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Fatty acids in thermoacidophilic algae

As has been published several times, three eukaryotic algae are present by the acid fumaroles in the world: one, with a spherical shape, has a diameter of 8-10 μ, divides into four, eight, sixteen or thirtytwo autospores, has a vacuole and can live in heterotrophy (*Pleurococcus sulphurarius* Galdieri) (De Luca, Gambardella and Merola 1979; De Luca and Taddei, 1976; Galdieri 1899); the second, also spherical, has a diameter of 5-6 μ, divides into four autospores, has no vacuole and is not able to grow in heterotrophy (*Cyanidium caldarium* Geitler) (De Luca, Gambardella and Merola 1979; De Luca and Taddei 1976; Geitler and Ruttner 1935); the third one has the shape of a club, is 3-3.5 μ wide and has a diameter of over 1-1.5 μ; divides into two by longitudinal fission, has no vacuole and is not able to grow in heterotrophy (*Cyanidioschyzon merolae* De Luca, Taddei, Varano) (De Luca, Moretti and Taddei 1978; De Luca, Taddei and Varano 1978).

Unfortunately most authors (as reported in De Luca and Taddei 1976) thought they observed in the acid fumaroles only one alga, which they called *Cyanidium caldarium* (Tilden) Geitler. Actually by this name there have been indicated either cultures of *Pleurococcus sulphurarius* or cultures of *Cyanidium caldarium* or mixed cultures of both species, containing even small quantities of *Cyanidioschyzon merolae.*

Because of this confusion, of course, there have been many contrasting results of ecology, physiology, chemotaxonomy experiments.

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Very contrasting are also the data concerning the composition in fatty acids. In this connection KLEINSCHMIDT and MC MAHON (1970) reported that their strains of « *Cyanidium caldarium* » presented different quantities of fatty acids when they were cultivated at 20° and 55°C. They also reported that linolenic acid was present only in the cells cultivated at 20°C.

ALLEN, GOOD and HOLTON (1970), studying three strains of « *Cyanidium caldarium* », cultivated at about 45°C, found that the quantities of fatty acids and their per cent ratios varied in the three strains; linolenic acid was present in two strains and was lacking in the third one.

IKAN and SECKBACH (1972) found in their strain of « *Cyanidium caldarium* », cultivated at 45°-50°C, quantities of fatty acids different from those found by the authors mentioned before.

BOENZI, DE LUCA and TADDEI (1977) studied fatty acids in monoalgal strains of *Cyanidium caldarium* Geitler (= *Cyanidium caldarium forma A*) (DE LUCA and TADDEI 1976) and of *Pleurococcus sulphurarius* (= *Cyanidium caldarium forma B*) (DE LUCA and TADDEI 1976), coming from the solfatara of Pozzuoli (Naples, Italy) and cultivated at 20°C and observed among other things that *Pleurococcus sulphurarius* contained linolenic acid, whereas *Cyanidium caldarium* Geitler was quite lacking it.

BEDORD, MCMAHON and ADAMS (1978), studying the biosynthesis of α-linolenic acid in « *Cyanidium caldarium* », maintained again that this fatty acid was abundant in cells cultivated at 20°C but was not present in cells cultivated at 55°C.

In order to solve definitively this problem of composition in fatty acids of « *Cyanidium* » we have assayed the fatty acids of the three thermoacidophilic algae, utilizing pure strains coming from fumaroles of the Yellowstone National Park (Wyoming, U.S.A.), making experiments at 20° and 55°C, that is at the same temperatures used by KLEINSCHMIDT and MCMAHON (1970) and by BEDORD, MCMAHON and ADAMS (1978) in their experiments.

MATERIAL AND METHODS

We have utilized the following strains coming from the algae collection of the Istituto di Botanica della Facoltà di Scienze della Università di Napoli (Italia): *Cyanidium caldàrium* Geitler (No. 139), *Pleurococcus sulphurarius* Galdieri (No. 140), *Cyanidioschyzon merolae* De Luca, Taddei, Varano (No. 205). The three strains had been isolated from material collected in the Yellowstone National Park (Wyoming, U.S.A.) in the course of a botanical expedition arranged by the Accademia Nazionale dei Lincei in 1971.

The three algae have been cultivated on ALLEN's medium (ALLEN 1959) at pH 1.5 in 500 ml cylinders in which air was being bubbled; lighting was obtained with Philips lamps TLD 30W/55, which gave a continuous light of 8000 lux. The algae were cultivated at 20° and 55°C; those placed at 55°C were gradually adjusted to this temperature, as *Pleurococcus sulphurarius* and *Cyanidioschyzon merolae* died if they were abruptly placed at 55°C.

The growth of algae was observed through the colorimeter, by measuring the absorption of algal suspensions at 550 nm.

The cells, collected during the exponential phase, were washed and lyophilized and the lipids were extracted by LITCHFILED's method (LITCHFIELD 1972).

