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Raman spectroscopy detection of biomolecules in biocrusts from differing environmental conditions

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ABSTRACT

Lichens and cyanobacteria colonize inhospitable places covering a wide climate range due to their different survival strategies, such as the synthesis of protective biomolecules. The effect of ecological factors on the synthesis of biomolecules has not been widely analysed. This study aimed to assess the effects of four factors (species, microclimate, seasonality and hydration state) and their interactions on the biomolecule frequency detected by Raman Spectroscopy. We included cyanobacterial biocrusts, and the lichens *Diploschistes diacapsis*, *Squamarina lentigera*, and *Lepraria isidiata*; two contrasted microclimates (typical and marginal), two contrasted seasons (hot and dry *vs* cool and wet) and two hydration states (dry and wet).
"Species" was the most influential factor in the identity and frequency of the main biomolecules. Microclimatic differences in the range of the local specific habitats only influenced the biomolecules in cyanobacteria. There was a quadruple interaction among the factors, the effects being different mainly depending on the species. At *D. diacapsis*, the production of their main biomolecules depended on microclimate, although it also depended on seasonality. Nevertheless, in L. *isidiata* and *S. lentigera* microclimatic differences did not significantly affect the production of biomolecules. In the lichen species, the microhabitats exposed to relatively larger incident radiation did not show significantly larger relative frequency of photoprotective biomolecules. No clear connection between higher production of oxalates and drier microhabitats was found, suggesting that the synthesis of oxalates is not related to water reserve strategy. The pros and cons of monitor biomolecules in biocrust by Raman spectrometry were also discussed.

1. Introduction

Biocrusts, which are often formed mainly by lichens and/or cyanobacteria, can make up the dominant coverage in drylands around the world. In these regions they play a critical role in ecosystem functioning and are one of the main sources of soil organic carbon [1,2], enriching the soil with carbohydrates [3] and enzymes that enable organic remains to be degraded [4,5]. They also modulate CO_2 fluxes in drylands [6–10].

Most of the organisms forming biocrusts are known as extremophiles because they are primary colonizers able to occupy inhospitable locations at "limit of life" [11]. In order to do so, these organisms develop survival strategies in which they produce biomolecules that protect them from hostile environmental conditions, such as desiccation, damaging short-wavelength radiation or excessive PAR (photosynthetically active radiation) and drastic temperature changes [12,13]. Some of these biomolecules have different roles, such as screen excess solar radiation, DNA repair agents, antidesiccants, and water-replacement molecules [14–16].

Cyanobacteria and lichens are an important cover in drylands, and their survival depends on the critical role of their protective biomolecules against environmental factors as harmful radiation in these extreme ecosystems. Some authors have studied the influence of environmental factors regulating the synthesis of biomolecules in lichens and cyanobacteria [17–24]. Previous studies have repeatedly indicated that solar radiation and stressful environmental conditions influence the production of photo-protective biomolecules in a wide variety of lichens and cyanobacteria [13,22,25–29]. The relative proportions and variation in concentration of such biomolecules have been associated with environmental and climate changes related to harmful solar radiation exposure [30] and specifically to excess of UV-B radiation and PAR [13,17]. Some authors have noted that the attenuation of UV radiation can reduce their need for production of UV protective biomolecules [27]. Nevertheless, temperature and moisture could play a more important role in biomolecules variation in lichens than light intensity

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[18,31–33]. Seasonal variations affect also the synthesis of such biomolecules [34,35].

Oxalates in lichens and cyanobacteria has been linked to water storage function [11,36–40] or waste disposal [26,38,40]. The presence of oxalates have been also related to light exposure (solar radiation intensity); Edwards et al. [41] and De Oliveira et al. [42] suggest that the synthesis of oxalates by lichens could also have an eco-morphological function protecting them against high light intensities which can damage photobiont cells.

The strong influence of climate and microclimate on biocrusts is well known and many directly or indirectly related studies have been carried out from a variety of viewpoints (for example, [43–50]). In the study area, the selected biocrusts showed clear differences in terms of incident PAR, the sunniest one being the primary colonizer cyanobacterial crust and the least sunny being the late-successional community of *Lepraria isidiata* [51].

Traditional analysis of biomolecules in lichens and cyanobacteria requires chemical and/or physical manipulation of the samples which destroys the sample for further analyses. An additional problem with chemical analyses could arise because terricolous lichens are usually intimately attached to the soil and their manual extraction could lead to contamination of the lichen with substratal detritus as described by Chu et al. [52] when studying the lichen, *Parmotrema praesorediosum*, by using standard techniques; the results were considered unreliable, because of the small quantities of material available and contamination from the substratum. Raman spectroscopy has recently awakened great interest for the study of biomolecules in complex samples (*e.g.*, micro and macro-organisms). It is a valuable analytical technique for identifying organic and inorganic compounds and their mixtures [53,54] and has been specifically used to assess biomolecules in lichens and cyanobacteria [12,27,55]. We have chosen Raman spectroscopy since there is no need for physical or chemical sample treatment and samples analysed by this technique can be completely recovered for eventual follow-up analyses.

