1 **The fitness of *Pseudomonas aeruginosa* quorum sensing signal cheats is influenced by the**

2 **diffusivity of the environment**

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15

16   Abstract

17   Experiments examining the social dynamics of bacterial quorum sensing (QS) have focused on

18   mutants which do not respond to signals, and the role of QS-regulated exoproducts as public

19   goods. The potential for QS signal molecules to themselves be social public goods has received

20   much less attention. Here, we analyse how signal-deficient (*lasI*) mutants of the opportunistic

21   pathogen *Pseudomonas aeruginosa* interact with wild-type cells in an environment where QS is

22   required for growth. We show that when growth requires a 'private' intracellular metabolic

23   mechanism activated by the presence of QS signal, *lasI* mutants act as social cheats and

24   outcompete signal-producing wild-type bacteria in mixed cultures, because they can use the

25   signals produced by wild type cells. However, reducing the ability of signal molecules to diffuse

26   through the growth medium, results in signal molecules becoming less accessible to mutants,

27   leading to reduced cheating. Our results indicate that QS signal molecules can be considered as

28   social public goods in a way that has been previously described for other exoproducts, but that

29   spatial structuring of populations reduces exploitation by non-cooperative signal cheats.

30

31   Importance

32   Bacteria communicate via signaling molecules to regulate the expression of a whole range of

33   genes. This process, termed quorum sensing (QS), moderates bacterial metabolism in many

34   environmental conditions, from soil and water (where QS-regulated genes influence nutrient

35   cycling) to animal hosts (where QS-regulated genes determine pathogen virulence).

36   Understanding the ecology of QS could therefore yield vital clues as to how we might modify

37   bacterial behaviour for environmental or clinical gains. Here, we demonstrate that QS signals act

38   as shareable public goods. This means that their evolution, and therefore population-level

39   responses to interference with QS, will be constrained by population structure. Further, we show

40   that environmental structure (constraints on signal diffusion) alters the accessibility of QS signals

41   and demonstrates that we need to consider population and environmental structure to help us

42   further our understanding of QS signaling systems.

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46   **Introduction**

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48   Bacterial quorum sensing (QS) is a cell-to-cell signalling mechanism that coordinates a range of
49   behaviours at the population level (1, 2). QS facilitates density-dependent production of
50   extracellular molecules including nutrient-scavenging enzymes and virulence factors. These
51   molecules have been termed 'public goods' because their benefits can be shared by all cells in
52   the local population (3-6). Because these QS-regulated exoproducts are metabolically costly for
53   cells to produce, QS can also be exploited by non-cooperating "cheats": cells that do not respond
54   to QS signals and so pay no costs, but which exploit wild type populations because they benefit
55   from the public goods produced by wild type neighbouring cells (4-7). Experiments into the
56   social dynamics of QS have traditionally focused on these "signal blind" mutants, and a number
57   of studies have shown that such mutants can arise in laboratory cultures and during infections (8-
58   14). In various laboratory conditions and *in vivo* infection models, they have been shown to act
59   as social cheats (6, 15-17).

60

61   However, little attention has been paid to whether QS signals themselves can act as exploitable
62   public goods, despite there being a metabolic cost associated with the production of QS signals
63   (18, 19). Here, we analyse how signal-negative (*lasI-*) mutants of the opportunistic pathogen
64   *Pseudomonas aeruginosa* socially interact with wild-type cells in an environment where growth
65   requires the cells to have a functional QS system, but where the fitness benefits of QS are
66   'private' to individual cells. *P. aeruginosa* regulates the production of many virulence factors
67   through two *N*-acyl homoserine lactone (AHL) based QS systems. These systems, termed the *las*
68   and *rhl* systems, produce and respond to the signals *N*-(3-oxododecanoyl)-L-homoserine lactone
69   (3O-C12-HSL) and *N*-butanoyl-L-homoserine lactone (C4-HSL) respectively (1, 20). We
70   conducted our experiments in a growth medium containing adenosine as a carbon source.
71   Adenosine is deaminated to form inosine, which is degraded inside the cell by a nucleoside
72   hydrolase (Nuh) to hypoxanthine plus ribose; hypoxanthine is then metabolised to produce
73   glyoxylate plus urea (21). QS is crucial for growth in this medium because the *las* system
74   (through the regulator LasR), positively regulates Nuh. Because Nuh acts intracellularly, any loss
75   of fitness due to mutation of the signal gene *lasI* will be directly due to the lack of signal – not to
76   any downstream effect on the production of extracellular enzymes. We demonstrate that, when
77   provided with adenosine as a carbon source, *lasI-* mutants act as cheats: they grow poorly in
78   monoculture but have a higher relative fitness than the signal-producing wild type in mixed
79   cultures. In contrast, *lasR* mutants, which cannot regulate Nuh in the presence of signal, do not

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80   gain any fitness benefits in mixed culture with wild type cells.

