

Original Research

pH-DEPENDENT GROWTH RESPONSES OF *Chlorella* STRAINS IN BG11 MEDIUM

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Abstract

Chlorella was among the first algae to be successfully cultured and remains one of the most well-known and extensively studied genera of microalgae. Several species are widely used as model organisms in environmental, industrial, and health-related research. Nevertheless, many strains remain unexplored and may possess valuable traits, both within described species and among novel isolates from extreme habitats such as caves. In this study, two *Chlorella* strains were examined: a commercially available strain and one isolated from lampenflora in Lazar Cave. Both showed notable resilience and the ability to grow across a range of initial pH conditions, with alkaline media proving most favourable for their growth.

Keywords: *Chlorella*, algal cultures, pH value, BG11 medium, lampenflora.

INTRODUCTION

Microalgae constitute a highly diverse and ubiquitous group of photosynthetic microorganisms with a global distribution, yet they also exhibit notable levels of endemism. They inhabit a wide range of aquatic and terrestrial environments, including many that are extreme, poorly explored, and understudied [1]. Despite their immense diversity, only a small fraction of microalgal species has been successfully isolated and maintained in culture collections worldwide. Of these, only a few hundred – mainly those of commercial relevance – have been thoroughly characterised in terms of genomic architecture, biochemical composition, and physiological traits [2].

Chlorella was the first alga to be successfully isolated in culture and played a pivotal role in early studies of photosynthesis and carbon dioxide assimilation in plants. It was also among the first microalgae investigated for mass cultivation and the earliest to be produced on a commercial scale. Intensive research on *Chlorella* began in the early 1950s, coinciding with

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the first large-scale cultivation efforts in the United States, while commercial production was established in 1961 [3,4].

Among *Chlorella* species, physiological and biochemical properties have been studied in considerable detail for *C. vulgaris* and *C. pyrenoidosa* [5]. In addition to these well-known taxa, other *Chlorella* strains have also been investigated and evaluated for various applications (e.g., Guccione *et al.* [5]). However, certain habitats remain underexplored as potential sources of novel representatives of the genus. Caves, in particular, represent such environments, where microalgae typically occur in entrance zones or in areas illuminated by artificial light in show caves, forming communities known as lampenflora.

This study focuses on two *Chlorella* strains, one commercial and one isolated from show cave lampenflora and examines their growth responses in culture media with varying initial pH values.

MATERIALS AND METHODS

***Chlorella* strains and cultivation**

For the purposes of this study, two *Chlorella* strains were used: a commercial strain obtained from a culture collection and a strain isolated from the lampenflora of Lazar Cave in eastern Serbia [6,7].

BG11 medium, prepared according to Rippka *et al.* [8], was used for algal cultures. The lampenflora-derived *Chlorella* strain was initially isolated in test tubes with solid BG11 medium, then cultivated and subcultivated on Petri dishes [6], and subsequently maintained in liquid medium. The commercial strain was maintained exclusively in liquid BG11 medium. Both strains were grown in a controlled growth chamber at 19–22 °C under a 12 h light: 12 h dark photoperiod. Cultures were regularly monitored by light microscopy using a Zeiss Axio Imager M1 microscope equipped with a digital camera, and images were analysed with AxioVision 4.8 software.

Experimental design

Prior to the experiment, a small aliquot of each *Chlorella* culture was transferred into liquid BG11 medium (pH 7.4) and incubated in a growth chamber until high cell density was reached. Subsequently, BG11 media with initial pH values of 3, 5, 7, 9, and 11 were then prepared, dispensed into 100 mL Erlenmeyer flasks (each containing up to 50 mL of medium), and sterilised by autoclaving at 114 °C for 25 minutes.

In the first part of the experiment, both *Chlorella* strains were inoculated into the prepared media with varying initial pH, with three replicates per pH level for each strain. Cultures were grown for two weeks on an orbital shaker at room temperature under a 12 h light: 12 h dark photoperiod. Growth was monitored by measuring optical density (OD) at 680 nm using a spectrophotometer, starting at the beginning of the experiment and subsequently every 2–3 days (three times per week) to generate growth curves.

In the second part of the experiment, the lampenflora-derived *Chlorella* strain was inoculated into the prepared media with varying initial pH values and cultivated for one month in a growth chamber at 19–22 °C under a 12 h light: 12 h dark photoperiod. Biomass production was assessed at the end of the experiment by determining chlorophyll *a*

concentration ($\mu\text{g L}^{-1}$) using the standard spectrophotometric method (ISO 10260:1992(E)) [9].

