MEMBRANE METABOLITES OF *ARTHRONEMA AFRICANUM* STRAINS FROM EXTREME HABITATS

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Summary. Two strains of scarcely studied *Arthronema africanum* (Cyanoprokaryota), originating from two desert habitats in Kuwait and Kathmandu (Nepal) were investigated. Both algae possess good adaptability and grow in a wide range of temperatures and light intensities. The lethal temperature for the Kuwait strain was 50 ± 1 °C while the one of Nepal origin died at 47 ± 1 °C. The content of phycobiliproteins enhanced at low light intensity while the content of chlorophyll and carotenoids increased at higher light intensity.

Fatty acids of monogalactosyldiacylglycerol, sulphoquinovosyldiacylglycerol, and phosphatidylglycerol as well as total lipids were analyzed. Percentage of linolenic acid decreased from 33 % at 16 °C to 0.5 % at 46 °C. In comparison to other algae and higher plants, *Arthronema* behavior represented a clearly expressed physiological response to temperature changes. Constantly high percentage of palmitoleic acid contributed to the endurance of the alga at stress temperature variations.

Keywords: *Arthronema africanum*, fatty acids, lipids, phycobiliproteins, stress.

INTRODUCTION

Blue-green alga *Arthronema africanum* was found in desert soil and marshes near to the sea in Kuwait, and in high plateaus in Nepal (Komarek and Lukavsky, 1988).

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Recently it has been also found in desert regions of Spain (Asencio and Aboal, 2003). Usually, this cyanophycean dwells in salt marshes. The alga ought to withstand salt concentration changes which are due to the often drought. It suffers the stress impact of wide range of temperature and light intensity. This alga shows clearly expressed qualities to survive the effect of several stressors in one and the same time. It is expected that naturally developed useful qualities of the wild alga might be kept when it is bred as an intensively growing dens culture.

Cultivation of *Arthronema* in diluted pig manure has been an object of successful experiments (Lepossa and Ördög, 2000). The chemical composition of *Arthronema* and its physiology have not been studied yet. Till now only cytokinin-like activity of the alga was described and isopentenyladenine isolated from its cultures was reported (Stirk et al., 1999).

Growth of the alga and its standard deviation at constant temperature in laboratory conditions was reported earlier (Iliev and Petkov, 2006). The aim of this work was to assess *Arthronema africanum* growth, its lipid and pigment composition, fatty acid profile, as well as temperature and light intensity influence on these parameters.

MATERIALS AND METHODS

Blue-green alga *Arthronema africanum* Komarek et Lukavsky (1988), strain Lukavsky 1981/1, isolated from desert salt marshes in Kuwait was kindly supplied by CCALA, Czech Academy of Sciences. The alga was grown intensively at a temperature range of 16 – 46ºC, under continuous light with intensity of 8 klx or 2 x 8 klx and aerated with 3 $cm^3.s^{-1}$ 0.5 % (v/v) CO_2. Algal density was maintained at about 2.5 ± 0.4 $g.dm^{-3}$

Proportion of the salts described by Allen and Arnon (1955) were used in $g.dm^{-3}$ as follows: 2.52 $NaNO_3$, 0.583 $K_2HPO_4.3H_2O$, 0.246 $MgSO_4.7H_2O$, 0,243 NaCl, 0.069 $CaCl_2.2H_2O$, 0.06 $Fe_2(SO_4)_3.9H_2O$. Microelements were used in $mg.dm^{-3}$, respectively 3.1 H_3BO_3, 2.230 $MnSO_4.4H_2O$, 0.088 $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.287 $ZnSO_4.7H_2O$, 0.146 $Co(NO_3)_2.6H_2O$, 0.125 $CuSO_4.5H_2O$ (Staub, 1961).