81

82   In contrast to experiments performed in well-mixed liquid medium in test tubes, interactions
83   between bacterial cells in natural environments (including infections) are affected by spatial
84   assortment and structuring (22-24). This affects how behaviours evolve (25-29). We tested how
85   simple spatial structuring, through the addition of agar to the growth medium, alters QS signal
86   diffusion and the social dynamics of wild-type and *lasI* cells. Consistent with work on other
87   bacterial public goods (27), we demonstrate that the ability of lasI- mutants to cheat is
88   significantly reduced in structured populations. These results have implications for understanding
89   how and why bacterial signaling evolves, and the likely evolutionary fate of different types of
90   QS mutant in varied environmental conditions (30).

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92

93   **Materials and Methods**

94

95   **Bacterial strains.** The strains used were the wild type *Pseudomonas aeruginosa* laboratory
96   strain PAO1 and isogenic mutants created via insertion of a gentamicin resistance gene in the QS
97   genes *lasI* (PAO1 *lasI*::Gm; referred to as *lasI*- (31)) or *lasR* (PAO1 *lasR*::Gm; referred to as
98   *lasR*- (16)). To test 3O-C12-HSL diffusion in different media, we used a reporter strain of the
99   *lasI*- mutant. This contains a chromosomal *luxABCDE* fusion to the promoter of the *lasB* gene,
100   which encodes the QS-dependent protease LasB (PAO1 p*lasB::lux* (31)).

101

102   **Growth conditions.** Quorum sensing medium (QSM) was modified from two previous studies
103   (5, 32). QSM consisted of M9 Minimal Salts supplemented with 6.8 g/L Na_2HPO_4, 3 g/L
104   KH_2PO_4, 0.5 g/L NaCl. 10μM NH_4Cl, 0.1 μM $CaCl_2$ and 1 μM $MgSO_4$. QSM was supplemented
105   with 0.1 % w/v of carbon sources as a mix of Casamino acids (CAA) and adenosine and the
106   medium was filter sterilized. The exact ratio of CAA and adenosine was varied as detailed in the
107   Results section. Liquid culture experiments were conducted in 24-well plates with a volume of 2
108   mL of media. Cultures were incubated overnight in LB medium at 37°C on an orbital shaker and
109   standardised to an OD_{600} of 0.8-0.9; 2 μL of pure or mixed inoculum was added to each
110   experimental culture. The starting frequency of the mutant was determined by diluting and
111   plating the starter cultures to determine the number of colony-forming units (CFU) of each

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112   genotype. Experimental cultures in QSM were incubated at 37°C with orbital shaking for 24h or

113   48 h. After that time, cultures were diluted and replica plated on LB and LB + gentamycin (25

114   μg/ml) agar to enumerate the CFU of PAO1 and *lasI* or *lasR* mutants in mixed culture.

115   Experiments in solid media were conducted in QSM + 2% w/v agar in 1 ml volumes in 48-well

116   plates. Inoculation and culture conditions were otherwise identical to experiments in liquid

117   medium. To break up agar prior to dilution and plating, the solid 1 ml agar cubes were retrieved

118   from the plate and divided into thirds with a sterile metal spatula; each third was placed in a

119   screw-cap tube containing 500 μl phosphate-buffered saline and 6 metal beads (Cambio) and

120   homogenised using a FastPrep-24 5G bead beater (MP Biomedicals).

121

122   **Measures of signal concentration.** To measure the concentration of QS signal (*N*-3-oxo-

123   dodecanoyl-L-homoserine lactone; 3O-C12-HSL) present in 48 h cultures, 100 μl of each culture

124   supernatant was mixed with 100 μl of a log phase culture of a luminescent *Escherichia coli*

125   bioreporter (pSB1075; (33)) in the wells of a 96-well plate. This mixture was incubated for 4 h in

126   a Tecan multimode plate reader and luminescence and OD_{600} recorded at 15-min intervals. To

127   estimate 3O-C12-HSL concentration, the luminescence of experimental samples was compared

128   against a calibration curve constructed using QSM supplemented with known concentrations of

129   purified 3O-C12-HSL.

130

131   **Assaying the effect of agar on QS signal diffusion**. Agar has been successfully used to retard

132   the diffusion of other bacterial exoproducts (27). To verify that agar affects 3O-C12-HSL

133   diffusion in QSM, and to determine the optimal agar concentration to use in further experiments,

134   we devised a "sandwich experiment" in which a population of bacteria that switch on a

135   luminescent reporter gene in response to QS signal, but which cannot themselves produce signal,

136   were separated from a reservoir of purified signal by a layer of agar-supplemented medium. By

137   measuring the time to expression of the luminescent reported, we can assess the extent to which

138   the agar barrier delays diffusion of the signal from the reservoir to the reporter population. 0.1

139   ml LB supplemented with 0.5% w/v agar and containing 0.5 μM purified 3O-C12-HSL was

140   added to the wells of a 48-well plate and allowed to solidify. A second layer of 0.8 ml LB

141   supplemented with 1, 2, 3 or 4% w/v agar was then added on top of the signal-containing layer.

