

Physiological and genetic potentials of microalgae and molecular biology aspects of photoautotrophic biosyntheses biotechnology

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1. Introduction

Photosynthesizing cells are the unique natural bioreactors which include the great processes of light energy transformation into biologically available forms, the reactions of carbon dioxide reduction, and primary organic compounds syntheses, specific biological catalysts for low- and macromolecular biosyntheses, and fine, delicate mechanisms for their permanent self-regulation, self-adaptation, and self-propagation. These bioreactors are under permanent genetic control and can be reconstructed by certain genetic manipulations.

That is why photoautotrophic microorganisms (microalgae and cyanobacteria) attracts the attention as the significant tool for the photosynthesis industrialization and introduction into biotechnology, as the sources of new physiologically active compounds, and also as potential recipients for foreign genes incorporation.

Physiological and genetic potentials of microalgae and cyanobacteria as objects of biotechnology are based on: (i) biological diversities of alga species; (ii) adaptive flexibility of metabolisms of alga cells; (iii) possibility of microalgae to mutagenesis and (iiii) gene engineering modifications of alga cells for production of specific compounds by transformants [1,2].

2. Biological diversities of alga species as the objects of biotechnology

The different species and strains of microalga can use for growth the wide range of physiological conditions (Tables 1, 2, 3).

Table 1. Growth sensitivity of alga strains to temperature.

Strain, collection index	Temperature, °C						
	5-10	20-30	30-35	35-40	40-45	45-50	55-60
<i>Scotiella nivalis</i> , IPPAS H-247	x						
<i>Chlorella pyrenoidosa</i> IPPAS C-2		x					
<i>Spirulina platensis</i> IPPAS B-256			x				
<i>Chlorella vulgaris</i> IPPAS C-1; CALU 246				x			
<i>Chlorella sorokiniana</i> SAG 211-32					x		
<i>Galdieria sulphuraria</i> SAG 107.79; UTEX 2393						x	
<i>Synechococcus elongatus</i> IPPAS D-267							x

Table 2. Growth sensitivity of alga strains to pH.

Strain, collection index	pH					
	1-2	3-4	5-6	7-8	9-10	11
<i>Dunaliella acidophila</i> IPPAS D-289;CAUP-G-301	x					
<i>Galdieria maxima</i> IPPAS P-507		x				
<i>Cyanidium caldarium</i> IPPAS P-509			x			
<i>Chlorella vulgaris</i> IPPAS C-1; CALU 246				x		
<i>Spirulina platensis</i> IPPAS B-256					x	
<i>Cyanospira capsulata</i> SAG B 2.90						x

Table 3. Growth sensitivity of alga strains to salinity level.

Strain, collection index	NaCl, M					
	0.1	0.5	1.0	2.0	3.0	4.0
<i>Porphyridium cruentum</i> IPPAS P-273; SAG 1380-1a	x					
<i>Chlorella stigmatophora</i> IPPAS C-114		x				
<i>Spirulina platensis</i> IPPAS B-410; ODESU-10			x			
<i>Dunaliella minuta</i> IPPAS D-204				x		
<i>Dunaliella salina</i> IPPAS D-209					x	
<i>Dunaliella salina</i> IPPAS D-209						x

This gives a great possibility for screening of natural forms and selections of the strains of specific properties fitting for special tasks of biotechnology.

3. Flexibility of metabolisms of alga cells and their biosynthetic potentials

The metabolism of alga cells can be changed by different growth conditions. These shifts in biosynthetic pathways are usually induced by the stress factors (light, temperature, salinity, nitrogen starvation and others) as figures 1 and 2 demonstrate [2-4].

