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Supplementary Materials for

Bacterial Quorum Sensing and Metabolic Incentives to Cooperate

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Materials and Methods:

Bacteria and growth conditions. We used the following *P. aeruginosa* strains: PAO1; Cl27 and CIG1, both cystic fibrosis clinical isolates; and the environmental isolates BE171, BE173, BE177, and PaE2 (*20, 21*). Quorum sensing mutants of these isolates have been described in (*15*). All experiments on the emergence or restraint of social cheaters were patterned after those described by Sandoz et al (*5*). We used a slightly different minimal medium (*22*) with carbon and energy sources added as indicated. For experiments with casein and adenosine as nitrogen sources we used M9 medium without (or with as a control) ammonium chloride. Briefly, to start an experiment, we used a starter culture grown in LB broth buffered with 50 mM MOPS, pH 6.8. The initial optical density was 0.01 at 600 nm. At 24 h intervals fresh medium was inoculated to a starting optical density of 0.01. We preserved day-25 samples from various experiments by freezing aliquots in an equal volume of 50% glycerol. We patched colonies onto skim milk and adenosine agar at 5-day intervals. These media have been described elsewhere (*5*).

Phenotypic profiling. We used a Phenotype MicroArray (PM) system (Biolog, Hayward, CA) for metabolic profiling according to the manufacturer's instructions to identify metabolic capabilities activated by addition of quorum sensing signals to quorum sensing-signal-generation mutants. Plates were incubated without shaking at 37^{o}C in an Omnilog instrument. Where indicated, cell suspensions used as inocula for the PM analysis were supplemented with 2 μM of C12-HSL and 10 μM C4-HSL. Respiration-dependent color changes in each well of the microtiter plate were measured at 15-min intervals for 48 h. Construction of the quorum sensing mutants is described elsewhere (*15*).

DNA sequencing. We grew cells overnight in 5 ml of LB broth, collected cells by centrifugation and extracted cellular DNA by using a Puregene cell extraction kit (Gentra). To sequence *lasR* we used primers described elsewhere to amplify full length *lasR* and flanking regions (1557669 - 1558902) (*13*).

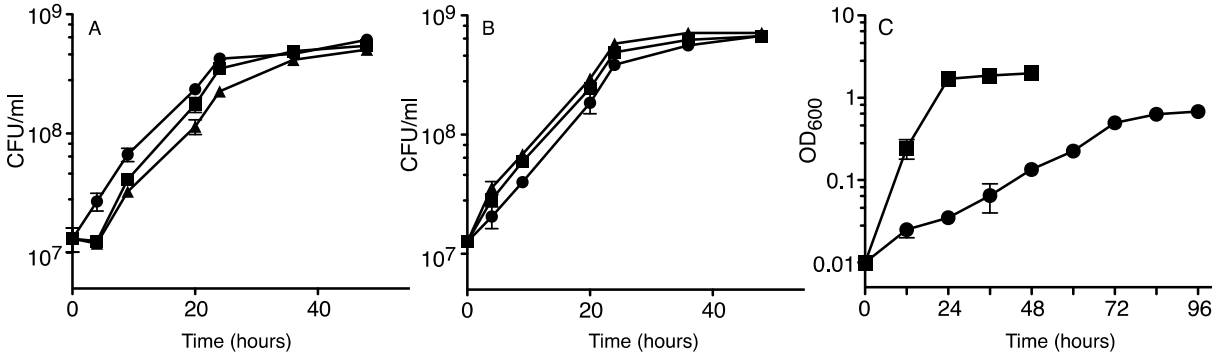

Figure S1. Growth curves of *P. aeruginosa* PAO1 stock culture or *P. aeruginosa* after 25 days of transfer and growth in different media. In all cases growth was logarithmic for at least 24 h. (A) *P. aeruginosa* PAO1 (from stock culture) grown in 1% casein (circles), 0.5% adenosine plus 0.5% casein (squares), and 0.25% casein plus 0.75% adenosine (triangles). (B) *P. aeruginosa* from a day-25 culture grown in 0.25% casein plus 0.75% adenosine (the symbols are the same as in panel A). (C) *P. aeruginosa* PAO1 from stock culture (circles) and an isolate from a day-25 0.25% casein-0.75% adenosine culture (squares) grown in 1% adenosine (without casein).

