The physical boundaries of public goods cooperation between surface-attached bacterial cells

Electronic Supplementary Material

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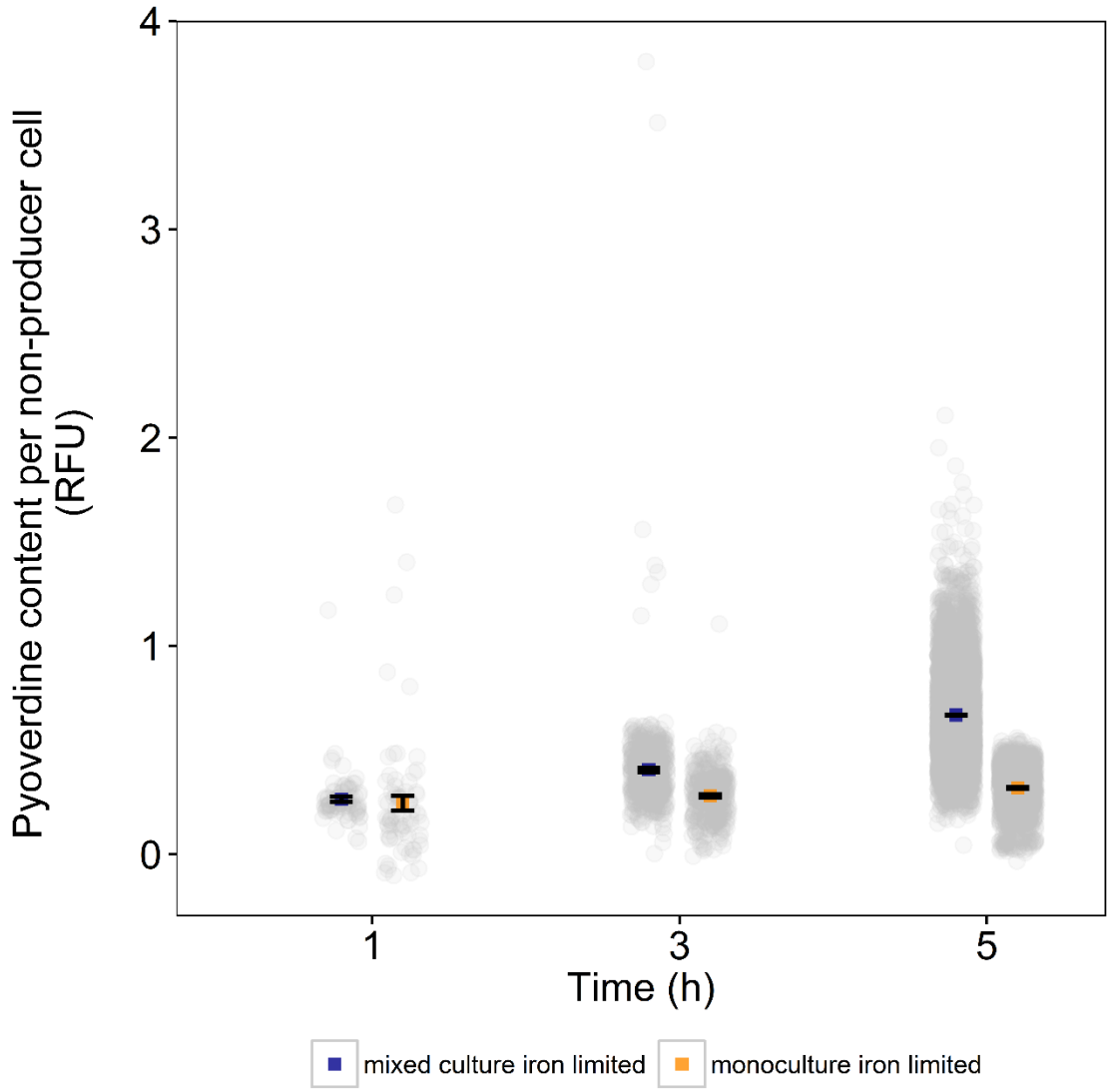

