Cercetări marine	I.R.C.M.	Nr.16	263 - 269	1983

INVESTIGATIONS ON THE GROWTH OF *Spirulina platensis* (GOM.) GEITLER IN THE PRESENCE OF THE PROCAINE

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ABSTRACT:

The paper concerns the growth of Spirulina platensis under laboratory culture conditions, in nutritive media containing different concentrations of procaine. It was stated that lower concentrations induce a slight increase of the biomass production; e.g.experimental variant containing 1 mg procaine per liter reached an algal production of 107.5% as related to the control cultures. This stimulative effect may be of interest for the large scale culture of Spirulina.

In the context of the present demographic growth,of the necessity to provide the food needed for a great part of the mankind affected now by chronic malnutrition, a major scientific and practical importance shows the turning to good account of several unconventional protein sources.

In this sense, a promising approach is also the mass culture of Spirulina platensis, a blue-green alga characterized by a very favorable biomass chemical content as related to nutritive needs: high amount of protein,essential aminoacids, vitamins a.o. (1, 2). In certain warm regions this alga is utilized either

by the collecting of the biomass grown under natural"water-bloom" conditions (small lakes in the Chad Lake region, Africa), or by its mass cultivation at industrial scale (Mexico,India, Thailand, Israel, USSR a.o.).

In Romania, the National Science and Technology Council sponsores a comprehensive research program on the large scale culture of Spirulina; this program is attended by research teams from the Institute of Biological Sciences, Bucharest, the Faculty of Biology from the Bucharest University, Biological Research Centres from Cluj-Napoca and from Iaşi, a.o.

The studies carried out at the Stejarul Research Station last years showed the possibility to grow this alga in outdoor basins, even at 47° Northern latitude and at an elevation of about 450 m a.s.l.; the yields obtained in these conditions are quantitatively lower to that produced under favourable climatic conditions, as a consequence not only of the lower temperatures during the day, but also, because of a relative short actual vegetative period, reaching here only 75-90 days per year.

One of the ways to rise the culture productivity was considered to be the utilization of certain growth stimulating substances which, introduced in small concentrations in the culture media could determine an increase of the amount of total biomass yielded from the culture.

Among the substances which showed a certain stimulating action upon algal growth under experimental conditions is considered the procaine. Interesting results were obtained by a plant physiology research team from Cluj-Napoca: it was shown that diluted aqueous solutions of procaine stimulate the growth of the green alga Stichococcus bacillaris,effect which becomes important at the concentration of 0.2 mg procaine litre. It is interesting that this growth of algal population density is accompanied by a slight diminution of the length of algal cells (3, 4, 5).

The physiological mechanism of growth stimulation is related, probably, to the modifications of the cell membrane permeability to different ions of the nutritive salts under the action of the procaine,as it was demonstrated for the animal cell during the studies on the membrane physiology performed at the

Biological Research Centre from Iaşi - Romania (NEACSU I., personal communication).

Our investigation aimed to put into evidence the effect of various concentrations of procaine on the growth of Spirulina platensis under laboratory conditions.

MATERIAL AND METHODS

The culture strain of Spirulina platensis utilized in our laboratory was isolated from an algal sample brought from Egypt, and it was kindly offered us by Professor Octavian Boldor, from Bucharest University.

The laboratory culture medium, usually coded UK-22, assured to the culture a quantitative evolution comparable to that determined by several specific"classical" media as Zarrouk's nutritive solution, or Aiba-Ogawa culture medium.

The experimental frame (Table 1) included two test series, each of them consisted in a control and four experimental variants, containing different procaine concentrations. Both series had been duplicated.

Table 1

The frame of the experimental cultures

Variant code	Culture medium	Culture volume (ml)	Culture time (days)	Procaine concentration (mg/l)	Observations
SN-256	UK-22	2000	35	0	control
SN-257	UK-22	2000	35	0.1	
SN-258	UK-22	2000	35	0.5	
SN-259	UK-22	2000	35	1.0	
SN-260	UK-22	2000	35	2.0	
ST-403	UK-22	3300	35	5.0	
ST-404	UK-22	3300	35	10.0	
ST-405	UK-22	3300	35	50.0	
ST-406	UK-22	3300	35	100.0	
ST-407	UK-22	3300	35	0	control

All the culture tests were made in laboratory sphaerical glass vessels. The cultivation was made in a special laboratory

installation, under continuous artificial illumination, using white light fluorescent tubes, 40 w each. The light intensity, measured at the surface of the culture vessels reached about 10,000 lux. The temperature varied between 28 and 30°C, corresponding to the ecological needs of this tropical alga.

