

Supplementary Material

Matrix is everywhere: extracellular DNA is a link between biofilm and mineralization in *Bacillus cereus* planktonic lifestyle

Lyubov A. Ivanova^{1,2}, Vladimir V. Egorov^{2,3}, Yana A. Zabrodsкая^{4,5}, Aram A. Shaldzhyan⁵, Alexander Ye. Baranchikov⁶, Natalia V. Tsvigun⁷, Anna N. Lykholay⁸, Alexey D. Yapryntsev⁶, Dmitry V. Lebedev¹, Anna A. Kulminskaya^{1,2}*

¹ Petersburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Center "Kurchatov Institute", 1, mkr. Orlova roshcha, Gatchina, Leningradskaya oblast, Russia.

² Kurchatov Genome Center - PNPI, 1, mkr. Orlova roshcha, Gatchina, Leningradskaya oblast, Russia.

³ Institute of Experimental Medicine, 12 Akademik Pavlov str., St. Petersburg 197376, Russia.

⁴ Peter the Great Saint Petersburg Polytechnic University, 29 Polytechnicheskaya str., St. Petersburg 194064, Russia.

⁵ Smorodintsev Research Institute of Influenza, Russian Ministry of Health, 15/17 Prof. Popova str., St. Petersburg 197376, Russia

⁶ Kurnakov Institute of General and Inorganic Chemistry of the Russian Academy of Sciences, 31, Leninskii av., Moscow 119071, Russia.

⁷ Federal Scientific Research Center "Crystallography and Photonics" of the Russian Academy of Sciences, 59, Leninskii av., Moscow 119333, Russia.

⁸ Centre for Molecular and Cell Technologies, Research Park of Saint-Petersburg State University, Saint-Petersburg, Petergof, 17, Botanicheskaya str., Russia.

* Corresponding author: Anna A. Kulminskaya, e-mail: kulminskaya_aa@pnpi.nrcki.ru.

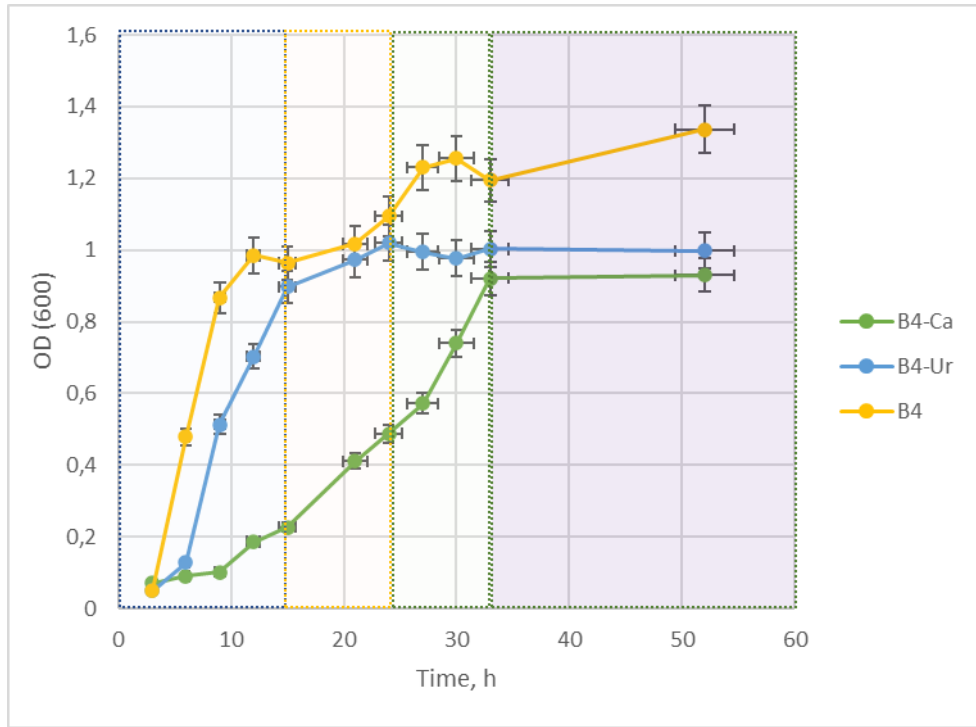


Fig. S1. Time-dependence of the bacterial biomass growth of *B. cereus* 4B in control media: B4_Ca (green), B4 (yellow) B4_Ur (blue). Time stages are highlighted in color: 0 – 15 hours in blue; 15 – 24 hours in orange; 24 – 33 hours in green; more than 33 hours in lilac.

