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Supplementary Materials for

Antithetic population response to antibiotics in a polybacterial community

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/10/eaaz5108/DC1)

Notebook S1 (.ipynb format). Code to reproduce the modeling results of Fig. 3.
Notebook S2 (.ipynb format). Code to reproduce the modeling results of Fig. 5.
Notebook S3 (.ipynb format). Code to reproduce the modeling results of fig. S6 (A and B).
Notebook S4 (.ipynb format). Code to reproduce the modeling results of fig. S6C.

Supplementary text

Modeling antibiotic pooling

The dynamics of two bacterial species growing together under the effect of a beta-lactam antibiotic can be described by the following model

$$\frac{dP_s}{dt} = (k_2 + \gamma_s)(P_{ts} - P_s) - k_1 P_s A_s \tag{11}$$
$$\frac{dP_c}{dt} = (k_2 + \gamma_c)(P_{tc} - P_c) - k_1 P_c A_c \tag{12}$$
$$\frac{dA_s}{dt} = k_2(P_{ts} - P_s) - k_1 P_s A_s + d_s(A_m - A_s) \tag{13}$$
$$\frac{dA_c}{dt} = k_2(P_{tc} - P_c) - k_1 P_c A_c + d_c(A_m - A_c) \tag{14}$$
$$\frac{dA_m}{dt} = D_s(A_s - A_m) + D_c(A_c - A_m) \tag{15}$$

where A_m represents the extracellular antiobiotic concentration and the subindices 's' and 'c' denote the two bacterial species. The coefficients d_i and D_i quantify the effect of the diffusion of the antibiotic through the cell membrane *(40)* on the intra- and extracellular antibiotic concentrations, respectively, for the two cell types.

Experimental measurements show that the intracellular and extracellular concentrations of beta-lactam antibiotics equilibrate in time scales of the order of, or smaller than, minutes *(32, 33)*. This allows us to eliminate adiabatically A_m, leading to a quasi-steady-state value of this variable

$$\frac{dA_m}{dt} \approx 0 \quad \Longrightarrow \quad A_m \approx \frac{D_s A_s + D_c A_c}{D_s + D_c} \tag{16}$$

Introducing the expression (16) into the intracellular antibiotic equations (13)-(14) leads to

$$\frac{dA_s}{dt} = k_2(P_{ts} - P_s) - k_1 P_s A_s + k_{as}(A_c - A_s) \tag{17}$$
$$\frac{dA_c}{dt} = k_2(P_{tc} - P_c) - k_1 P_c A_c + k_{ac}(A_s - A_c) \tag{18}$$

where $k_{as} \equiv d_s D_c/(D_s+D_c)$ and $k_{ac} \equiv d_c D_s/(D_s+D_c)$ are the effective diffusion coefficients. The coefficients d_s and d_c differ on the values of the cell volumes of the two species, which are similar to one another (see e.g. BNIDs 114921 and 114924 *(41)*). In turn, d_i and D_i differ on the cell density *(40)*. Since here we are only interested in the moment at which the population of either bacterial species begins to grow, we can assume that the cell densities of the two species (which were chosen identical initially) are the same throughout the period of interest. In those conditions, we can consider the two effective diffusion coefficients to be the same, $k_{as} = k_{ac} \equiv k_a$.

