

# Supporting Information: Engineering Bacterial Polymers for Biomanufacturing: Characterization and Manipulation of *Sphingomonas sp. LM7* Extracellular Polysaccharide

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# 1 Supporting Figures

## 1.1 Uniaxial Tensile Tests

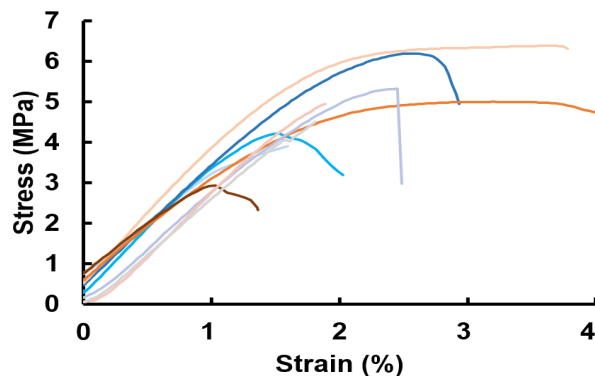


Figure S1: Tensile testing trials for films showing variation in the fracture strength of the material. Young's Modulus is consistent among tests.

## 1.2 The Effect of Water Content on Rheological Properties: Strain Sweep

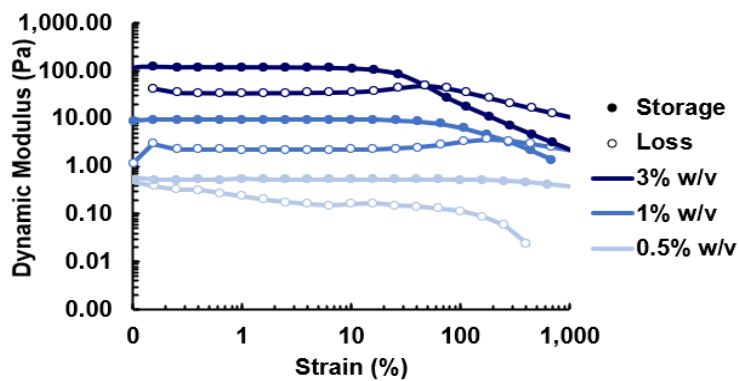


Figure S2: Strain sweep results comparing sample water content showing the gel-like behavior. Dynamic moduli increase for higher w/v ratios.

### 1.3 How Bivalent and Monovalent Ions alter Rheological Properties: Strain Sweep

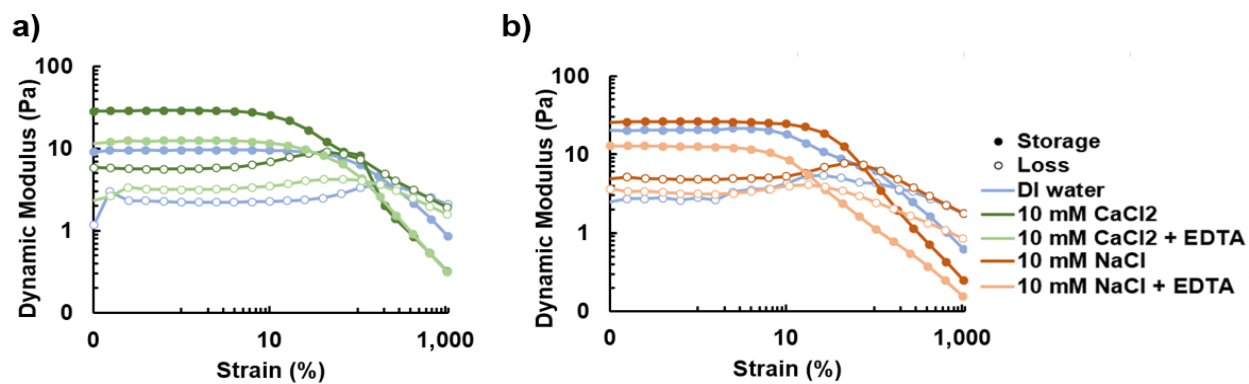


Figure S3: Strain sweep results comparing the addition of a) bivalent ions and b) monovalent ions.

