Science Bulletin 62 (2017) 764–766

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Science Bulletin
www.scibull.com

Short Communication

Seasonal variations and characteristics differences in the fluorescent components of extracellular polymeric substances from mixed biofilms in saline lake

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ARTICLE INFO

Article history:
Received 8 February 2017
Received in revised form 14 March 2017
Accepted 17 March 2017
Available online 21 April 2017

Cyanobacteria are the Earth's oldest oxygenic photoautotrophs and have had major impacts on shaping the biosphere. Their long evolutionary history (~3.5 bya) has enabled them to adapt to geochemical and climatic changes and, more recently, anthropogenic modifications of aquatic environments, including nutrient over-enrichment (eutrophication), water diversion, withdrawal, and salinisation [1]. Owing to increased mineralisation and alkalinity, the biota of saline lakes predominantly consists of prokaryotic microorganisms whose metabolism is distinguished by a number of specific features determined by the specifics of the conditions of existence [2]. Salinisation has increased worldwide due to summer droughts, rising sea levels and increased use of freshwater for agricultural irrigation [1]. The salt-tolerant cyanobacterial species are present in brackish systems, apparently spurred on by a combination of nutrient over-enrichment, climatic changes and salinisation. Microbial communities often form aggregates, biofilm, or mat communities in response to environmentally stressful conditions, such as hypersalinity [3].

The biofilm matrix can be considered as an important property of microorganisms, allowing them to form stable synergistic consortia, supporting interaction by signalling molecules and horizontal gene transfer and, eventually, being activated by extracellular enzymes, which turn the matrix into an external digestion system [4]. Extracellular polymeric substances (EPS) can vary greatly between biofilms, depending on the microorganisms present, the shear forces experienced, the temperature and the availability of nutrients [5]. How similar types of EPS influence biofilm development in different bacterial species is largely unknown. Furthermore, the amount and the temporal sequence of EPS formation and its constituents in response to various physical and biological conditions are typically unknown for environmental biofilms [5].

Specifically, different molecular-level compositions of EPS with seasonal changes are important for a better understanding of microbial biofilms and linked microorganisms. Such information could be examined using fluorescence (excitation-emission matrix, EEM) spectra in combination with parallel factor (PRAFAC) modelling, which is a three-way multivariate analysis that is widely used currently to separate and characterise the individual fluorescent components from total organic matter in water [6,7]. It was reported that cyanobacterial blooms were tracked by examining the possible correlations between the different EEM maxima and the amount of excreted toxins of various fluorescence signals, including protein-like and humic-like substances [5,8].

Floating biofilm mat samples from the saline pond west of Lake Bosten (42°01′N and 86°47′E) was collected over two summer seasons (early May) and in between early winter season (November). Hydrology and other surrounding environmental details of the Lake Bosten were previously reported [9]. A large steel mug of long handle was used to collect the floating biofilm mat from the lake water. The biofilm mats were large in chunks which were put in drum of 30 L with lake water. After transportation to laboratory they were put in a plastic zip bag of 500 mL and stored at −20 °C. To extract EPS, biofilm samples were washed ten times with Milli-Q pure water and dispersed into suspension by a stirrer followed by sonication at 40 W in an ice bath for two minutes. The

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http://dx.doi.org/10.1016/j.scib.2017.04.016
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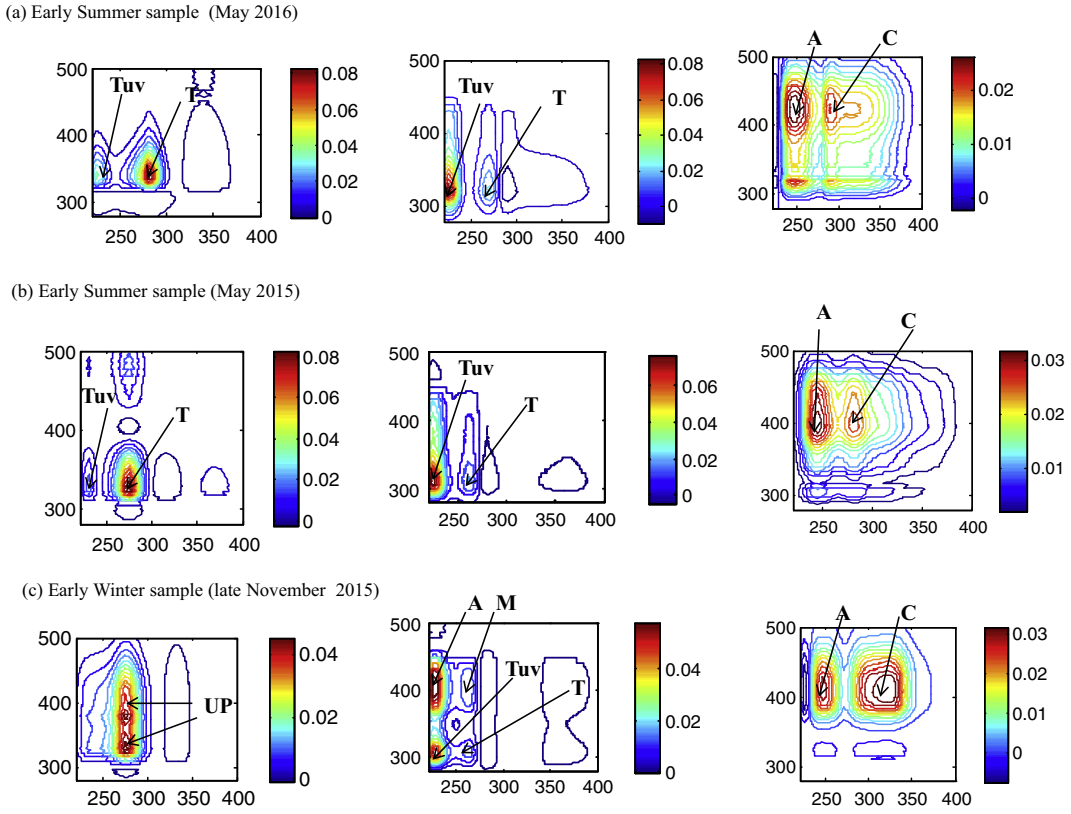