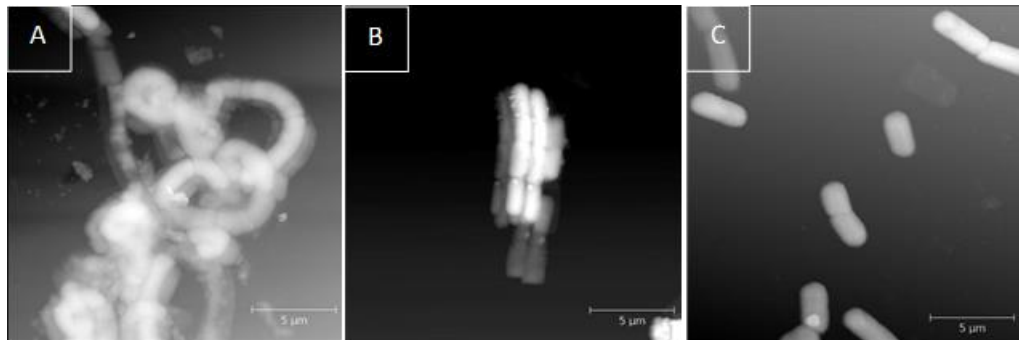


Fig. S2. AFM microscopy image of *B. cereus* after 6 hours of cultivation in media: A: B4_Ca; B: B4_Ur; C: B4.

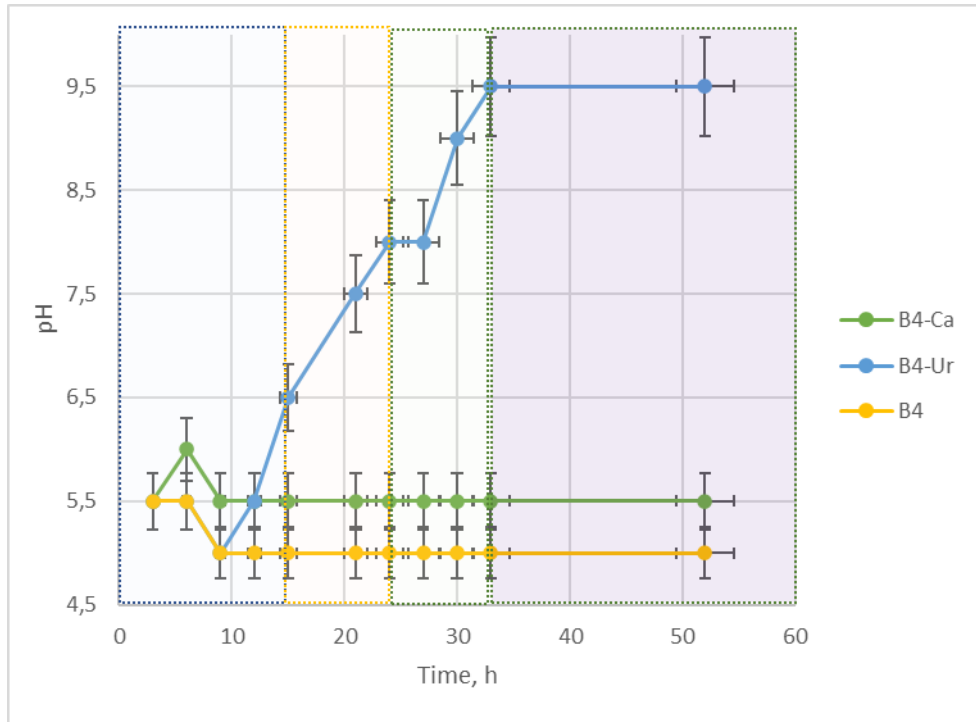


Fig. S3. Time-dependence of the medium acidity during the incubation of *B. cereus* 4B in the control media: B4_Ca (in green), B4 (in yellow), B4_Ur (in blue). Time stages are highlighted in color: 0 – 15 hours in blue; 15 – 24 hours in orange; 24 – 33 hours in green; more than 33 hours in lilac.

