SUPPLEMENTAL INFORMATION FOR:

Spatial and temporal features of the growth of a bacterial species colonizing the zebrafish gut

Contents:

SUPPLEMENTARY TEXT

Comparing bacterial abundance from fluorescence measurements and plating

To determine the extent to which our fluorescence signal arises from viable (living) bacteria, we performed a set of experiments that quantify bacterial numbers by fluorescence imaging (as in the main manuscript) and by plating; the latter will report a signal only from living microbes. We apply both methods to the same specimens, enabling direct comparisons. In brief: population values derived from imaging and from plating are very similar to one another.

We inoculated larval zebrafish with 10^6 CFU/ml of GFP-labeled A. *veronii* and imaged these samples at either 3 (n = 10) and 24 (n = 10) hours post inoculation in order to image a wide range of bacterial abundances. Zebrafish intestines were dissected immediately after imaging (within 5 minutes) and placed in sterile embryo medium at room temperature for 30-45 minutes, and then kept at 4 °C until homogenization and plating. The short time between the imaging and dissection and the low temperature limited the potential for growth post-imaging. Counting of colony forming units (CFUs) by dilution and plating was done by standard methods.

In Fig. S1 we show the fluorescence-based and plating-based measures of bacterial population, N_f and N_p respectively. The data show a similar range spanned by both, implying that fluorescence arises largely from live bacteria. If all bacteria are viable and are correctly identified by imaging, we would expect two properties for the relationship between N_f and N_p: (i) linearity, and (ii) a proportionality of 1 for the linear relationship. Fitting $\log(N_f)$ vs $\log(N_p)$ gives a slope, i.e. a power law exponent, of 0.9 ± 0.5, i.e. consistent with a linear relationship. The proportionality, i.e. the slope of a linear regression of N_f vs. N_p, is 2.0 ± 1.0, (barely) consistent with 1, which we comment on below. As "order of magnitude" measures, the values from plating and fluorescence are very consistent with each other.

It is interesting to note that the plating values are highly dissimilar to the numbers of isolated individual bacteria (N_i) identified from fluorescence. The crosses in Fig. S1 shows N_p plotted against N_i. Only considering individual bacteria severely underestimates the number of viable bacteria in the intestine. This also rules out the possibility that the clusters observed during imaging are primarily aggregates of dead cells.

As noted, there is a small (2x) difference between the counts derived from our plating and fluorescence-based bacterial population measurements. Several reasons for this are possible:

- Not all the bacteria detected in our imaging-based approach are living.
- In the process of gut dissection, some bacteria are ejected from the gut and as a result do not contribute to the plating population. Relatedly, the homogenization of the gut after dissection may damage some fraction of the bacteria.
- Our estimate of the mean fluorescence intensity of single bacterium is too low. This number is used to translate the overall cluster intensity into a cluster population. Supporting this, the scatter in the data shown in Fig. S1 is smaller if the average bacteria intensity across specimens, rather than each specimen's average intensity, is used for this normalization.

Regardless of the reason for the small discrepancy between these two measurements of population, it does not affect the conclusions of our manuscript, which are concerned with bacterial growth rates and spatial distributions in the intestine. Neither the rates nor the distributions are altered by a rescaling of population size.