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142   Each agar concentration was replicated in 6 wells. This layer was allowed to solidify and a final

143   layer of LB containing 0.5% w/v agar and the reporter PAO1 *lasI⁻* p*lasB*::*lux* (overnight culture

144   at OD_{600} of 0.2) added. The plate was incubated in a Tecan multimode reader for 8 h and

145   luminescence read at 10 min intervals. As shown in Figure S1, increasing agar concentration

146   progressively delayed and reduced expression of luminescence. In order to check if higher

147   luminescence was due to increased bacterial numbers, bacteria were retrieved and CFU were

148   counted by plating. Median CFU was similar when 1 % or 2% agar was used (approx. 1.410^7),

149   but decreased by 30% when more agar was added (to approx. 110^7). It was difficult to determine

150   whether this was due to agar retarding growth at high concentrations, or simply due to the

151   increased difficulty of thoroughly homogenising media rich in agar. 1% agar was therefore

152   chosen for use in further experiments.

153

154   **Statistical analysis**. Relative fitness of mutants, *v*, was calculated as $x_2(1\text{-}x_1) / x_1(1\text{-}x_2)$, where x_1

155   is the starting frequency of the mutant and x_2 is the end frequency. It follows from the definition

156   that a relative fitness <1 signifies a decrease in mutant frequency, while a relative fitness >1

157   signifies an increase in mutant frequency. Statistical analysis of the results was conducted in R

158   3.2.3 (34) using generalized linear models assuming an underlying gamma distribution, with

159   adenosine treated as a continuous variable and block and treatment (liquid/solid medium) fitted

160   as factors. Raw data for all analyses reported is supplied as Supplemental Material (Data S1).

161

162   **Results**

163

164   *las* **mutants grow poorly in an environment where QS is required for growth**. Previous work

165   has shown that *las* mutants grow poorly in media where QS is required for growth (5, 7, 35). We

166   first confirmed that both *lasI⁻* and *lasR⁻* mutants were reduced in fitness in the specific medium

167   we chose for our experiments. We grew PAO1 and each mutant in a minimal medium base

168   containing 0.1% w/v carbon source. The carbon source was composed of casamino acids (CAA,

169   available for use by all cells, regardless of genotype) and adenosine (which can only be

170   metabolised when QS is functional in cells), in varying ratios. As the relative amount of

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171   adenosine increased and cells were increasingly dependent on QS, the total cell density after 48 h

172   was reduced, and this effect was more pronounced in *lasI* monocultures than in wild-type

173   monocultures (Figure 1, CFU pure adenosine of CFU pure CAA: 4.6% for wild type, 0.1-0.2%

174   for *las* mutants). When all the available carbon was supplied as adenosine, *lasI⁻* monocultures

175   grew to approximately 7% of the density of wild-type monocultures.

176

177   **Signal-negative *lasI⁻* mutants act as social cheats in adenosine-based growth medium, but**

178   **signal-blind *lasR⁻* mutant do not.** We next tested whether adding purified 3O-C12-HSL, or co-

179   culturing with signal-producing wild-type bacteria, could restore the growth of *lasI⁻* mutants. We

180   calculated the fitness of *lasI⁻* mutants relative to the wild type (i) in pure culture with or without

181   exogenous 3O-C12-HSL, and (ii) in 1:1 co-culture with wild-type PAO1. Experiments were

182   conducted in quorum-sensing media with varying ratios of CAA and adenosine as above. A

183   relative fitness of 1 signifies similar growth of mutant and wild type bacteria, while values <1

184   reflect relatively poorer growth of the mutant and values >1 reflect better growth of the mutant.

185   Figure 2 shows fitted models describing how the relative fitness of *lasI⁻* (a,b) and *lasR⁻* (c,d)

186   mutants are affected by culture conditions. The raw data to which the models were fitted are

187   plotted in Figure S2.

188

189   Pure *lasI⁻* cultures became progressively less fit than the wild type as access to carbon depended

190   more on quorum sensing (negative correlation between relative fitness and % adenosine:

191   coefficient -21.6, *p* < 0.001). However, when 10 μM exogenous 3O-C12-HSL was supplied,

192   *lasI⁻* mutants surpassed the wild type in growth (positive correlation between relative fitness and

193   % adenosine: coefficient 8,74, *p* = 0.003; Figure 2a, S2a) This result is consistent with previous

194   work demonstrating a cost to 3O-C12 production (18). As predicted, this ability of *lasI⁻* mutants

195   to use exogenous signal, combined with the cost of signal production to the wild type, means that

196   *lasI⁻* mutants grown in co-culture with wild type bacteria act as social cheats: the average relative

197   fitness was consistently > 1 and did not decline as % adenosine increased (coefficient 1.8, *p* =

198   0.88 Figure 2b, S2a). There was, however, a slight drop in relative fitness when all carbon was

199   supplied as adenosine (Figure S2a. This is most likely attributable to the wild-type bacteria

200   growing more slowly and taking longer to fully switch on QS responses. Both wild-type growth

201   (Figure 1) and the pool of available signal (Figure S3) are reduced in this condition, leaving less

202   opportunity for exploitation by cheats.