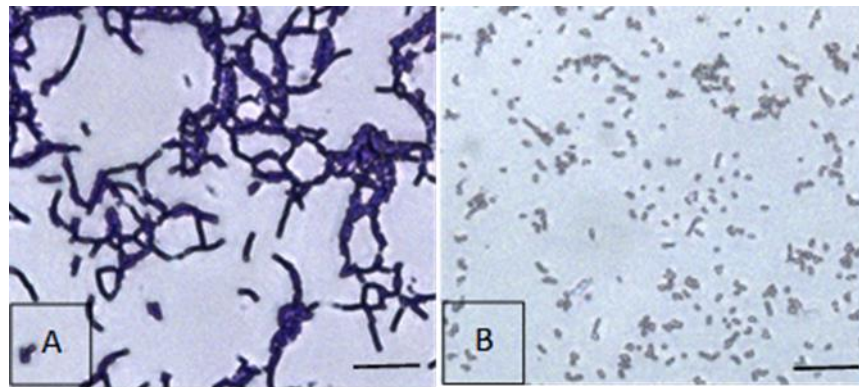


Fig. S4. Image of light microscopy with crystal violet staining of *B. cereus* cells at the fourth stage of growth (52 hours of inoculation). A: In the B4_Ur medium B: in the B4 medium. Dimensional designations correspond to 5 μ m.

