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Changes of C18 fatty acids content in phospholipids of synchronously cultured *Chlorella vulgaris*

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Chlorella cells can desaturate monoenoic acids and, unlike higher plants, stearic acid [1]. This is why Chlorella cells contain only small amounts, if any, of stearic acid (18:0) but high quantities of C18 unsaturated acids.

As described by many authors, the quantities of particular fatty acids in Chlorella cells are very different and depend on culture conditions, on the stage of developmental cycle and on genotype of the organism tested. Comparisons of published data concerning qualitative and quantitative composition of fatty acids are difficult because of incomplete information on cultivation conditions and the developmental cycle phase of the cells tested. Synchronized cultures of Chlorella cells on strictly defined media and under carefully controlled conditions may clarify the relationship between fatty acid content in cells and the life cycle stage.

In the presented work we determined C18 fatty acids present in the phospholipid fraction of *Chlorella vulgaris* Beijerinck 1890, genus A-8, harvested at different life cycle stages as described previously [2].

Conditions for synchronic cultivation of Chlorella cells were as follows: cell concentration 5.0×10^6 per ml of Lorenzen medium at 30°C, light administration of 15000 lux on the reactor surface during 10 hours, followed by 14 hours of dark period. During the illumination period, the culture was aerated by air supplemented with by 2% CO_2, at a rate of 30 l/h, and during the dark period by air without CO_2. At the beginning of the experiments (t = 0 h, when the illumination started) only aplanospores were present, whereas after 10 h of illumination the culture contained only mother cells [2]. The cytokinesis was completed and the cycle ended with the separation and release of autospores in the dark period of cultivation (Fig. 1).

All stages of the cell life cycle were monitored by using a scanning microscope "Morphoquant" with the computer program Microscan-80 describing the cell shape, size and intracellular arrangement by 21 parameters detailed elsewhere [3, 4]. In the present paper, only one parameter was chosen, i.e. SUEX, which describes the total intracellular extinction (400 - 800 nm) of Chlorella cells at chosen time of the cell life cycle (Fig. 2). The SUEX parameter reflected the intensity of synthesis of organic material during the illumination period (t = 0 - 10 h). The values of SUEX parameter, characteristic for aplanospores (t = 0 h), were in the range of 66 - 117, whereas those for mother cells (t = 10 h) were 674 - 1472. The SUEX values (Fig. 2) indicate that the development of Chlorella cells took place only during the illumination period of the experiment, whereas the aplanospores growth (stage G_o in Fig. 1) in the absence of illumination (t = 14 h - 24 h) was completely inhibited. On the basis of this morphometric analysis, cells from the time range t = 0 h to t = 10 h were harvested for fatty acid analysis.

The Chlorella cells harvested at the cell cycle stage shown in Fig. 2 were homogenized by sonication in distilled water and the lyophilized powder was extracted at room temperature for 1 h with chloroform:methanol (2:1, v/v) under stirring. The extract was concentrated by evaporation at room temperature and dissolved in 1 ml of chloroform. Phospholipids were precipitated from the concen-

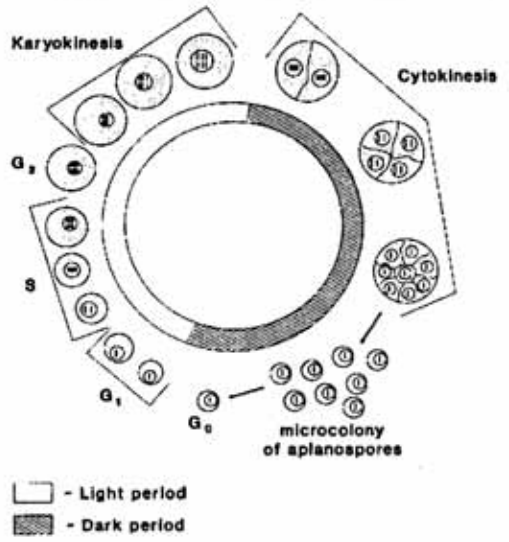

Fig. 1. Scheme of the cell life cycle of Chlorella vulgaris, Beijerinck 1890.
The development and growth of all stadia from G1 to cytokinesis is strongly dependent on illumination and culture conditions. From the very beginning of the dark phase, cytokinesis and sporulation take place

trated lipid fraction by addition of cold acetone to a final concentration of 90%, and then redissolved in petroleum ether, mixed with 1 M NaOH (1:1, v/v) and heated for 3 min in a boiling water bath. The fatty acids in the petroleum ether layer were esterified using the BCl_3-methanol-Supelco method. In brief, fatty acids were measured using gas chromatography on OV-1 Fused Silica Capillary Column as described by Hashimoto *et al.* [5].

The results show that the fatty acid content in Chlorella cells depends on the stage of the cell cycle. In aplanospores (t = 0 h) only linoleic (18:2) and linolenic (18:3) acids were present, while in mother cells (t = 10) stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids were found. However, stearic acid (18:0) was recorded only in the t = 8 - 10 h phase, whereas oleic acid (18:1) at t = 5 - 10 h. Linoleic and linolenic acids were found in all tested stages of development, but at t = 0 h, 1 h and 5 h linolenic acid was present in small amounts only (Fig. 3). Fatty acid content varied not only qualitatively but also quantitatively depending on the cell cycle stage of the examined organism. In aplanospores 18:2 was the characteristic fatty acid while 18:3 was typical for mother cells.

