Mixotrophy of few strains of cyanobacteria and algae isolated from lampenflora communities

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Abstract. In the current study abilities for the mixotrophic growth of phototropic cave strains were observed. The influence of organic substrates on the growth rate on Chlorella vulgaris, Stichococcus bacillaris, Leptolyngbya foveolara, Scytonema were studied. In most cases glucose, maltose, glycerine, malate, acetate, sucrose, and peptone showed a stimulating effect on the culture growth rate by the dark and by the light incubation. Specific abilities for mixotrophic carbon consumption were proved.

1 Introduction

Caves are not a favorable habitat for phototrophic species, due to the limit of light [1]. However, the development of phototrophic communities is noted in entrance photic zones and under artificial light. The question of species adaptation to cave habitats remains disputable. There are several survival strategies that make phototrophs adapt to cave environmental conditions.

Firstly, morphological adaptations provide long-term viability during low light periods. For example, a precipitation of calcium carbonate crystals on the cell membrane by cyanobacteria of the genus Scytonema. Such phenomena were observed in cave zones with low light intensity. Organisms with calcified trichomes survived better than those that do not have $CaCO_3$ deposits [2]. In addition, glycans, the UV-absorbing pigments, and water stress proteins were detected in the extracellular polymer matrix of biofilms [3]. Studies of the ultrastructure of representatives of the genera Chroococcidiopsis, Cyanosarcina, Leptolyngbya, Phormidium and Pseudocapsa isolated from caves in Murcia, Spain [4], indicate a well-developed system of thylakoids. Moreover, the presence of all the most widely known cellular inclusions (lipids, globules, polyphosphate) was also observed.

Secondly, phototrophs undergo modification in the photosynthetic apparatus and get other physiological adaptations. Changes in the quantity of pigments and associated pigment-forming proteins as a response to a change in the level of illumination are

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described [5,6]. For example, a decrease in photon flux density often leads to an increase in the concentration of chlorophyll and phycobilins. The ability of cells to synthesize auxiliary photosynthetic pigments to increase proton capture efficiency in low light has been noted [7].

Thirdly, mixotrophy can be one of the strategies helping phototrophs to survive in the dark. Mixotrophy is a metabolic strategy of some organisms that combines the features of auto- and heterotrophy, that is the consumption of various sources of carbon and energy [8]. Mixotrophic growth seems to be very energy-intensive, and the organism must always maintain an optimal balance between physiological costs and benefits. On the one hand, the cell needs to maintain the photosynthetic apparatus and the entire enzyme sets of the Calvin cycle. On the other hand, heterotrophic growth requires membrane transporters of simple organic substances and ecto- and exoenzymes for extracellular hydrolysis of organic polymers.

The experiment [9] with cultures of micro-algae Chlorella and Coccomixa showed their ability to grow on an organic substrate. Mixotrophy was described for several phototrophic species [10] including Plectonema boryanum, that is widespread in caves [11]. The use of exogenous sugars for heterotrophic and photoheterotrophic growth of Scytonema coactile isolated from the twilight zone of one of the Indian caves was shown [12].

The aim of the present study was to establish the capacity of cyanobacteria and algae isolated from caves to grow using mixotrophic adaptation strategy.

Cyanobacteria and algae pre-culture. The strains of the dominant phototrophic species isolated from the lampenflora communities of the Caucasus caves were objects of the study: Chlorella vulgaris NA05.05.2018 (Novoafonskaya cave), Stichococcus bacillaris Ach05.05.2019 (Akhshtyrskaya cave), Leptolyngbya foveolara NA05.05.2018 (Novoafonskaya cave), Scytonema drilosiphon NA05.05.2018 (Novoafonskaya cave). The last one is a cultivated strain without carbonate cases.