Figure S1: Pyoverdine is taken up by non-producing cells in a time-dependent manner, demonstrating pyoverdine sharing between physically separated, surface-attached micro-colonies. Time-course measures on natural pyoverdine fluorescence units (RFU) shows constant background fluorescence in non-producer cells grown in monocultures (orange squares), whereas pyoverdine fluorescence significantly increased in non-producer cells grown in mixed cultures with producers (blue squares). Mean relative fluorescence values ± standard errors are scaled relative to producer monocultures after one hour of growth. Important to note is that only apo-pyoverdine (i.e. iron-free) is fluorescent, and therefore the measured fluorescence intensities represent a conservative measure of the actual pyoverdine content per cell. Furthermore, the fluorescence intensity in producer cells is always higher than in non-producer cells because it represents the sum of pyoverdine taken up from the environment and newly synthesized pyoverdine, whereas for non-producers, fluorescence represents pyoverdine uptake only.

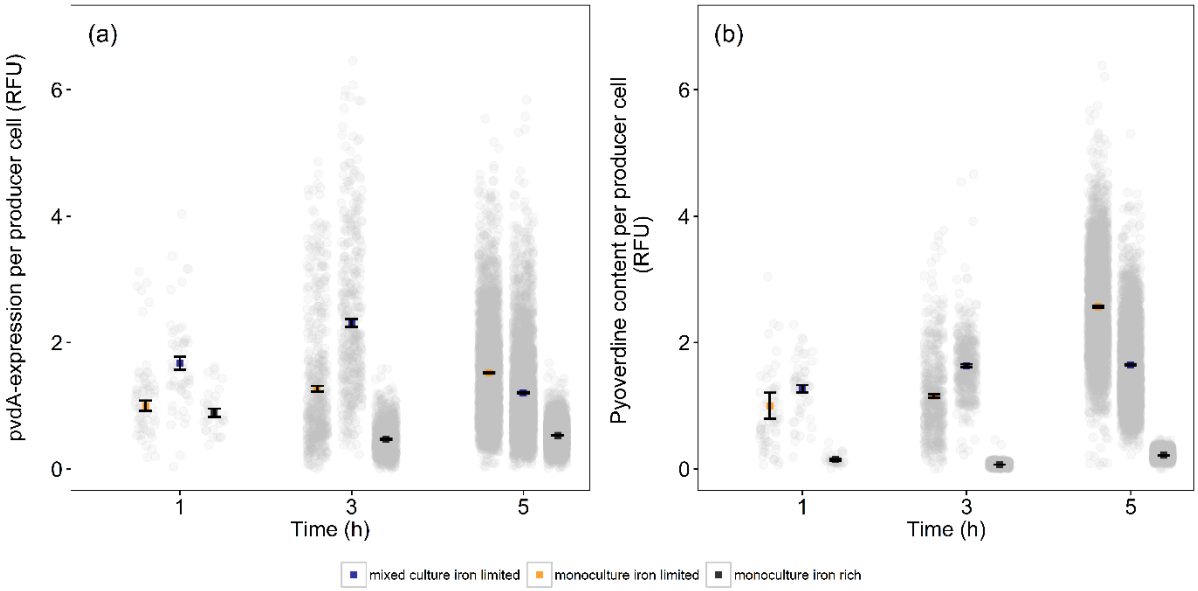

Figure S2: Producer cells adjust their pyoverdine investment level in response to changes in the social environment. **(a)** Time-course data show that *pvdA*, a gene encoding an enzyme involved in pyoverdin synthesis, is down-regulated in iron-rich media (grey squares), but up-regulated in iron-deplete media. Importantly, producers exhibited different *pvdA* expression patterns depending on whether they grew together with non-producers (blue squares) or as monoculture (orange squares). While producers showed increased gene expression in mixed compared to monoculture after one and three hours, the pattern flipped after five hours. **(b)** The same qualitative pattern was observed when measuring pyoverdine content per cell, as relative fluorescence units (RFU). Fluorescence values are scaled relative to the producer monocultures after one hour of growth. Important to note is that only apo-pyoverdine (i.e. iron-free) is fluorescent. Error bars indicate standard errors of the mean.