## 1.4 Dialysis-Based Purification Process

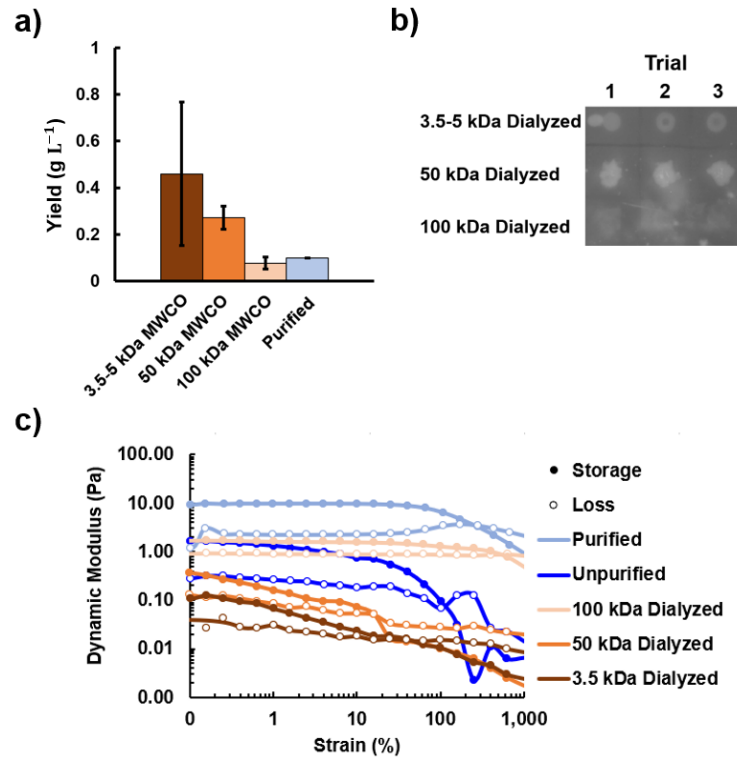


Figure S4: a) Magnified view of sample yields from varying dialysis MWCO values compared to the yield of the full enzymatic purification process. b) A dot blot showing lectin-glycan binding for dialysis groups confirming the presence of polysaccharide c) Strain sweep of samples at various levels of purification.

## 1.5 The Effect of Ultracentrifugation on Lectin Binding and Rheological Properties

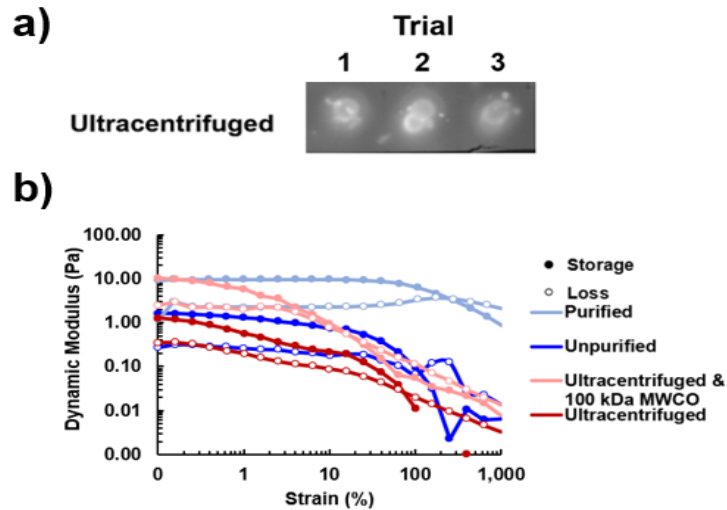


Figure S5: a) A dot blot showing lectin-glycan binding in the sample confirming the presence of polysaccharide post ultracentrifugation. b) Strain sweep of purified, unpurified, and ultracentrifuged samples.

