

## **Supplementary Information for**

# **Biopolymer-enriched *B. subtilis* NCIB 3610 biofilms exhibit increased erosion resistance**

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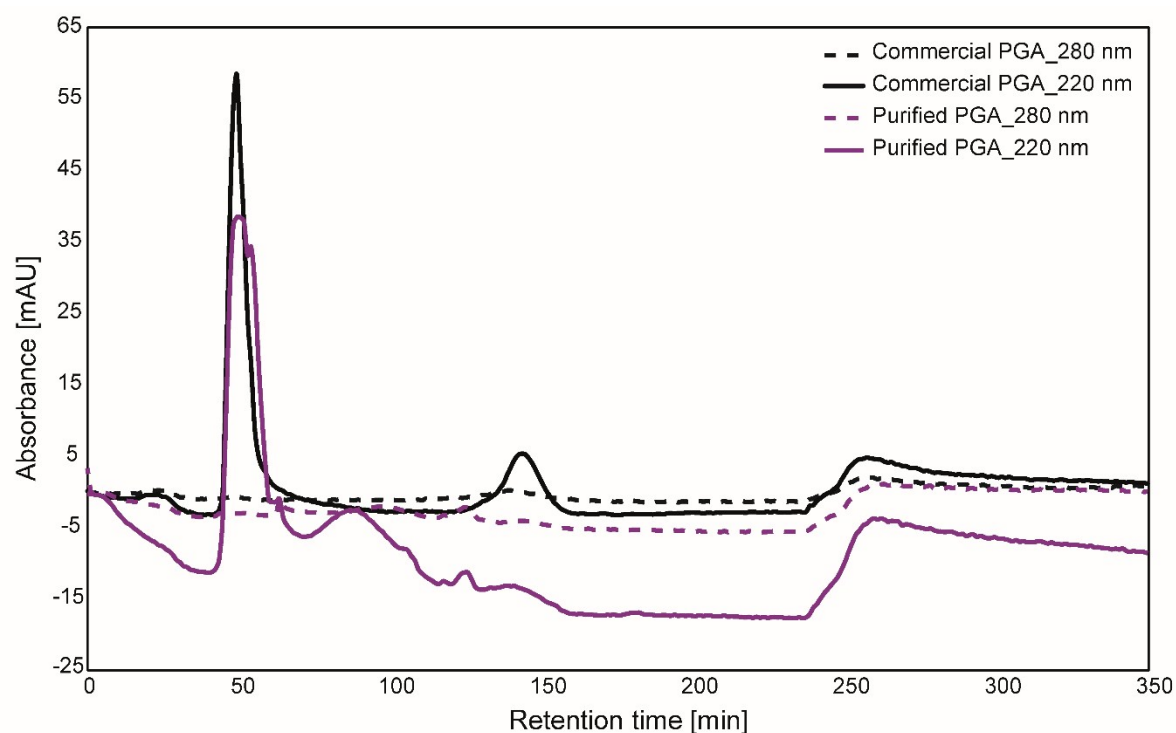
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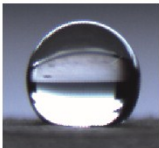
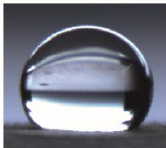
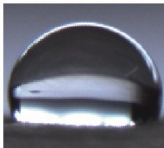

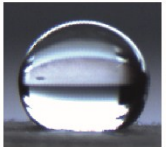
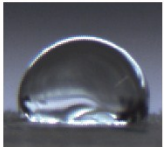
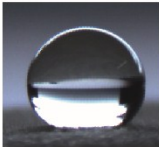
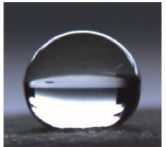
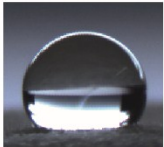
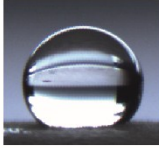