203

204 Post-hoc comparisons confirmed that *lasI* relative fitness was significantly increased in mixed
205 populations vs. pure cultures in all media containing adenosine (*t*-tests, *p* < 0.01). When all
206 carbon was supplied as CAA and signal is not required, there was no significant effect of pure vs.
207 mixed culture on fitness (*p* > 0.95) Taken together with the fact that mixed cultures grew to a
208 lower density than the wild-type cultures (Figure S5), these results indicate that *lasI* mutants
209 have an increased fitness when grown in the presence of wild-type bacteria under conditions
210 requiring social interaction, while in turn decreasing wild-type fitness.

211

212 To ensure that the effect described above was due to the social dynamics of signal production,
213 and not to the well-documented social dynamics of downstream exoproducts, this experiment
214 was repeated using a *lasR⁻* mutant. *lasR⁻* mutants are unable to respond to 3O-C12-HSL and
215 should therefore not be able to derive fitness benefits from exogenous signal in our setup. We
216 found the same negative correlations between % adenosine and CFU (Figure 1) and % adenosine
217 and fitness relative to the wild type (Figure 2c, S2b, coefficient -12.8, *p* = 0.001) as with the *lasI*
218 mutant for *lasR⁻* monocultures. Crucially, *lasR⁻* relative fitness was not rescued by adding 3O-
219 C12-HSL or by co-culturing with the wild type (coefficient -10.7, *p* < 0.001; Figure 2c,d and
220 S2b): i.e. these mutants could not exploit wild type bacteria.

221

222 **Slowing signal diffusion reduces *lasI* mutant cheating.** As a last step, we tested whether
223 impeding the diffusion of signal molecules would make the *lasI* mutant less effective as a cheat.
224 Reduced diffusion was achieved by adding agar to solidify the growth medium(Figure S1). *lasI*
225 monocultures showed comparable declines in fitness in liquid and solid medium (Figure 3a, S4a.
226 ANOVA: liquid/solid $F_{(1,124)}$ = 6.13, *p* = 0.01; adenosine $F_{(1,123)}$ = 117.58, *p* < 0.001; interaction
227 $F_{(1,122)}$ = 0.1658, *p* = 0.68). In mixed cultures, the relative fitness of the *lasI* mutant was
228 positively correlated with the percentage of carbon available as adenosine in liquid cultures, as
229 expected under cheating: but when the medium was solidified, *lasI* relative fitness actually
230 showed a modest negative correlation with percentage adenosine (Figure 3b, S4b. ANOVA:
231 liquid/solid $F_{(1,123)}$ = 11.1324, *p* = 0.001; adenosine $F_{(1,124)}$ = 0.0236, *p* = 0.88; interaction
232 $F_{(1,122)}$ = 0.9760, *p* = 0.33). This demonstrates that there is less opportunity for cheating in an
233 environment where signal molecules cannot diffuse freely.

234

235 **Discussion**

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236

237   While there has been some discussion of QS signals as public goods (e.g. (36)), most published
238   work on the social evolution of QS focuses on signal-blind mutants and the benefits of cheating
239   on the production of QS-regulated exoproducts(5, 7, 14, 32). Here we provide the first direct
240   evidence that, in addition to regulating the production of public goods, QS signal molecules are
241   themselves capable of acting as public goods. Social cheating in the context of QS can therefore
242   take multiple forms, depending on the environmental circumstances in which bacteria find
243   themselves. Previous research had shown that (a) signal-blind mutants can be cheats when
244   growth depends on the production of QS-dependent extracellular enzymes; and (b) cheating by
245   signal-blind mutants can be constrained when some 'private' QS-controlled processes contribute
246   to growth (35). Following recent confirmation that QS signals are costly to make (18), we now
247   add two new perspectives to the social evolution of QS: (c) that signal-negative mutants can be
248   cheats when growth depends entirely on private goods, regardless of any downstream effects on
249   exoenzyme production; and (d) that this signal cheating can only occur when the environment
250   permits sufficient diffusion of signal molecules.

