Supplementary Information

Digital Plating: A Universal and Versatile Microbiological Technique

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S1. Quantitative characterization of microbial interactions using the DP platform

To quantitatively assess the strength of the interaction between the two microbes, *e.g.*, the degree of the antagonism of *Bacillus* against *Salmonella* in this study, we can calculate the ratio of the area of the inhibition zone induced by a *Bacillus* microcolony to the area of the *Bacillus* microcolony itself and use it as an evaluation index. A higher ratio indicates a stronger antagonistic interaction between the two tested strains, while a lower ratio suggests a weaker interaction. The DP platform, due to its "digital" nature, enables precise quantification of microbial interactions by counting the number of picowells occupied by the *Bacillus* microcolony and those occupied by the inhibition zone, and subsequently calculating the ratio between them. This task can be easily accomplished with the help of image analysis software, *e.g.*, ImageJ (https://imagej.net). The detailed procedure is shown below:

1) Load an image which is needed to be analyzed. To do this, select: *File* > *open.*
2) Mark and Count the picowells occupied by the *Bacillus* microcolony and the picowells occupied by the inhibition zone by going to *Plugins* > *Analyze* > *Cell Counter*.
3) Click “Initialize” and then select “Type”: Type 1 and 2 for marking and counting the picowells occupied by the inhibition zone and the picowells covered by the *Bacillus*

microcolony or microcolonies, respectively.

4) Click “Results” to display the counting results.

Fig. S3 shows representative marked images of the picowells to be counted, in which the yellow dots represent the picowells occupied by the inhibition zone and the pink dots represent the picowells covered by the *Bacillus* microcolonies. By comparing the ratios of the number of yellow dots to that of pink dots obtained from **Fig. S3**a and b**,** we can infer that the *Bacillus* microcolony in **Fig. S3**a exerts a stronger antagonistic effect against *Salmonella* than the microcolony in **Fig. S3**b. This suggests that there is a heterogeneity of antagonistic effect against *Salmonella* in *Bacillus* populations. In addition, it can be found that the number of the picowells occupied by the inhibition zone in the region between the two *Bacillus* microcolonies is more than that in the external regions on the sides of each *Bacillus* microcolony, suggesting a synergistic effect of neighboring *Bacillus* microcolonies against *Salmonella*.

S2. Demonstration of the compatibility of the DP method with commercial ready-to-use agar plates

Due to its assemblability, our DP platform allows compatibility with commercial ready-to-use agar plates. By conformally attaching the sample-loaded PicoArray device upside down to a commercial ready-to-use agar plate (**Fig. S4**a), different experiments or assays, such as bacterial culture, identification, or antibiotic susceptibility testing, can be facilely carried out, depending on the specific properties of the agar plate. To evaluate if the DP method is compatible with commercial ready-to-use agar plates, we conducted a number of bacterial selective enrichment and identification experiments by combining four PicoArray chips with four typical commercial ready-to-use agar plates, respectively. These plates included an Eosin Methylene Blue (EMB) agar plate, a MacConkey agar (MAC) plate, a Hektoen Enteric (HE) agar plate, and a Rappaport Vassiliadis Soya (RVS) agar plate. EMB and MCA agars selectively promote the growth of Gram-negative bacteria and allow for differentiation of gram-negative bacteria based on their ability to ferment lactose; HE agar is designed to isolate and differentiate members of the species *Salmonella* and *Shigella* from other *Enterobacteriaceae*; RVS agar is used for the enrichment and isolation of *Salmonellae* species from food, animal feed, and environmental samples. First, each PicoArray chip was loaded with a microbial mixture consisting of *Salmonella* and *E. coli* and then the chips were conformally attached to the surfaces of four commercial ready-to-use agar plates upside down, respectively. After assembly, the chip/plate assemblies were incubated at 37 ºC for 6 h. Finally, the assemblies were observed *via* microscopy. Note that, to achieve better image

contrast and resolution, the EMB agar-covered chip and HE agar-covered chip were observed using dark-field microscopy, the MCA-covered chip was observed using an oblique illumination-assisted bright-field microscopy, and the RVS agar-covered chip was observed using bright-field microscopy. As expected, only *E. coli* microcolonies were observed in the picowells covered with the EMB agar plate and MCA plate while only *Salmonella* microcolonies were observed in the picowells covered with the HE agar plate and RVS agar plate (**Fig. S4b)**. These results are similar to those obtained by the conventional plating methods, confirming that the DP platform is well compatible with commercially available ready-to-use agar plates.