Supplementary figures

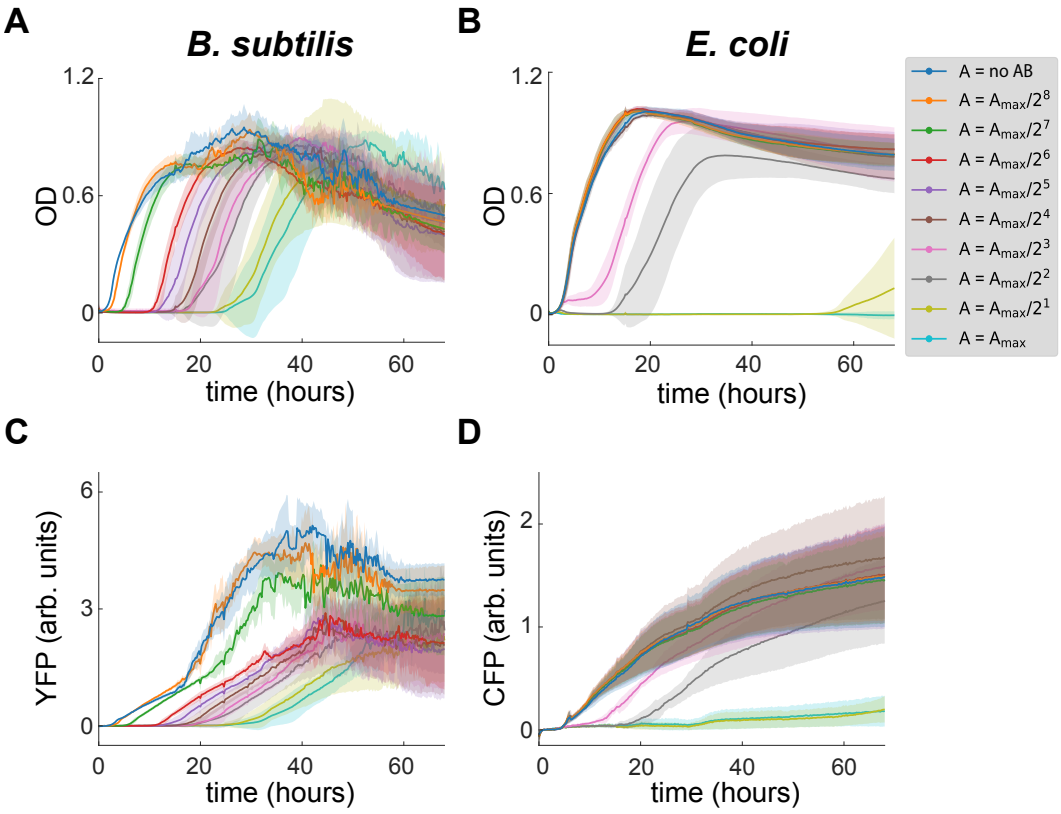

Fig. S1. Growth curves of monocultures of *B. subtilis* and *E. coli* in the presence of increasing concentrations of ampicillin. (**A** and **B**) OD as a function of time (hours) for *B. subtilis* (A) and *E. coli* (B) monocultures. Solid lines show the mean of eight independent measurements for each concentration. The shaded area corresponds to the standard error. The maximum antibiotic concentration is $A_{\max} = 50$ μg/ml in both cases. (**C** and **D**) YFP and CFP signals (arb. units) as a function of time for the corresponding growth curves in panels (A) and (B), respectively.

