SUPPLEMENTARY INFORMATION

INTERACTIONS OF BIOFILM POLYSACCHARIDES PRODUCED BY HUMAN INFECTIVE BACTERIA WITH MOLECULES OF THE QUORUM SENSING SYSTEM. A MICROSCOPY AND NMR STUDY

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Production and purification of the polysaccharide samples used in this study

Burkholderia multivorans strain C1576 (LMG 16660) is a reference strain from the panel of *Burkholderia cepacia* Complex strains [1] and was purchased from BCCM™bacteria collection (Dept. of Biochemistry and Microbiology, Faculty of Sciences of Ghent University, Belgium). Colony biofilms were grown on cellulose membranes (Sigma, cut-off 12.400 Da), cut in circles the size of a Petri dish, washed first in boiling 5 % Na_2CO_3, then in boiling distilled water for 15 min. After autoclave treatment, the membranes were layered over Petri dishes containing Müller Hinton (MH) agar, and the excess liquid was dried. An overnight liquid culture of bacteria in MH broth was diluted 100 times and deposited onto the membranes, in three spots of 10 μL each. After a 7-day incubation at 30 °C, the bacterial product was collected with 0.15 M NaCl, 1 M NaOH was added to obtain 0.1 M final concentration of base, and the suspension was stirred at 10 °C for 3 h, before centrifuging it at 48000×g for 60 min at 4 °C. The supernatant was dialyzed first against 0.1 M NaCl, and then against distilled water, before adjusting the pH to neutrality. After lyophilization, the product was dissolved in distilled water (10 mg/mL), treated with 20 % w/v (final concentration) of trichloroacetic acid for 30 min in ice, and centrifuged at 48000×g for 30 min at 4 °C to remove the precipitated proteins. The supernatant was adjusted to neutral pH, dialyzed against distilled water, filtered (Millipore membranes 0.45 μm), and recovered by lyophilization.

The Δ*bcsB*/pBerA strain [2], a kind gift of Prof. Tim T. Nielsen (Costerton Biofilm Center, Department of Immunology and Microbiology, University of Copenhagen, DK-2200 Copenhagen, Denmark), was derived from *Burkholderia cenocepacia* H111, a clinical isolate from a cystic fibrosis patient [3], and was used for polysaccharide production [4]. Bacteria were spread from a −80 °C glycerol stock culture directly onto four agar plates containing the nutrient-yeast extract-glycerol (NYG) medium (0.5 % peptone, 0.3 % yeast extract, 2 % (w/v) glycerol, and 1.5 % agar) and kanamycin (100 μg/mL final concentration), and incubated for four days at 30 °C. The biofilm produced by bacteria was removed from the plates in one piece, washed with 15 mL of 0.9 % NaCl, and centrifuged (1900×g 10 °C for 15 min) three times. The pellet was treated with 0.3 M NaOH for 3 h at 10 °C and then centrifuged at 22400×g for 30 min at 4 °C. This treatment solubilizes EPOL H111-SOL, together with the water-insoluble EPOL H111-INS. The latter was removed from the solution through extensive dialysis against water prompting its precipitation in the dialysis bag [5] and was separated by centrifugation at 1900×g at 4 °C for 30 min. The supernatant, containing the EPOL H111-SOL, was treated with 20 % w/v (final concentration) trichloroacetic acid in ice for 30 min, centrifuged at

48000×g at 4 °C for 30 min to remove proteins, and the supernatant was dialyzed against distilled water. The solution was treated with 4 volumes of ethanol to precipitate the polysaccharide, followed by centrifugation at 1900×g at 10 °C for 20 min. The pellet was dissolved in distilled water and dialyzed; the supernatant was taken to dryness by using a rotary evaporator and re-dissolved in distilled water. Both solutions were filtered on 0.8 µm membranes and lyophilized after adjusting their pH to 7.0. The EPOL H111-SOL was present both in the supernatant and in the pellet, due to its amphiphilic character [4]; for experiments described in this work, the PS recovered from the supernatant was used.

Klebsiella pneumoniae KpB-1 (Sequence Type 12) was isolated from the pleural fluid of an inpatient in an Italian hospital. Bacteria growth and CPS purification were achieved as previously described [6]. Bacterial cells were grown on Worfel-Ferguson solid medium for 4 days at 30 °C, the material was collected with 0.9 % NaCl (about 6 mL per dish), gently stirred at 10 °C for 2 h, and the suspension was centrifuged at 22400×g for 30 min at 6 °C. Four volumes of cold ethanol were added to the supernatant causing the precipitation of the PS. The pellet was recovered by centrifugation at 1900×g at 4 °C for 30 min, dissolved in 60 mL of distilled water, dialyzed first against 0.1 M NaCl, and then distilled water. After adjusting the pH to 7.3, the solution was filtered (Millipore membranes 0.45 µm) and lyophilized.

References

[1] E. Mahenthiralingam, T. Coenye, J.W. Chung, D.P. Speert, J.R.W. Govan, P. Taylor, P. Vandamme, Diagnostically and experimentally useful panel of strains from the *Burkholderia cepacia* Complex, J. Clin. Microbiol. 38 (2000) 910–913, https://doi.org/10.1128/JCM.38.2.910-913.2000

[2] M. Fazli, Y. McCarthy, M. Givskov, R.P. Ryan, T. Tolker-Nielsen, The exopolysaccharide gene cluster Bcam1330–Bcam1341 is involved in *Burkholderia cenocepacia* biofilm formation, and its expression is regulated by c-di-GMP and Bcam1349, MicrobiologyOpen 2 (2013) 105–122. https://doi.org/10.1002/mbo3.61.

[3] A. Carlier, K. Agnoli, G. Pessi, A. Suppiger, C. Jenul, N. Schmid, B. Tümmler, M. Pinto-Carbo, L. Eberl, 2014. Genome sequence of *Burkholderia cenocepacia* H111, a cystic fibrosis airway isolate, Genome Announc. 2, e00298-14. https://doi.org/10.1128/genomeA.00298-14.

[4] B. Bellich, I.A. Jou, C. Buriola, N. Ravenscroft, J. W. Brady J.W., M. Fazli, T. Tolker-Nielsen, R. Rizzo, P. Cescutti, 2021. The biofilm of *Burkholderia cenocepacia* H111 contains an exopolysaccharide

composed of rhamnose and L-mannose: structural characterization and molecular modelling. Carbohydr. Res. 499, 108231. https://doi.org/10.1016/j.carres.2020.108231.

[5] B. Bellich, et al., *Burkholderia cenocepacia* H111 produces a water-insoluble exopolysaccharide in biofilm: structural determination and molecular modelling, Int. J. Mol. Sci. 21 (2020) 1702–1714, https://doi.org/10.3390/ijms21051702.

[6] B. Bellich, C. Lagatolla, R. Rizzo, M.M. D'Andrea, G.M. Rossolini, P. Cescutti, Determination of the capsular polysaccharide structure of the *Klebsiella pneumoniae* ST512 representative strain KPB-1 and assignments of the glycosyltransferases functions, Int. J. Biol. Macromol. 155 (2020) 315–323. https://doi.org/10.1016/j.ijbiomac.2020.03.196.