It has been shown by Nichols *et al.* [6] that under autotrophic conditions *Chlorella vulgaris* cells contain only 2.2 - 2.9% of oleic and 12.8 - 20.1% of linolenic acid, whereas under photoheterotrophic conditions as much as 28.3 - 30.2% and 2.5 - 4.0% and in heterotrophic conditions 10.8 - 22.3% and 1.3 - 6.5% of total fatty acids content, respectively. The type of feeding did not influence the content of 18:0 and 18:2 acids [6]. Changes in illumination intensity caused a lack of 18:0 acid while the amount of unsaturated C18 acids were 2%, 17 -

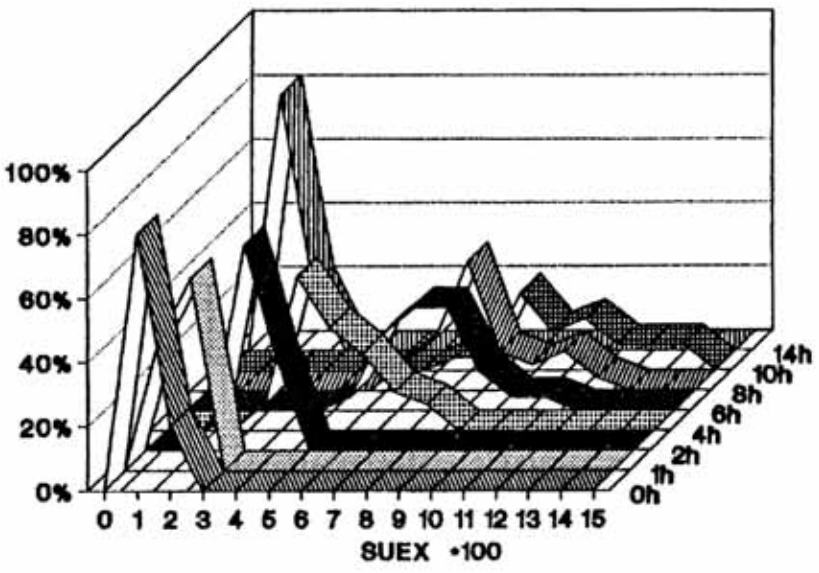

Fig. 2. The total intracellular extinction (SUEX) presented as three-dimensional histogram.
SUEX x 100, values of total intracellular extinction in the range of 400 - 800 nm; %, percentage of Chlorella cells; h, hour of cell cultivation. At t = 0 h and t = 14 h aplanospores are only present and at t = 10 h only mother cells are present

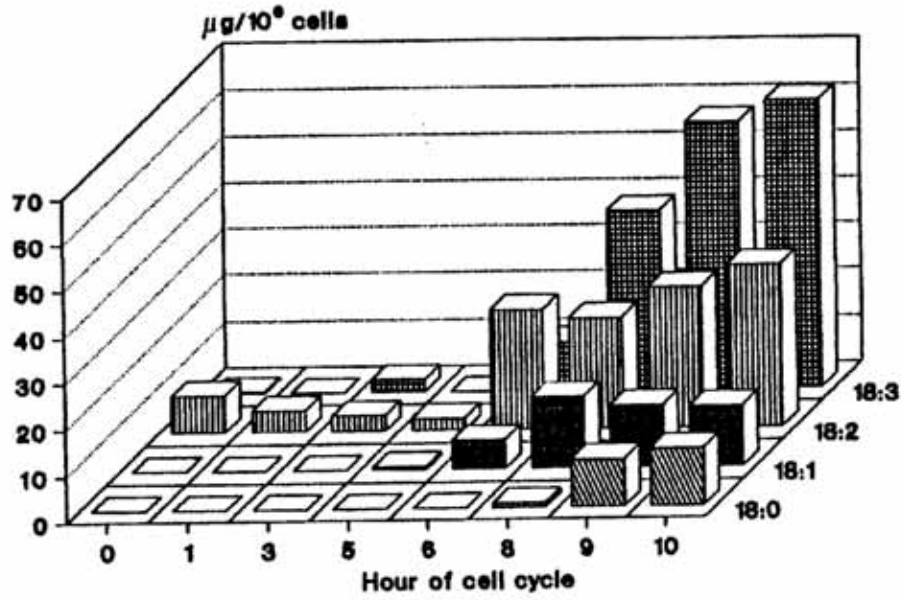

Fig. 3. Changes in C18 fatty acid content at various stages of the life cycle of Chlorella vulgaris, Beijerinck 1890, cultivated synchronously

29% and 16 - 30% for 18:1, 18:2 and 18:3, respectively [7].

Generally, in aplanospores only the 18:2 fatty acid was present in the phospholipid fraction. Trace amounts of 18:3 were also detected. The illumination produced a decrease of 18:2 content till the 5th hour of the cultivation period followed by an increase of 18:3; 18:2; 18:1 and 18:0 thereafter. The most dramatic change occurred between t = 5 h and t = 6 h. This is possibly related to the complete rearrangement of the cell which takes place at this time as observed under the microscope, as described in detail elsewhere [8, 9].

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