The fatty acids, both free and combined were methylated by MORRISON and SMITH's method (MORRISON and SMITH 1964). The methyl esters were observed through the gas chromatograph PYE. Unicam supplied with glass columns (1.5 m X 4 mm) containing PEGA 10% 100-120 mesh. The operative conditions were the following: column temperature 190°C, injector and detector temperature 230°C, carrier gas nitrogen 30 ml/min.

Methyl esters of standard fatty acids of the Carlo Erba and Sigma have been utilized to detect the various fatty acids; methyl arachidate has been utilized as internal standard.

RESULTS AND DISCUSSION

Upon examination of the chromatograms (Fig. 1) and Table I we notice that at 20°C *Cyanidium caldarium* and *Cyanidioschyzon merolae* have a very similar composition in fatty

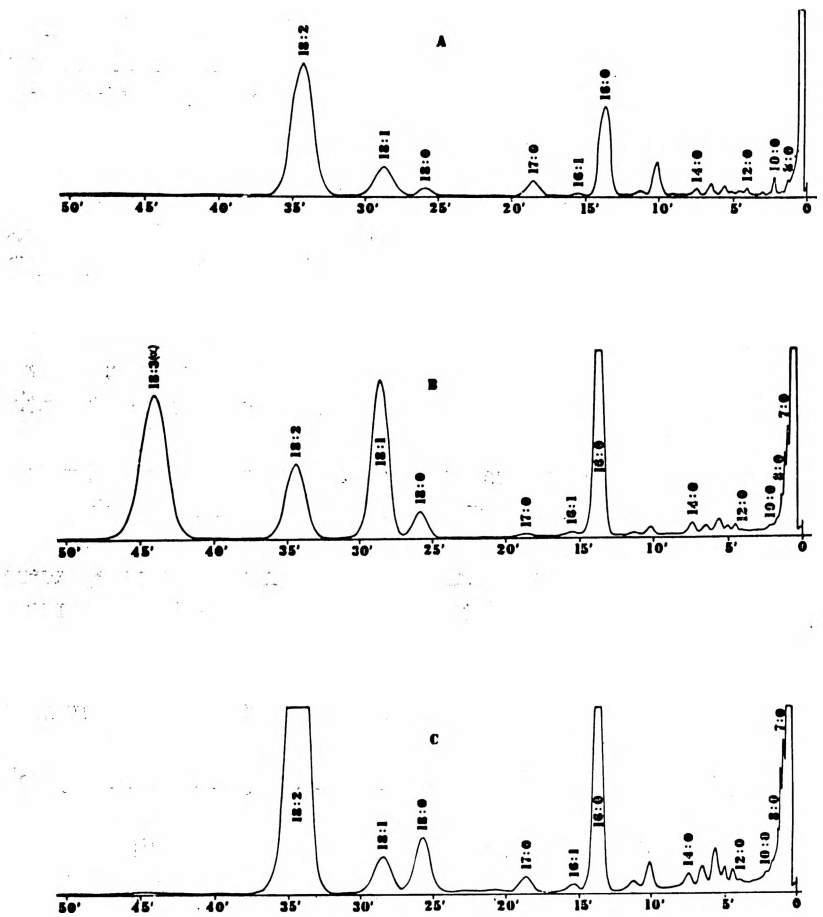

FIG. 1. Gas chromatograms of fatty acids of thermoacidophilic algae at 20°C.

A = *Cyanidium caldarium* B = *Pleurococcus sulphurarius*

C = *Cyanidioschyzon merolae*

acids: in both species linoleic and palmitic acid are the fatty acids most abundant. The difference between the two algae consists in the inversion of the ratio between oleic and stearic acid and in the absence of enantic acid in *Cyanidium caldarium*. *Pleurococcus sulphurarius*, at 20°C, presents a different composition of fatty acids: first of all α-linolenic acid, which is lacking in the other two species, is present in it; furthermore its most abundant acids are α-linolenic, oleic and palmitic acid.

Table I - Percentage values of fatty acids present in thermoacidophilic algae at 20° and 55°C

Acid	*Cyanidium*		*Pleurococcus*		*Cyanidioschyzon*	
	20°C	55°C	20°C	55°C	20°C	55°C
	%		%		%	
Enantic	...	...	0.15	...	0.39	...
Caprylic	1.41	0.73	trace	trace	trace	6.02
Capric	1.90	1.75	trace	trace	trace	7.70
Lauric	0.76	0.64	trace	trace	0.03	trace
Myristic	0.76	2.76	0.52	2.99	0.82	7.98
Palmitic	20.55	26.10	23.65	27.77	24.82	8.96
Palmitoleic	0.76	3.49	0.18	3.42	0.20	6.86
Heptadecanoic	4.57	8.27	0.21	2.56	1.58	10.92
Stearic	5.71	15.26	3.37	10.68	7.59	29.69
Oleic	12.56	13.05	24.33	22.65	5.69	trace
Linoleic	51.01	27.94	13.57	22.65	58.87	21.85
α-Linolenic	...	...	34.03	7.26	...	...

Upon examination of Tables I and II it turn out that the three species, when grown at 55°C, have a smaller quantity of unsatured fatty acids; the only exception being palmitoleic acid which is present in larger percentage in the cells of the three species cultivated at the higher temperature.

