Journal of Microbiological Methods 239 (2025) 107324

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

Note

Microscale assay for the quantification of total polysaccharides to estimate extracellular polymeric substances (EPS) in soil

G. Bogar [a,*], J.T. Lennon [b], H.M. Vander Stel [c], S.E. Evans [c]

[a] Department of Plant Biology, University of California, Davis, 1002 Green Hall, Davis, CA 95616, USA
[b] Department of Biology, Indiana University, 1001 E. 3rd St, Bloomington, IN 47405, USA
[c] Department of Ecology, Evolutionary Biology, and Behavior, Michigan State University, 567 Wilson Road, East Lansing, MI 48824, USA

ARTICLE INFO

Keywords:
Biofilm
Extracellular polymeric substances
EPS
Polysaccharides
Carbohydrates

ABSTRACT

We present an inexpensive and practical microscale spectrophotometric acid-phenol assay to quantify polysaccharides in aqueous solution. The instant reaction within disposable materials is designed and benchmarked for use in quantifying extracellular polymeric substances (EPS) in soils. Precipitation recovery was high across expected ranges of soil EPS content.

1. Introduction

Soil microbes and plant roots influence soil physical (Benard et al., 2019; Blankinship et al., 2016; Zheng et al., 2018) and chemical (Cotrufo et al., 2019) properties through the synthesis of mucilage and biofilms. These compounds, described as extracellular polymeric substances (EPS), bind to clay minerals (Lin et al., 2016) and their deposition or loss may be important for soil carbon sequestration (Cotrufo et al., 2019; Sher et al., 2020). Soil EPS can be estimated by quantifying polysaccharides extracted with a method that preserves and excludes intact cells (Redmile-Gordon et al., 2014). However, studies quantifying polysaccharides in soil often rely on a logistically challenging spectrophotometric assay (DuBois et al., 1956). This assay features 4–7 mL of superheated sulfuric acid and phenol, requiring glass vessels and significant hazard mitigation. Assay sensitivity seems dependent on high momentary temperature during the exothermic reaction of concentrated acid added to aqueous solution (Taylor, 1995); reducing reaction volume seemingly makes the method less sensitive (Rasouli et al., 2014). Other microscale versions feature long, near-boiling incubation steps to increase sensitivity (Sher et al., 2020), adding difficulty.

2. Methods, results, and discussion

We present a modified microscale assay, designed to quantify total and precipitated polysaccharides to estimate extracellular polymeric substances (EPS) in soil extracts (Supplementary Material 1 – Verbose Protocol). Our method is based on previous work (Rasouli et al., 2014) that performed reactions using water in glass tubes with a 95 °C incubation step. In our method, reactions are performed using 1× PBS to pair with soil EPS extraction (Redmile-Gordon et al., 2014), in sealed 2.0 mL polypropylene tubes, without incubation. We present additional steps and benchmarks to aid accuracy and interpretation of soil EPS quantification. Data processing, analysis, and graphing was conducted using R (R Core Team, 2024), including packages 'tidyverse' (Wickham et al., 2019), 'magick' (Ooms, 2024), and 'ggpmisc' (Aphalo, 2025).

Our method balances safety and sensitivity, occurring at a temperature low enough to be conducted in sealed disposable tubes, without a heated incubation step, while being more sensitive than the previous microscale assay by a factor of 2.8 (as estimated by comparing slopes of standard curves), with a lower detection limit (Fig. 1). As in previous versions of this assay, reaction-to-reaction variability is high; we recommend analytical duplicates or triplicates to improve precision (Supplementary Fig. 1). We also recommend that a new standard curve be calculated for each lot of reaction tubes; we found variable baseline μg-scale reactable material between fresh bags of PCR-grade consumables. The order and rate of reagent combinations affects assay outcomes, and care must be taken when adding acid and mixing reaction tubes (see Supplementary Material 1). We advise extreme caution if using materials and volumes other than those specified; the specific combination of a small reaction volume and polypropylene reaction tube prevents the plastic tube from failing.

