Peer Community In Ecology

Disentangling microbial exopolymer dynamics in intertidal sediments

Ute Risse-Buhl and **Nils Rädecker** based on peer reviews by 2 anonymous reviewers

Cédric Hubas, Julie Gaubert-Boussarie, An-Sofie D'Hondt, Bruno Jesus, Dominique Lamy, Vona Meleder, Antoine Prins, Philippe Rosa, Willem Stock, Koen Sabbe (2023) Identification of microbial exopolymer producers in sandy and muddy intertidal sediments by compound-specific isotope analysis. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.1101/2022.12.02.516908

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The secretion of extracellular polymeric substances (EPS) enables microorganisms to shape and interact with their environment [1]. EPS support cell adhesion and motility, offer protection from unfavorable conditions, and facilitate nutrient acquisition and transfer between microorganisms [2]. EPS production and consumption thus control the formation and structural organization of biofilms [3]. However, in marine environments, our understanding of the sources and composition of EPS is limited. In this study, **Hubas *et al.* [4]** compare the carbon and nitrogen isotope ratios in EPS with the carbon isotope ratios of fatty acid biomarkers to identify the main EPS producers in intertidal sediments. The authors find pronounced differences in the diversity, composition, isotope signatures, and production/consumption dynamics of EPS between muddy and sandy environments. While the contribution of diatoms was highest in the bound fraction of EPS in muddy environments, diatom contribution was highest in the colloidal fraction of EPS in sandy environments. These differences between sites likely reflect the functional differences in EPS dynamics of epipelic and episammic sediment communities. Taken together, the innovative approach of the authors provides insights into the diversity and origin of EPS in microphytobenthic communities and highlights the importance of different microbial groups in EPS production. These findings are vital for understanding EPS dynamics in microbial interactions and their role in the functioning of coastal ecosystems.

References:

- Flemming, H.-C. (2016) EPS-then and now. Microorganisms 4, 41 https://doi.org/10.3390/microorganisms4040041

- Wolfaardt, G.M. et al. (1999) Function of EPS. In Microbial Extracellular Polymeric Substances, pp. 171–200, Springer Berlin Heidelberg https://doi.org/10.1007/978-3-642-60147-7

- Flemming, H.-C. et al. (2007) The EPS matrix: the "house of biofilm cells." J. Bacteriol. 189, 7945–7947 https://doi.org/10.1128/jb.00858-07

- Hubas, C. et al. (2022) Identification of microbial exopolymer producers in sandy and muddy intertidal sediments by compound-specific isotope analysis. bioRxiv, ver. 2 peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.1101/2022.12.02.516908

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2022.12.02.516908
Version of the preprint: 1

Authors' reply, 14 July 2023

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Decision by Ute Risse-Buhl, posted 27 March 2023, validated 27 March 2023

Dear Dr. Hubas and co-authors,

both reviewers and I very much enjoyed reading your preprint. You illustrate a very interesting and novel approach. The reviewers highlighted that your preprint contains much needed information on EPS and is of high impact. You will see that both reviewers raised a number of suggestions on how to improve the manuscript. I highly recommend revising the pre-print and considering the comments of both reviewers.

Best regards,

Ute Risse-Buhl

Reviewed by anonymous reviewer 1, 27 February 2023

GENERAL COMMENT: I really enjoyed reading this paper. I think this research is very novel and of high impact. However, authors should revise carefully the results and put more emphasis on the fatty-acids results (table ST1). If they really want to assess the difference between Mud and Sand, then some statistical analyses comparing these two groups should be more evident. I hope that my specific comments (see below) help to resolve these more general issues.

Introduction:

I miss information about fatty acids. Specifically,

1) line 97, which are the fatty acid biomarkers?

2) lines 103-105, which ratios to which bacteria or algal groups?

3) lines 112-115: authors should specify which are the fatty acid biomarkers of diatoms and bacteria

Materials and methods:

Lines 137-147, please specify the references for the colloidal EPS extraction and for the bound EPS extraction.

Results and discussion:

Lines 264-267, what about C, N and P from other origins different than bacteria and algae but attached to the EPS (e.g. detritus)? Could they modify the isotopic fractionation?

Section Elemental EPS compositions:

For me it is not clear the comparison between mud and sand. This section puts a lot of emphasis between Ms1 and Ms2 but what about mud and sand? From Fig.2 b, we can see that the signature 13C is useful to differentiate between bound and colloidal EPS but not between mud and sand environments... variability among sites Ms1, Ms2, Ms3, Ms4, Ss1, Ss2 and Ss3 is higher than differences between the Ms and Ss as grouped factor.

Lines 304-306, have you analysed the biological composition of your sites? Your both sites (muddy and sand) are diatom-dominated, right? So then you should not expect differences due to bacterial-dominated sites...

Section EPS isotopic compositions:

Lines 330-333: could differences in 13C between bound and colloidal EPS be also due to deposition/adsorption of organic matter in the EPS?

Section Carbon isotope ratio of fatty acid classes:

Lines 340-350: if you are aiming to compare muddy sites from sandy sites, why don't you test if there are significant differences between muddy and sandy sites per each fatty acid group separately? (i.e. compare BFA in mud vs. BFA in sand). This would allow the reader to understand better if there are differences between sites.

Section Biomarkers revealed contrasting EPS producers between sites:

Explain the link between fatty acids and bacteria, and between fatty acids and diatoms. This section is very interesting but the core of the article is explained in the supplementary information... I would suggest to include a detailed explanation about fatty acids and EPS producers, otherwise a lot of information is missing.

Section Epipelic and epipsammic diatoms contributed differently to the EPS chemistry:

Have you characterized them? Somewhere in the paper you should include the characterization of these biofilms (at least density of bacteria and chl-a) to reinforce your idea that they are different.

Lines 405 – 416: what about the other fatty acids that show an alignment between their 13C and bound and colloidal EPS 13C in mud and sand (fig. 4)? E.g.:

15:0anteiso, 15:0iso and 24:0 in sand EPS bound

17:0iso in Mud EPS colloidal

17:0, 18:1n-9 in Mud EPS bound

Lines 446-448: in several times in the discussion the author points towards the difference in composition between epipsammic (i.e. diatoms, cyanobacteria, green algae and bacteria) and epipelic biofilms. In this line, it could be very useful if the authors can provide some results (even if they are semi-quantitative) regarding these different biological groups between mud and sand study sites.

ABSTRACT

Line 9: the authors have not analysed EPS degradation, so this concept should not be here.

Line 10: "very different communities in muddy and sandy sediments". The authors have not shown the communities in muddy and sandy sediments, so there are not results to know if the communities are really different. What the author could say is that "are supported by different fatty acid composition suggesting different communities".

Line 11: “EPS sources are more diverse in the sand”. I do not agree. What I see in the results (table ST1) is that EPS colloidal sources are more diverse in the sand and that EPS bound sources are more diverse in the mud.

Lines 12-15: I do not agree with the description of these results, they are not in agreement with table ST1. Revise please.

Reviewed by anonymous reviewer 2, 20 March 2023

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