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Studying photosynthesis under simulated M-dwarf star light.

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Abstract. Several recently discovered rocky exoplanets (Earths and super-Earths) are orbiting the Habitable Zone of M-dwarf stars suggesting them as possible cradles for alien life. Anyway, such stars are less luminous and colder than the Sun with an emission maximum at redder wavelengths. These characteristics do not seem suitable for most oxygenic photosynthetic organisms we know from Earth, that evolved to absorb only visible light. Many researchers discussed the possibility of oxygenic photosynthesis in these worlds so far, but no experimental research has been done testing organisms under simulated M-dwarf spectra. To achieve these aims, a collaboration between the Department of Biology, the Astronomical Observatory (INAF) and the Institute of Photonics and Nanotechnology (IFN-CNR) led to the construction and the development of a new experimental tool. Such a setup allows us to monitor photosynthetic microorganism's growth and gas exchange performances under selected conditions of light quality and intensity, temperature, and atmospheres simulating non-terrestrial environments. All parameters are detected by remote sensing techniques. We initially focused on cyanobacteria as target microorganisms, due to their extraordinary capacities to withstand every kind of environment on the Earth as well as their ability to acclimate to Far-Red light.

Key words. Stars: M-dwarfs – Super–Earths – Photosynthesis

1. Introduction

Among all the planetary hosts, low mass stars, mainly M spectral type stars, are the main targets of the extrasolar planet surveys due to both their high density in the Galaxy and their small radii that provide higher amplitude transit signals than solar-like stars (Dressing & Charbonneau 2015). Even more striking, the frequency of super-Earths found in the habitable zone (HZ) of M dwarfs (with a period between 10 and 100 days) is about 50% (Bonfils et al. 2013; Kopparapu et al. 2013). A plenty of habitable planets.

In these years, scientists argued a lot about the possibility to have photosynthesis at work on these kind of planets, mainly due to the M star spectral radiation and its difference by that of the Sun (see Figure 1). Despite of this, no experiments have been conducted to validate or denied this hypothesis. At the best of the knowledge of the authors, we performed for the first time such an experiment with the help of novel instrumentation.

The experiment is based on the exposure of photosynthetic microorganisms at simulated exoplanetary environments, combined with the spectroscopic detection of their biosignatures, namely pigment reflection and photosynthetic oxygen evolution. Here, the (unavoidable) working hypothesis is that the evolution of extra-terrestrial life converged to pigment production and photosynthetic mechanisms similar to that of terrestrial extremophiles under non-Earth conditions.

2. The method and the materials

Our research plan foresaw four steps: *i)* set-up of the laboratory instrumentation needed for the simulations and choice of the oxygenic photosynthetic microorganisms (OPs) for the experiments; *ii)* validation of the setup performing fiducial experiments by irradiating the selected OPs with simulated solar light within a terrestrial atmosphere *iii)* experiments exposing the OPs to a simulated M star irradiation in a terrestrial atmosphere; *iv)* experiments exposing OPs to simulated M star irradiation in modified atmospheric compositions. The first 3 goals are already reached and partially published (Claudi et al. 2016; Battistuzzi et al. 2020; Claudi et al. 2021), while the last experiments are undergoing and are considering data obtained by the 1-D model of the atmosphere of super-Earths described by Petralia et al. (2020).

The innovative part of the experimental setup is composed by a Star-Light Simulator (SLS), an Atmosphere Simulator Chamber (ASC) and a Reflectivity Detection System (RDS) (Battistuzzi et al. 2020; Claudi et al. 2021). The SLS is a PC controlled LED-based device (see Figure 2 a, b, and c) that produces a low-resolution star spectrum of a selected spectral type in the wavelength range between 350 and 900 nm. The ASC (see Figure 2 d) is a thermally controlled environmental growth chamber, with a volume of 0.5 l, that can be filled with different atmospheric compositions. The chamber is designed to growth OPs in an open glass Petri dish exposed to the selected atmosphere and to simultaneously irradiate them by the SLS. Sensors also allow to monitor the content of CO_2 and O_2 without interfering with the experiments. The growth of the OPs can be monitored by remote sensing through the RDS. The RDS comprises a custom-made baffling system, embedding the optical fiber probe connected to the spectrometer (see Figure 2 a). It allows the measure of the reflectivity of OPs growing inside the ASC, and subsequently to obtain the Normalized Difference Vegetation Index (NDVI), a parameter that can be correlated to the growth of the culture (Battistuzzi et al. 2020). Some of the setup validation data are presented in the results section of this paper.