To benchmark assay effectiveness, we extracted EPS (Redmile-

* Corresponding author.
E-mail addresses: gbogar@ucdavis.edu (G. Bogar), lennonj@iu.edu (J.T. Lennon), evanssa6@msu.edu (S.E. Evans).

https://doi.org/10.1016/j.mimet.2025.107324
Received 25 June 2025; Received in revised form 19 October 2025; Accepted 9 November 2025
Available online 10 November 2025

Gordon et al., 2014) from 68 field-moist agricultural soil samples, quantified glucose-equivalent polysaccharide per oven-dry mass of soil, and compared these with total soil carbon (Fig. 2). Soil was sampled from the top 20 cm of fields then growing corn (*Zea mays*), across three states of the U.S. Midwest from September–October 2021. Soils were transported and stored at 4 °C until sieving, extraction, and quantification. Average distance between sites was 290 km (maximum 790 km), and soil physical and chemical conditions were diverse (3.2–35.1 % clay, 3.5–81.3 % sand, 0.7–3.8 % carbon, and pH 4.08–6.99). Total extracellular polysaccharide concentrations ranged from 52 to 237 μg glucose-equivalent per gram oven-dry soil, with an average of 113 μg glucose-equivalent per g soil. This is approximately one-third the range and mean of water-extractable organic carbon (WEOC) from comparable soil samples (Boyer and Groffman, 1996). There was a significant correlation (r = 0.67, p = 2.5 e-10) between %C and total extractable extracellular polysaccharides (Fig. 2), comparable to the strength of the relationship between %C and other commonly measured water-extractable C fractions, such as potassium permanganate oxidizable carbon (Culman et al., 2012).

We include steps to improve accuracy and utility when measuring polysaccharides in soil extracts. First, we corrected for soil pigments, which may not degrade during the reaction. Second, we quantified the correlation of total extractable extracellular polysaccharides with ethanol-precipitated polysaccharides from which soluble sugars had been removed. When measuring polysaccharides as a proxy for large polymers (e.g., microbial biofilm, root mucilage), extractions are generally precipitated to exclude small sugars. We extracted and quantified total and precipitated polysaccharides both in a mineral medium (81 % sand, 19 % clay) spiked with xanthan, and from a representative subsample of soils in which corn (*Z. mays*) had grown, at the conclusion of a greenhouse drought stress experiment, to test the efficacy and requirement of polysaccharide precipitation. Solutions were precipitated by adding ethanol to extractions to a final concentration of 80 % at room temperature, followed by immediate centrifugation and resuspension in extraction solution (Alves et al., 2007; Brunchi et al., 2021).

Precipitation recovered almost all large polysaccharides from extracts, but precipitation may not be necessary to estimate EPS in soil, given the strength of correlation we found between precipitated and total polysaccharides. We measured an average precipitation recovery of 92.5 % from all xanthan concentrations above 10 μg glucose equivalent/

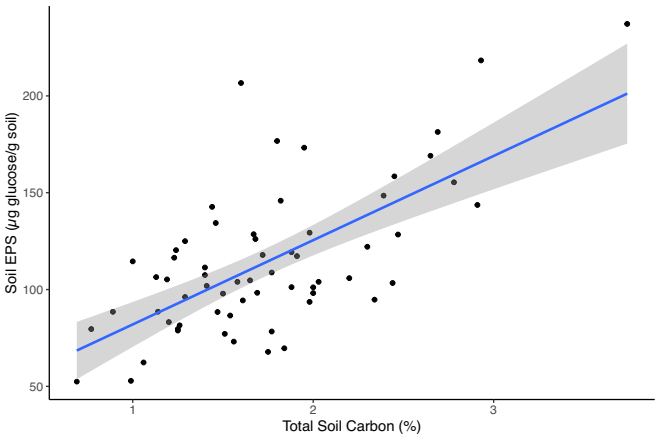

Fig. 2. Linear regression of percent total soil carbon vs. total extracellular polysaccharides, with 95 % confidence interval. $F_{66} = 55.5$, $P = 2.5 * 10^{-10}$, $r^2 = 0.45$.

g soil (Supplementary Table S1), and a strong linear correlation between extracted and precipitated polysaccharides in soils (r = 0.98, Fig. 3). In very low-carbon soils or sediments, analytical triplicates or quadruplicates are recommended to account for inherent assay variability. Further research is needed to determine the environmental contexts in which precipitation is required; precipitation is likely needed in circumstances where concentrations of soluble sugars are expected to vary between samples.

