Characterization of Extracellular Polymeric Substances in Hypersaline Cyanobacterial Mats and Mat-forming Cyanobacterial Isolates

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Introduction

Photosynthetic microbial mats are highly structured laminated microbial communities that can host all the major biogeochemical cycles in a few millimeters of depth and their activity likely dominated biogeochemical cycling throughout Earth's history. More recently they have generated interest because of their ability to produce hydrogen gas. While most microbial life exists as biofilms (of which extracellular polymeric substances (EPS) are a major component), our understanding of nutrient exchange and energy flow through the EPS in these mats is not well understood.

In this work we designed methods to extract EPS from both natural mats from Elkhorn Slough in Monterey Bay, CA (Fig. 1) and a diazotrophic cyanobacterium isolated from these mats (Fig. 2) and profiled proteins present in the isolate EPS.

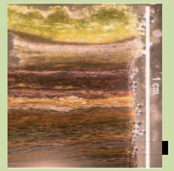


Fig. 1 Cross-section from Elkhorn Slough mat

ESFC-1, a filamentous oscilliatorian cyanobacterium

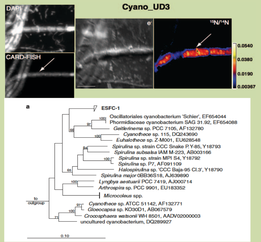
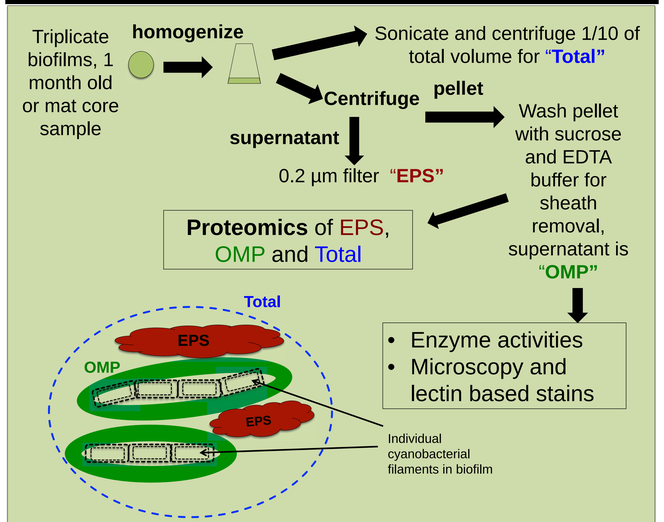
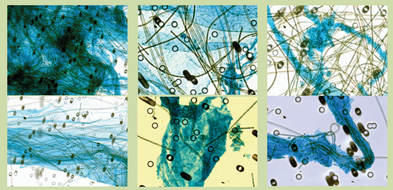


Fig. 2 Woebken *et al.* 2012, ISME J

Methods

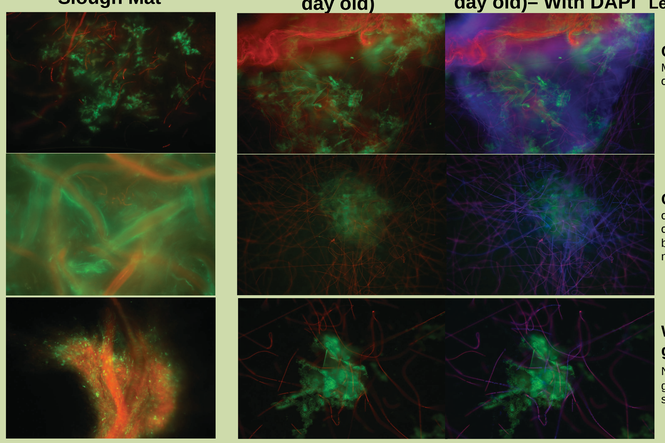


Results: Microscopy and Enzyme activity



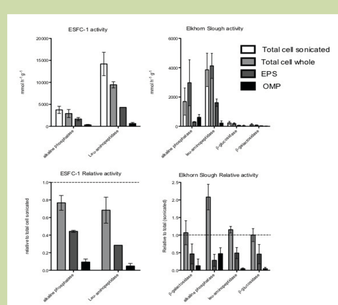
Alcian blue staining after each step of extraction:

- We remove some of the EPS, but not all
- Low percentage of lysed cells (also verified by G6PDH enzyme activity not detectable in EPS and OMP fractions)



Lectin-based probe staining in natural mat community and cultures. Green represents lectin binding, red represents autofluorescence and blue is DAPI DNA stain. Natural mat stains were done on homogenized fixed samples and were gravity filtered. Culture stains were done directly in 6-well plates with 6 day old cultures.

