PM376 Monitoring biological soil crust reactivation by flow cytometry to set up virome analysis

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Background: Biological soil crusts (BSCs) are one of the dominant community types on Earth, composed of micro and macro poikilohydric organisms. Particularly in drylands, BSCs play a vital role in biogeochemical cycles and geomorphological processes, mainly due to the microbial activity, which are reactivated only upon moisture uptake. In these communities, the diversity and role of viruses remain poorly understood.

Objectives: The aim of this study was to reactivate the BSCs in order to isolate the associated virus-like particles (VLPs).

Methods: An innovative protocol was developed for the BSCs reactivation monitoring. It was based on flow cytometry (FCM) and a biological marker: the amount of live cells released from BSCs after a controlled hydration and day-light exposure. Living microbial cells were monitored by FCM after a SYTO™ 24 and propidium iodide (PI) dual staining and a carboxyfluorescein diacetate succinimidyl ester (CFDAse) single staining. The reactivated BSCs were subjected to the isolation of VLPs through a tangential flow filtration and cesium chloride ultracentrifugation steps, followed by DNA extraction suitable for shotgun metagenomic virome analysis.

Results: An innovative FCM-based protocol was for the first time applied for the BSCs reactivation monitoring. It was useful for the isolation of VLPs from BSCs samples. The results obtained allowed the isolation of 200 ng of viral DNA from 300 g of BSCs. The controlled BSCs reactivation protocol developed in this study will be useful in all the studies focused on the characterization of microbial activities of BSCs.

ABSTRACT BOOK

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