## 1.6 Rheological Properties of Protein Knockout Material

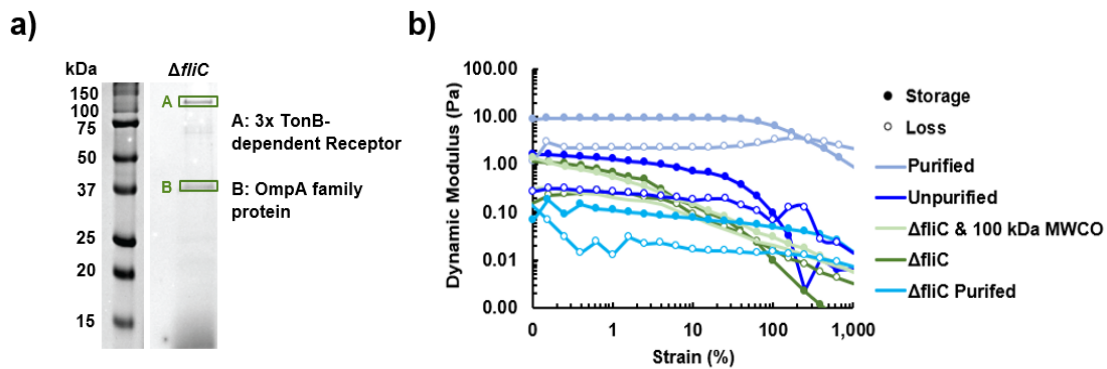


Figure S6: a) Secreted proteins from the  $\Delta prmJ \Delta fliC$  strain b) Strain sweep of purified, unpurified, and protein knockout material samples.

## 1.7 Extracellular DNA Analysis

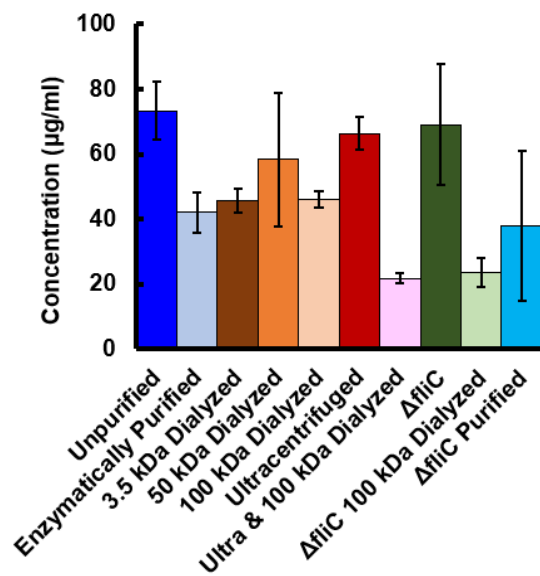


Figure S7: Extracellular DNA abundance in samples tested.