251

252   A *lasI⁻* mutant grew poorly in monoculture, but growth could be rescued by adding either
253   purified 3O-C12-HSL signal or by co-culturing with wild-type, signal-producing bacteria. In co-
254   cultures, the fitness of *lasI⁻* mutants relative to the wild type increased as we forced the bacteria
255   to rely more on adenosine for carbon. As the population became more reliant on QS, *lasI* mutants
256   gained a greater fitness pay-off from exploiting costly, diffusible signal produced by the wild-
257   type. *lasR⁻* mutants did not gain a similar advantage from co-culture with the wild type in
258   adenosine medium. These signal-blind mutants can take up signals, but cannot respond to them
259   and so cannot switch on expression of the *nuh* hydrolase required for growth on adenosine (21,
260   35). The contrasting results for the two different QS mutants confirm that, in this environment,
261   the QS signal itself acts as a public good (Figure 4)

262

263   Adding agar to the growth medium lowered both the diffusion of signal molecules and the
264   relative fitness of cheats in mixed culture. Thus adding simple spatial structuring into our system
265   had a significant impact on the ability of signal-negative mutants to cheat on the wild type. There
266   was no effect of structuring on relative fitness in pure cultures: even though agar enhanced the
267   overall growth rate of the bacteria, the basic costs and benefits of signalling remained the same
268   in liquid and solid media. This final observation is consistent with work on other bacterial public

269 goods (27). We thus predict that the evolution of QS signalling strategies will be influenced by

270 population genetic and spatial structure, and that signal-negative cheats might only rise to

271 appreciable frequencies in environments where signals diffuse freely. For example, the thick,

272 adhesive mucus and bacterial biofilm polymers that block the airways of cystic fibrosis patients

273 with chronic lung infection may partially protect producers from cheating by signal-negative

274 mutants (24). Spatial dynamics play a huge role in the real-life ecology of environmentally and

275 clinically important microbial ecosystems, and are therefore of considerable interest to

276 microbiologists investigating the roles of bacteria in processes as diverse as geochemical cycling,

277 soil health, fouling and infection (29, 37, 38).

278

279 Our work opens up new avenues for exploring how, when and why bacterial signalling evolves

280 in different environments and why we find a variety of QS mutant genotypes and phenotypes in

281 natural environments (8-14, 39, 40). Given what we are now learning about the evolution of

282 traits such as QS and how spatial structure changes the evolutionary dynamics, we suggest that

283 there is a need to carefully consider the experimental design of *in vitro* experiments to increase

284 their relevance to actual infections (41). To be forewarned is to be forearmed: a more accurate

285 understanding of microbial ecology and evolution, gained from more realistic lab experiments,

286 will be a vital weapon in the fight against antibiotic resistant infection.

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289 **Acknowledgements**

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409   **69:**2371-2383.
410 40. **Feltner, J.B., Wolter, D.J., Pope, C.E., Groleau, M.-C., Smalley, N.E., Greenberg,**
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415   *vitro* experimentation in understanding biofilms and chronic infection. Journal of
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417

Figure legends

419

Figure 1. Population density (colony-forming units: CFU) after 48h of growth in quorum sensing medium with varying ratios of casamino acids:adenosine supplied to a total concentration of 0.1% w/v carbon source. Black dots: indvididual wild-type cultures; grey dots: indicate *lasI⁻* cultures*;* white dots: *lasR* cultures.

424

Figure 2. Results of fitting generalized linear models to relative fitness data from experiments with *lasI⁻* (a,b) and *lasR⁻* (c,d) mutants. Lines show fitted models, shaded areas denote standard deviation. Relative fitness is compared between pure cultures of each mutant with added 3O-C12-HSL or with a solvent-only control (a,c), and between pure cultures and mixed culture with wild-type bacteria (b,d). Raw data for these experiments are shown in Figure S2.

430

13

431   **Figure 3.** Comparison of *lasI⁻* relative fitness in pure culture (a) or mixed culture with the wild
432   type (b). Results of fitting generalized linear models to relative fitness data from experiments in
433   liquid *versus* agar-supplemented medium. Lines show fitted models, shaded areas denote
434   standard deviation. Raw data for these experiments are shown in Figure S4.

435

436   **Figure 4.** Schematic of the effects of LasIR quorum sensing on the production of intracellular
437   and extracellular enzymes by *Pseudomonas aeruginosa*.

438

439   **Figure S1**. Supplementing growth medium with agar retards diffusion of 3O-C12-HSL. (a)
440   Expression of a 3O-C12-HSL-dependent luminescent reporter construct is delayed and reduced
441   when signal-negative reporter bacteria are separated from a reservoir or purified signal by an
442   agar barrier. Lines and shading show means and s.d. of 15 replicates. (b) The experimental set-
443   up, as described in the Materials and Methods, is easily visualised by replicating the experiment
444   on a larger scale and adding red food colouring in place of bacterial signal.

445

446   **Figure S2**. Relative fitness of (a) *lasI⁻* mutants and (b) *lasR⁻* mutants when in pure culture (black
447   circles), grown in 1:1 mixture with wild type (grey circles) and in pure culture supplemented
448   with 10 μM 3O-C12-HSL (white circles).