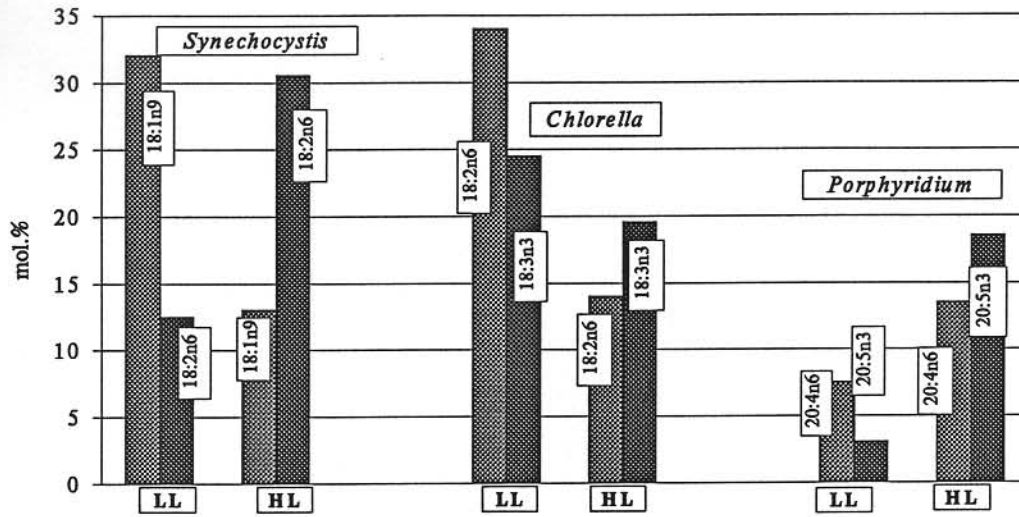


Fig.1. Light-dependent changes of fatty acid levels responding to light intensity. LL, low light, HL, high intensity

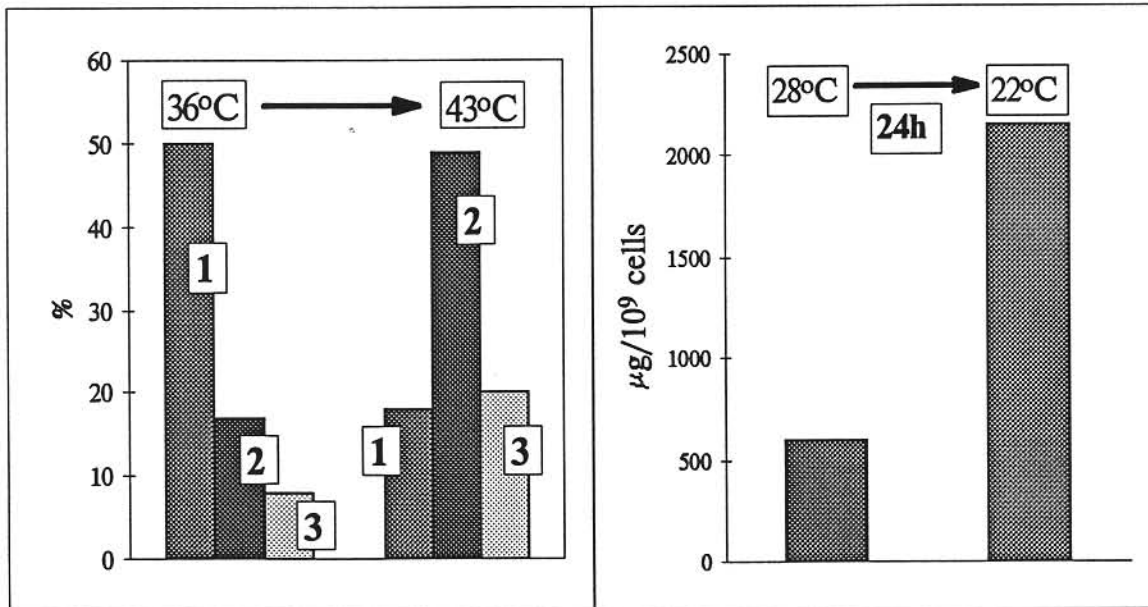


Fig. 2. Temperature induction of carbohydrate accumulation in *Chlorella* cells (left) and β-carotene in *Dunaliella salina* cells (right) [1,3].

The plasticity of metabolisms and the cell capability for directed changes in the biosynthesis give the possibility to elaborate the specific technologies (Fig. 3) for production of aim-products or the biomass with modified biochemical composition.