To isolate and cultivate cyanobacterial and algal strain samples of biofilms were collected by scraping the cave substratum with a sterile scalpel. Samples originate from the areas with artificial lighting. The obtained material was transferred to the laboratory. Samples were investigated for their morphology using a light microscope Leica DMLS (Germany) and Biolam MBS-9 (Russia). Species identification was carried out using a described method [13]. Systematics of cyanobacteria and algae is provided by AlgaBase [14].

Isolation of pure cultures was carried out using the method of fouling glasses and cultivation on the agar Bristol medium. Cultures were stored at the temperature of 25 °C and light intensity of 30-40 $\mu m \times m^{-2} \times s^{-1}$.

Culture axenization was performed without antibiotics by adding sodium oleate, followed by centrifugation and purification using bidistillated water.

Experimental conditions. Cyanobacteria and algae samples were grown in Bristol medium. Addition of organic substates such as glucose, maltose, glycerin, malate, acetate was conducted to determine the possibility of algae and cyanobacteria using organic substances as a carbon source. All substrates were used at a concentration of 0.5% (mass / volume).

After measuring the initial cell density (starting point), cell cultures were divided into two groups. The first group was treated with organic substates and incubated apart by the light and apart in the dark (control). The second group was incubated in the same way, but without the addition of organic substrates. Each incubation was carried out for 3 days. After that, cell density was measured using a Shimadzu UV-1280 spectrophotometer. The specific growth rate (μ) was calculated (1) where Nt and No are the cell densities at the start and the end of the experiment, t is the experiment duration.

2 Materials and methods

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$$\mu = \frac{(N_t - N_o)}{t} \quad (1)$$

3 Results

The influence of additional organic substrates on the growth of *Chlorella vulgaris, Stichococcus bacillaris, Leptolyngbya foveolara, Shizotrix vaginata* strains was revealed. Each strain was tested in four groups. Cell culture with and without the addition of organic substrates in light. Cell culture with and without addition of organic substrates in the dark. The obtained data was compered using a t-test.

In the case of Chlorella vulgaris (figure 1) only glucose and maltose caused a significant increase in the specific growth rate in the dark. The other studied organic substrates did not provide significant changes of the culture growth rate in the dark experiment. Maltose, glycerin, malate and peptone stimulated the development of cell cultures in the light. At the same time, glucose, acetate and sucrose significantly reduced the growth rate in the presence of lighting.

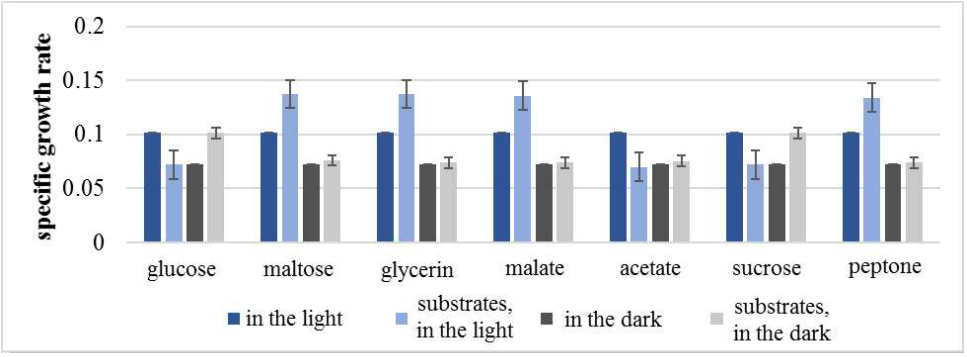

Fig. 1 The specific growth rate of *Chlorella vulgaris* on media with the addition of glucose, maltose, glycerin, malate, acetate, sucrose, and peptone.