For the experiments, the OPs selected were cyanobacteria, expected to be able to perform oxygenic photosynthesis under simulated M star light radiations, considering that the minimum light level for photosynthesis is about 0.01 μmole m^{-2} s^{-1}, i.e., less than 10^{-5} of the direct solar flux at Earth in the PAR wavelength range (2000 μmole m^{-2} s^{-1}) (McKay 2000; Cockell & Raven 2004, and references therein). We tested different strains so far and here we present the results about *Chlorogloeopsis fritschii* PCC6912 able to grow utilizing Far Red (FR) light, which is abundant in M spectral type stars (Gan & Bryant 2015; Wolf & Blankenship 2019), and *Synechocystis* sp. PCC6803 a cyanobacterium unable to utilize it as a control organism.

3. Results

First of all, we checked the ASC and its capability to maintain stable conditions over time, monitoring delta pressure, temperature, CO_2, and O_2. Then we moved to perform several experiments with the cyanobacterium *S. sp.* PCC6803. We grew liquid cultures in the ASC, set at 30° C temperature, 1 atm pressure of an atmosphere composed of 75%N_2, 20%O_2, 5%CO_2. Cultures were illuminated with a solar light spectrum generated by the SLS at two different light intensities: 30, and 95 μmole m^{-2} s^{-1}. We demonstrated *a*) the ability of the

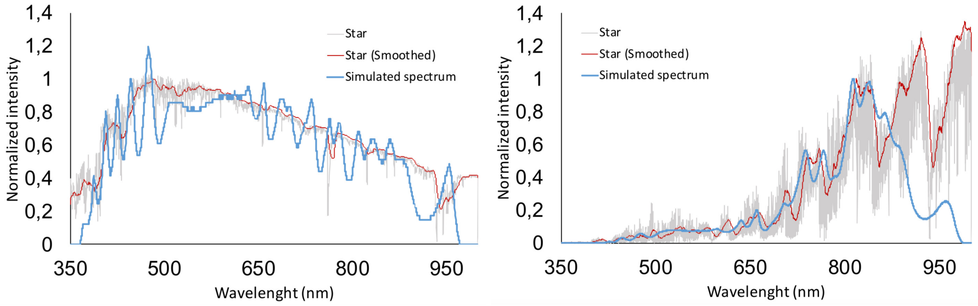

Fig. 1. left: The spectrum of the Sun (G2V) as it is (gray), smoothed (red) and simulated by the SLS (cyan) **right**: the same of the left panel, but for an M7V star.

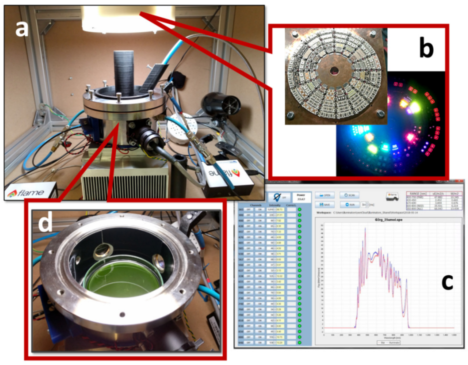

Fig. 2. The experimental setup: **a**: The inside of the of the SLS box. The ASC with the gas sensors and the reflectiviy detection system mounted are visible. **b**: The 25 dimmable channels with their 312 LEDs mounted **c**: The user interface of the SLS control software Salasnich et al. (2018) **d**: The ASC with the Petri dish with target organisms inside.

setup to continuously record CO_2 and O_2 levels due to the photosynthetic activity of the cultures during each experiment (see Figure 3 top and middle panels) and *b*) the ability to monitor the growth of the cultures by utilizing the RDS to obtain a NDVI values that were linearly correlated with physiological parameters of the cultures obtained at the beginning and at the end of the experiments (see Battistuzzi et al. (2020)).