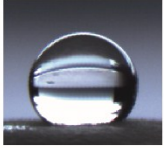





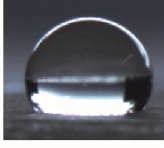



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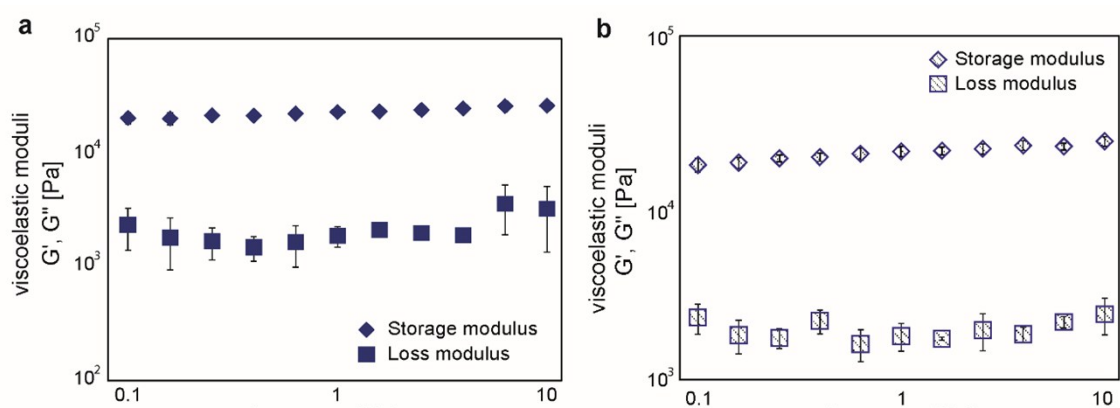
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**Figure S1. Gel filtration chromatograms of commercial and in-lab purified PGA.** The same amount of a commercial or purified PGA solution (concentration 0.05% (w/v)) was run through a Sepharose 6FF XK50/100 column, and the absorbance at 220 nm and 280 nm was compared. Both PGA variants give a strong peak at 220 nm and at the same retention time. Moreover, neither sample exhibits significant absorption at 280 nm, which shows that the purity of both samples is comparable.

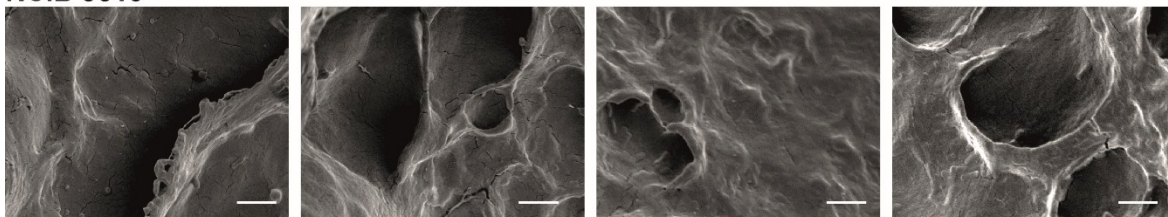
Biofilm	Initial CA	Advancing CA	Receding CA	CA Hysteresis [°]
NCIB 3610				41
Dilution				46
PGA				22
Alginate				21
PEG 10000				16
PEG 600				16
B-1				2

**Figure S2. Exemplary images of water droplets on biofilm samples as used for the determination of contact angles.** Initial (5  $\mu$ L), advancing (10  $\mu$ L) and receding (5  $\mu$ L) contact angle images of water droplets on each biofilm colony were acquired, and the contact angle hysteresis was calculated by subtracting the receding contact angle from the advancing contact angle.

