THE CUSPER BIOREPORTER – MEASURING REPRODUCTIVE SUCCESS OF INDIVIDUAL BACTERIA IN THE PHYLLOSPHERE

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THE CUSPER BIOREPORTER – MEASURING REPRODUCTIVE SUCCESS OF INDIVIDUAL BACTERIA IN THE PHYLLOSPHERE

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One of the challenges in the exciting and emergent field of individual-based microbial ecology is the development of experimental tools that reliably quantify the individual contributions of microorganisms to population size and structure in natural environments. With CUSPER, we introduce a novel bioreporter tool that is capable of determining the reproductive success of individual bacteria in simple and complex environments. The concept of CUSPER is based on the dilution of pre-formed stable green fluorescent protein (GFP) from dividing bacteria, which inversely relates GFP concentration to reproductive success (reproductive success, or repsuc reads CUSPER backwards). To assess the effect of environmental heterogeneity on the reproductive success of individual bacteria, we first exposed this bioreporter, based on the bacterium *Erwinia herbicola* 299R, to LB broth, which represents a condition of low environmental heterogeneity. Cells that were recovered from this environment and analyzed by epifluorescence microscopy and image cytometry, exhibited a normal distribution of reproductive success at all sampling times. This suggests that each individual bacterium in the broth culture contributed equally to the observed population increase. In contrast, cells that were exposed to leaf surfaces, which represent a more complex environment and which are the natural habitat for *E. herbicola* 299R, showed increasingly larger deviations from the normal distribution over time, suggesting that some initial colonizers of the leaf surface were more successful in creating offspring than others. These results demonstrate the usefulness of CUSPER as a tool to assess reproductive success of individual bacterial cells. The compatibility of CUSPER with other single-cell interrogation techniques offers future promise for linking individual reproductive success to specific individual bacterial behaviors and/or environmental experiences.

Theoretical background:

The reporter consists of a bacterial strain (*Erwinia herbicola*), that harbors a gene for the very stable GFPmut3 and a constitutively expressed repressor, which can be de-repressed by adding IPTG to the growth medium.

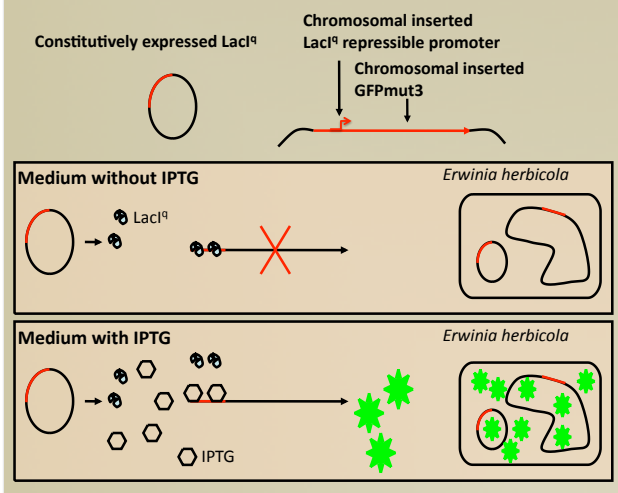

***Figure 1*: Construction and basic principle of the bioreporter**
In a growth medium without IPTG LacI^q is inhibiting GFP expression. Thus, no GFP is produced.
If IPTG is present, it is de-repressing GFP expression. This leads to GFP-filled, bright-fluorescent cells.

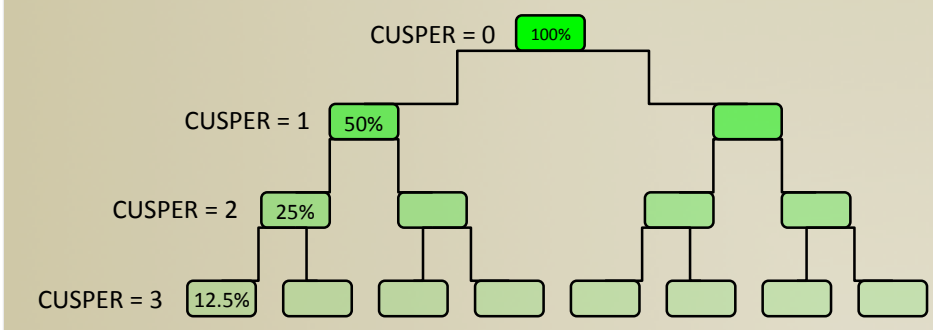

***Figure 2*: The CUSPER principle**
In the absence of IPTG growth of GFP-filled cells leads to a decrease in GFP intensity. The dimmer a cell, the more reproductively successful the cell's ancestor. GFP fluorescence in each cell becomes a measure for reproductive success. CUSPER is defined by $\ln^2(GFP^0/GFP)$.