We exposed cyanobacteria solid cultures to three different light spectra, maintaining a terrestrial atmosphere: Sun light (G2), M star light (M7), and a monochromatic far red light (FR). In detail, we arranged in plates several spots of 20 μl of cultures of *C. fritschii* PCC6912 and *S. sp.* PCC6803, at different cell concentrations. The plates were then placed under the different light spectra for 72 hr. All the experiments were performed in three biological replicates. Results (see Figure 3, bottom panel) demonstrated the ability of both cyanobacteria to survive and grow similarly under M7 and G2 light conditions, as could be seen through visual inspection of the plates and fluorescence measurements (Fv/Fm) of photosynthetic efficiency which were indicative of the fitness of the strains (Claudi et al. 2021). Under FR light only *C. fritschii* PCC6912 could grow also in that condition maintaining similar values of F_v/F_m, while *S. sp.* PCC6803 did not grow and had lower values of F_v/F_m, demonstrating its incapability to utilize such a light.

4. Conclusions

We realised an experimental setup using original laboratory devices (e.g. the SLS) and novel measurement methods that allow us to reproduce in the laboratory an alien environment and monitor constantly the OPs we want to test. For the first time, we present the experimental data obtained directly through exposing photosynthetic organisms to a simulated M dwarf spectrum. We compared the results to responses of those species under solar and far-red simulated lights. As expected, in far-red light, only the cyanobacterium able to utilize it could grow. Surprisingly, all strains, both able or un-

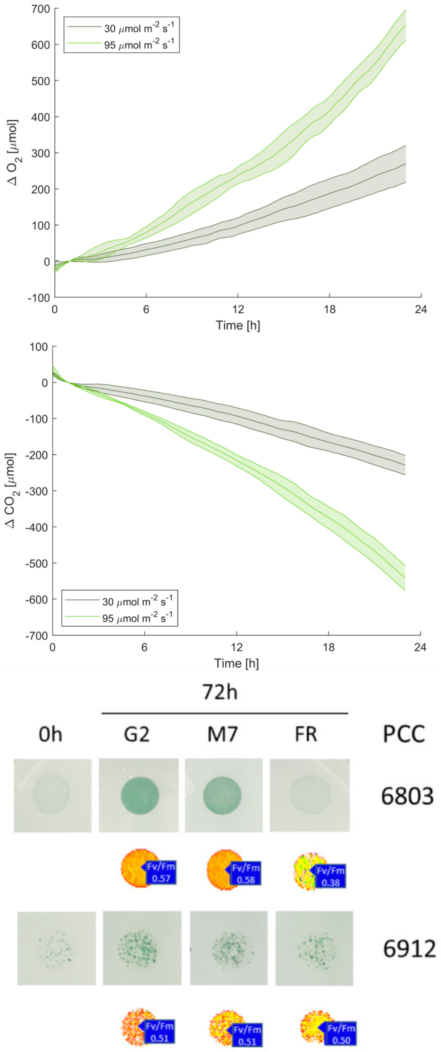

Fig. 3. Top and Middle panels: *Synechocystis* sp. PCC6803 O_2 production and CO_2 consumption and under two different solar light intensities. **Bottom panel**: comparison between the different phenotypes and photosynthetic efficiency of *Synechocystis* sp. PCC6803 and *C. fritschii* PCC6912 after 72 hr of irradiation with the three different light sources.

able to use far-red light, grew and photosynthesized under the M dwarf generated spectrum in a similar way to the solar light. We are carrying out now more extensive experiments on OPs to verify their capacity of thriving and acclimating to extraterrestrial conditions. To prepare for the next step of our research plan (point *iv* in section 2), we have already produced several models of stable super-Earth atmospheres to be used in the laboratory.

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