RESULTS AND DISCUSSION

The experiment assessing the growth response of two *Chlorella* strains was conducted over a two-week period and included the exponential phase of algal growth. As shown in Figure 1, the lampenflora-derived *Chlorella* strain exhibited a slightly longer lag phase, with more pronounced growth beginning after the second measurement, whereas the commercial strain showed detectable growth from the first measurement. Despite this difference in initial adaptation, both strains displayed a broadly similar growth pattern across media with different initial pH values. No growth was observed in either strain at pH 3. Among the remaining pH treatments, the lowest optical density (OD) values were recorded at pH 5, while the highest values were observed at pH 11 for both strains. Although the overall growth trends were comparable, final OD values differed slightly between the strains, with the commercial strain (Figure 2b) consistently exhibiting higher values across all pH conditions.

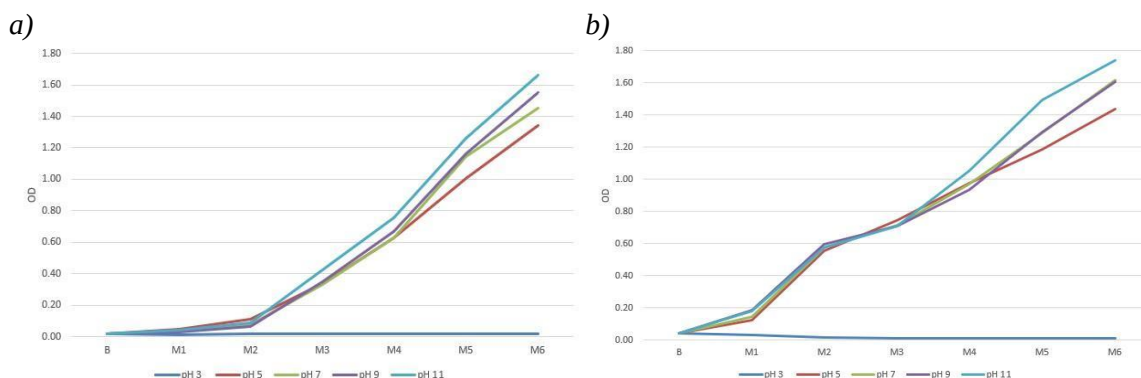


Figure 1 Growth response of (a) the *Chlorella* strain isolated from lampenflora (left) and (b) the commercial *Chlorella* strain (right) after two weeks of cultivation. OD – optical density; B – initial measurement of OD at the beginning of the experiment; M1–M6 – subsequent OD measurements

The second experiment involved cultivating the lampenflora-derived *Chlorella* strain in a growth chamber, with primary production assessed after one month. The results were consistent with those of the previous experiment: no growth was observed at pH 3, while among the remaining treatments, the lowest biomass production occurred at pH 5 and the highest at pH 11.

Many studies have identified pH as a critical parameter to optimise when aiming to increase biomass yield [10]. Reported optimal pH values for *Chlorella* growth vary among strains, indicating that species within this genus can tolerate a wide range of pH conditions. Gaysina [11] demonstrated that *C. vulgaris* exhibits high resistance to both acidic and alkaline environments. During cultivation, it maintained its basic morphological characteristics across a pH range of 4–11.5. At pH 3.5, approximately 30% of the cells lost their green pigmentation, while more pronounced discolouration was observed at pH 2–3 and pH 12. In contrast, El-Sheekh *et al.* [12] evaluated the growth of *C. vulgaris* at pH values of 6, 7, 9, 11, and 12, reporting the highest optical density at pH 12. Similarly, Gong *et al.* [10] observed a

favourable response of *C. vulgaris* to alkaline conditions, identifying a pH range of 10–10.5 as optimal compared to pH values of 7, 8, and 9. According to Gaysina [11], it is also recognised that algal strains capable of tolerating extreme pH conditions have considerable potential for biotechnological applications.

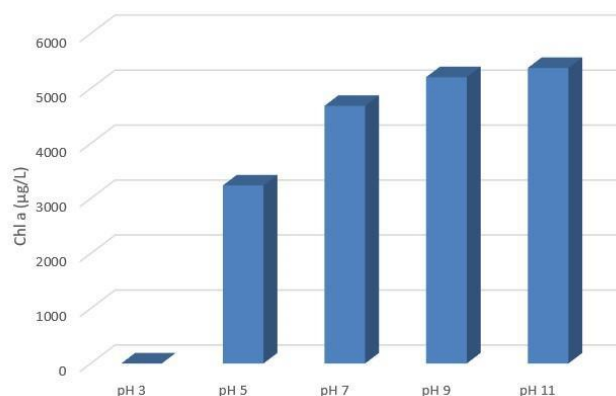


Figure 2 Chlorophyll a content of *Chlorella* culture isolated from lampenflora after one month of cultivation

CONCLUSION

Although *Chlorella* is one of the most extensively studied genera of microalgae, a considerable proportion of its species remain uncultured or insufficiently characterised. These unexplored or understudied strains may possess unique traits, such as tolerance to extreme environments or novel metabolic capabilities, with significant ecological and biotechnological potential. In this context, exploring extreme habitats, such as caves, is particularly valuable, as it can reveal previously unknown diversity and expand our understanding of the genus.

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