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UV–C irradiation as a tool to eradicate algae in caves

Fabien Borderie [a], Alaoui-Sehmer Laurence [a], Raouf Naoufal [a], Bousta Faisl [b], Orial Geneviève [b], Rieffel Dominique [a], Alaoui-Sosse Badr [a,*]

[a] Laboratoire Chrono-Environnement, UMR UFC/CNRS 6249 USC INRA Université de Franche-Comté, 2 Place Leclerc, F-25030 Besançon Cedex, France
[b] LRMH, 29 rue de Paris F-77420, Champs sur Marne, France

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ABSTRACT

Algal proliferation has commonly been reported to occur on monuments, such as crypts, churches, and caves, as soon as artificial lighting is used. In this work we study the effects of UV–C irradiation on algae collected in different caves in Dordogne (southwest of France). First, the effect of UV–C irradiation was tested on algal cell suspensions during increasing exposure times. After treatment, the photosynthetic capacity was assayed using a polarometric method, and algal cell viability was then estimated using a Trypan blue test after a rest period of 15 h. UV–C irradiation was then studied on algal cells cultivated on a solid support consisting of pieces of calcareous stone. Drops of concentrated algal cells were inoculated on stone and exposed to UV–C radiation for 3, 6, or 9 h. After this irradiation, half of the samples were submitted to a high white light intensity (1400 μmol m^{-2} s^{-1} of photosynthetically active radiation, PAR) for 6 h while the other half were incubated in the culture room. Subsequently, algal macroscopic parameters such as covering rate and colonized area were measured by macro photography. Both experiments led to the conclusion that UV–C irradiation has deleterious effects on photosynthetic parameters and growth of algal cells.

1. Introduction

Caves, crypts, or shelters under rock constitute particular ecosystems defined by their biotic and abiotic factors. They are characterized by the fragile dynamic balance linked to environmental factors such as temperature, moisture, carbonic gas content, charged salt water circulation, and animal and plant populations (Brunet et al., 2000). Such places, and particularly prehistoric caves in the south-west of France (Lascaux, Dome, Jovelle, Laugerie-Haute, Montignac, etc.) with mural paintings, are often considered part of humankind's cultural heritage, and as such receive thousands of visitors per year. Painting illumination associated with the great number of visitors led to serious biological disturbances. Development of algae on walls of the Lascaux cave was reported for the first time in 1960 (Lefèvre, 1974), and was called "the green sickness" or "Lampenflora."

Many chemical treatments have been used to remove algae or to control their spread. Major disadvantages of these compounds are their persistance over the years and contamination of the whole ecosystem through lixiviation, a widespread phenomenon in humid places. Therefore, among new methods explored to limit those negative environmental impacts, the use of UV irradiation appears to be a suitable method for killing or at least limiting algae proliferation. Ultraviolet radiations are part of the light solar spectrum with intermediate wavelengths between visible light and X-rays. The UV–C part of these radiations, (250–280 nm), is entirely filtered by the ozone layer of the earth's atmosphere. The UV–B, (280–315 nm), is partially filtered, while the UV–A, (315–400 nm), entirely reaches the terrestrial surface. Over the past decades, the effect of ultraviolet rays on photosynthetic organisms due to the depletion of the ozone layer has been extensively studied. Hermann et al. (1996) and Prasad and Zeeshan (2004) have analyzed the effect of UV radiation on two species of marine phytoplankton (*Dunalliela salina* and *Ochromonas danica*) and on one species of Cyanobacteria (*Plectonema boryanum*). They have shown that ultraviolet radiation (mainly UV–B) causes serious damage to the photosynthetic apparatus, especially a degradation of the protein D1, a main component of photosystem II. The DNA is also affected (DNA laddering), as reported by Holzinger and Lutz (2005).

This study aims to investigate the effect of UV–C irradiation on survival, proliferation, and photosynthetic rates of cave-harvested algae as a function of time of exposure.

2. Materials and methods

2.1. Biological material and sampling procedure

The organisms studied are a mixture of unicellular green algae strains harvested in three caves (Domme, Laugerie-Haute, and

* Corresponding author. Tel.: +33 0 381 665 730; fax: +33 0 381 665 797.
E-mail address: badr.alaoui-sosse@univ-fcomte.fr (A.-S. Badr).

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Jovelle) of the Dordogne département (southwest of France). This mixture is principally composed of *Klebsormidium flaccidum* (Klebsormidiales, Charophyceae), a filamentous green algae that colonizes terrestrial and freshwater habitats, and other unicellular green algae belonging to the classes Chlorophycea and Diniphycea. The precise identification of those species has not yet been achieved. The algal samples were collected with a sterile scalpel and preserved until use.

2.2. Algal culture and UV–C exposure

The growth of the algal cells was performed in a complete culture medium adapted to algal development (cyanobacteria BG11 freshwater solution, C3061, SIGMA) to avoid any contamination by other organisms. The Erlenmeyer flasks (Fig. 1) were continuously horizontally agitated at 70 rpm for proper oxygenation of the medium and exposed to a photoperiod of 13 h at 130 μmol photons m^{-2} s^{-1} and 11 h in the dark with a thermoperiod of 24 °C and 18 °C, respectively.