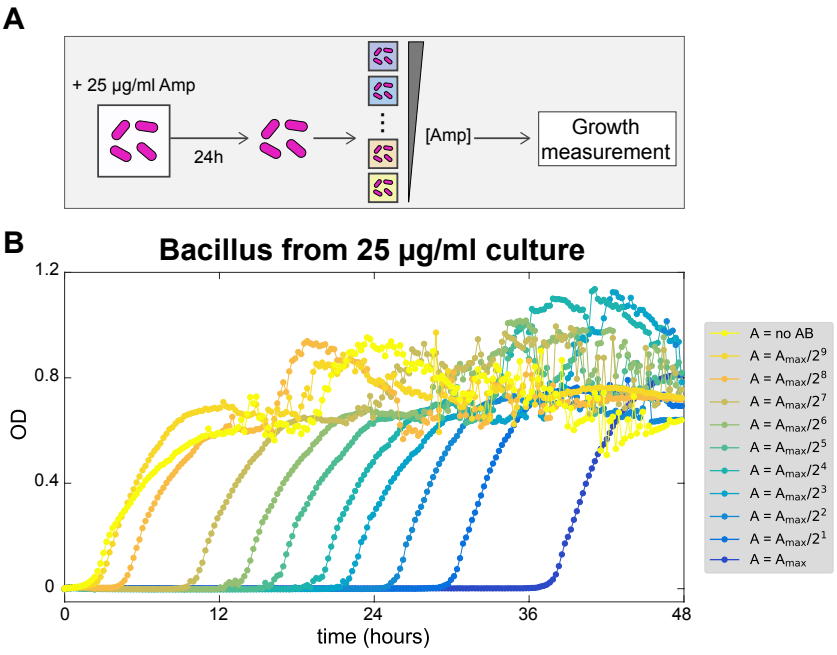

Fig. S2. ***B. subtilis*** **tolerance to ampicillin is not due to gain of resistance.** (**A**) Scheme of the experimental setup to test a possible gain of resistance of *B. subtilis* after growing in the presence of ampicillin. Cells grown 24 hours under 25 µg/ml of ampicillin were extracted from the culture and placed in different new media with increasing concentrations of ampicillin. The growth of the different cultures was then measured. (**B**) OD as a function of time (hours) for *B. subtilis* monocultures after 24 hours of growth in the presence of 25 µg/ml ampicillin, under increasing concentrations of the antibiotic.

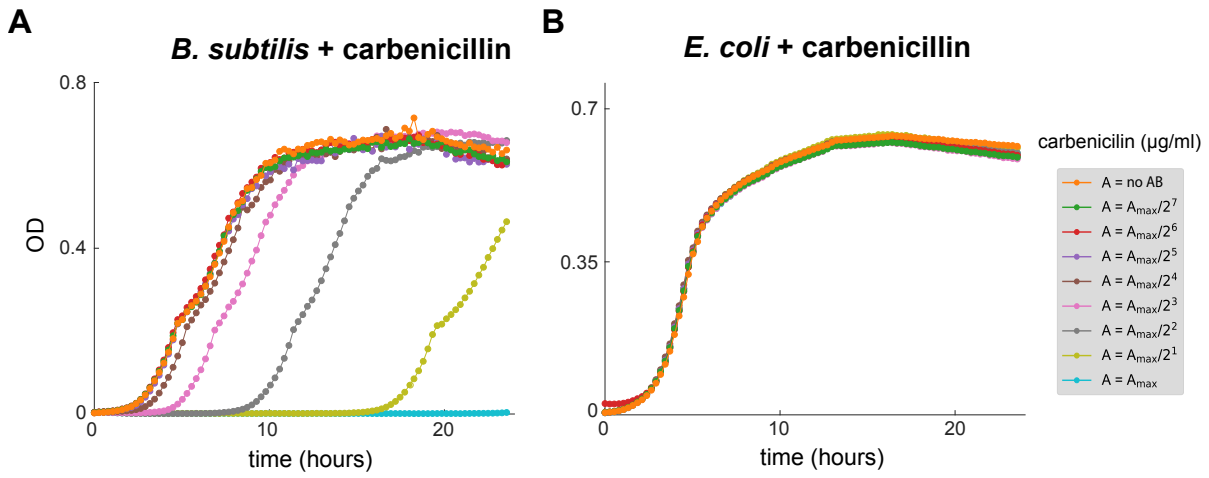

Fig. S3. Growth curves of monocultures of *B. subtilis* and *E. coli* in the presence of low concentrations of another β-lactam antibiotic. (**A** and **B**) OD as a function of time (hours) for *B. subtilis* and *E. coli* monocultures, under increasing concentrations of carbenicillin, respectively. The maximum antibiotic concentration used in both cases is 6.25 μg/ml.