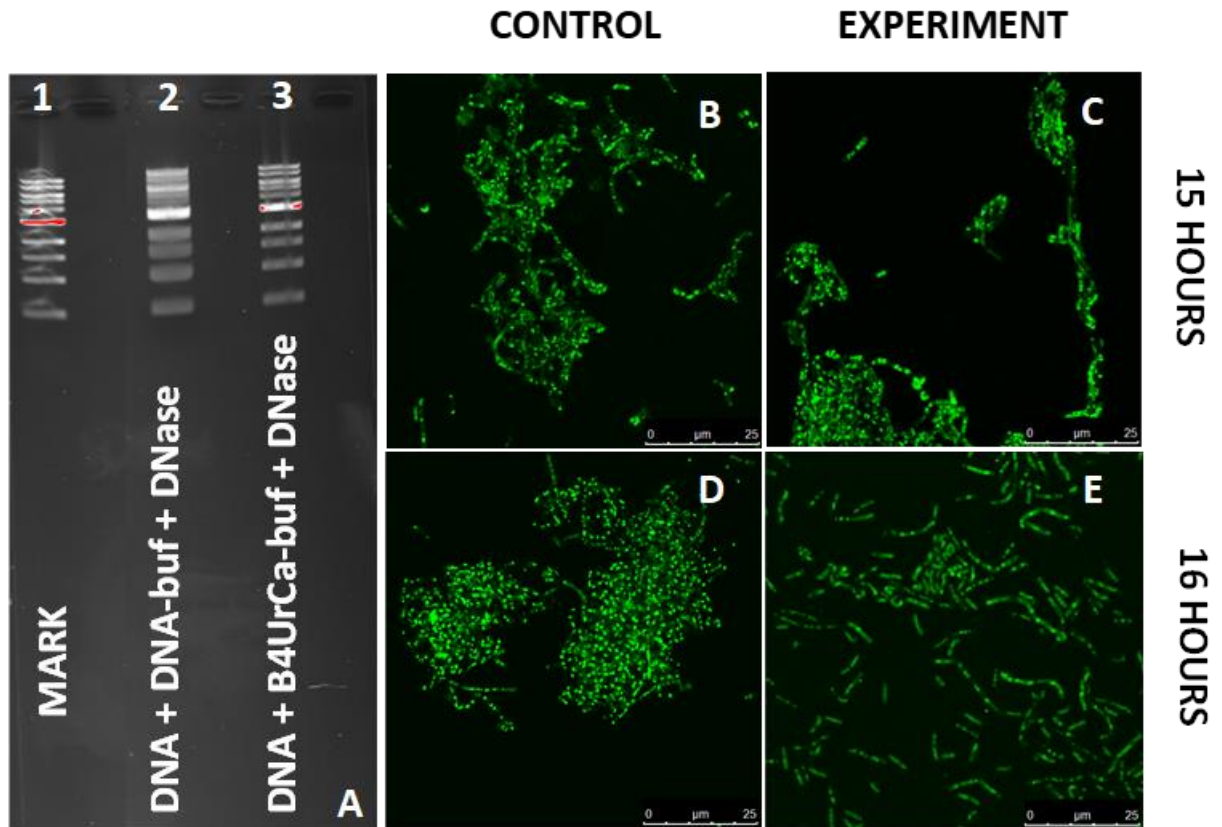


Fig. S5. DNA-DNase experiments. **A:** Image of agarose gel after PAGE, where **1:** DNA marker; **2:** DNA fragments obtained by hydrolysis of a salmon sperm DNA molecule with DNase (I) in a commercial DNA buffer; **3:** DNA fragments obtained by hydrolysis of a salmon sperm DNA molecule with DNase (I) in **B4-CaUr** medium. **B-E:** Confocal microscopy image with Sytox green (eDNA) staining of *B. cereus* samples in **B4_CaUr** medium before addition of the DNase (I) and cultured for 15 hours (**B, C**) and one hour after addition of DNase (I) in experimental sample (**E**) compared to a control sample without enzyme (**D**).

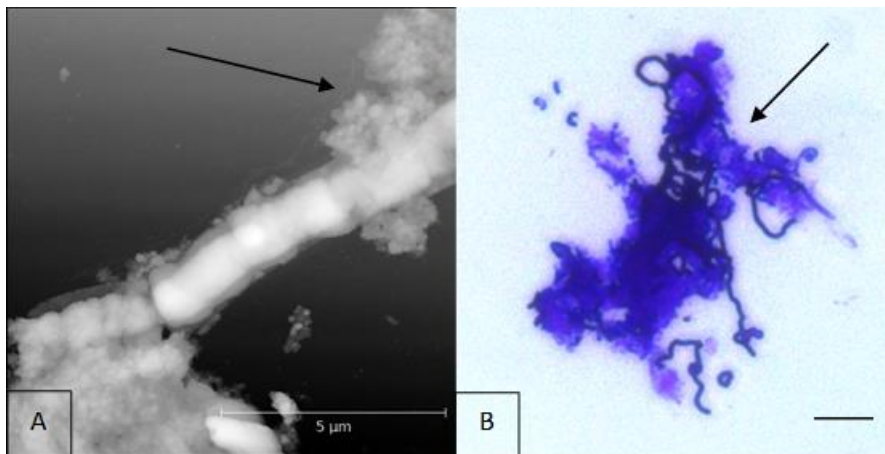


Fig. S6. A: AFM microscopy image of *B. cereus* after 9 hours of cultivation in medium **B4_Ca**. Dimensional designations correspond to 5 μm . Black arrows indicate polysaccharides. **B:** Image of light microscopy with crystal violet staining of *B. cereus* in **B4_Ca** medium at the first stage of growth (15 hours after inoculation).

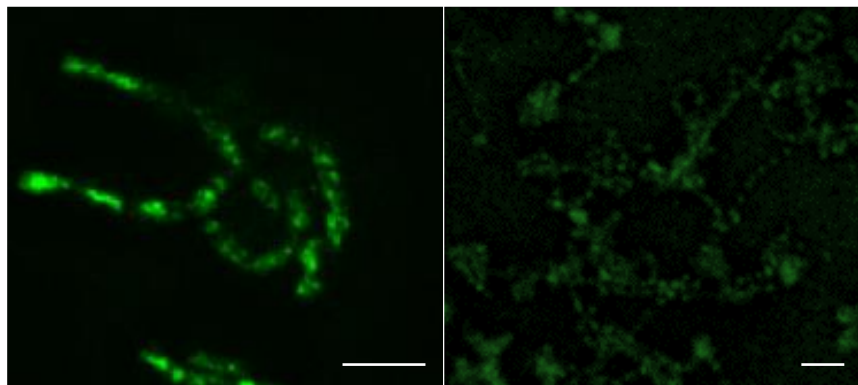


Fig. S7. Confocal microscopy image with Sytox green (eDNA) staining of *B. cereus* after 30 hours of cultivation in **B4_Ur** (at the left) and **B4_Ca** (at the right) media.