Table S1. Growth of *Pseudomonas aeruginosa* MW1 (*LasI*$^-$*RhlI*$^-$) with or without added signal in the presence of 380 nitrogen sources.

Nitrogen sources that do not support growth with or without added signal
D-Ala-Gly-Gly, D,L-α-Amino-Caprylic acid, D,L-α-Amino-N-Butyric acid, e-Amino-*N*-Caproic acid, *N*-Amylamine, D-Aspartic acid, Biuret, *N*-Butylamine, L-Cysteine, Ethylamine, Ethylenediamine, D-Galactosamine, γ-D-Glu-Gly, D-Glucosamine, Gly-D-Asp, Gly-D-Thr, Gly-D-Val, Gly-Gly-D-Leu, L-Homoserine, Hydroxylamine, Ile-Met, Ile-Trp, Leu-D-Leu, D-Leu-D-Leu, D-Leu-Gly, Leu-Leu-Leu, D-Leu-Tyr, D-Mannosamine, Met-Glu, Met-Ile, Met-Leu, Met-Met, Met-Phe, Methylamine, Phe-Trp, Phe-Tyr, Pro-Hyp, Pro-Trp, Thymidine, Trp-Asp, Trp-Glu, Trp-Phe, Tyr-Ile, Val-Tyr-Val

Nitrogen sources that support growth only in the absence of added signal
Gly-D-Ser

Nitrogen sources that support growth only in the presence of added signal
Glu-Tyr, His-Trp, Tyr-Gly-Gly, Tyr-Phe, Tyr-Trp, Tyr-Tyr

Nitrogen sources that support growth in the presence or absence of signal
Acetamide, N-Acetyl-D-Galactosamine, N-Acetyl-D-Glucosamine, N-Acetyl-D-Mannosamine, N-Acetyl-L-Glutamic acid, Adenine, Adenosine, Agmatine, Ala-Ala, Ala-Ala-Ala, Ala-Arg, Ala-Asn, Ala-Asp, Ala-Asp, D-Ala-D-Ala, Ala-Gln, Ala-Gln, Ala-Glu, Ala-Glu, D-Ala-Gly, Ala-Gly, Ala-Gly, β-Ala-His, Ala-His, Ala-His, Ala-Ile, D-Ala-Leu, Ala-Leu, Ala-Leu, Ala-Lys, Ala-Met, Ala-Phe, Ala-Pro, Ala-Ser, Ala-Thr, Ala-Thr, Ala-Trp, Ala-Tyr, Ala-Val, L-Alanine, Allantoin, Alloxan, γ-Amino-N-Butyric acid, α-Amino-N-Valeric acid, d-Amino-N-Valeric acid, Ammonia, Arg-Ala, Arg-Arg, Arg-Asp, Arg-Gln, Arg-Glu, Arg-Ile, Arg-Leu, Arg-Lys, Arg-Met, Arg-Phe, Arg-Ser, Arg-Trp, Arg-Tyr, Arg-Val, L-Arginine, Asn-Glu, Asn-Val, Asp-Ala, Asp-Asp, Asp-Gln, Asp-Glu, Asp-Gly, Asp-Leu, Asp-Lys, Asp-Phe, Asp-Trp, Asp-Val, D-Asparagine, L-Asparagine, L-Aspartic