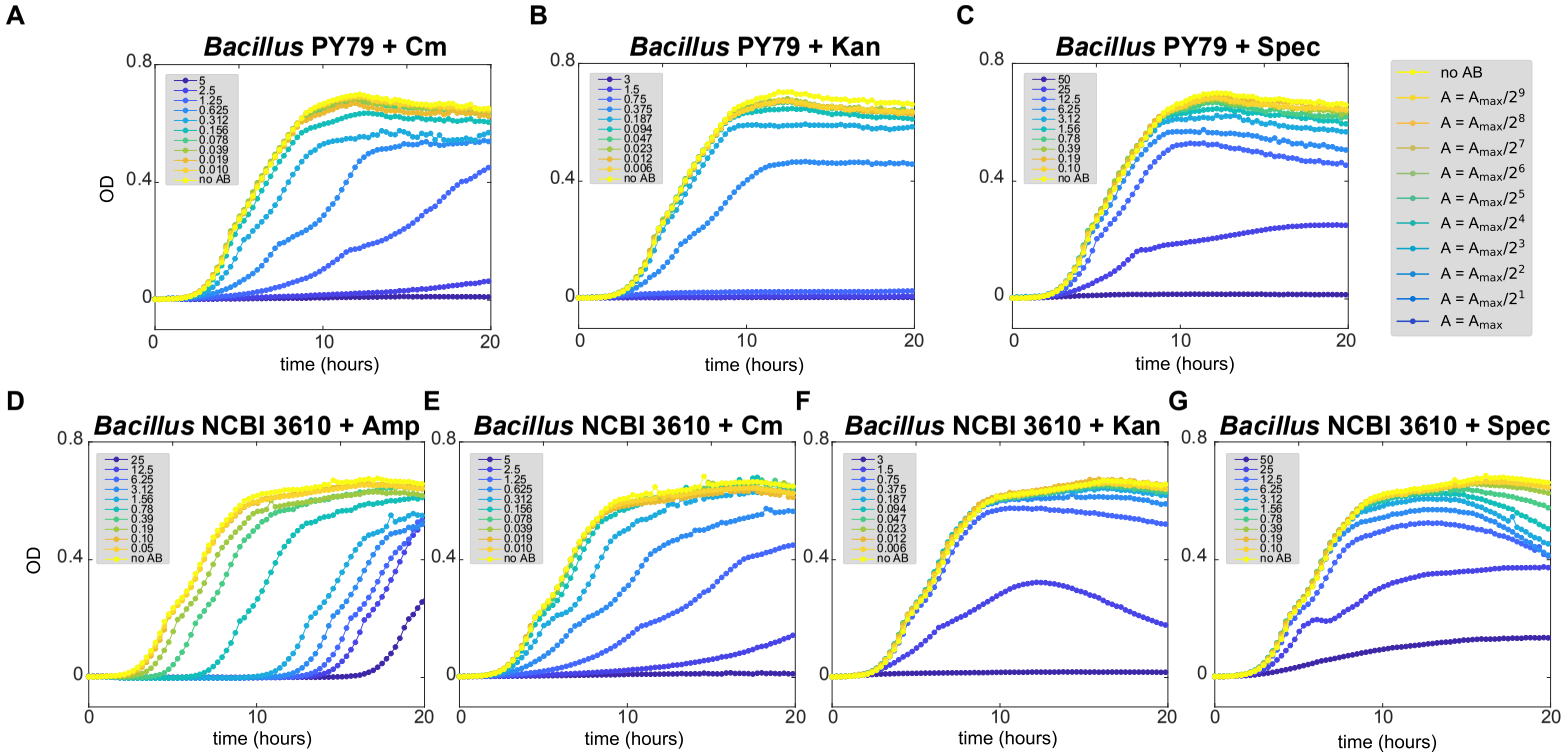

Fig. S4. Growth curves of monocultures of *B. subtilis* PY79 and NCBI 3610 in the presence of increasing concentrations of different antibiotics. (**A**,**B** and **C**) OD as a function of time (hours) for *B. subtilis* PY79 monocultures, under increasing concentrations of chloramphenicol, kanamycin and spectinomycin, respectively. The maximum antibiotic concentrations used for each condition are: 5 µg/ml chloramphenicol, 3 µg/ml kanamycin and 50 µg/ml spectinomycin. (**D**,**E**,**F** and **G**) OD as a function of time (hours) for B. subtilis NCBI 3610 monocultures, under increasing concentrations of ampicillin, chloramphenicol, kanamycin and spectinomycin, respectively. The maximum antibiotic concentrations used for each condition are: 25 µg/ml ampicillin, 5 µg/ml chloramphenicol, 3 µg/ml kanamycin and 50 µg/ml spectinomycin.