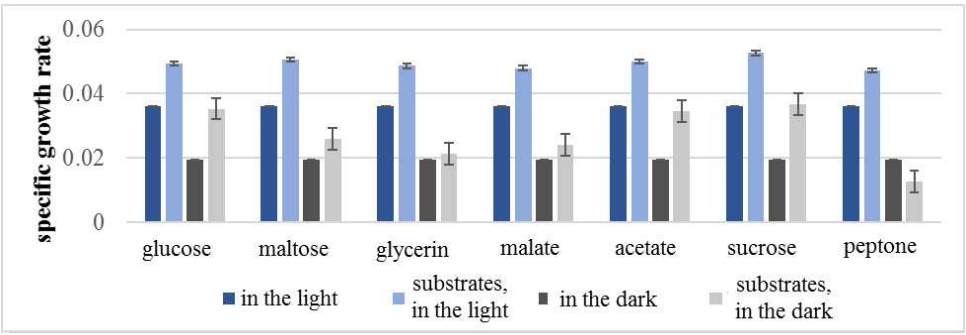

Fig. 2 The growth rate of *Stichococcus bacillaris*. With and without the addition of glucose, maltose, glycerin, malate, acetate, sucrose, and peptone.

All studied organic substrates except peptone increased the growth rate of *Stichococcus bacillaris* (figure 2) in the dark. The highest increase was observed in the presence of glucose, acetate and sucrose. The addition of peptone slightly decreased the cell culture growth rate. In all studied cases, the addition of organic substances had a stimulating effect in light. An increase of up to 20% was observed.

The presence of organic compounds in culture media also caused the increase of the growth rate of *Leptolyngbya foveolara* (figure 3) in the dark. The greatest effect was noted in the presence of glucose, sucrose and glycerol. All studied organic compounds provided a stimulating effect on the growth rate. In comparison to other studied microorganisms, *Leptolyngbya foveolara* showed the most powerful response to the added organic substrates. An almost 2-3 fold increase in the growth rate was observed.

A similar effect was observed after treating *Scytonema drilosiphon* (figure 4) with organic substrates (glucose, maltose, glycerin, malate, acetate, sucrose, and peptone). *Scytonema drilosiphon*'s growth rate was the most sensitive for the lack of light. However, glucose, malate, acetate and sucrose fully compensate the drop in of the growth rate in the absence of light.

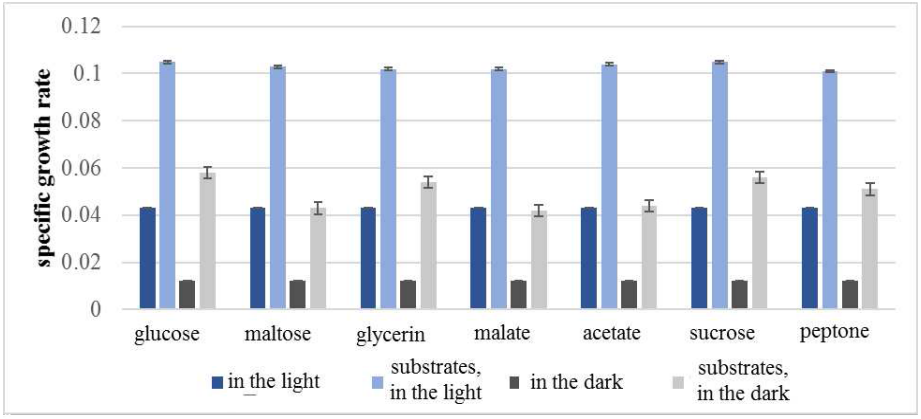

Fig. 3 The growth rate of *Leptolyngbya foveolara*. With and without the addition of glucose, maltose, glycerin, malate, acetate, sucrose, and peptone.