Algae were centrifuged at 3000g. Dry weight was evaluated gravimetrically. The fresh algal biomass was extracted 3 times with chloroform – methanol (2:1 v/v) for 0.5 h under reflux. The extract was evaporated under vacuum and the residue was re-extracted with chloroform. The total lipids were estimated gravimetrically. Individual lipid classes were separated on TLC in mobile phase chloroform – methanol – water (65:25:4 v/v/v). Parts of the lipid samples were converted to fatty acid methyl esters by heating in methanol containing 6 % (m/m) anhydrous HCl at 60ºC for 1.5 h. Fatty acid methyl esters were extracted with hexane and purified by TLC on silica gel with hexane – diethyl ether (10:1 v/v). Solvents and TLC plates from Merck

(Germany) were used. Gas chromatography (GC) of fatty acid methyl esters was carried out on a Perkin-Elmer 3920B, using 180 cm column, 10 % DEGS at 190ºC and FID. Nitrogen was carrier gas at a 0.8 $cm^3.s^{-1}$flow rate. Fatty acids were identified using reference substances. Pigment content was measured and calculated according to usual adopted methods (Mackinney, 1941). Phycobiliproteins were extracted and measured as described elsewhere (Minkova et al., 2003).

RESULTS AND DISCUSSION

Two strains of scarcely studied *Arthronema africanum* (Cyanoprokaryota), originating from two desert habitats, in Kuwait and Kathmandu (Nepal), were investigated. Both algae possess good adaptability and grow in wide ranges of temperature and light intensity. Lethal temperature for the Kuwait strain was 50 ± 1 ºC and that from Nepal died at 47 ± 1 ^{0}C. Growth of strain from Kuwait is presented on Fig. 1. A mean daily increase of 0.44 $g.dm^{-3}$ *A. africanum* was accepted as well-yielded for the conditions of intensive cultivation.

0	24	48	72	96	h
0.8 ± 0.02	1.37 ± 0.11	1.95 ± 0.14	2.26 ± 0.11	2.57 ± 0.41	$g.dm^{-3}$.

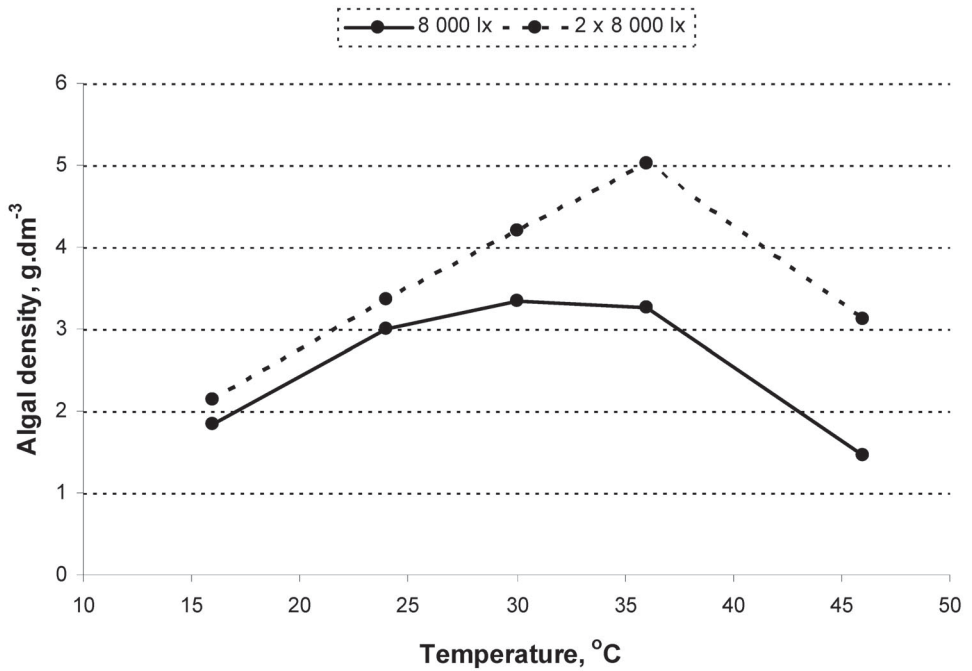

Fig. 1. Growth of *A. africanum* at different temperatures and light intensities.