Our total polysaccharide assay, designed to be conducted directly on soil EPS extracts using sealed, disposable tubes and cuvettes, and benchmarked across diverse soil conditions, allows any researcher with a flow hood and cuvette spectrophotometer to study microbial biofilms and plant exudates in soils and sediments. It may also be useful to researchers measuring non-structural carbohydrates (NSC) in plant tissues, or μg/mL-scale polysaccharides in any other cells or tissues.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

The authors used ChatGPT to aid in writing code in R to extract and

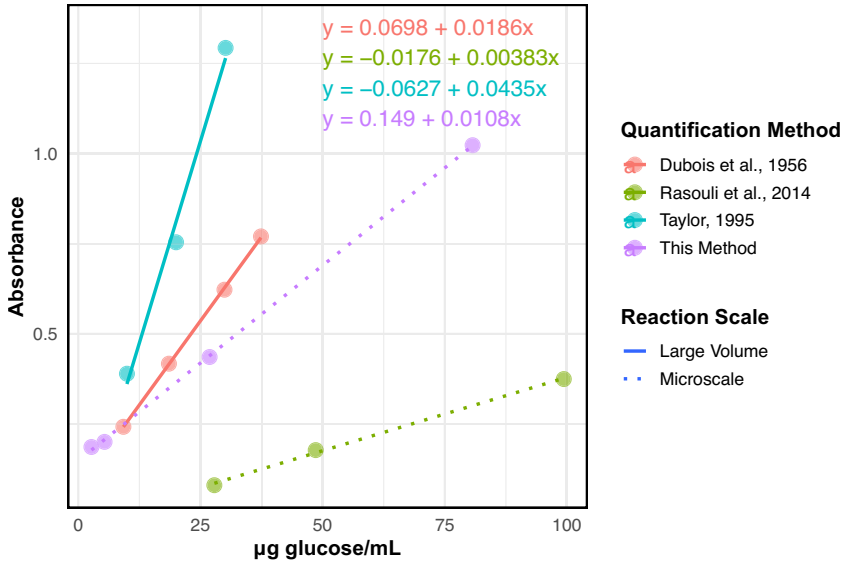

Fig. 1. Comparison of glucose standard curves from four phenol-acid assays: the original (DuBois et al., 1956, final reaction volume = 7.05 mL), an optimized version (Taylor, 1995, final reaction volume = 4.05 mL), an optimized microscale version (Rasouli et al., 2014, final reaction volume = 0.7 mL), and the version presented here (all datapoints shown in Supplementary Fig. 1, final reaction volume = 0.7 mL). Modified assay is more sensitive, with a lower detection limit, than previous microscale methods. Data extracted and reproduced from figures in manuscripts using R packages magick() and tcltk().

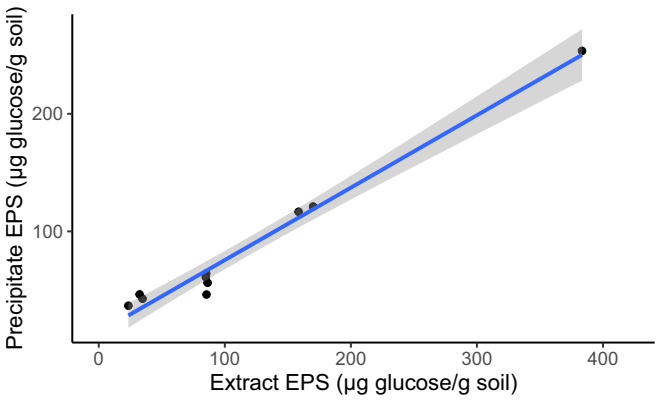

Fig. 3. Linear regression of total extracted extracellular polysaccharide vs. precipitated extracellular polysaccharide with 95 % confidence interval, extracted from soil in pots of corn (*Z. mays*) at the conclusion of a greenhouse drought stress experiment. $F_8 = 349$, $P = 7.0 * 10^{-8}$, $r^2 = 0.97$. Precipitate EPS = 14.0 + 0.616(Extract EPS).

visualize data in Fig. 1. The authors reviewed and edited this code for accuracy, and take full responsibility for the content of the published article.