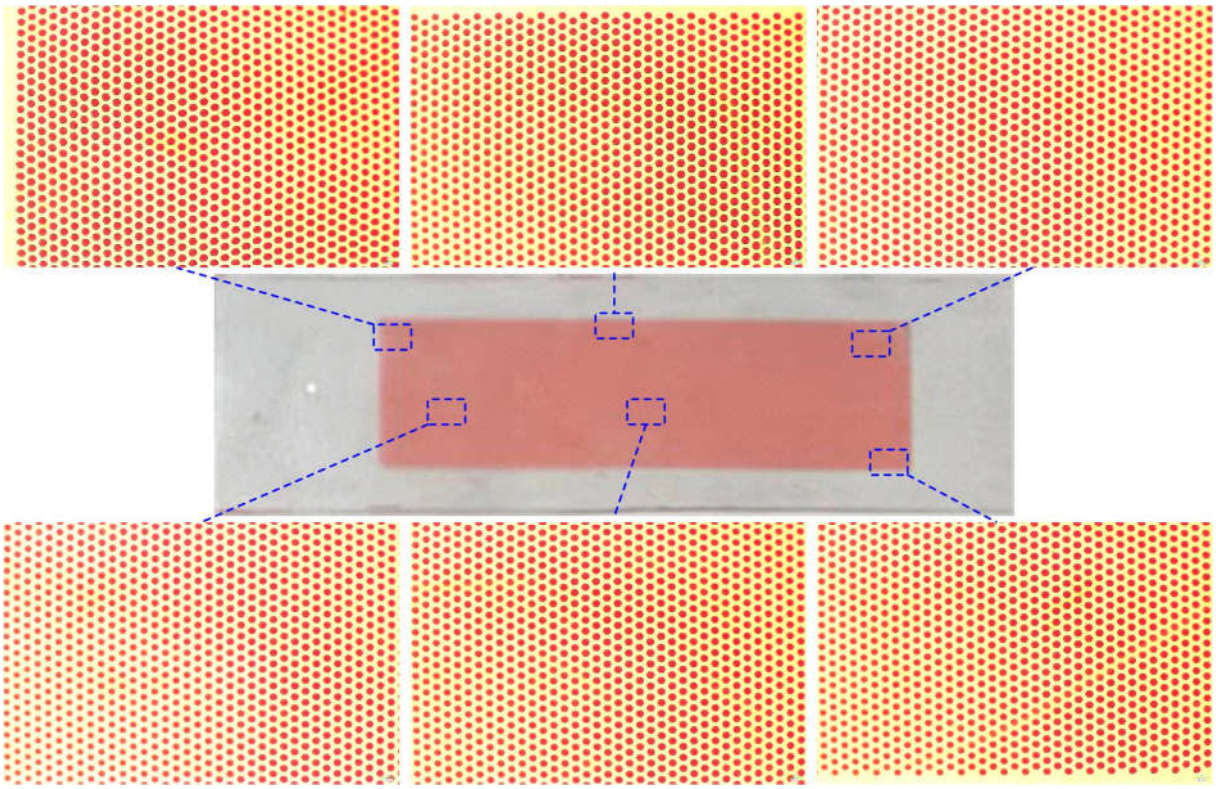

Fig. S1 Microscopic images of the picowells from six random positions on the DP chip after 15 min of covering with a red dye-mixed agar sheet.