Fig. 1. Fluorescent components of EEM-PARAFAC identified by three-component analysis for (a) and (b) all raw EPS collected from summer biofilm (c) all raw EPS collected from early winter biofilm sample (from late November) from saline Lake Bosten, Xinjiang, China.

sonicated suspension was then centrifuged for 20 min at 16,000 r/min at 4 °C. The supernatants were then filtered through a 0.22 μm polycarbonate filters, which was immediately stored at −20 °C until further use as EPS solution. EPS stock solution was used for chemical analysis. Several dilutions of raw EPS samples were prepared to identify EEM spectra with repetition.

EEM-PARAFAC analysis of EPS sample collected from the saline Lake Bosten over two seasons revealed various components. The early summer biofilm EPS sample (collected in early to mid May) was rich in protein-like (peak T at *Ex*/*Em* = 275–290/328–407 nm and peak Tuv at *Ex*/*Em* = 225–230/328–407 nm), tyrosine-like or tryptophan-like (peak T at *Ex*/*Em* = 270/310–321 nm and peak Tuv at *Ex*/*Em* = 225/310–321 nm, respectively) and humic-like (peak M at 280–290/397–426 nm and peak A at *Ex*/*Em* = 245–250/397–426 nm) substances (Fig. 1a, b). On the other hand, the early winter (collected in late November) biofilm EPS sample showed combined fluorescent components composed of protein-like (peak T at *Ex*/*Em* = 275/328 nm and peak Tuv at *Ex*/*Em* = 230/328 nm) and humic-like (peak M at *Ex*/*Em* = 275/380 nm and peak A at *Ex*/*Em* = 230/380 nm) substances along with individual humic-like substances (peak C at *Ex*/*Em* = 320/406 nm and peak A at *Ex*/*Em* = 245/406 nm) and an unknown fluorescent component with four fluorescent peaks (265/307 nm, 225/307 nm, 265/399 nm and 225/399 nm) denoted as unknown peak, UP (Fig. 1c). These fluorescent components except unknown fluorescent peaks were reported previously [7,8,10].

It is therefore evident that at the early stage of summer, when biofilm development starts, protein-like and tyrosine- or tryptophan-like components were more prevalent, along with humic-like substances. With the maturation of biofilm in the winter with seasonal change, a strong backbone of EPS is generated with molecular compositions forming combined fluorescent components, including protein-like and humic-like substances, individual humic-like substances with strong fluorescence intensity at peak C region and unknown fluorescent components. Such effects could result from high contents of EPS extracted from microbial biofilm during summer season. It is obvious that with the changes in seasonal variation from summer to the progression of winter, the mat's vertical layered structure would further fluctuate along with the molecular composition of the EPS. Sampling from the saline pond waste of Lake Bosten in summer showed that water pH varied from 9.08 to 10.85 in different locations with total dissolved solids (TDS) values of 72.7 g/L and dissolved organic carbon (DOC) value ranging from of 84.5 ± 0.6 mg/L to 100.8 ± 0.1 mg/L. With the onset of winter, the temperature and illumination intensity dropped, and the physico-chemical parameters of the saline pond of Lake Bosten changed with pH values ranging from 8.08 to 8.5, and TDS values of 57.1 g/L, thereby increasing the DOC concentration (167.7 ± 0.6 mg/L). Decrease in TDS along with increase in DOC concentration during season changes from summer to winter was linked with the release of DOC from TDS, including microbial biofilm under both photoinduced and microbial respiration [7].

Such changes in TDS and DOC could not be directly related to the variation of EPS from cyanobacteria. These results indicate that typical differences in fluorescent components in EPS from algal-cyanobacterial biofilm mat are caused by the seasonal changes upon timescale. This could be useful for getting appropriate sampling to attain maximum performances of future EPS linked researches. The simultaneous influence of increased mineralisation, alkalinity, pH, and temperature and illumination intensity with seasonal variation might have influences on the molecular composition of the biofilm mats and subsequently changes in the EPS production and its molecular composition which require further investigation. Furthermore, the differences of EPS composition

in saline and freshwater lakes could be interesting and warranted for further studies.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was financially supported by National Natural Science Foundation of China (U1503281 and U1403181). This study was also partly supported by the Key Construction Program of the National "985" Project, Tianjin University, China. Partial funding for this research *Search Project and Mashura Shammi was supported by* the CAS–TWAS President's Fellowship program 2013.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scib.2017.04.016.

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