CRediT authorship contribution statement

G. Bogar: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **J.T. Lennon:** Writing – review & editing. **H.M. Vander Stel:** Writing – review & editing. **S.E. Evans:** Writing – review & editing, Supervision, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data available at https://github.com/gdbogar/eps_method_manuscript

Acknowledgements

This work was funded by NSF Award number 2009125 and 8685. Support for this research was also provided by the NSF Long-term Ecological Research Program (DEB 2224712) at the Kellogg Biological Station and by Michigan State University AgBioResearch. This is Kellogg Biological Station contribution #2419. Thanks to Dr. Laura Bogar and the Dept. of Plant Biology at University of California, Davis, for laboratory equipment, resources, and helpful comments. Thanks to Dr. Jennifer Jones for preliminary assay validation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2025.107324.

References

Alves, L.A., Almeida, E., Silva, J.B., Giulietti, M., 2007. Solubility of D -glucose in water and ethanol/water mixtures. J. Chem. Eng. Data 52, 2166–2170. https://doi.org/10.1021/je700177n.

Aphalo, P., 2025. ggpmisc: Miscellaneous Extensions to "ggplot2.".

Benard, P., Zarebanadkouki, M., Brax, M., Kaltenbach, R., Jerjen, I., Marone, F., Couradeau, E., Felde, V.J.M.N.L., Kaestner, A., Carminati, A., 2019. Microhydrological niches in soils: how mucilage and EPS alter the biophysical properties of the rhizosphere and other biological hotspots. Vadose Zone J. 18, 180211. https://doi.org/10.2136/vzj2018.12.0211.

Blankinship, J.C., Fonte, S.J., Six, J., Schimel, J.P., 2016. Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem. Geoderma 272, 39–50. https://doi.org/10.1016/j.geoderma.2016.03.008.

Boyer, J.N., Groffman, P.M., 1996. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. Soil Biol. Biochem. 28, 783–790. https://doi.org/10.1016/0038-0717(96)00015-6.

Brunchi, C.-E., Morariu, S., Bercea, M., 2021. Impact of ethanol addition on the behaviour of xanthan gum in aqueous media. Food Hydrocoll. 120, 106928. https://doi.org/10.1016/j.foodhyd.2021.106928.

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nat. Geosci. 12, 989–994. https://doi.org/10.1038/s41561-019-0484-6.

Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirksy, S.B., Spargo, J.T., Wander, M.M., 2012. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. Soil Sci. Soc. Am. J. 76, 494. https://doi.org/10.2136/sssaj2011.0286.

DuBois, Michel, Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, Fred, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017.

Lin, D., Ma, W., Jin, Z., Wang, Y., Huang, Q., Cai, P., 2016. Interactions of EPS with soil minerals: a combination study by ITC and CLSM. Colloids Surf. B Biointerfaces 138, 10–16. https://doi.org/10.1016/j.colsurfb.2015.11.026.

Ooms, J., 2024. magick: Advanced Graphics and Image-Processing in R.

R Core Team, 2024. R: A Language and Environment for Statistical Computing.

Rasouli, M., Ostovar-Ravari, A., Shokri-Afra, H., 2014. Characterization and improvement of phenol-sulfuric acid microassay for glucose-based glycogen. Eur. Rev. Med. Pharmacol. Sci. 2020–2024.

Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T., Hirsch, P.R., 2014. Measuring the soil-microbial interface: extraction of extracellular polymeric substances (EPS) from soil biofilms. Soil Biol. Biochem. 72, 163–171. https://doi.org/10.1016/j.soilbio.2014.01.025.

Sher, Y., Baker, N.R., Herman, D., Fossum, C., Hale, L., Zhang, X., Nuccio, E., Saha, M., Zhou, J., Pett-Ridge, J., Firestone, M., 2020. Microbial extracellular polysaccharide production and aggregate stability controlled by switchgrass (Panicum virgatum) root biomass and soil water potential. Soil Biol. Biochem. 143, 107742. https://doi.org/10.1016/j.soilbio.2020.107742.

Taylor, K.A.C.C., 1995. A modification of the phenol/sulfuric acid assay for total carbohydrates giving more comparable absorbances. Appl. Biochem. Biotechnol. 53, 207–214. https://doi.org/10.1007/BF02783496.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the Tidyverse. JOSS 4, 1686. https://doi.org/10.21105/joss.01686.

Zheng, W., Zeng, S., Bais, H., LaManna, J.M., Hussey, D.S., Jacobson, D.L., Jin, Y., 2018. Plant growth-promoting Rhizobacteria (PGPR) reduce evaporation and increase soil water retention. Water Resour. Res. 54, 3673–3687. https://doi.org/10.1029/2018WR022656.