## 1.8 Thermogravimetric Analysis

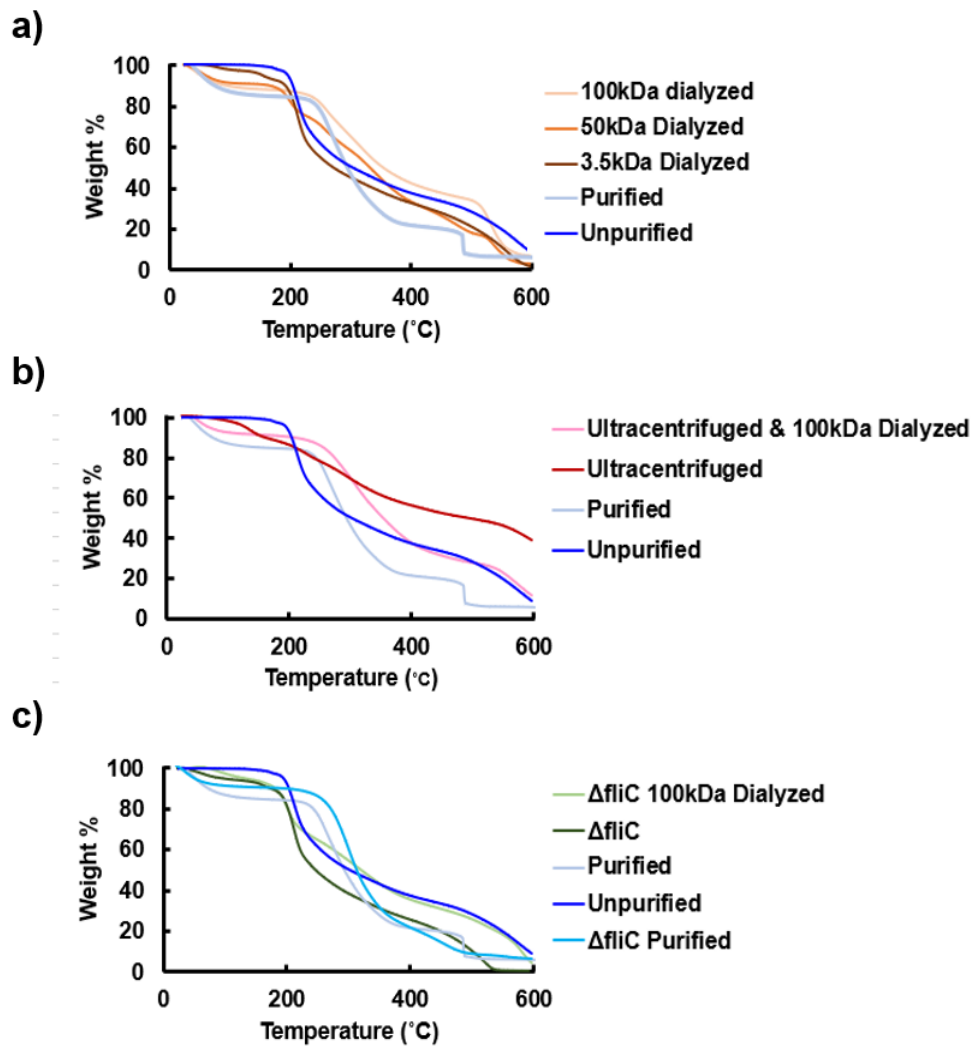


Figure S8: Thermogravimetric analysis for a) dialyzed material, b) ultracentrifuged material and c) protein knockout material.



## 2 Supporting Tables

### 2.1 Strains Used in this Study

Table S1: Strains used in this Study

Strain	Organism	Genotype	Description	Source
DH173	<i>Sphingomonas sp.</i> LM7	LM7	Wild Type	Hershey Lab[1]
DH313	<i>Sphingomonas sp.</i> LM7	$\Delta prmJ$	In frame deletion of BXU08_RS00395	Hershey Lab[1]
DH1312	<i>Sphingomonas sp.</i> LM7	$\Delta fliC$	In frame deletion of BXU08_RS13585	This work
DH1313	<i>Sphingomonas sp.</i> LM7	$\Delta prmJ \Delta fliC$	In frame deletion of BXU08_RS13585 in DH313 background	This work

### 2.2 Plasmids Used in this Study

Table S2: Plasmids used in this Study

Plasmid	Description	Antibiotic	Reference
pNPTS138	Suicide plasmid for deletion in <i>Sphingomonas sp.</i> LM7; carries <i>sacB</i> for counter-selection	Km	M. Alley[1]
pDH298	To delete <i>prmJ</i> ; Gibson cloning of fused upstream and downstream regions of BXU08_RS00395	Km	Hershey Lab[1]
pDH1329	To delete <i>fliC</i> ; commercial synthesis of fused BXU08_RS13585 upstream and downstream regions, inserted into SpeI/HindIII site of pDH100	Km	Hershey Lab[1]
pDH1331	To delete <i>fliC</i> ; commercial synthesis of fused BXU08_RS13585 upstream and downstream regions, inserted into SpeI/HindIII site of pDH100	Km	This work

## References

- [1] Alexandra G. Goetsch et al. *A novel exopolysaccharide pathway from a freshwater Sphingomonas isolate*. Nov. 4, 2023. DOI: 10.1101/2023.11.03.565537. URL: <https://www.biorxiv.org/content/10.1101/2023.11.03.565537v1> (visited on 11/13/2023).