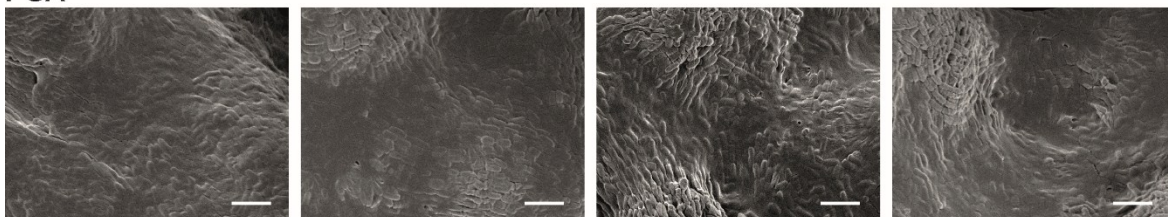


**Figure S3. Viscoelastic properties of different *B. subtilis* biofilms.** Frequency spectra showing the storage ( $G'$ ) and loss ( $G''$ ) moduli of **a)** standard, **b)** diluted, as well as **c)** PGA-, **d)** alginate-, **e)** PEG 10000-, or **f)** PEG 600-enriched NCIB 3610 and **g)** B-1 biofilms. The error bars denote the standard deviation as obtained from  $n = 5$  independent samples which were generated from  $N = 3$  different growth batches.