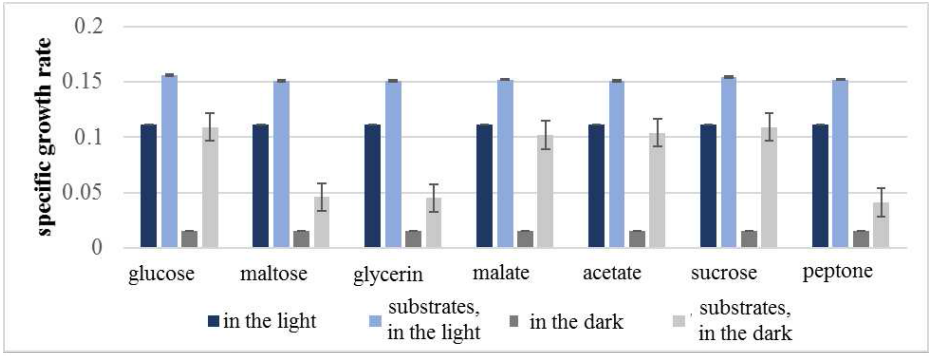

Fig. 4 The specific growth rate of *Scytonema drilosiphon*. With and without the addition of glucose, maltose, glycerin, malate, acetate, sucrose, and peptone.

4 Discussion

The stimulating effect of all organic compounds (glucose, maltose, glycerin, malate, acetate, sucrose, and peptone) on the development of algae and cyanobacteria in the dark conditions was proved. Glucose and sucrose caused the most significant increase in specific growth rate of all investigated strains. This corresponds to the data [15] that shows that the green algae *Mychonastes homosphaera* (Skuja) Kalina et Punc and diatom *Nitzschia palea* (Kütz.) W. Sm. isolated from cave Propashhaya yama (Republic of Bashkortostan) assimilate the glucose and can be determined as mixotrophic strains. However, *Chlorella vulgaris* provided a negative response to the addition of glucose in the light experiment. Thus, may be associated with a rapid increase in the population at the initial stages. Another decrease in the growth rate in response to organic substrate was observed in the dark experiment with *Stichococcus bacillaris*. A complex composition of peptone mixture can differently influence various microorganisms.

Mechanisms of mixotrophy can be very specific. For some species, it may include phagotrophy, the engulfing of other organisms, or osmotrophy - the uptake of dissolved organic carbon from the environment. Osmotrophy was shown in the example of *Chlorella*

vulgaris [17]. The amount of assimilated carbon also varies between species and conditions of cell culture growth [18].

The other explanation of the "inhibitory effect" of peptone on the *Stichococcus bacillaris* growth rate in the dark is a time gap that is needed to adopt to new conditions. According to Sauer and Tanner (1989) Mixotrophic growth requires the presence of appropriate uptake proteins such as Hup1 in *Chlorella kessleri*, and the ability to metabolize the carbon source [19]. That means that the time required to enhance the regulation and expression of metabolic proteins can lead to a lag-phase in population growth. A two-day lag-phase was observed for *Galdieria sulphuraria* [20]. A 24-hour lag-phase was observed for *Cyclotella cryptic* [21]. Peptone is a complex mixture of proteins, fatty acids, and micro-elements. Therefore, it is possible to expect a long adjustment process. The absence of a response to other organic additives also does not mean the impossibility of a substance to be consumed. The microorganism may require a longer period of adaptation.

The source of organic matter for mixotrophy in cave conditions remains questionable. After all, underground habitats are oligotrophic. The source of organic matter for cyanobacteria and algae can be substances in the exopolymer matrix of biofilms. According to recent publications, mixotrophy can be a potential mechanism to reduce the costs of microalgal culturing [22]. Mixotrophic cultivation has sufficient advantages over photrophic growth of microalgae: a theoretical lower cost [23], increased amount of biomass, increased cell density and an increase in the target biochemical component [24]. Therefore, isolation of unique potentially mixotrophic species can be perspective direction of investigation.

5 Conclusion

The source of organic matter for mixotrophy in the conditions of the cave remains questionable. However, the evidence for mixotrophic mechanisms of carbon consumption were obtained for phototrophic microorganisms. The dynamic of the cell culture development in the presence of various organic compounds was studied. Further study of this issue is necessary to provide more information about the influence of different substrates and adaptation mechanisms.

Acknowledgment

The current article was supported by RUDN University (R.4-N-2022).

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