SUPPLEMENTARY FIGURES

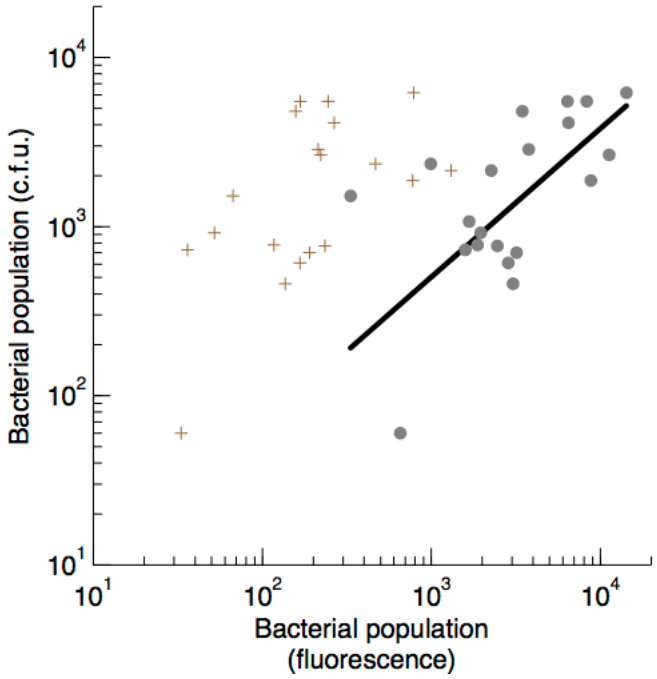

Figure S1. Bacterial populations derived from plating and colony counting (CFU) versus population values derived from fluorescence imaging and image analysis. Gray circles: total fluorescence-derived values. Crosses: Fluorescence-derived populations of only isolated individual bacteria, excluding clusters. Line: Best fit to a power-law relationship between the plating- and fluorescence-based values.

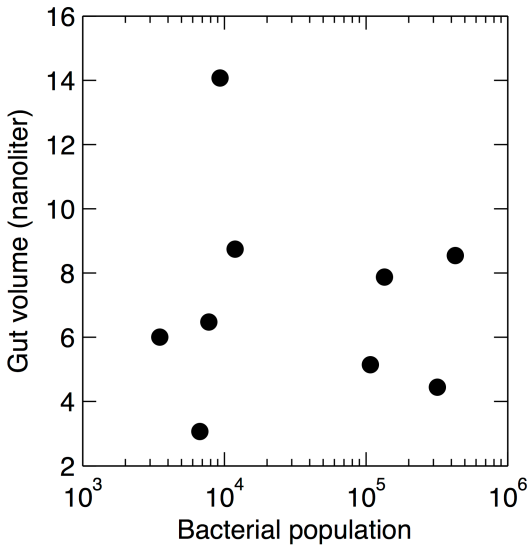

Figure S2. Approximate gut volume vs. bacterial populations for representative scans near the end of the time series for each fish.

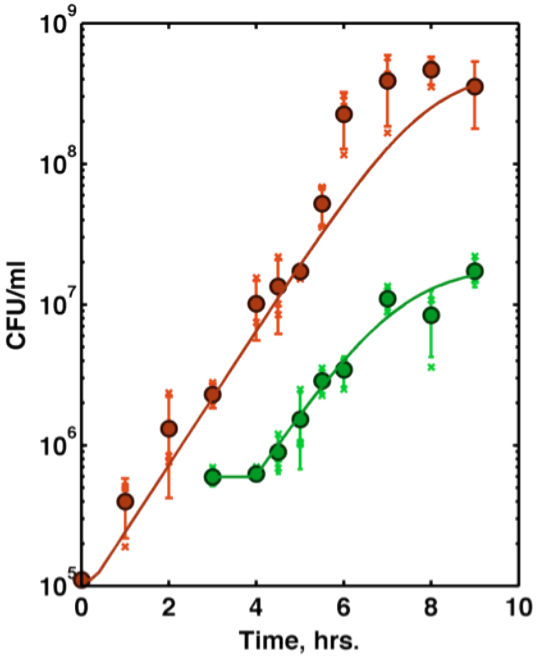

Figure S3. In vitro growth rate of dTomato-labeled (red) and EGFP-labeled (green) A. veronii, with a three hour time delay in which these derivatives were respectively added to the medium. Error bars give standard deviation over three replicates.

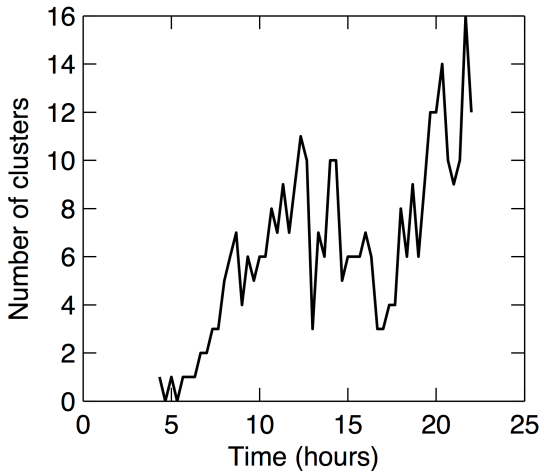

Figure S4. Number of clusters vs. time for a single fish.