In this work, biomolecules (pigments and oxalates) of a cyanobacterial community and three different lichen species were analysed by Raman spectroscopy. We studied the influence of two areas of the microclimatic gradient of each species on the biomolecule production, in two contrasted seasons: cooler and wetter (winter) *vs* hotter and dryer (summer), and under two hydration states: dry and wet. This involved several specific objectives: i) assess the ability of Raman spectroscopy to identify biomolecules in cyanobacteria and different lichens in their dry and wet states; ii) estimate the frequency of occurrence of the biomolecules under different conditions considering different species or biocrust types, and iii) find out what ecological factors, considering the species or biocrust type, season, microclimate and hydration state, have significant effect on the presence of each biomolecule, and what factor or interaction among them has the main effect.

2. Material and methods

2.1. Site description

The Tabernas Desert is a badlands area located in southeastern Spain (Province of Almería, fig. 1) in the Sorbas–Tabernas basin, mainly filled by soft Miocene rock, mostly marls and calcareous sandstone, and surrounded by mountain ranges in the Betic Chain, the Gador, Nevada, Filabres and Alhamilla Mountains. Climate is semiarid warm Mediterranean with a particularly strong water deficit during the summer months. The first three ranges intercept most rainfall fronts, which come mainly from the west, explaining the low mean annual precipitation of around 230 mm in the study area, which shows also a high inter-annual and intra-annual rainfall variability [56]. The average annual temperature is 17.9 °C, with an absolute maximum of 45 °C, an absolute minimum of −4.5 °C [57]. The geomorphology at the experimental site consists of a series of parallel SE-NW catchments, where NE-facing hillslopes have gradients of <30° [58] with more or less developed soils (Endoleptic Regosols or Lithic–xeric Torriorthent) covered by grasses, annuals and dwarf shrubs, mainly near the hillslope bottom, and an important cover of biological soil crust, including many species of terricolous lichens (*Diploschistes diacapsis* (Ach.) Lumbsch., *Squamarina lentigera* (Weber) Poelt and *Lepraria isidiata* (Llimona) Llimona & A. Crespo being among the most frequent) and patches dominated by cyanobacterial communities, mainly near the top of the hillslope. Separating some catchments are old residual hanging pediments, which are more or less flat and exposed to the sun, where soils are thicker (Haplic Calcisol or Xeric Haplocalcid), and there are scattered perennial plants with biological soil crusts in open areas, often dominated by cyanobacteria, and patches of annual plants (dominated by *Stipa capensis* Thunb). SW-facing slopes are steeper (gradients up to 70°), with poorly developed soils, Epileptic Regosols or Lithic Torriorthent, which are bare or, in the few stabilized spaces, scarcely

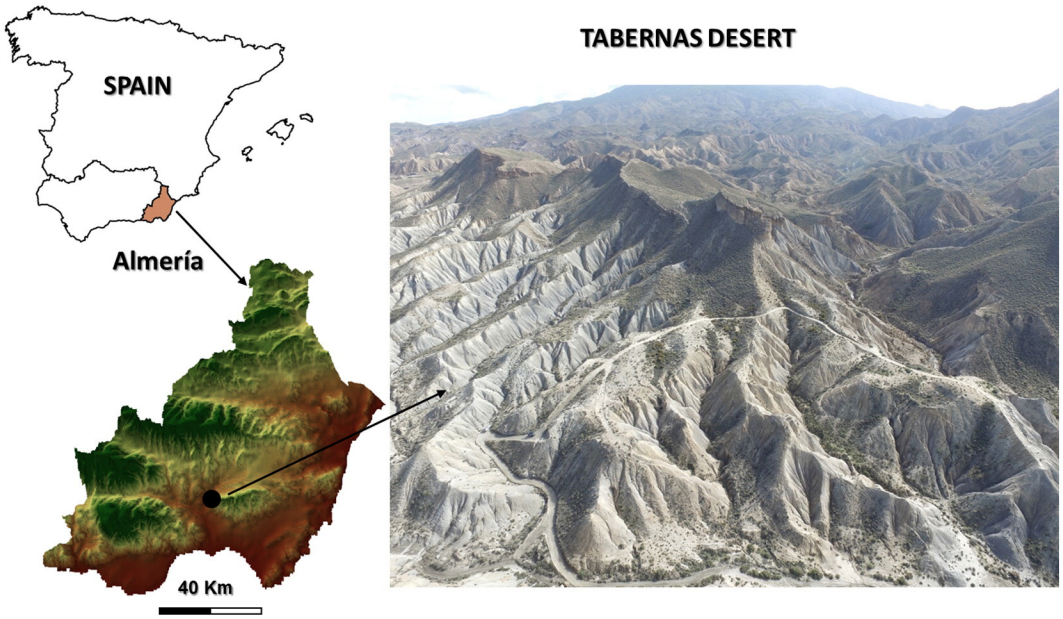

Fig. 1. Study area location.