



## Optimizing N-Fixing cyanobacteria culture to restore arid degraded soils

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Cyanobacteria present several metabolic activities and mechanisms of adaptation which enable them to colonize different habitats, in almost all biome and continents, especially under extreme environmental conditions, as on the surface of the most arid soils and under the highest temperatures. In drylands, they are usually found among plants, cohabiting with organisms such as algae, lichens, mosses, bacteria and fungi, and in association with soil surface particles, forming communities known as biocrusts. Because they can survive under water stress and are considered ecosystem engineers, facilitating the establishment of other organisms, they can play a key role in the development of a successful restoration approach to recover the functionality of soils in arid and semiarid regions. In addition cyanobacteria can be cultured “ex-situ” obtaining high quantities of biomass to be used as soil inoculum at large scale. For these reasons, the inoculation of degraded soils with cyanobacteria can be considered an alternative to traditional restoration. This approach is expected to promote: the stabilization of the soil surface and the decrease of water and wind erosion; the increase of soil fertility by fixing N and C; and the succession of more developed organisms as mosses or vascular and annual plants.

The objectives were: to evaluate the potential of a soil native cyanobacteria strain to be artificially cultured and the optimization of the process, and to analyze the effects of the inoculation of the biomass on soil under laboratory conditions. Cyanobacteria were isolated from biocrusts sampled on a limestone quarry located at the southeastern edge of the Sierra de Gádor massif (Spain). It was genetically and morphological identified as belonging to the nitrogen-fixing genera *Nostoc*. Essays were accomplished in bubble columns reactors (0.25 L), using different culture media: BG11+N, BG11<sub>0</sub>, and two media made with fertilizers. Illumination simulated a circadian cycle with a maximum irradiance of  $1035 \mu\text{E m}^{-2}\text{s}^{-1}$ . Absorbance, chlorophyll fluorescence and dry weight were measured daily. The produced biomass was inoculated ( $6 \text{ g m}^{-2}$ ) on Petri dishes with 80g of sterilized soil coming from the limestone quarry. Soils were watered below field capacity twice a week during three months, under constant illumination of  $70 \mu\text{E m}^{-2}\text{s}^{-1}$  and  $25^\circ\text{C}$ .

The growth rate and biomass productivity obtained for each culture media verified that this strain can be successfully cultured under laboratory conditions. The best results were obtained with BG11+N, nevertheless results from media made with fertilizers were very similar, key to develop a low-cost culture strategy. The culture process has been optimized simulating a continuous mode, in order to produce biomass on a large scale, obtaining an optimal productivity of  $0.41 \text{ g L}^{-1} \text{ d}^{-1}$ , with a dilution rate of 20% and a concentration of  $2.06 \text{ g L}^{-1}$ .

Soils inoculated with cyanobacteria biomass obtained from laboratory culture showed an increase in biocrust cover and soil organic carbon content with time. Thus our results demonstrate that inoculation with native cyanobacteria cultured “ex-situ” represent a very promising and “low-cost” tool for the restoration of arid degraded soils.