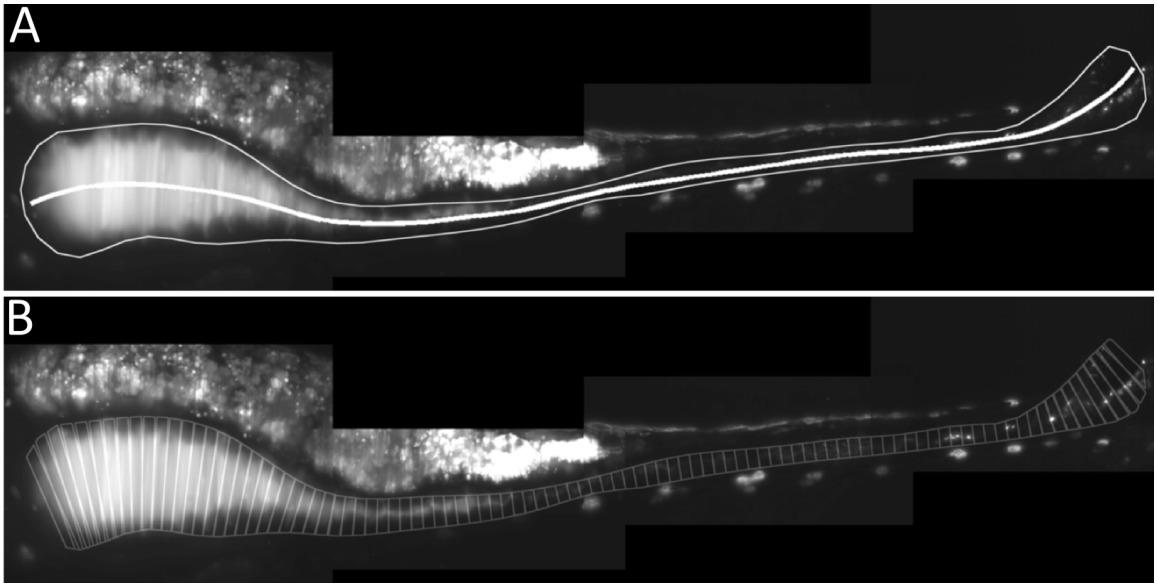

Figure S5

(A) Maximum Intensity Projection (MIP) of larval zebrafish intestine showing both the manually drawn outline and the center of the gut. Scale bar: 100 microns.

(B) MIP of a larval zebrafish intestine showing boxes created to bin microbial population data along the long axis of the intestine.

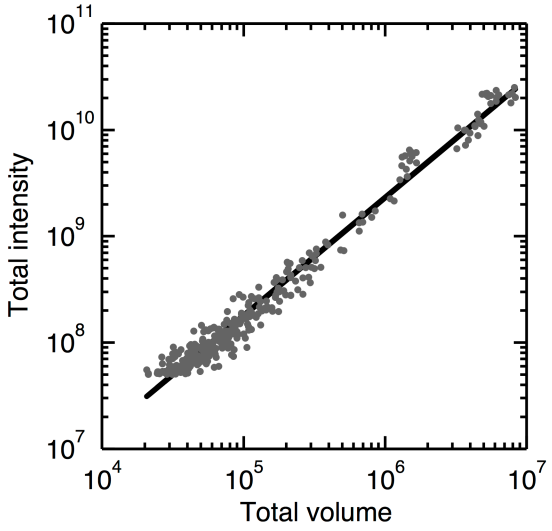

Figure S6. Total fluorescence intensity vs. volume for all clusters identified, for the same fish shown in Figures S4. The line indicates the best-fit power-law to the data, which gives a scaling exponent of 1.103 ± 0.004.

SUPPLEMENTARY VIDEO CAPTIONS

Supplementary movie 1

MIP of colonization of the zebrafish intestine by EGFP-labeled *A. veronii.* Time is measured from the start of inoculation. Scale bar: 100 microns.

Supplementary movie 2

Bacterial population for a sub-region of the scan shown in Figure 4A, showing clusters and individuals of dTomato-labeled *A. veronii.* Scale bar: 100 microns. Rendering was done using *FluoRender (*software funded by the NIH: Fluorender: An Imaging Tool for Visualization and Analysis of Confocal Data as Applied to Zebrafish Research, R01-GM098151-01).

Supplementary movie 3

Bacterial population for a sub-region of the scan shown in Figure 4B, showing clusters and individuals of EGFP-labeld *A. veronii*. Same sub-region as is shown above for Movie S2. Scale bar: 100 microns.

Supplementary video 4

Overlay of the renderings from Movie S2-3. Scale bar: 100 microns.