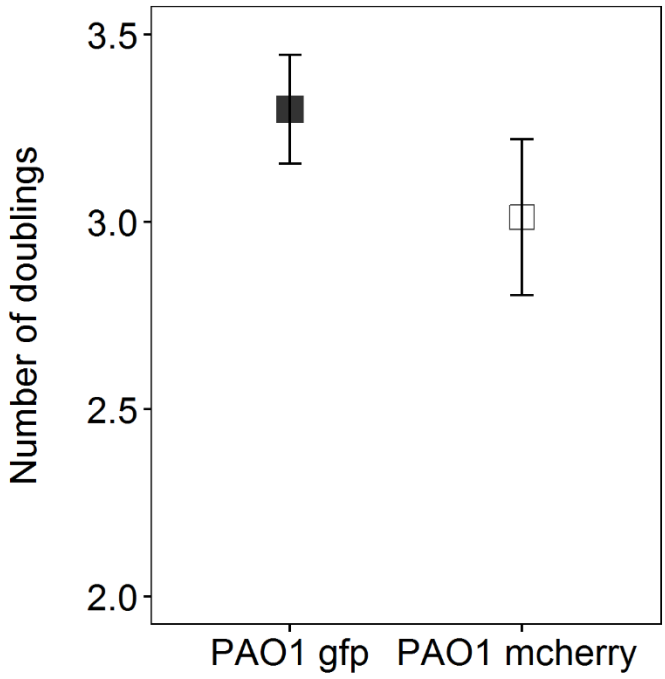

Figure S3: Growth did not differ between strains tagged with GFP or mCherry. We grew the wildtype PAO1 strain, either tagged with GFP or mCherry, on iron-limited agarose pads and calculated the number of doublings over 5 hours. Doubling numbers did not significantly differ between the two strains (t-test: $t_{94} = 1.14$, $p = 0.258$). Thus, we can be confident that growth differences observed in our experiments are due to biological and not tag effects. Symbols and error bars indicate means and standard errors of the mean, respectively.

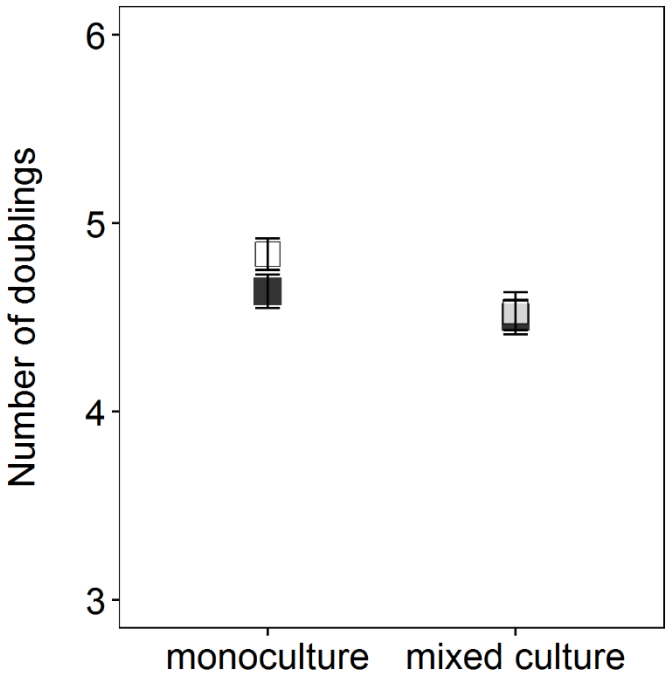

Figure S4: There are no growth differences between the pyoverdine-producing strain (filled squares) and the non-producing strain (open squares) on agarose pads supplemented with 200 µM $FeCl_3$. Growth of the two strains was neither different in monoculture (t-test: t_{78} = -1.61, p = 0.11) nor in mixed culture (t-test: t_{71} = -0.23, p = 0.82). These results are in line with the view that pyoverdine production is completely stalled when iron is plentiful (46), such that there is no more difference in the strains' phenotype. This also means that the fitness effects we observed in iron-depleted media (Figures 3 - 6) are attributable to pyoverdine-mediated social interactions. The number of doublings was calculated over a growth period of 3 hours. Symbols and error bars indicate means and standard errors of the mean, respectively.