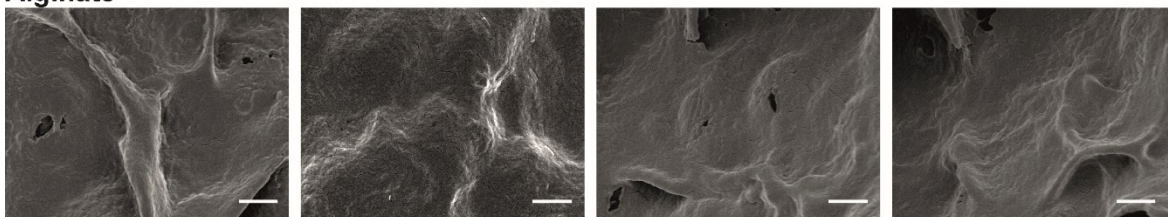
**NCIB 3610**



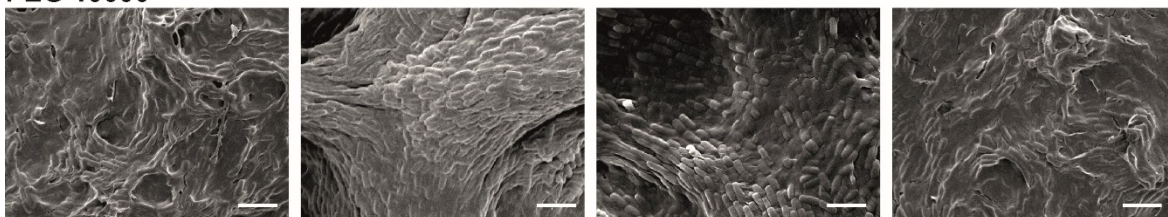
**PGA**



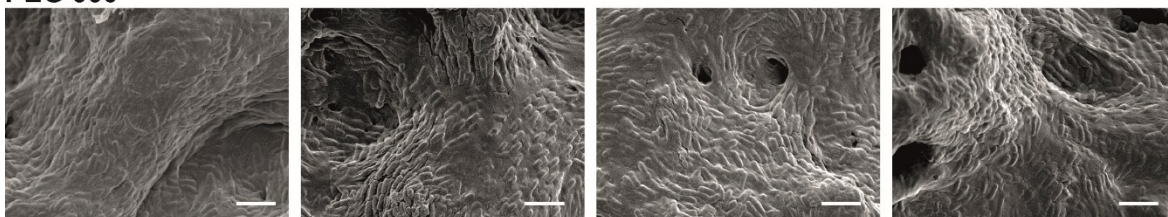
**Alginate**



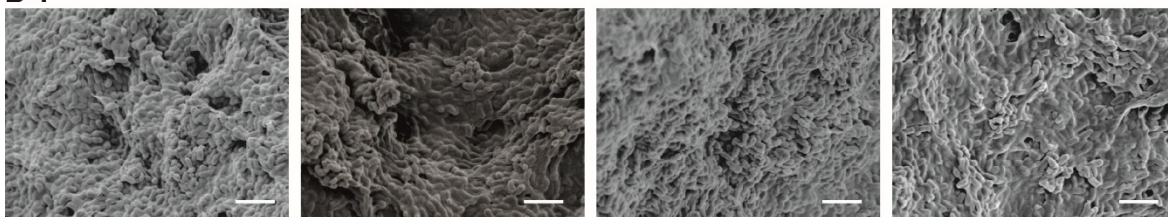
**PEG 10000**



**PEG 600**



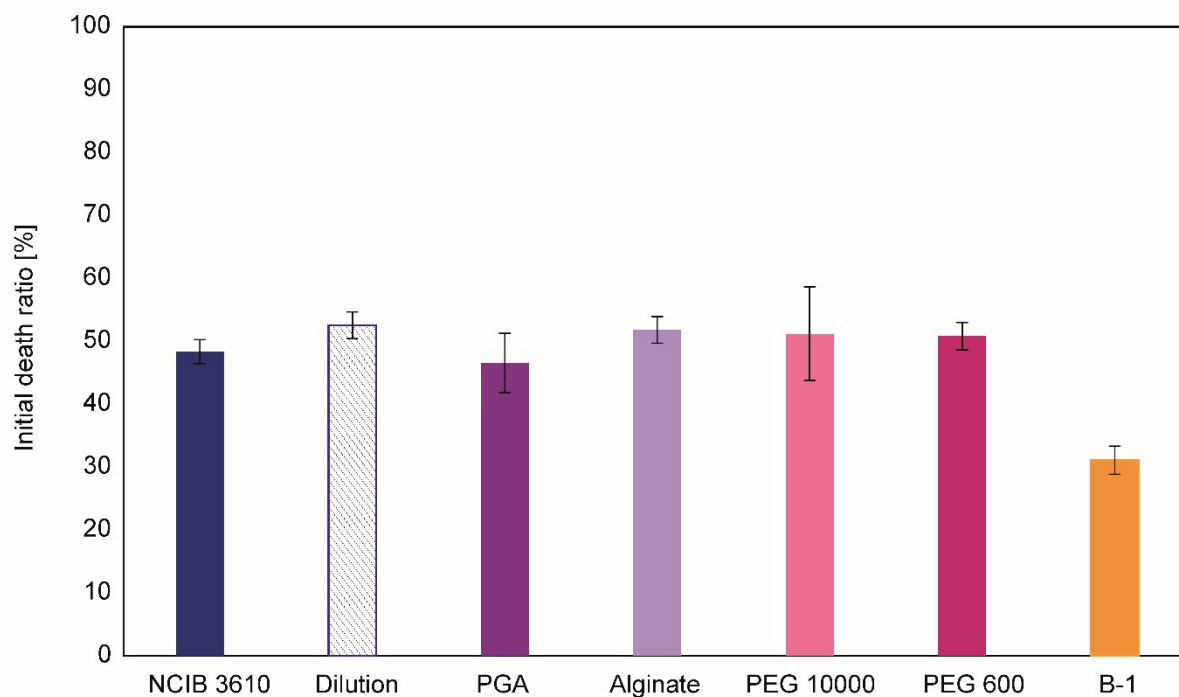
**B-1**



**Figure S4. SEM images of obtained from the surface of different *B. subtilis* biofilm variants.** All images shown were acquired at 3500x magnification; the scale bars represent 5  $\mu\text{m}$ . “PGA”, “Alginate”, “PEG 600” and “PEG 10000” labels refer to NCIB 3610 biofilms enriched with these



(bio)polymers.



**Figure S5. Fraction of dead biofilm cells before antibiotic treatment.** The viability of the bacterial cells was tested by a two-color staining method using a LIVE/DEAD BacLight viability kit (see main paper for details). “PGA”, “Alginate”, “PEG 600” and “PEG 10000” labels refer to NCIB 3610 biofilms enriched with these (bio)polymers. The buffer-diluted NCIB 3610 sample is labelled as “Dilution”. The error bars denote the standard deviation as obtained from  $n = 9$  independent samples, created from  $N = 3$  distinct growth batches each.