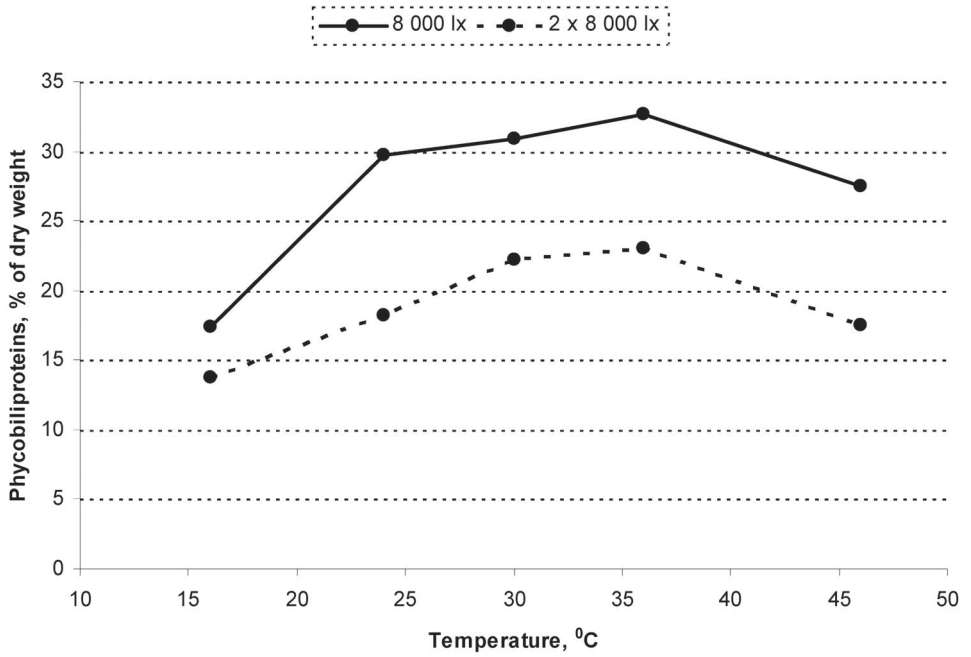

Fig. 2. Phycobiliproteins at different temperatures and light intensities.

A higher increase at constant temperature was observed between 24 and 48 h from the beginning of the cultivation period. Exhausted nutrition medium was clear and translucent after separation of algal biomass. At higher density the filaments of the alga were twisted in the form of plaits or interwoven filaments. A filament was usually bent and twisted round itself like a rope with loop. These fairly looking microscopic images are very informative from taxonomic point of view.

At low light intensity, the content of phycobiliproteins increased in accordance with their function as additional light-harvesting antenna complexes (Fig. 2). Higher light intensity provoked stimulation of chlorophyll and carotenoids biosynthesis.

Main lipid constituents in the cultivation media were monogalactosyldiacylglycerol (MGDG), sulphoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) (Table 1). Fatty acids of MGDG, SQDG, PG and total lipids were analyzed (Table 2). Changes of fatty acid ratio in the temperature range 16-46 ºC were monitored: percentage of linolenic acid being 33 % at 16 ºC decreased to 0.5 % at 46 ºC (Table 3). The qualitative composition of fatty acids of *A. africanum* is typical for blue-green algae. The trend of fatty acid changes at variable conditions was similar, as well. In comparison to other algae and higher plants, the *Arthronema* behavior represented a clearly expressed physiological response to temperature changes. The melting point of fatty acids mixture depended on the percentage of the

Table 1. Percentage of membrane components in *A. africanum*

Substances	% (m/m)
Lipids in dry biomass	8.8 ± 0.9**
Substances in the lipids	
MGDG	22*
SQDG	16*
PG	7*
Chlorophyll a	18
Carotenoids	6
Total fatty acids	24
Unsaponifiable	9.6

* Tentative values

** Results are mean values and SD of 5 repetitions.

Table 2. Fatty acid percentages of individual lipids of Arthronema at 27 ± 1°C

Fatty acids	Total*	MGDG	SQDG	PG
14:0	0.2 ± 0.1	0.2	1.5	0.2
16:0	26 ± 4	24.5	24.5	48.8
16:1	27 ± 3	34.1	37.7	9.5
18:0	0.3 ± 0.1	tr.	tr.	0.8
18:1	4 ± 1	2.0	tr.	2.7
18:2	27 ± 3	19.2	9.8	22.0
18:3^{9,12,15}	16 ± 5	19.9	26.5	16.0

* Results are mean values and SD of 3 repetitions.