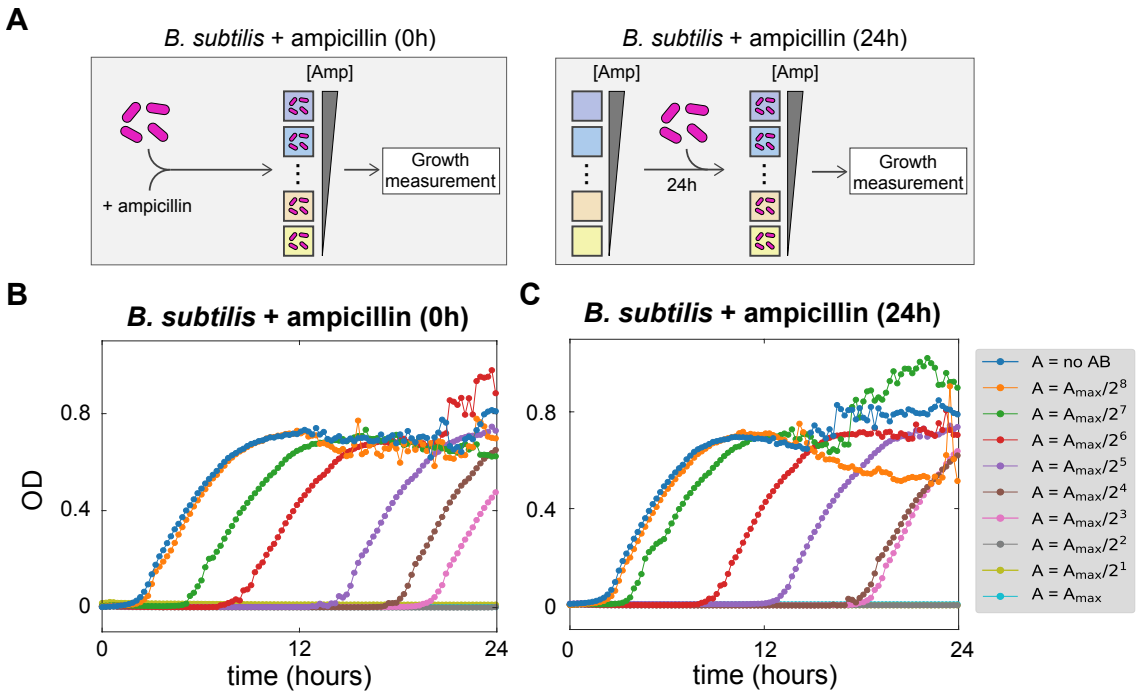

Fig. S5. Ampicillin is not degraded passively in the medium. (**A**) Scheme of the experimental setup to compare growth curves of *B. subtilis* monocultures in media supplemented with ampicillin at t = 0 hours (left) and ampicillin cultured for 24 hours (right). (**B**) OD as a function of time for *B. subtilis* monocultures, under increasing concentrations of ampicillin added at t = 0 h. (**C**) OD as a function of time for *B. subtilis* monocultures, under increasing concentrations of ampicillin, cultured previously for 24 hours.