In order to mimic the natural conditions in caves, the algae were inoculated on limestone blocks (Fig. 1) of 6 cm in length, 4 cm in width, and 6 mm in thickness provided by the LRMH (Laboratoire de Recherche des Monuments Historiques, Champs-sur-Marne, France). A standardized protocol of algae inoculation was developed to homogenize the number of algal cells deposited on the blocks. The maximum amount of culture medium containing the algae was removed by centrifugation at 500 g for 5 min. The algal cell concentration of the pellet was determined using a spectrophotometric method (Barberousse et al., 2006). The OD measured at 750 nm wavelength can be related to a concentration of algal cells per μl, using a linear regression equation previously established with the same algal strain. Usually, the pellet concentration was around of 5×10^6 cells μl^{-1}. Aliquots of 150 μl, 350 μl, or 1000 μl were deposited within a 1-cm-diameter circle drawn on the block. The deposited volume corresponds, respectively, to 0.75×10^9, 1×10^9, and 5×10^9 algal cells. The thickness of the algal stain increases with the volume of algal suspension deposited on the block and ranges between at least 0.5 mm for the thin layer (150 μl) and 2.5 mm for the thick layer (1000 μl). The blocks were then transferred to the culture room until UV–C exposure.

For all UV–C treatments, samples were placed in a 0.2-m^3 closed chamber (Fig. 1) at 30 cm from the UV–C lamp (GTE Sylvania G30T8, 80 W).

For algal cells cultivated in liquid medium, 3 ml of algal suspension were added to petri dishes (4 cm in diameter) without a lid and were exposed to UV–C at 20, 30, 60, and 180 min. Triplicates were used for measurements.

Analyses were carried out directly after UV exposure and after a rest period of 15 h in a culture room.

For algal cells cultivated on solid support, the different light treatments are summarized in Table 1. Exposure to UV–C was performed intermittently: Ninety minutes of UV–C followed by a rest period of 15 min in the dark. The increase of algal volume was correlated with an increase of exposure time. For treatment IV (see Table 1), 6 h of high light (HL, 1380 μmol m^{-2} s^{-1}) provided by CL 1500 ECO SCHOTT (150 W) were added to test a photo-inhibition effect (Gevaert et al., 2002).

2.3. Biological and physiological parameters

Algal cell viability after UV–C exposure was assayed by the Trypan blue method after a rest period of 15 h. An aliquot (100 μl) of the algal suspension previously exposed to UV–C was mixed with 250 μl of Trypan blue solution (2,7-naphtalenedisulfonic acid, 3,3′-((3,3′-dimethyl (1,1′-biphenyl) 4,4′-diyl-) bis (azo), bis (5-amino 4-hydroxy-) tetrasodium)) and 650 μl of BG11 and introduced into a Malassez cell. Living algal cells appeared uncoloured while dead cells showed a typical blue coloration due to their inability to exclude the Trypan blue. Dead and live cell counting was then performed under a microscope with a manual counter. The survival rate was calculated in comparison to the control.

The effect of UV–C irradiation on the photosynthetic capacity was assessed by a polarometric method (Oxygraph System, Hansatech). After standardization of algal cell concentration

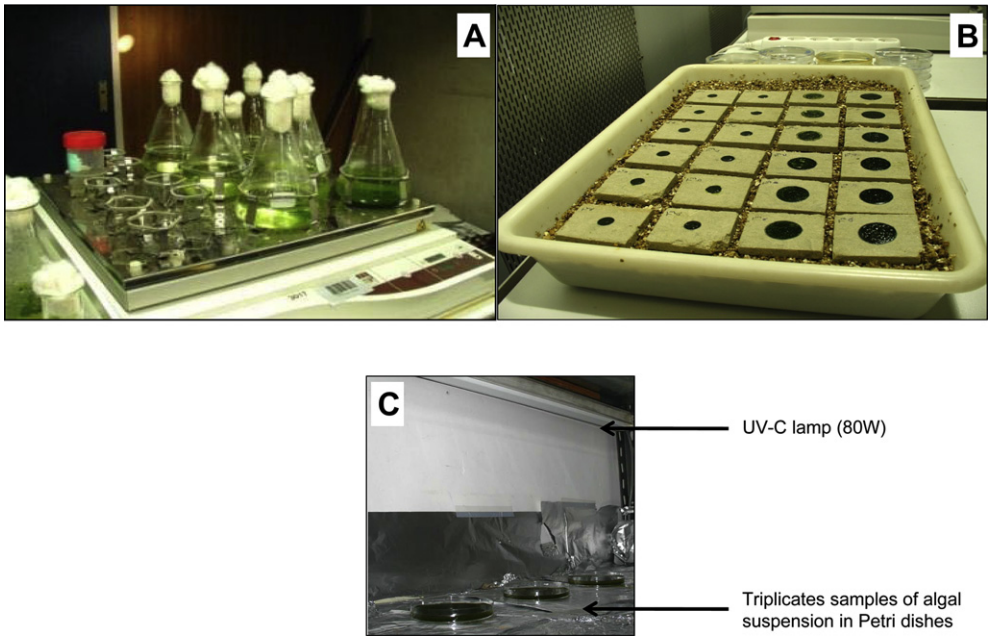

Fig. 1. Technical cultures of algae and exposure chamber. A: algal in freshwater BG11 in Erlenmeyer on agitator, B: algal inoculation on limestone blocks disposed on humid vermiculite for keep constant humidity rate, C: exposure chamber and irradiated samples of algae.