449

450   **Figure S3.** Measurements of 3O-C12-HSL concentration in wild-type supernatant after 48h. (a)
451   The total concentration of 3O-C12-HSL declines with increasing adenosine. (b) After dividing
452   the signal concentration by the CFU of the producing culture, one observes the reverse trend –
453   concentration of 3O-C12-HSL per CFU increases with adenosine level and reaches its maximum
454   in pure adenosine.

455

456   **Figure S4.** Relative fitness of *lasI⁻* mutants in liquid medium (black circles) *versus* agar-
457   supplemented medium (grey circles), (a) in pure culture and (b) in mixed culture with the wild
458   type.

459

460   **Figure S5.** Population density (colony-forming units: CFU) after 48h of growth in quorum
461   sensing medium, Blue dots: indvidibual wild-type cultures; yellow dots: indicate *lasI* and wild-

14

462   type mixed cultures*; red dots: *lasI* cultures.

463

464   **Data S1.** Data for all analyses reported in the text will be made available on with the published
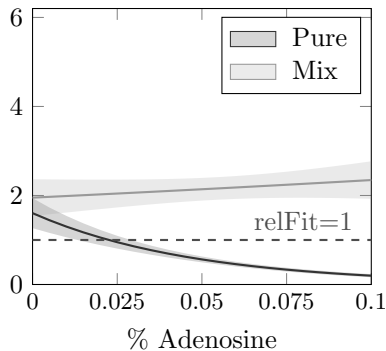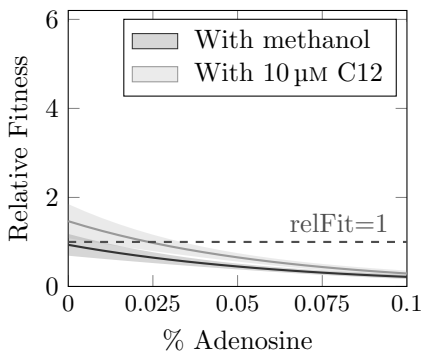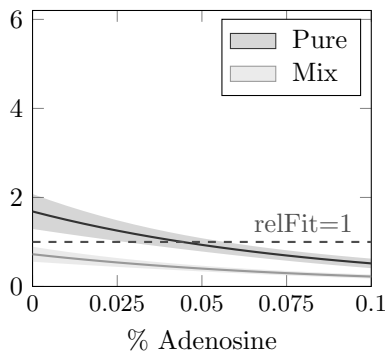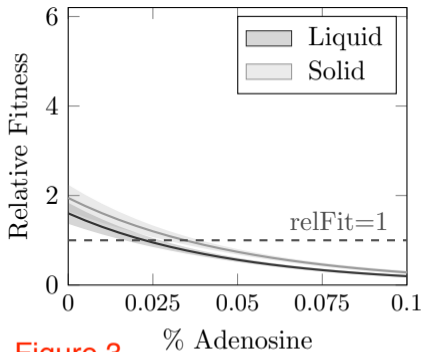
465   version of this article.

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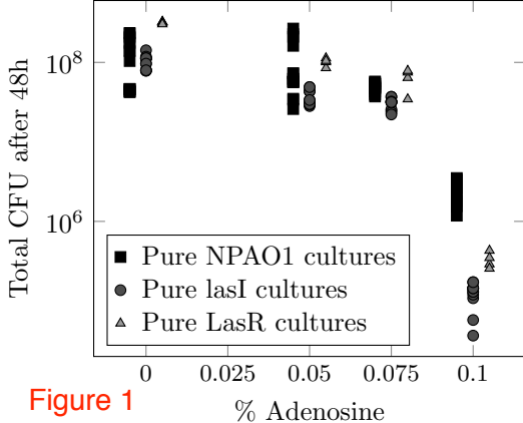
Figure 1
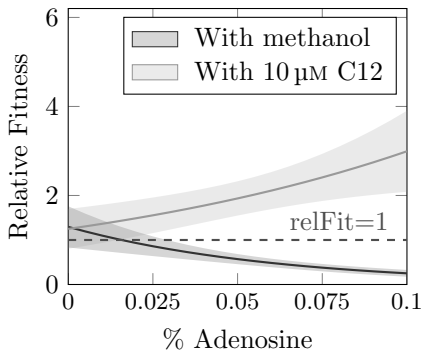
(a) lasI fitness with 10 μM C12
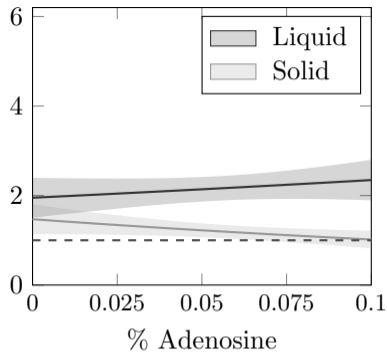
(b) lasI fitness in mixed/pure culture