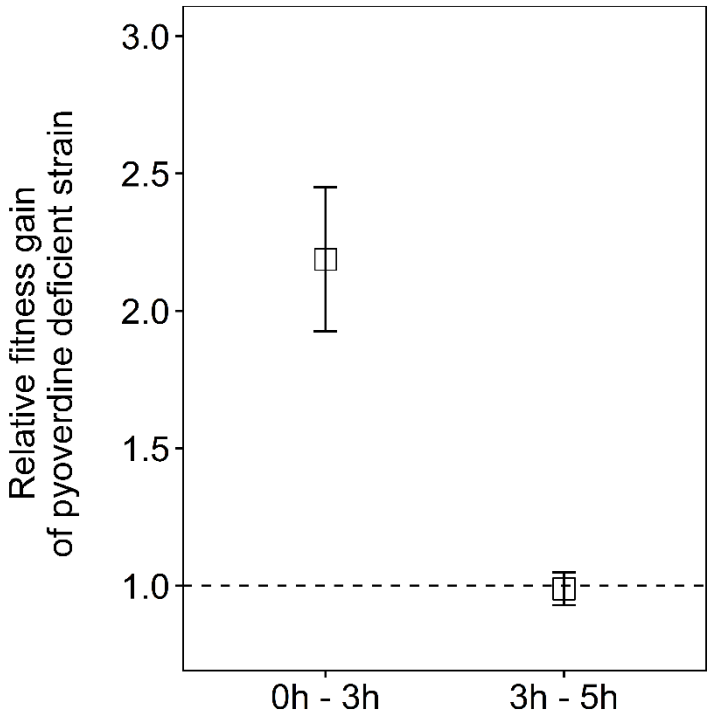

Figure S5: Non-producers experience a significant relative fitness advantage during the first three hours of competition (zero to three hours; one sample t-test: $t_{20} = 4.53$, $p < 0.001$), but not during the later competition phase (three to five hours; one sample t-test: $t_{41} = -0.18$, $p = 0.85$). We used cell numbers to calculate strain frequencies at time point zero, three and five hours and to estimate the relative fitness of non-producers as $v = [q_2(1 - q_1]/[q_1(1 - q_2]$, where q_1 and q_2 are the initial and final frequencies of the non-producer (Ross-Gillespie et al. 2007)[a]. The dotted line represents the fitness equilibrium, where no strain has a relative fitness advantage over the other. Symbols and error bars indicate means and standard errors of the mean, respectively.

[a] Ross-Gillespie A, Gardner A, Buckling A, West SA, Griffin AS. Frequency dependence and cooperation: theory and a test with bacteria. Am Nat. 2007;170(3):242–331. DOI: 10.1086/519860

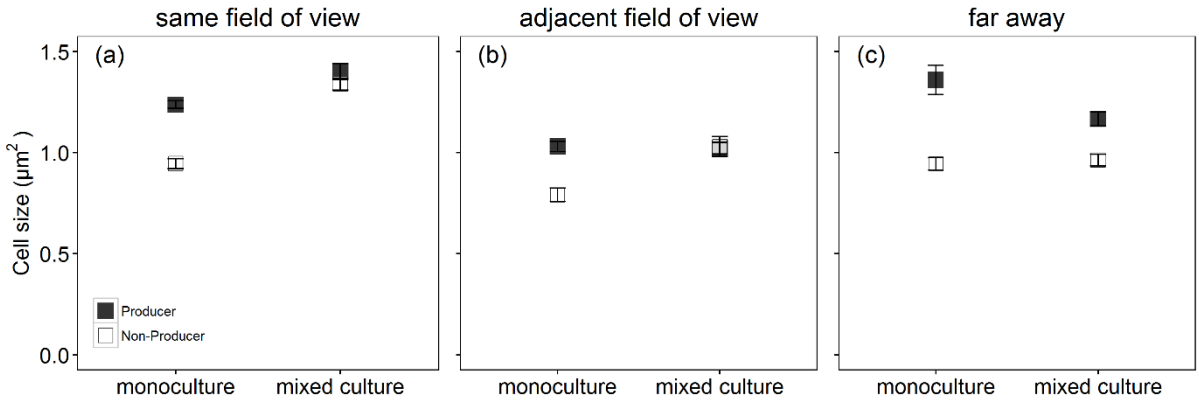

Figure S6: Pyoverdine sharing affects cell size. While non-producer cells (open squares) were significantly smaller than producer cells (closed squares) in monocultures, non-producer cell size was restored to wildtype level in mixed cultures when the producer microcolony was (a) within the same field of view (average distance between cells 36 μm), (b) in an adjacent field of view (minimal distance ~ 100 μm), but not when producers were far away (on opposite ends of the agarose pad) (c). Cell size was measured after three hours of growth. Error bars indicate the standard error of the mean.