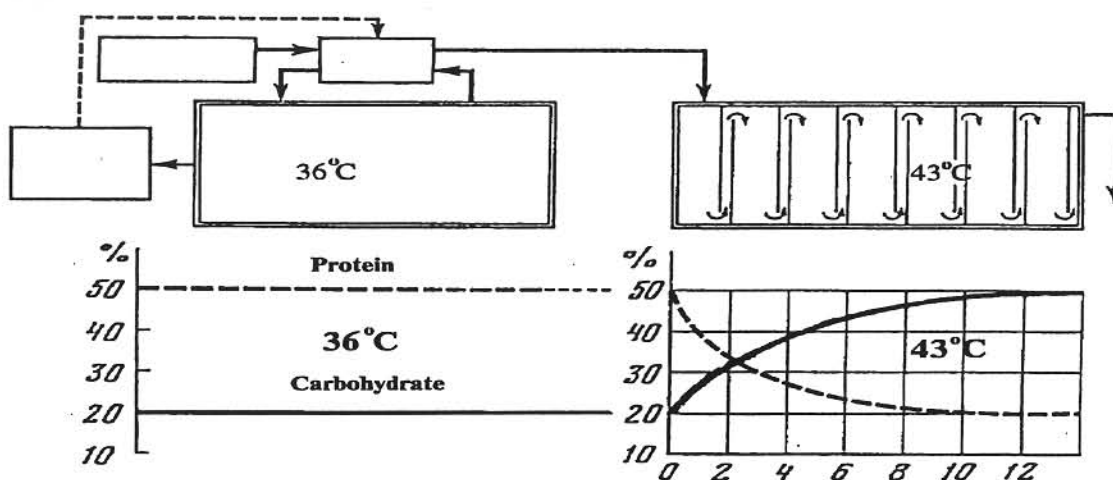


Fig.3. The scheme of chemically transformed *Chlorella* biomass production with the aim of continuous two photobioreactor system [1].

4. Potentials of alga mutants as overproducers of different compounds

The mutagenesis of alga cells can be thought to be one of the effective tools to obtain the mutant-overproducers of various compounds. Tables 4-6 demonstrate the current progress in this field.

Table 4. *Chlamydomonas reinhardtii* mutants - overproducers of ζ -carotene [5].

Strains	Carotene contents, % of total carotenes			
	α -carotene	β -carotene	ζ -carotene	unidentified carotene
Wild type (WT)	5,3 \pm 1,4	94.7 \pm 2.1	0	0
Mutant C-41	0	43.0 \pm 2.5	37.9 \pm 4.1	19.1 \pm 3.7

Table 5. *Chlorella* mutants-overproducers of cysteine [6].

Strains	Cysteine content		
	% of total amino acids	mg/100 mg DW	% to WT
Wild type (WT)	1,19 \pm 0,12	0,51 \pm 0,03	100
Mutants:			
K-75	6,79 \pm 1,62	2,70 \pm 0,56	529
K-94	4,95 \pm 0,88	2,25 \pm 0,30	441
K-96	4,94 \pm 0,44	2,12 \pm 0,23	415

Table 6. Regulatory *Chlorella* mutants-overproducers of starch.

Strains	Starch contents, % to dry weight	
	optimal growth conditions	stress conditions
Wild type	16	40
Mutant CRS-03	15	70

However, the mutant obtaining had to be guided to a definite goals, taking into account the requirements of industry and the opportunities of technology.

5. Glucose feed-back regulation of photosynthetic genes expression and obtaining of alga mutants with non-restricted photosynthesis

The glucose feed-back mechanism of photosynthesis negative regulation was demonstrated (Fig.4) in our Laboratory [1,7,8]. This mechanism provides a basis for the sink-source intracellular interactions and restricts the photosynthetic gene transcription [8,9].