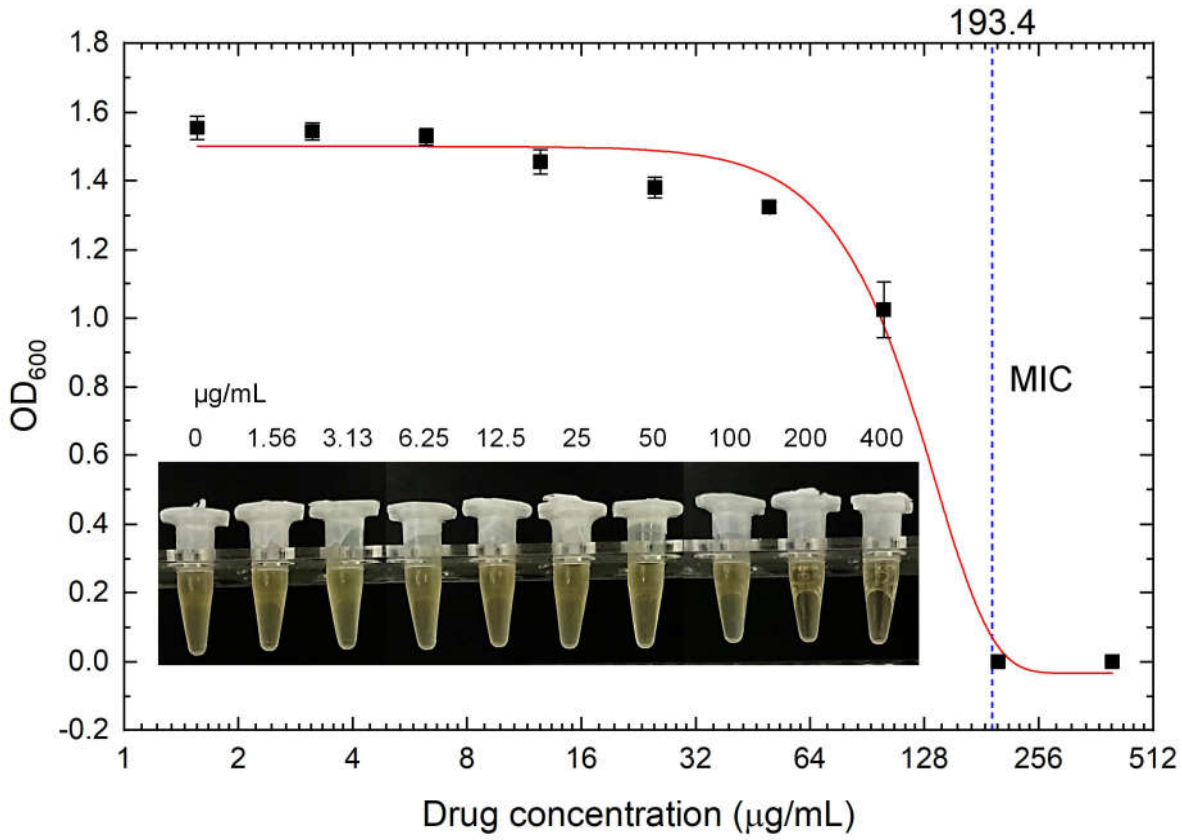

Fig. S2 Dose-response curve of penicillin G sodium toward *E. coli* obtained by the broth dilution technique. Calculation of MIC using a Gompertz function fit. Blue vertical dashed line shows the position of the MIC.

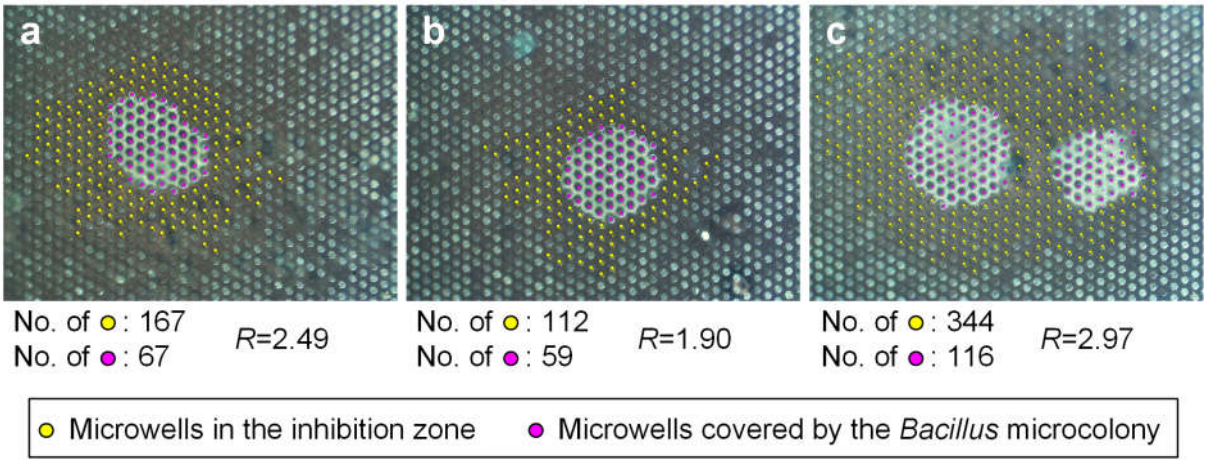

Fig. S3 Digital assessment of the antagonistic effect of *Bacillus* against *Salmonella* using the DP platform. (a-c) Representative images of the picowells in the inhibition zone and those covered by the *Bacillus* microcolonies after being marked in ImageJ, which are used for counting the picowells in the inhibition zone and those covered by the *Bacillus* microcolonies and quantitatively assessing the antagonistic effect of *Bacillus* against *Salmonella*.