Proof of principle:

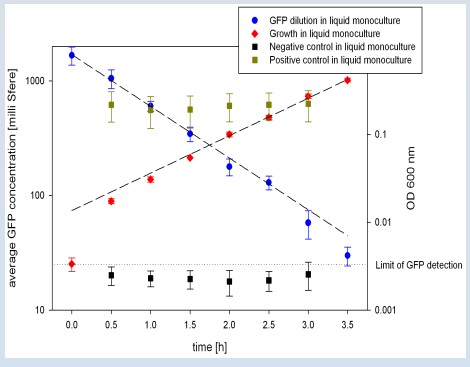

***Figure 3*: Growth of a GFP-filled culture in shaken LB-Medium.**
Single-cell GFP intensities, determined by epifluorescence microscopy, were averaged and plotted as a function of time. Optical density was measured by spectrophotometry at 600nm. Observed growth rate matched the observed GFP-dilution rate almost exactly.

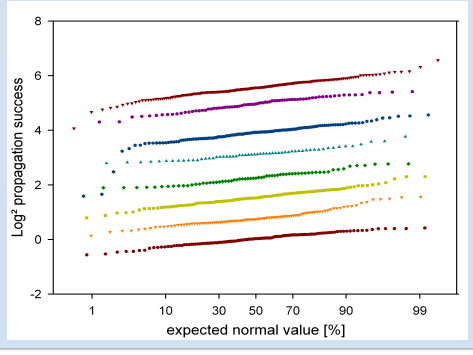

***Figure 4*: Single-cell CUSPER in LB-grown cultures**
30-min intervals from t=0$^{(purple\ dots)}$. The CUSPER-value of each individual bacterium was plotted as a function of its expected normal value.

Conclusions

●In a shaken liquid culture, individual bacteria were equally successful in terms CUSPER. This is most easily explained by assuming that all cells in the culture experience the same growth conditions. Under such conditions, growth rate and GFP-dilution rate are inversely proportional, and measurements of one can be used to predict the other.

●We hypothesized that bacteria in environments with micro-scale differences in habitability would show much more heterogeneity in CUSPER.

●Application of the bioreporter to the phyllosphere indeed revealed that this environment offers a wide range of growth-supporting conditions. Bended lines suggest a sliding scale of habitability. CUSPER pattern could be used to predict growth curves established by CFU from the same leaves.

Application of the Bioreporter:

The phyllosphere was chosen for application of the bioreporter because of the heterogeneity it exhibits. Bean plant leaves were inoculated with a suspension of GFP-filled bioreporter cells. Plants were incubated under high humidity conditions. Bacterial cells were recovered from leaves and analyzed for GFP fluorescence by epifluorescence microscopy.

***Figure 5*: Experimental system**
Phaseolus vulgaris – Green snap bean

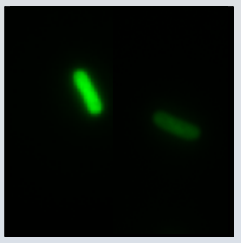

***Figure 6*: GFP-filled Erwinia herbicola**
Two bacteria with different CUSPER-values. Epifluorescence-microscopy picture

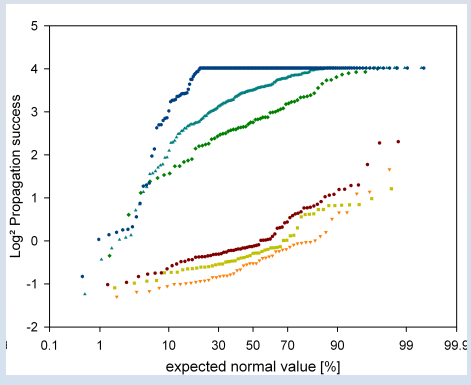

***Figure 7*: CUSPER distribution of bacterial on bean leaves**
CUSPER distributions after 0$^{(orange)}$; 1$^{(olive)}$; 3$^{(dark\text{-}red)}$; 6$^{(green)}$; 9$^{(turquoise)}$; and 24$^{(blue)}$ hours after inoculation. Initial parallel almost straight distributions became more bended during the course of the experiment. This indicates an initial rather homogeneous growth followed by decrease in partial growth success. Overall the phyllosphere seems to offer a sliding scale of habitability.

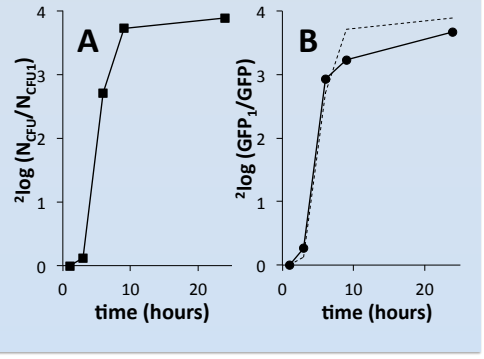

***Figure 8*: Prediction of bacterial growth by using CUSPER**
A: Bacterial growth on the leaf surface determined by CFU counts **B**: Average cells GFP_1/GFP. Owing to the unavailability of comparable colony counts for *t*=0, both graphs compare population sizes relative to time point *t*= 1h. The stippled line in panel **B** serves as a reference representing the shape of the curve in panel **A**.

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