The number of filaments per ml at the end of experimentation was used to describe dynamics of Spirulina populations. Final biomass production (g of dry matter/l) was estimated by weighing total algal yield, after its desalinization and drying.

RESULTS AND DISCUSSION

The two experimental series of cultures carried out covered a large amplitude of variation of the procaine concentration (Table 1).

Table 2

Growth peculiarities of Spirulina in media additioned with different procain concentrations

Variant code	Population density on the day: 0	2	4	7 (filaments per litre of medium)	Final yield (g dry biomass)	Culture productivity (mg dry biomass/l/day)
SN-256	500	2091	2958	5577	3.075	43.93
SN-257	500	1394	2944	6818	2.837	40.53
SN-258	500	2169	3873	7183	2.557	36.55
SN-259	500	1901	4803	7669	3.307	47.24
SN-260	500	2115	4718	6257	3.197	45.67
ST-403	180	221	893	1668	2.611	22.61
ST-404	180	323	925	2443	2.625	22.73
ST-405	180	179	1040	2103	2.545	22.03
ST-406	180	298	423	830	0.000	0.00
ST-407	180	111	884	1881	3.388	29.33

It must be noticed that within the two experimental tests, two different starting densities of algal populations were realized (Table 2); so, the evolution of the numerical density of Spirulina filaments must be considered and compared directly only

within each of the series. The comparison of the variants of the whole experimental investigation may be made only through the estimation of the multiplication factor of the algal density; this parameter represents the ratio between the algal population density at a certain moment of the culture and the algal density in the culture immediately after the inoculation.

As it may be seen from the data (Table 3), on the second day of cultivation, the best growth of algae population was recorded within the variants SN-258 and SN-260, respectively at a procaine concentrations of 0.5 and 2.0 mg/litre. The worst growth was determined for the procaine concentration of 50 mg/l.

Table 3

Dynamics of the experimental cultures as defined by the evolution of multiplication factor of initial algal density

Variant code	Population density in the day of cultivation: 0 (start)	2	4	7
	(Multiplication factor)			
SN-256	1	4.182	5.916	11.154
SN-257	1	2.788	5.888	13.636
SN-258	1	4.338	7.746	14.366
SN-259	1	3.802	9.606	15.338
SN-260	1	4.226	9.436	12.514
ST-403	1	1.227	4.961	9.266
ST-404	1	1.794	5.139	13.572
ST-405	1	0.994	5.778	11.683
ST-406	1	1.655	2.350	4.611
ST-407	1	0.616	4.911	10.450

After other two days of culture, the situation was a little changed, the best growth of Spirulina was determined within the variants containing 1.0 and 2.0 mg procaine/litre, the algal density in these variants depassed that of the control culture by 1.62 and 1.59, respectively. Within the larger concentrations of procaine (the second test series), the best growth was observed for the cultures containing 50 mg procaine/l; the algal density

in this variant was over the control by 1.17.

On the seventh day of culture, the maximal growth of population density was recorded within the variants containing 0.5 and 1.0 mg procaine/l, which surpassed by 1.288 and 1.375, respectively, the multiplication factor of the control cultures of the series.

Higher concentrations of procaine induced a certain inhibition of the Spirulina culture; this is more evident within the variant ST-406, containing 100 mg procaine/litre, where the culture grew very slowly, much under the growth rate of the control cultures (the ratio of the multiplication factors was of 4.611 : 10.450).

For defining the influence of the procaine concentrations on the culture productivity, a major importance has also the final production of algal biomass within each experimental variant. Generally, higher values correspond to the lower concentrations of procaine; the maximum algal production was recorded at a concentration of procaine of 1 mg per litre of culture medium: 3.307 grams of dry and desalinized biomass, which represented 107.5% as related to the control cultures (considered as 100 %).The higher procaine concentrations the lower final biomass was obtained: in 50 mg procaine/l variant algal productivity was over the control only with 75.11%, when procaine concentration reached 100 mg/l,the death of algae was noticed; thus the biomass production was zero.

In the conclusion of this preliminary investigation on the growth of Spirulina platensis on nutritive media containing procaine, it may be noticed that several low concentrations could show a stimulative effect upon the culture productivity, effect that, at a procaine concentration of 1 mg/l means an increase of 7.5 % as related to the control laboratory variant. Even at this stimulation level, this fact may be of interest for the large scale culture of this valuable alga.

For the further phases of the investigation, it seems to be useful the testing of the procaine effect on Spirulina growth under different culture conditions, both in laboratory and in pilot plant.

ACKNOWLEDGEMENTS

The Author expresses his gratitude to Professor O.Boldor (Bucharest University), and to Dr.Al.Marton (Laboratory of Animal Nutrition, COMTIM, Timişoara), for providing the necessary amount of procaine and for his useful suggestions.

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