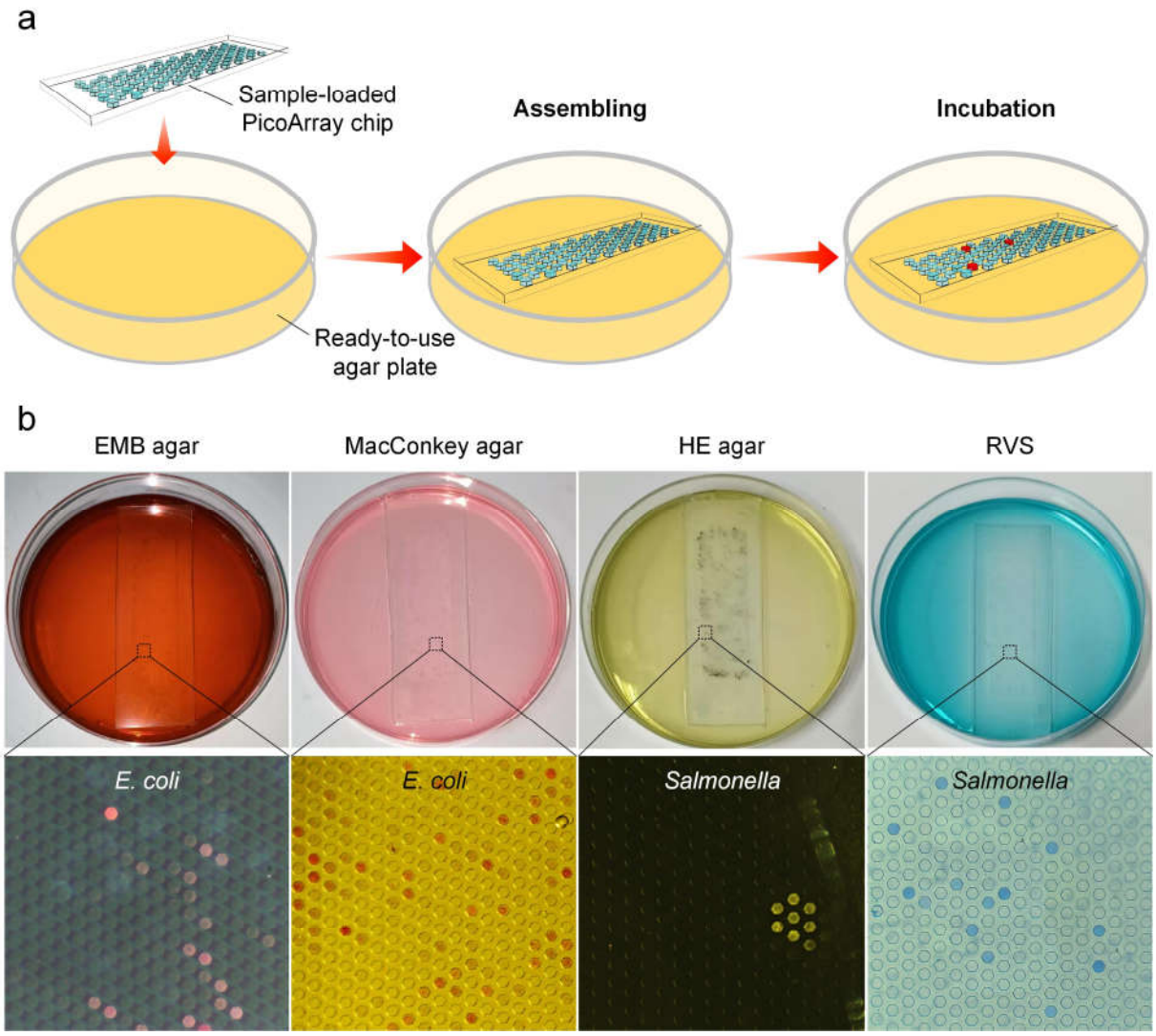

Fig. S4 Coupling the DP method with conventional agar plates. (a) Experimental workflow for coupling the DP method with conventional agar plates. (b) Examples of combining the DP chip with the commercial ready-to-use agar plates for the selective enrichment and identification of specific bacteria.

Supplementary Movies Legend

Movie S1: Demonstration of the operation of the DP platform.

Movie S2: Example video of the *E. coli* population in picowells covered with a blank agar medium sheet for 6 h. Nearly all cells were actively swimming.

Movie S3: Example video of the *E. coli* population in picowells covered with an ampicillin sodium-mixed agar medium for 6 h. No cell was moving.