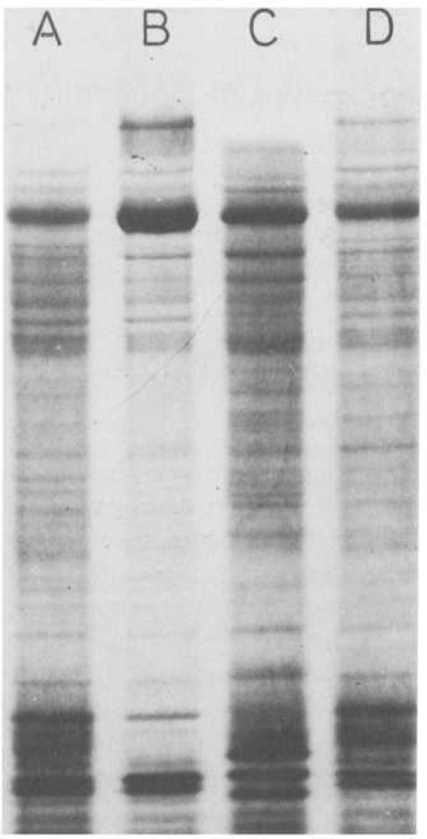

Fig. 2. Polyacrylamide gel electrophoretogram of photosynthetic-membrane proteins isolated from four different strains of *Spirulina*.

death. So far we have been able to isolate *Spirulina* strains with high light-saturation values and showing reduced inhibition of growth at high light intensities. When tested outdoors some of those strains reach higher production rates than the more sensitive strains.

2 – Dark respiration: outdoor cultures are not artificially irradiated during the night, and preliminary measurements in outdoor cultures of *Spirulina* have revealed that up to 35% of the total biomass produced during the day is lost through respiration at night. Algal strains with a low dark-respiration rate or a high ratio of light-dependent O_2 evolution to O_2 uptake in the dark should make good candidates for outdoor mass cultivation.

3 – Sensitivity to high O_2 concentration: maintaining adequate turbulence is a significant problem in large ponds. One of the purposes of inducing turbulence is to remove oxygen evolved during photosynthesis. In relatively small ponds where high water-flow rates can be maintained, the oxygen concentration can be kept at levels no higher than 200% of saturation. In large ponds where the water-flow is relatively low ($10\ cm \cdot s^{-1}$), the O_2 concentration may reach 500% of saturation when high photosynthetic rates exist. High concentrations of O_2 inhibit photosynthesis and growth and sometimes may lead to total loss of the culture. Naturally, existing strains as well as induced mutants that can tolerate high oxygen concentrations may be isolated for mass production.

4 – Diurnal fluctuation in temperature: many arid zones are suitable for algal mass culture since high temperatures and light intensities, and a large land area, are available. However, diurnal fluctuations in temperature of up to 20 °C are frequently

Table 1. Fatty acid content[a] in *Spirulina* strains[b] (Cohen *et al.*, 1987).

Strain	Fatty Acids						
	16:0	16:1	18:0	18:1	18:2	18:3	Total[c]
SB	44.6	4.4	0.5	6.4	17.1	27.0	5.16
Mad	47.0	0.5	0.7	9.3	10.8	31.7	4.23
Cat	47.6	2.5	1.0	8.0	15.3	25.6	5.07
Art. B	46.1	1.0	1.6	10.9	13.6	26.8	4.74
1928	47.3	2.0	1.0	2.9	18.1	28.7	4.25
L1	45.0	1.4	1.0	15.5	16.4	20.7	5.59
AR	49.1	2.2	1.0	6.4	15.7	25.6	4.27
B4	49.6	2.1	0.7	5.0	16.5	26.1	3.94
B2	47.3	3.4	0.8	5.8	20.7	20.7	3.80
G	49.2	2.9	0.9	8.0	15.7	23.3	3.97
PC	52.5	2.4	0.8	7.2	14.0	23.2	4.04
B3	52.9	2.2	1.1	7.6	13.7	22.5	4.10
Art. A	48.5	2.4	1.3	6.0	15.8	26.0	3.37
Eth	54.1	2.6	1.0	7.7	13.5	21.3	4.08
L2	50.7	1.1	0.8	7.3	14.3	25.8	2.96
Minor	46.8	1.2	1.5	12.0	18.4	20.1	3.63
2342	47.5	1.6	0.5	9.3	21.8	19.3	3.75
2340	49.3	2.2	1.2	8.6	30.7	8.0	3.19

[a] Weight percent of total fatty acids.
[b] Cultures were grown at 35 °C.
[c] Weight percent of total ash-free dry weight.

recorded. During the morning when the light is intense enough to support high growth rates, the water temperature in large ponds is usually significantly below the air temperature and below the optimum for growth. At this stage the algal cells still have to recover from the low night temperature. Algal strains with a wide optimum temperature range for growth may be advantageous in outdoor cultures.

5 – Sensitivity to high osmoticum: in large algal ponds evaporation rates of 1 – 2 cm · d^{-1} are usually recorded. In 15 – 20 cm-deep cultures operated in a continuous mode by recycling the culture medium, this means a continuous increase in the salt concentration. After about two months the salinity may reach twice the original level. Such change may cause severe osmotic stress. We have demonstrated that *Spirulina* can be grown under elevated NaCl concentrations without a significant reduction in the rate of photosynthetic oxygen evolution. Nevertheless a reduction in the output rate can be observed due to elevated activity of dark respiration (Vonshak & Guy, 1985). Thus an algal strain that will tolerate the increasing osmoticum without an increase in respiration activity will be of great advantage.

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