

1 Supplementary Information

2 Title: A microfluidic co-cultivation platform to investigate microbial 3 interactions at defined microenvironments

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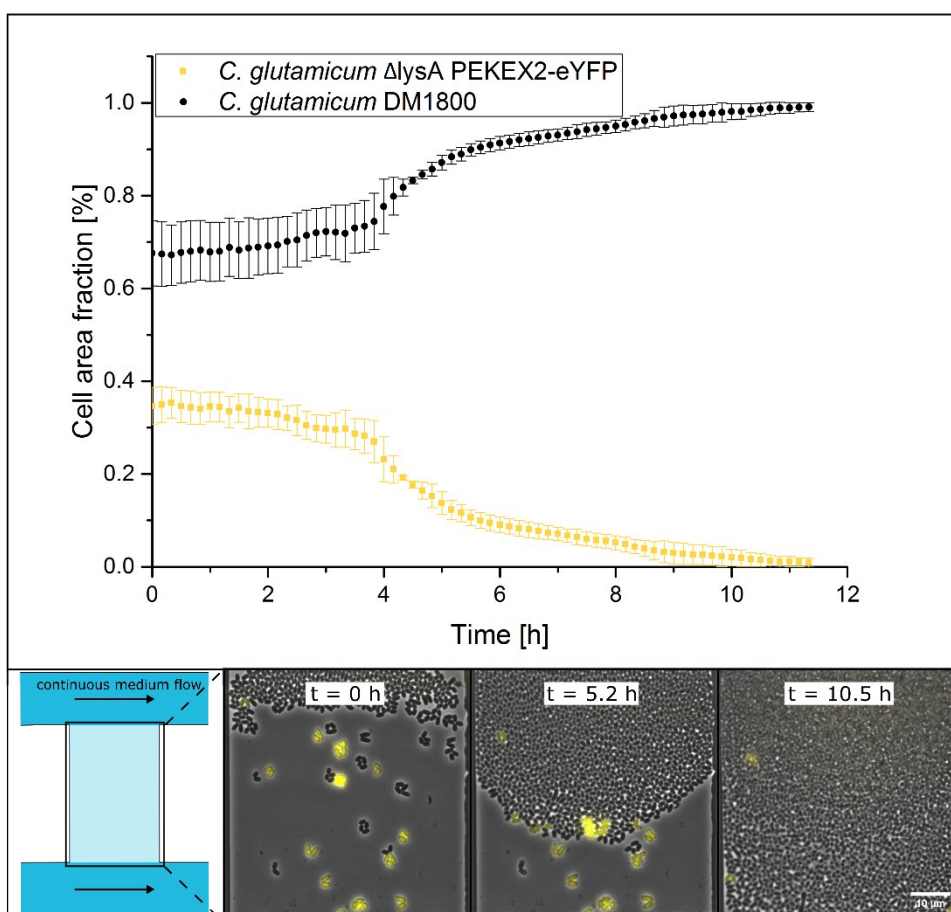
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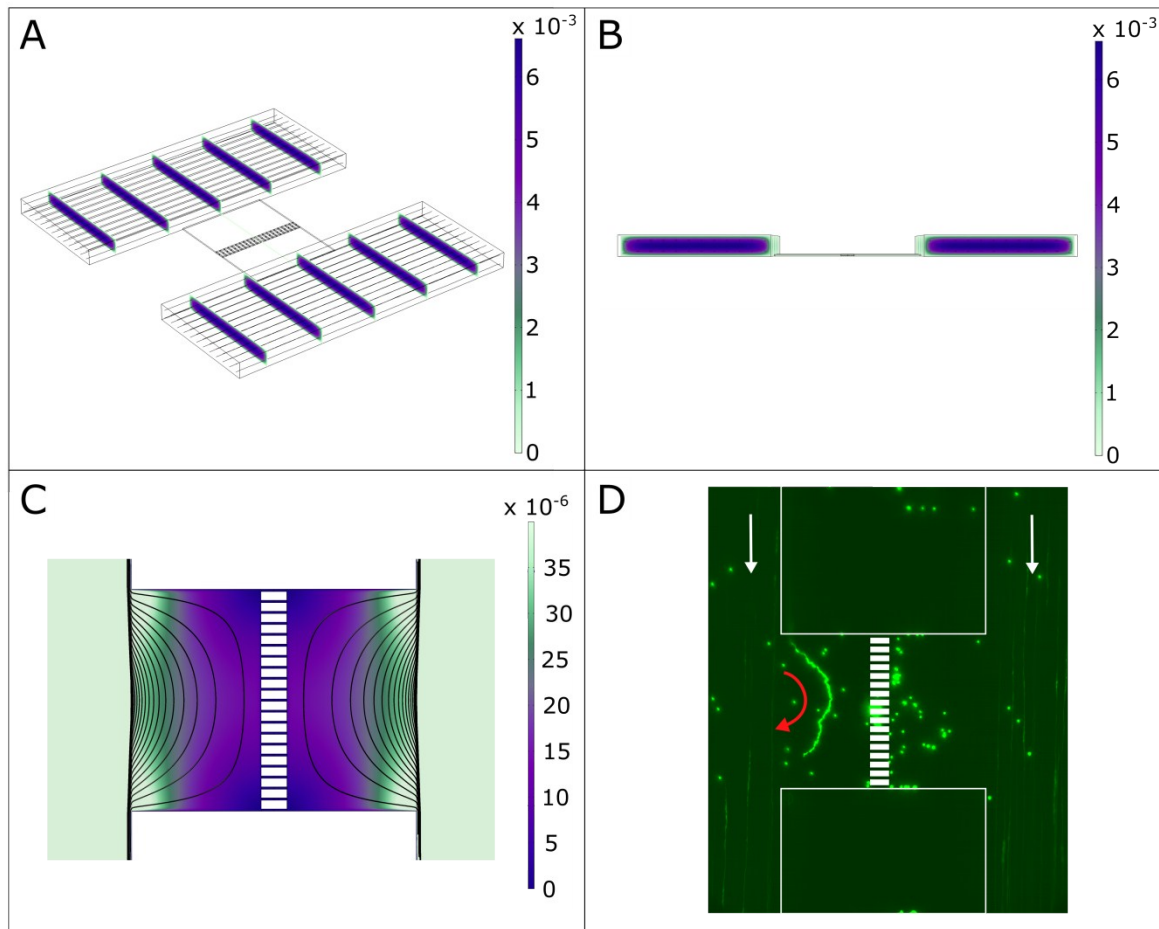
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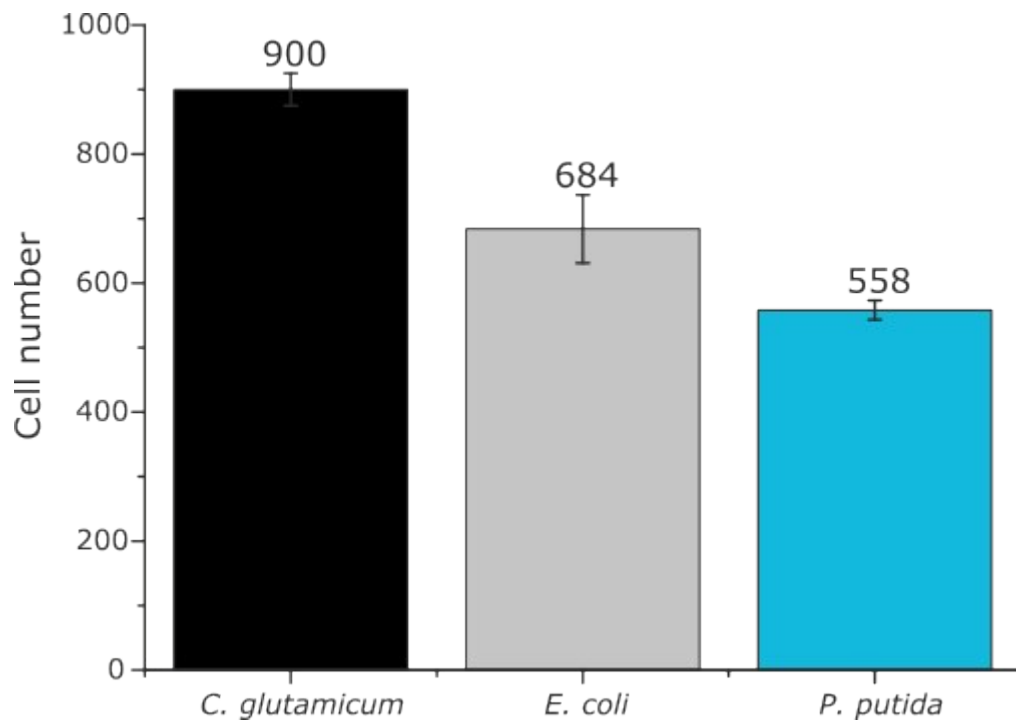
19 Figure S1 Wash-out of auxotrophic strain in monolayer growth chambers²⁷. In a mixed culture consisting of a yellow
20 fluorescing L-lysine auxotrophic *C. glutamicum* strain and the non-fluorescent L-lysine producer *C. glutamicum* DM1800,
21 the auxotrophic strain was rapidly overgrown by the fast-growing *C. glutamicum* DM1800. After 10.5 hours nearly all L-
22 lysine auxotrophic cells were washed out of the growth chamber and analysis of bacterial interaction is not possible.