- ESFC-1 lacks an outer sheath that many other filamentous cyanobacteria have, seen in the natural mat ConA stain.
- DAPI stain indicates that most of the early EPS secretion is polysaccharidic, although some possible eDNA can be seen in the top panel

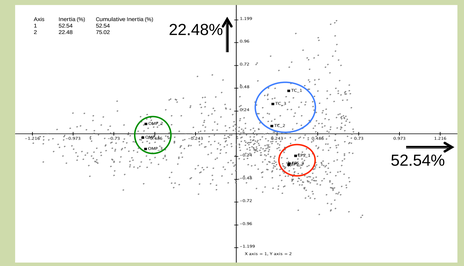


Enzyme activities:

- Activity levels were higher in the cultures than in the natural mat samples
- No glucosidase or galactosidase activity was detected in ESFC-1 cultures but was reliably detected in the natural mats
- Ratios between the different fractions showed alkaline phosphatase may be more abundant in the matrix than in natural mats

Results: Isolate Proteomics

1. Fractions cluster separately:

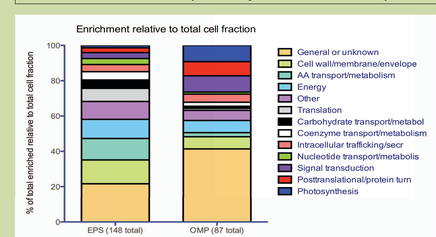


Correspondence analysis of all detected proteins

- Quantitation based on normalized spectral counts (Scaffold™ v3)
- 848 proteins total identified in at least one fraction of biological triplicates (>95% identity, 2+ unique peptides)

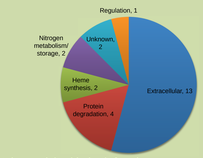
2. Functional categories:

- EPS fraction has a diverse set of enriched proteins and a higher total number than the OMP fraction.
- OMP enriched in photosynthetic related proteins



Functional categories of enriched proteins. Enrichment calculated using a t-test (p>0.05) relative to total fraction. Categories based on KEGG classification.

3. Protein enriched in both OMP and EPS fractions



- 24 proteins enriched in both fractions
- 17 are predicted to be extracellular based on GramLocNeg and CELL0; 13 with unknown function:

Description	EPS mean	OMP mean	TC mean	Functional prediction
hypothetical protein	38.71	55.25	13.51	ABC transport gene cluster
Family description./Domain of unknown function (DUF4114).	7.90	40.80	2.76	Adhesion
Putative peptidoglycan-binding domain-containing protein	15.97	12.98	2.67	Cell wall degradation
hypothetical protein	4.39	5.02	1.00	Cell wall biogenesis cluster
Domain of unknown function (DUF4384)./Caspase domain.	12.75	14.77	1.00	Cell wall biogenesis cluster
hypothetical protein	9.38	61.59	3.28	Cellulose synthase similarity
Domain of unknown function (DUF4347)./Domain of unknown function (DUF4114)	11.97	12.75	4.09	Cluster with efflux transport
Secreted and surface protein containing fasciclin-like repeats	29.33	75.33	4.38	Nostoc homolog in EPS with galactosidase activity (Morsy, 2008)
TPR repeat./Tetratricopeptide repeat.	8.84	22.47	1.00	Protein aggregation in some homologs
Protein of unknown function (DUF3747)./S-layer homology domain.	22.41	20.32	1.00	S-layer binding
hypothetical protein	24.08	30.07	15.21	GramLocNeg only
Uncharacterized conserved protein	3.35	8.46	1.00	GramLocNeg only
Tic22-like family.	7.39	25.78	1.52	GramLocNeg only

4.

Proteases and peptidases were highly enriched in the EPS and OMP fractions indicating extracelullar protein degradation

Description	Enrichment (log2) EPS	OMP
Predicted Zn-dependent peptidases	**2.27**	**3.42**
Subtilisin-like serine proteases	**2.33**	**3.30**
Predicted Zn-dependent peptidases	**3.34**	**2.99**
Membrane proteins related to metalloendopeptidases	0.42	**1.97**
Bacterial pre-peptidase C-terminal domain.	0.66	**1.48**
Predicted Zn-dependent proteases and their inactivated homologs	**1.53**	0.13
Predicted Zn-dependent proteases and their inactivated homologs	**0.48**	-0.31
Predicted Zn-dependent proteases and their inactivated homologs	**1.52**	-0.86
Dipeptidyl aminopeptidases/acylaminoacyl-peptidases	**1.39**	-1.24
Transglutaminase-like enzymes, putative cysteine proteases	**0.88**	-1.59

Conclusions

- Fractionation of cultures and natural mats results in distinct fractions with different proteins in each fraction
- Lectin based stains provide visualization of EPS to compare natural mats to cultures
- Extracellular enzyme activity is more diverse in the natural mats
- Proteomics reveal many secreted proteins of unknown function in the EPS and OMP fractions with diverse putative functions such as protein degradation and adhesion

Acknowledgements

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