acid, β-Ala-Ala, β-Ala-Gly, β-Ala-Phe, L-Citrulline, Cys-Gly, Cytidine, Cytosine, D-Alanine, Ethanolamine, Formamide, Gln-Gln, Gln-Glu, Gln-Gly, Glu-Ala, Glu-Asp, Glu-Glu, γ-Glu-Gly, γ-Glu-Gly, Glu-Gly, Glu-Ser, Glu-Trp, Glu-Val, Glucuronamide, D-Glutamic acid, L-Glutamic acid, L-Glutamine, L-Glutamine, L-Glutamine, L-Glutamine, Gly-Ala, Gly-Arg, Gly-Asn, Gly-Asn, Gly-Asp, Gly-Cys, Gly-D-Ala, Gly-Gln, Gly-Glu, Gly-Gly, Gly-Gly-Ala, Gly-Gly-Gly, Gly-Gly-Ile, Gly-Gly-Leu, Gly-Gly-Phe, Gly-His, Gly-Ile, Gly-Leu, Gly-Lys, Gly-Met, Gly-Met, Gly-Phe, Gly-Phe-Phe, Gly-Pro, Gly-Ser, Gly-Thr, Gly-Trp, Gly-Tyr, Gly-Val, Glycine, Guanine, Guanosine, His-Ala, His-Asp, His-Glu, His-Gly, His-His, His-Leu, His-Lys, His-Met, His-Pro, His-Ser, His-Tyr, His-Val, Histamine, L-Histidine, Ile-Ala, Ile-Arg, Ile-Asn, Ile-Gln, Ile-Gly, Ile-His, Ile-Ile, Ile-Leu, Ile-Phe, Ile-Pro, Ile-Ser, Ile-Tyr, Ile-Val, Inosine, L-Isoleucine, D,L-Lactamide, Leu-Ala, Leu-Arg, Leu-Asn, Leu-Asp, Leu-β-Ala, Leu-Glu, Leu-Gly, Leu-Gly-Gly, Leu-His, Leu-Ile, Leu-Leu, Leu-Met, Leu-Phe, Leu-Pro, Leu-Ser, Leu-Trp, Leu-Tyr, Leu-Val, L-Leucine, Lys-Ala, Lys-Arg, Lys-Asp, Lys-Glu, Lys-Gly, Lys-Ile, Lys-Leu, Lys-Lys, Lys-Met, Lys-Phe, Lys-Pro, Lys-Ser, Lys-Thr, Lys-Trp, Lys-Tyr, Lys-Val, D-Lysine, L-Lysine, Met-Ala, Met-Arg, Met-Asp, Met-β-Ala, Met-Gln, Met-Gly, Met-His, Met-Lys, Met-Pro, Met-Thr, Met-Trp, Met-Tyr, Met-Val, L-Methionine, Nitrate, Nitrite, L-Ornithine, Parabanic acid, Phe-Ala, Phe-Asp, Phe-β-Ala, Phe-Glu, Phe-Gly, Phe-Gly-Gly, Phe-Ile, Phe-Met, Phe-Phe, Phe-Pro, Phe-Ser, Phe-Val, L-Phenylalanine, β-Phenylethylamine, N-Phthaloyl-L-Glutamic acid, Pro-Ala, Pro-Arg, Pro-Asn, Pro-Asp, Pro-Gln, Pro-Glu, Pro-Gly, Pro-Leu, Pro-Ile, Pro-Lys, Pro-Phe, Pro-Pro, Pro-Ser, Pro-Tyr, Pro-Val, L-Proline, Putrescine, L-Pyroglutamic acid, Ser-Ala, Ser-Asn, Ser-Asp, Ser-Gln, Ser-Glu, Ser-Gly, Ser-His, Ser-Leu, Ser-Met, Ser-Phe, Ser-Pro, Ser-Ser, Ser-Tyr,