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25 **Figure S2 (A)** Simulated flow velocity profile inside the supply channels of our microfluidic co-cultivation device. (B) Cross
 26 section of supply channels with flow velocity profile. While the flow velocity reaches 6 mm s^{-1} in the center of the channel,
 27 the velocity near the walls converges to 0 mm s^{-1} . (C) Flow velocity gradient inside co-cultivation chambers. At the chamber
 28 entrance the velocity is around $30\text{-}40 \mu\text{m s}^{-1}$ and decreases to $0 \mu\text{m s}^{-1}$ in the center. (D) Flow profile experiment with
 29 fluorescent beads. Results agree with the simulation shown in (C). Single beads from the main channels could slowly flow
 30 through the chambers in a semicircle. Unit of scale bars is m s^{-1} .



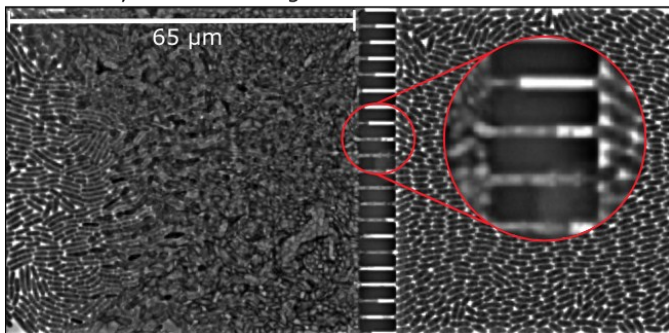
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32 Figure S3 Maximum cell numbers of different species in completely filled microfluidic co-cultivation chambers with a size
 33 of 35x60 µm.

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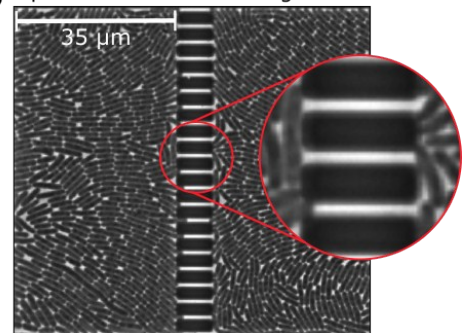
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A Preliminary chamber design



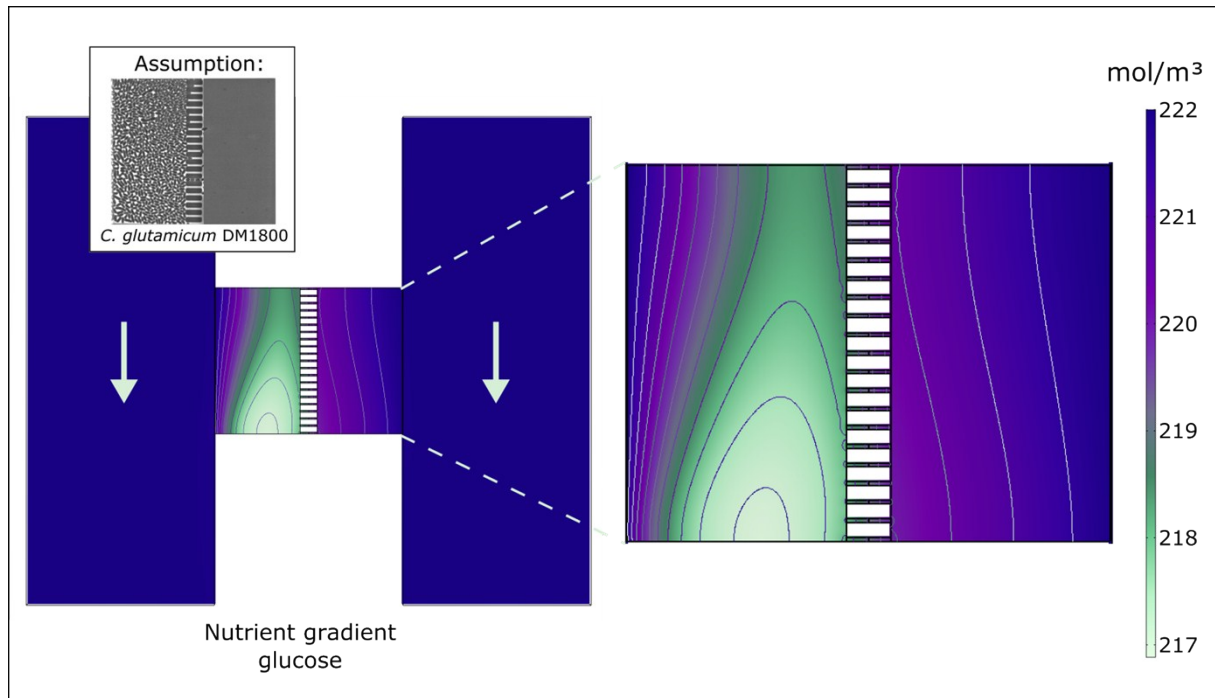
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B Optimized chamber design



37 Figure S4 (A) Cultivation chambers with a length of 65 µm resulted in great pressure on the barrier structure and cells were
 38 squeezed through the nanochannels. (B) A reduction of the chamber length to 35 µm decreased the pressure on the
 39 nanochannels and cells could not pass them.

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42 **Figure S5** CFD simulation of the glucose gradient inside the microfluidic co-cultivation chambers with one chamber
43 compartment covered with cells. Only minor concentration differences of around 5 mmol L⁻¹ are expected to occur, even
44 for 10 times higher than expected uptake rates.

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