α-Linolenic acid, as it has been said, is present only in *Pleurococcus sulphurarius* and does not disappear in this alga at 55°C, even if its percentage decreases. This contrasts with

Table II - Ratio between various fatty acids present in thermoacidophilic algae at 20° and 55°C

Ratio	*Cyanidium*		*Pleurococcus*		*Cyanidioschyzon*	
	20°C	55°C	20°C	55°C	20°C	55°C
18:1/18:0	2.20	0.85	7.22	2.12	0.75	...
18:2/18:0	8.96	1.83	4.03	2.12	7.76	0.74
18:3/18:0	...	...	10.10	0.68	...	...
18 Unsat./18:0	11.14	2.69	21.34	4.92	8.51	0.74
Unsat./Sat.	1.80	0.80	2.58	1.27	1.84	0.40

what KLEINSCHMIDT and MCMAHON (1970) and BEDORD, MCMAHON and ADAMS (1978) observed. These authors probably worked on mixed strains and during the experiments made at 55°C *Pleurococcus sulphurarius* disappeared, being less thermoresistent.

The absence of α-linolenic acid, in one of the three cultures of « *Cyanidium caldarium* » utilized by ALLEN, GOOD and HOLTON (1970), is certainly due to the fact that *Pleurococcus sulphurarius* was lacking in it; the finding of linolenic acid in the other two cultures is instead explainable with the presence of this alga. DE LUCA, GAMBARDELLA and MEROLA (1979), among other things, have demonstrated that one of these two last strains, the one isolated by M. B. ALLEN and classified *Cyanidium caldarium* (Tilden) Geitler (ALLEN 1959), is actually *Pleurococcus sulphurarius* Galdieri (DE LUCA and TADDEI 1976).

The synthesis of large amounts of α-linolenic acid, that BEDORD, MCMAHON and ADAMS (1978) have reported for « *Cyanidium caldarium* » cultivated at 20°C in heterotrophy, is to be attributed to the fact that *Pleurococcus sulphurarius* was present in the culture utilized; this alga, in fact, is the only one in the group of thermoacidophilic algae which can grow in heterotrophy (DE LUCA, GAMBARDELLA and MEROLA 1979; DE LUCA, MUSACCHIO and TADDEI 1972).

The identical composition in fatty acids of *Cyanidium caldarium* and *Cyanidioschyzon merolae* confirms the existence of an affinity between these two species, as it has already been supposed in the past (De Luca, Moretti and Taddei 1978; De Luca, Taddei and Varano 1978). Furthermore no trienoic acids have been found in these two species and this indicates they are much more primitive than *Pleurococcus sulphurarius,* which instead presents trienoic acids like all other eukaryotes (Hitchcock and Nichols 1971).

As far as the classification of the three thermoacidophilic algae is concerned, being eukaryotes with chloroplasts provided with chlorophyll a and c-phycocyanin, they should be inserted in a taxon near to Rhodophyta (De Luca and Taddei 1976); but we must notice that they are quite different from representatives of this group in their composition in fatty acids: in fact whereas *Pleurococcus sulphurarius* presents only α-linolenic acid, and *Cyanidium caldarium* and *Cyanidioschyzon merolae* have no trienoic acids, the Rhodophyta present always γ-linolenic acid (Hitchcock and Nichols 1971); this last fatty acid has been found recently in *Porphyridium cruentum* (Siniscalco Gigliano pers. com.), the unicellular Rhodophyta more related to the three thermoacidophilic algae.

RIASSUNTO

Abbiamo esaminato la composizione degli acidi grassi, a due differenti temperature (20° e 55°C), delle tre alghe termoacidofile *Cyanidium caldarium* Geitler, *Pleurococcus sulphurarius* Galdieri e *Cyanidioschyzon merolae* De Luca, Taddei, Varano.

Abbiamo riscontrato delle differenze qualitative nella composizione lipidica delle tre alghe; inoltre, nell'ambito di ogni singola specie, sono risultate delle differenze quando abbiamo lavorato alle due differenti temperature di cultura.

Il dato più rilevante riguarda la mancanza dell'acido α-linolenico in *Cyanidium caldarium* e *Cyanidioschyzon merolae* e la sua presenza in *Pleurococcus sulphurarius.*

Cyanidium caldarium e *Cyanidioschyzon merolae* sono gli unici eucarioti conosciuti che non presentano acidi trienoici.

SUMMARY

We have examined the composition in fatty acids, at two different temperatures (20° and 55°C), of the three thermoacidophilic algae *Cyanidium caldarium* Geitler, *Pleurococcus sulphurarius* Galdieri and *Cyanidioschyzon merolae* De Luca, Taddei, Varano.

We have found differences in quality in the lipid composition of the three algae; differences within one single species have also been found, when we have worked at the two different temperatures of culture.

The most relevant datum is the lacking of α-linolenic acid in *Cyanidium caldarium* and *Cyanidioschyzon merolae* and its presence in *Pleurococcus sulphurarius.*

Cyanidium caldarium and *Cyanidioschyzon merolae* are the only known eukaryotes lacking in trienoic acids.

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