Ser-Val, D-Serine, L-Serine, Thr-Ala, Thr-Arg, Thr-Asp, Thr-Gln, Thr-Glu, Thr-Gly, Thr-Leu, Thr-Met, Thr-Phe, Thr-Pro, Thr-Ser, L-Threonine, Thymine, Trp-Ala, Trp-Arg, Trp-Gly, Trp-Leu, Trp-Lys, Trp-Ser, Trp-Trp, Trp-Tyr, Trp-Val, L-Tryptophan, Tyr-Ala, Tyr-Gln, Tyr-Glu, Tyr-Gly, Tyr-His, Tyr-Leu, Tyr-Lys, Tyr-Val, Tyramine, L-Tyrosine, Uracil, Urea, Uric acid, Uridine, Val-Ala, Val-Arg, Val-Asn, Val-Asp, Val-Gln, Val-Glu, Val-Gly, Val-His, Val-Ile, Val-Leu, Val-Lys, Val-Met, Val-Phe, Val-Pro, Val-Ser, Val-Tyr, Val-Val, D-Valine, L-Valine, Xanthine, Xanthosine

Table S2. Growth of *Pseudomonas aeruginosa* MW1 (*LasI-RhlI-*) with or without added signal in the presence of 190 carbon sources.

Carbon sources that do not support growth with or without added signal
Acetamide, Acetoacetic acid, N-Acetyl-D-Galactosamine, N-Acetyl-D-Glucosaminitol, N-Acetyl-D-Mannosamine, N-Acetyl-Neuraminic acid, Adenosine, Adonitol, Ala-Gly, L-Alaninamide, β-D-Allose, Amygdalin, D-Arabinose, L-Arabinose, D-Arabitol, L-Arabitol, Arbutin, D-Aspartic acid, sec-Butylamine, 2, 3-Butanediol, 2, 3-Butanone, Capric acid, D-Cellobiose, Chondroitin Sulfate C, Citraconic acid, α-Cyclodextrin, β-Cyclodextrin, γ-Cyclodextrin, 2-Deoxy-D-Ribose, 2`-Deoxyadenosine, Dextrin, Dihydroxyacetone, Dulcitol, i-Erythritol, Formic acid, D-Fructose-6-Phosphate, D-Fucose, L-Fucose, D-Galactonic acid-γ-Lactone, L-Galactonic acid-γ-Lactone, D-Galactose, 3-0-β-D-Galactopyranosyl-D-Arabinose, D-Galacturonic acid, Gelatin, Gentiobiose, D-Glucosaminic acid, D-Glucosamine, L-Glucose, D-Glucose-1-Phosphate, D-Glucose-6-Phosphate, D-Glucuronic acid, Glucuronamide, Gly-Glu, Gly-Asp, D,L-α-Glycerol Phosphate, Glycine, Glycogen, Glycolic acid, Glyoxylic acid, L-Homoserine, 2-Hydroxybenzoic acid, γ-Hydroxybutyric acid, 3-Hydroxy-2-butanone, α-Hydroxybutyric acid, α-Hydroxyglutaric acid-γ-Lactone, m-Hydroxyphenyl Acetic acid, m-Inositol, Inulin, α-Ketobutyric acid, 5-Keto-D-Gluconic acid, α-Keto-Valeric acid, D-Lactic acid Methyl Ester, D-Lactitol, α-D-Lactose, Lactulose, Laminarin, L-Lyxose, D-Malic acid, Maltitol, Maltose, Maltotriose, Mannan, D-Mannose, D-Melezitose, Melibionic acid, D-Melibiose, L-Methionine, α-Methyl-D-Galactoside, β-Methyl-D-Galactoside, α-Methyl-D-Glucoside, β-Methyl-D-Glucoside, β-Methyl-D-Glucuronic acid, 3-Methylglucose, α-Methyl-D-Mannoside, β-Methyl-D-Xyloside, Mucic acid, Oxalic acid, Oxalomalic acid, Palatinose, Pectin, Phenylethylamine, 1, 2-Propanediol, D-Raffinose, L-Rhamnose, D-Ribono-1, 4-Lactone, D-Ribose, D-Saccharic acid, Salicin, Sedoheptulosan, D-Serine, L-Serine, D-Sorbitol, Sorbic acid, L-Sorbose, Stachyose, Sucrose, D-Tagatose, D-Tartaric acid, L-Tartaric acid, m-Tartaric acid, Turanose, D-Threonine, Thymidine, Tricarballylic acid, Uridine, L-Valine, Xylitol, D-Xylose

Carbon sources that support growth only in the absence of added signal
Butyric acid, L-Lysine, L-Phenylalanine, Sebacic acid, L-Threonine

Carbon sources that support growth only in the presence of added signal
Inosine, D-Trehalose

Carbon sources that support growth in the presence or absence of signal
Acetic acid, N-Acetyl-D-Glucosamine, N-Acetyl-L-Glutamic acid, γ-Amino-N-Butyric acid, d-Amino Valeric acid, D-Alanine, L-Alanine, 2-Aminoethanol, L-Arginine, L-Asparagine, L-Aspartic acid, Bromosuccinic acid, Caproic acid, D,L-Carnitine, D,L-Citramalic acid, Citric acid, D-Fructose, Fumaric acid, Glycerol, α-D-Glucose, D-Gluconic acid, L-Glutamic acid, L-Glutamine, Gly-Pro, L-Histidine, 4-Hydroxybenzoic acid, β-Hydroxybutyric acid, p-Hydroxyphenyl Acetic acid, Hydroxy-L-Proline, L-Isoleucine, Itaconic acid, α-Ketoglutaric acid, L-Lactic acid, L-Leucine, L-Malic acid, D,L-Malic acid, Malonic acid, D-Mannitol, Methylpyruvate, Mono-Methylsuccinate, D,L-Octopamine, L-Ornithine, L-Proline, Propionic acid, D-Psicose, Putrescine, L-Pyroglutamic acid, Pyruvic acid, Quinic acid, Succinamic acid, Succinic acid, Tween 20, Tween 40, Tween 80, Tyramine

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