(c) lasR fitness with 10 μM C12

(d) lasR fitness in mixed/pure culture

Figure 2

Figure 3

(a) Fitted model for relative fitness of pure cultures
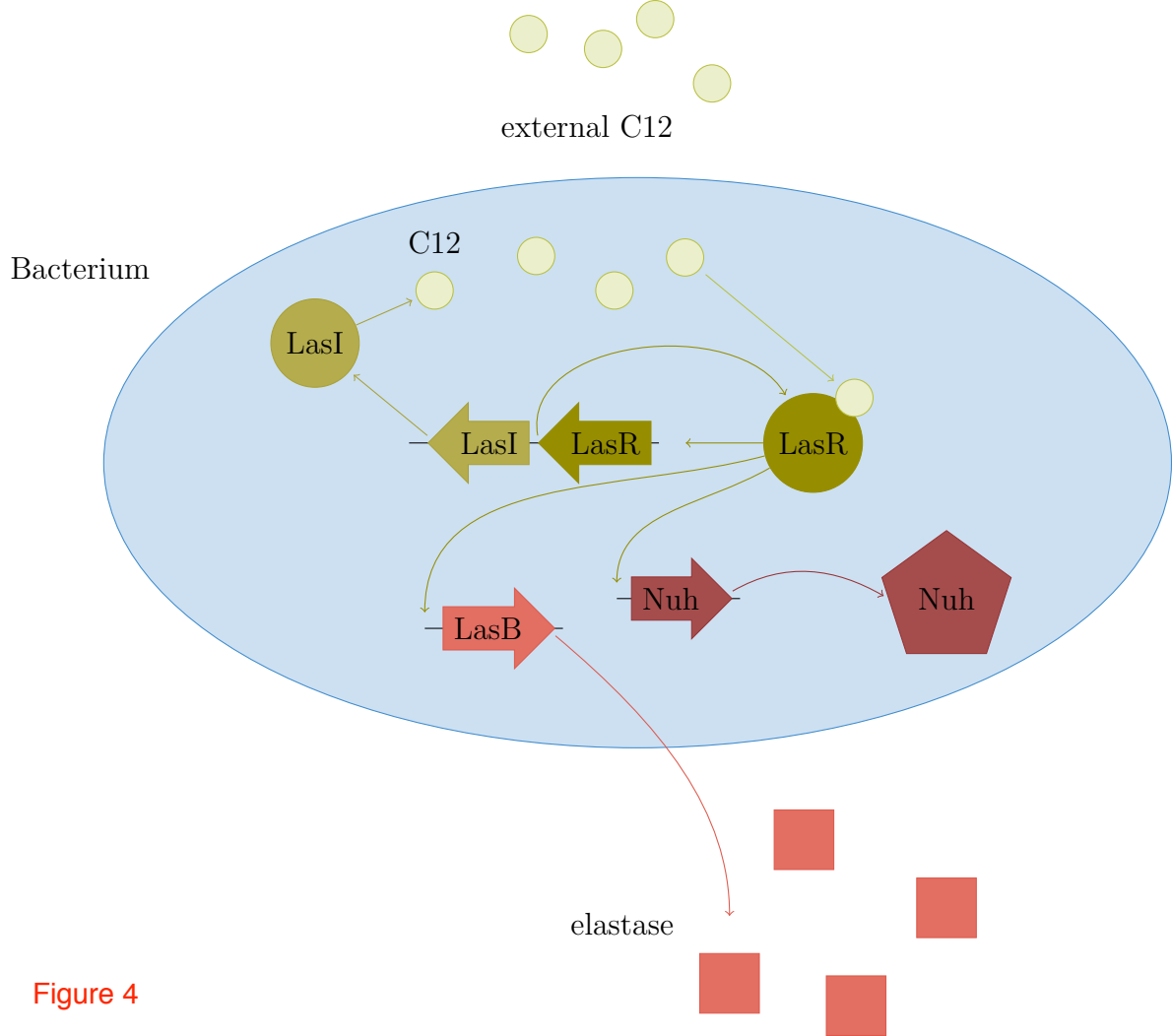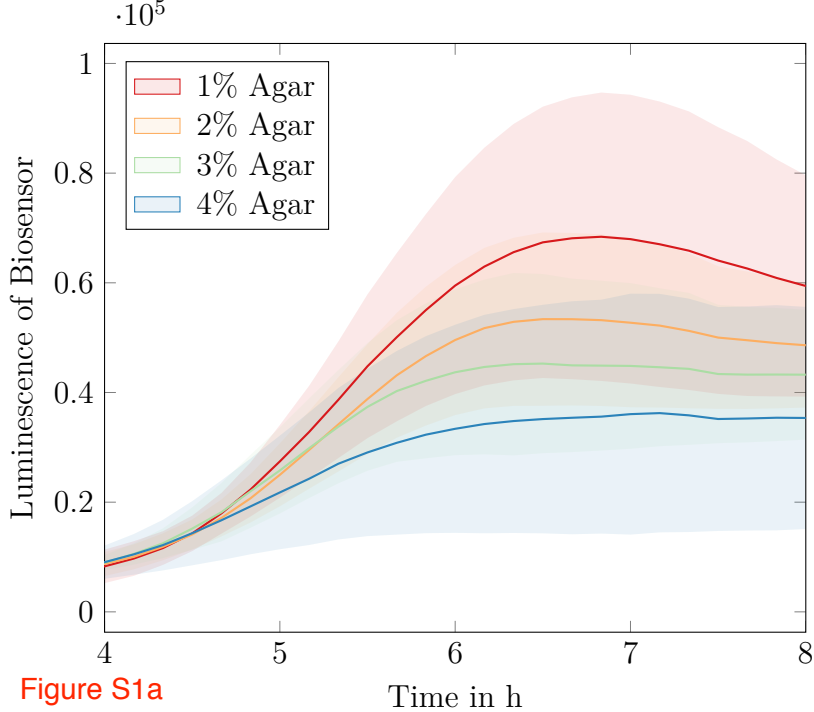
(b) Fitted model for relative fitness of mixed cultures

