

Short Communication

Unravelling Hidden Metabolic Capabilities in Microbial Mat Ecosystems by Combining *In Situ* Measurements and Omic Approaches

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Abstract

Microbial mats are unique ecosystems that harbor diversified metabolic capabilities raging from photosynthesis in the upper layer to fermentation and organic material degradation accompanied with methane production in the deepest layer. Microorganisms in a microbial mat are successful to flourish in extreme environments because they can shift their metabolism in response to the changes in their environment. These metabolic flexibility and capabilities of the microorganisms are underestimated because most of the studies so far have focused on measuring changing in the behavior of the inhabiting microorganisms, or on certain biochemical indicators, or on the shift of microbial community structure. However, to be able to broaden our understanding of the metabolic capabilities, an intensive study of the underlying molecular mechanisms should be carried out. Therefore, the best approach to be adopted is by combining *in situ* measurements with omics analysis (i.e., matatranscriptomic and/or metaproteomic) in response to changes in the environmental conditions.

Microbial mats are complete ecosystems on a millimeter scale that harbor diversified metabolic processes [1-5]. In the upper most layer, photosynthesis is the dominant process because of light, while fermentation and methane production are the most abundant metabolism in the deepest layer [6,7] because of absence of both O, and light. On the metabolic level, this is indeed comparable to other ecosystems that expand to meters scale such as the marine water column and rainforest [8]. Thriving in extreme habitat (i.e., extreme temperature, high salinity, extreme alkalinity, etc.) is another interesting characteristic that distinguishes microbial mat ecosystem from other complete ecosystems. Apparently, the microorganisms in microbial mat are successful in combating these extreme conditions because they are equipped with unique metabolic capabilities and flexibility. For example, cyanobacteria, which is a ubiquitous biotic component of microbial mats, can switch its metabolism from performing photosynthesis under light availability to aerobic respiration under the absence of light. In addition, cyanobacteria has the ability to perform fermentation under the absence of both oxygen and light [9,10]. The metabolic flexibility or persistence of cyanobacteria to suit changes to their dynamic environment explains why they confer fitness advantage. Comparative genomics of different strains of cyanobacteria indicate that they are capable of retaining or shredding genes which improves microbial fitness.

Most of the studies on microbial mats either investigated the behavioral response of their biotic component [11-13], or measured physicochemical indicators such as O_2 , H_2S , pH, and production of Extracellular Polymeric Substances (EPS) [14,15]. Others investigated the shift in microbial community structure using basic molecular biology techniques or high-throughput sequencing [16,17]. Stronger hypotheses and conclusions are derived from studies that use complimentary approaches to study microbial mats [18,19]. However, these studies have missed an important jigsaw to the puzzle as previous research so far is underestimating the metabolic capabilities of each microorganism living in the microbial mats. This knowledge gap can be filled by combining measurements in response to the dynamic changes, together with omics approach (metagenomics, metatranscriptomic, metaproteomic, and metabolomics).

So far, there have been limited studies that adopted this multipronged approach. Subsequently, the results of these studies were

very interesting as they have revealed new metabolic possibilities for some of the microorganisms in the microbial mats. Recently, Stuart et al. [20] used a suit of techniques that includes, but not limited to, cell culture, ¹³C-labeling combined with NanoSIMS analysis, EPS separation, detailed biochemical and sugar analysis, and proteomic analysis to conclusively show that cyanobacteria is utilizing the EPS. This interesting finding expands our knowledge about the metabolic flexibility of cyanobacteria and it extends the list of the carbohydrate that they can consume. Such result wouldn't be clear and wellsupported if there was no support from proteomic analysis showing the expression of EPS degrading enzymes that are cyanobacteria-specific enzymes.

Rajeev and his colleagues [21] investigated the biological desert crusts (BDC) and used combined microsensor measurements of photosynthesis and time-series transcriptional analysis after rewetting the dried crust. Their ultimate goal was to understand how cyanobacteria can reactivate its photosynthetic activity. They have shown that cyanobacteria can retain its photosynthetic activity in one hour and this was correlated with raising the anabolic activity of cyanobacteria as shown from their transcriptome. This study provided insight into retaining photosynthesis after rehydrating the BDC, but overlooked the fast resynthesis of chlorophyll (Chl). This important question about rapid Chl synthesis in response to rehydration has been raised recently from the work of Chennu et al. [13]. In their study, Chennu and his colleagues were able to detect Chl, using combination of HPLC and hyperspectral imaging, in a matter of minutes after rehydrating dried marine microbial mat. In their study, they pointed out that there is a

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need to conduct proteomic analysis to show if cyanobacteria retain the Chl in an inactive form when the mat is dehydrated or if they are able to retain precursor(s) of Chl synthesis.

All of these interesting studies and research questions that are still open can be best approached by conducting complimentary approaches that show measurements correlated with transcriptomic or proteomic analysis. This combined approach will allow the discovery of novel metabolic capabilities and may help to discover new enzymes that might have very important biotechnological applications.

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