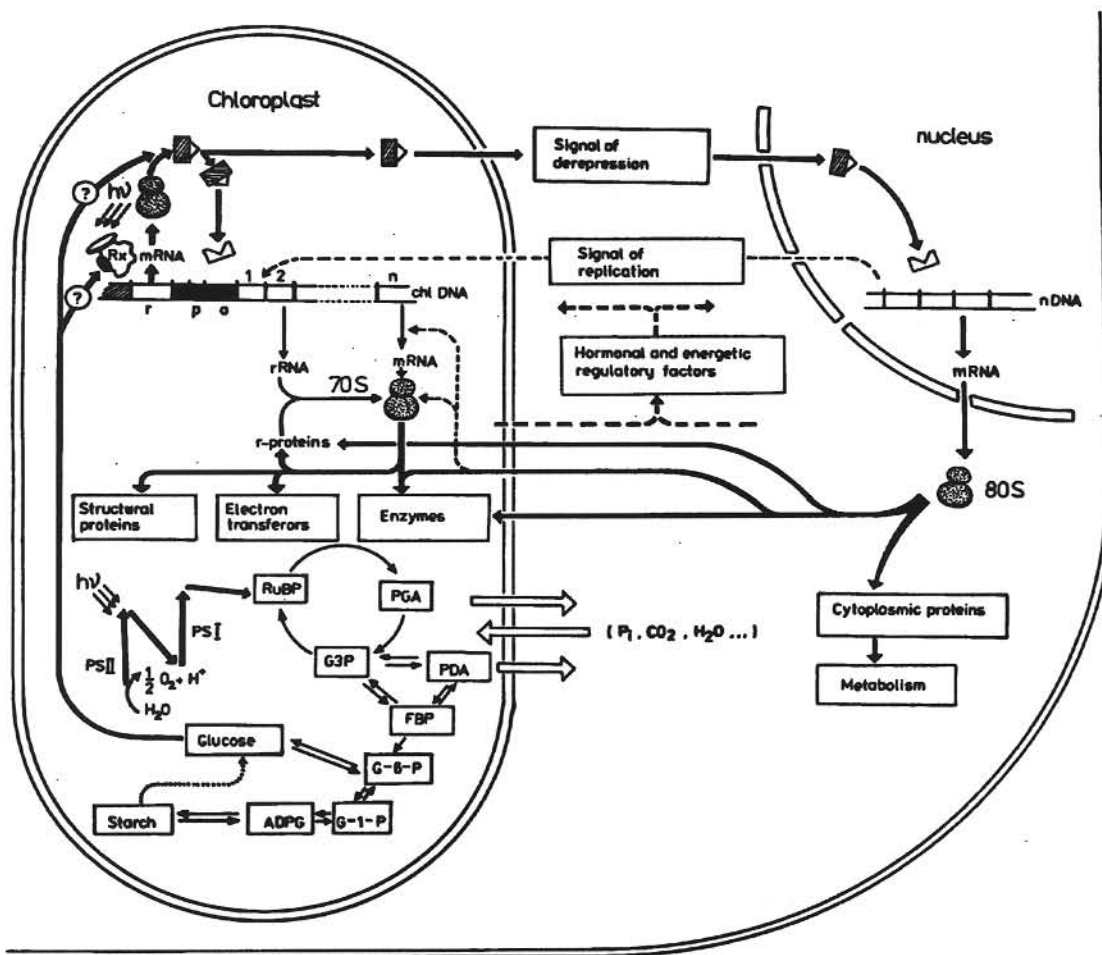


Fig 4. Glucose feed-back control of photosynthetic genes expression.

The glucose feed-back control works in cooperation with the photoregulation of photosynthetic gene expression [8,10]. In our Laboratory on the basis of these findings the method of selection was elaborated to obtain the regulatory mutants of *Chlorella* [11]. Due to disturbances in the glucose feed-back control, these mutants are characterized by the super accumulation of photosynthetic products (carbohydrates) (Table 6).

6. The gene engineering of alga strains for the biotechnology of photoautotrophic biosyntheses

The transformation of algal cells by the gene engineering manipulations is based on the achievements of contemporary molecular genetics. We have now some examples [12,13] of the successful transformation of algae (Table 7).

Table 7. Gene engineering algae strains.

Strains	Foreign gene	Vectors	Expression of foreign gene by transformant
<i>Anacystis nidulans</i> IPPAS B-434 PCC 6301 UTEX 625 ATCC 27144 SAG 1402/1 CCAP 1405/1	h-SOD The chemically synthesized gene encoding human CuZn superoxide dismutase.	pBAX 18 The shuttle cloning vector for <i>A.nidulans</i> and <i>E.coli</i> , include regulatory elements of RuBisCO promoter from <i>A.nidulans</i>	Stable expression. h-SOD expression levels of transformant increased more than 18-fold by light. Accumulation of the SOD protein <i>ca.</i> 3% to total soluble protein [12].
<i>Anacystis nidulans</i> R2-SPc	desA gene The gene from <i>Synechocystis</i> PCC6803 encoded desaturase of 18:1 fatty acid (Δ 12).	pUC 303 The shuttle vector between <i>A.nidulans</i> and <i>E.coli</i> .	Stable expression. 18:2 (9,12) fatty acid accumulation increases the tolerance of transformant to low temperature [13].

The use of regulatory elements of adaptive plant genes for the activation of foreign gene expression in algal cells is of a particular interest [12,14]. The elaboration of technology for the ecologically permissible cultures of algal transformants is also of a great importance.

Some examples, demonstrated above, can serve as foundation for contemporary development of alga biotechnology and for its progress in future.

7. Strategy of alga cells transformation for biotechnology purpose

The construction of alga strains for biotechnology by the methods of gene engineering would imply the following strategy:

1. Selection of alga strains for transformation with reference to:
 - a) organization of alga cells (procaryotic or eucaryotic cells, structure of all wall);
 - b) activity of site-specific endonucleases (high or low activity);
 - c) source of codon.

2. Selection of foreign gene taking into account:
 - a) the homologous or heterologous structure of gene;
 - b) antigenic activity of the foreign gene products
3. Selection of vectors for foreign gene transfer:
 - a) self-replicating;
 - b) integrating;
 - c) shuttle vectors
4. Optimization of foreign gene expression taking into account:
 - a) activity of promoters;
 - b) regulatory elements of transcription and translation;
 - c) light-, temperature-, stress-responsive elements.
5. Investigation of transgenic microalgae physiological parameters.

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