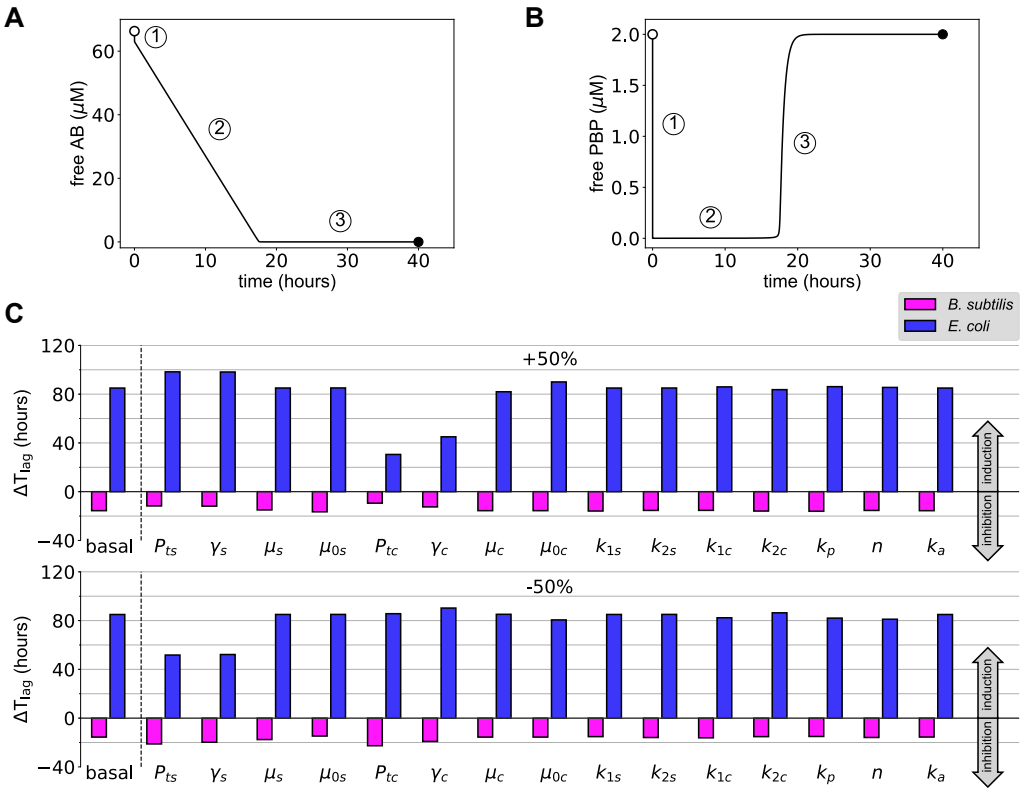

Fig. S6. Model dynamics and sensitivity analysis. Time traces of free antibiotic (**A**) and free PBP (**B**) corresponding to the trajectory shown in Fig. 3A. The three labels 1-3 correspond to the three portions of the trajectory indicated in Fig. 3A. (**C**) Time lag difference between the mixed species and the two isolated species, for a total antibiotic concentration $A_t = 100\,\mu M$, when the basal parameters (left bars in the two rows) are increased (top) and decreased (bottom) 50%.

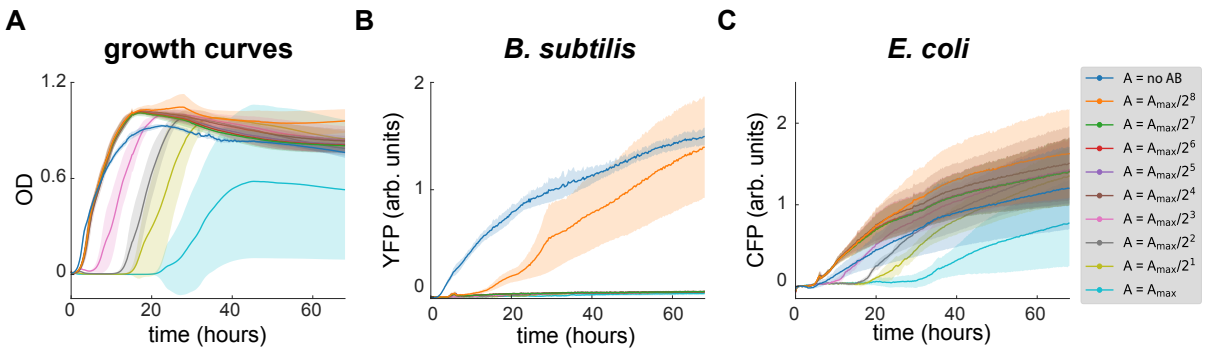

Fig. S7. Growth curves and fluorescent signal of mixed culture in the presence of increasing concentrations of ampicillin. (**A**) OD as a function of time for mixed culture of *B. subtilis* and *E. coli*, under increasing concentrations of the antibiotic. Solid lines show the mean of eight independent measurements for each concentration. The shaded area corresponds to the standard error. The maximum antibiotic concentration is $A_{\max} = 100\ \mu/ml$. (**B**) YFP signal (arb. units) reporting *B. subtilis* as a function of time for the corresponding growth curves in panel (A). (**C**) CFP signal (arb. units) reporting *E. coli* as a function of time for the corresponding growth curves in panel (A).

Table S1. Strains used in this study.

Strain	Genotype	Source
E. coli CFP	*E. coli* K-12 MG1655, pSB1C3-BBa_K880005-*cfp*	This study
B. subtilis Wild-type	*B. subtilis* NCBI 3610	Gürol Süel, UCSD
B. subtilis YFP	*B. subtilis* PY79, *amyE::*P_{hyp}*-yfp*	*(42)*

Supplementary notebook captions

Notebook S1. Code to reproduce the modeling results of Fig. 3.

Notebook S2. Code to reproduce the modeling results of Fig. 5.

Notebook S3. Code to reproduce the modeling results of fig. S6 (A and B).

Notebook S4. Code to reproduce the modeling results of fig. S6C.