external C12

Bacterium

C12

LasI

LasI

LasR

LasR

Nuh

Nuh

LasB

elastase

Figure 4

Figure S1a

Figure S1b   time
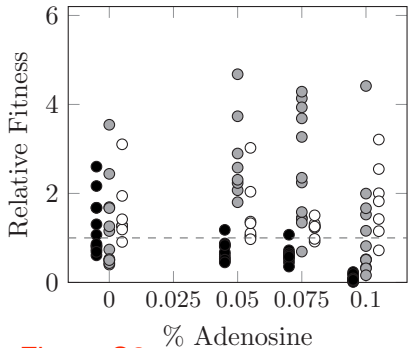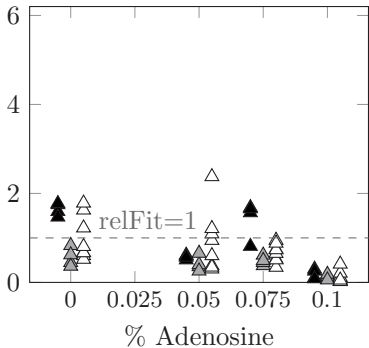
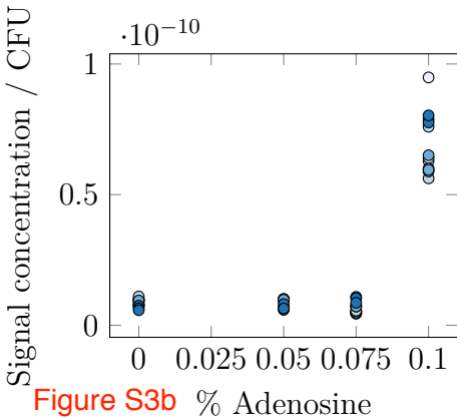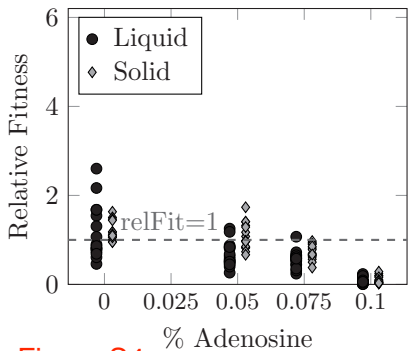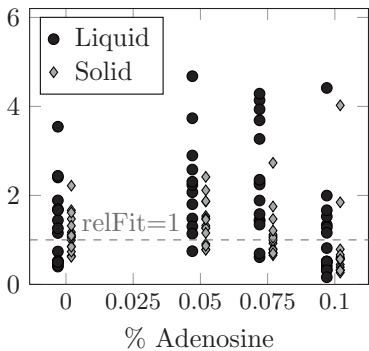(a) Relative fitness of lasI⁻   (b) Relative fitness of lasR⁻
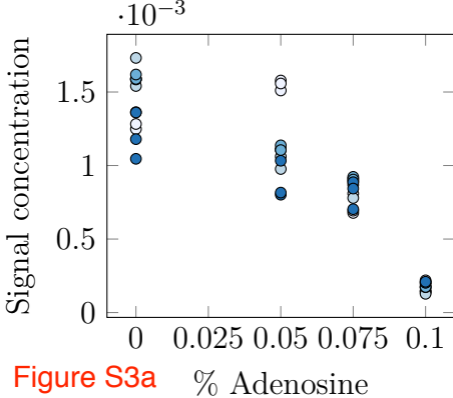
Figure S3a

Figure S3b

Figure S4

(a) Pure lasI⁻ cultures   (b) Mixed cultures

Figure S5