Table 3. Influence of temperature on fatty acid profile of *A. africanum*

Fatty acid	16°C	20°C	28°C	32°C	35°C	40°C	46°C
14:0	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.4
16:0	32 ± 3	32 ± 3	35 ± 4	35 ± 4	35 ± 4	40 ± 10	40 ± 10
16:1	26 ± 2	23 ± 3	27 ± 3	36 ± 3	22 ± 3	18 ± 2	23 ± 2
18:0	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.7 ± 0.3	1 ± 0.5	2 ± 1	3 ± 2
18:1	4 ± 1	3 ± 1	3 ± 1	5 ± 1	7 ± 2	9 ± 4	20 ± 6
18:2	4 ± 1	9 ± 2	17 ± 3	24 ± 3	27 ± 5	20 ± 5	16 ± 10
18:3^{9,12,15}	33 ± 3	30 ± 3	17 ± 5	5 ± 1	3 ± 2	0.5 ± 0.4	0.5 ± 0.4

* Results are mean values and SD of 3 repetitions.

separate compounds presented. The steady state of the membranes was due to the high variability of the percentage of linolenic acid in total lipids. The fluidity of membranes remained relatively constant despite of diurnal variations of temperature.

The palmitoleic acid, presented with high and relatively constant percentage, contributed to the endurance of the alga at stress temperature variations. This fatty acid was a constant component regardless the alterations of the environmental con-

ditions. It predominated in the lipids of thylacoid membranes, such as MGDG and SQDG.

Toxic substances were not found in *Arthronema* and this was expected. This alga survives stress impact withstanding mainly against high variations of physical agents. Other blue-green algae which produce poisons ought to survive against organisms. It could be concluded that blue-green algae have improved mechanisms of their primary metabolism through resisting the variations in the physical factors of their habitat by quick biochemical reactions. Very closed to the characteristics of *Arthronema* presented here, is the physiological response of *Plectonema* (Chaneva and Furnadzieva 1997). Similar behavior has also been demonstrated by *Spirulina* which dwells at high pH due to Na^+. These blue-green algae react to the change of light intensity with a wide set of changes in carotenoid pigments and phycobiliproteins.

Unlike the blue-green algae mentioned above, algae which are involved in complicated relationships with other species elaborate better developed secondary metabolism. Cyanophycean such as *Anabaena*, *Microcystis*, *Aphanizomenon*, *Lyngbya*, which dwell steady waters at relatively constant temperate conditions, develop more abundant secondary metabolism (Shimizu, 1993; Baker and Humpage, 1994; Carmichael et al., 1997; Beltran and Neilan, 2000). These algae produce poisons, which most probably help the organisms to survive in highly competitive environment inhabited by bacteria, fungi, and many other algae. On the other hand, the desert tolerant species of *Nostoc* do not produce poisons. Ability to produce toxins has been reported for other species, e.g. *N. rivulare*, which are involved in more complex relationships with other organisms (Davidson, 1959; Sivonen, 1990). The generally established idea that particularly rich secondary metabolism is not a typical characteristic of blue-green algae from extreme habitats still needs further research in order to be confirmed.

It could be concluded that cyanophycean *A. africanum* is worth studying under both natural conditions and biotechnology processes. It inhabits very extreme habitats being an important part of their common ecology. This alga takes part in nutritional chains and in soil formation. Having highly expressed qualities to withstand adverse conditions of deserts and high altitude, *A. africanum* could be adopted as a prospective photoautotrophic object for biotechnological studies.

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