Cell distribution and habitat fragmentation affecting the spread of plasmids in soil bacterial populations

Author(s):
Tecon, Robin; Or, Dani

Publication date:
2019-01-30

Permanent link:
https://doi.org/10.3929/ethz-b-000338408

Cell Distribution and Habitat Fragmentation Affecting the Spread of Plasmids in Soil Bacterial Populations

Robin Tecon and Dani Or

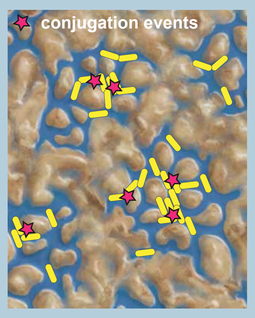
Soil & Terrestrial Environmental Physics
Department of Environmental Systems Science , ETH Zurich

contact
robin.tecon@usys.ethz.ch
www.step.ethz.ch

1. Introduction and Objectives

Transfer of conjugative plasmids among soil bacteria is an important evolutionary driver for adaptation to environmental stresses such as antibiotics or xenobiotic contamination. However, basic mechanisms of plasmid spread in soil environments (often spatially heterogeneous) are not well understood. We hypothesize that unsaturated water conditions prevailing in most soils lead to habitat fragmentation fostering plasmid spread through high local cell densities and reduction in competition.

Our specific objectives are i) to assess the effects of cell density and antibiotic concentration on transfer events and plasmid selection in homogeneous systems, ii) to develop a new heterogeneous environment (PDMS micromodel) in order to study spatial processes (especially aqueous habitat fragmentation) controlling plasmid spread at the local and system scales.



2. Materials and Methods

Soil bacteria exchanging a conjugative plasmid

Pseudomonas putida KT2440 serves as donor and recipient of the conjugative plasmid pIPO2 carrying a tetracycline resistance gene. A tagging system based on fluorescent proteins allows us to visually discriminate donors and transconjugants (Fig. 1, for details see Klümper *et al.*, 2015).

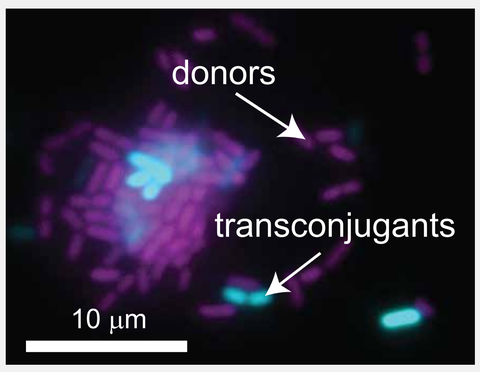


Fig. 1. *Micrograph show bacteria expressing mCherry (magenta) and eGFP (cyan) after conjugation on agar surface*

Connected microhabitats in PDMS micromodel

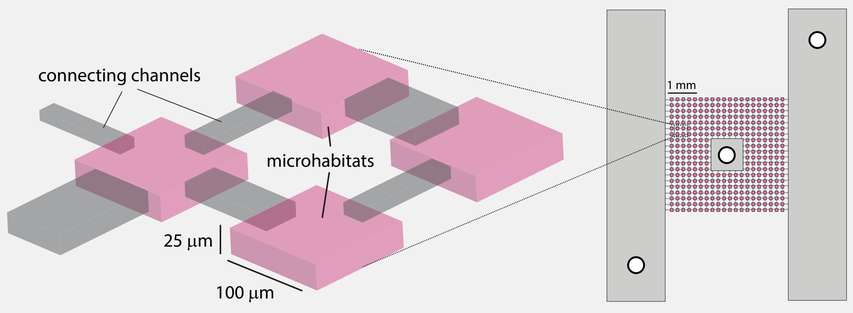


Fig. 2. *Schematic view of the chip containing 364 connected microhabitats of dimensions 100 X 100 X 25 μm. The connecting channels are 15 μm deep and 15, 30 or 60 μm wide. Microhabitats are connected to side reservoirs by nanoslits*

We specifically designed microchips with connected microhabitats (Fig. 2). The distribution of connecting channels of various sizes permits different levels of system fragmentation (Fig. 3).

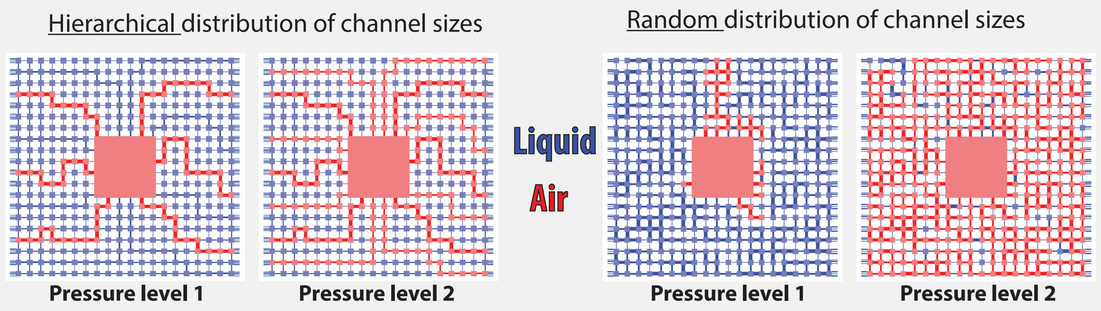


Fig. 3. *Mathematical modeling predictions of air invasion in PDMS chips at two levels of pressure applied to the central square. The 'hierarchical' chip has paths of preferential flow that produces nested fragmentation patterns, unlike the 'random' chip*

3. Results

Plasmid transfer on homogeneous (agar) surfaces

Transfer rates and plasmid selection increase with cell density (Fig. 4) and with increasing sub-inhibitory antibiotic concentrations (Fig. 5).

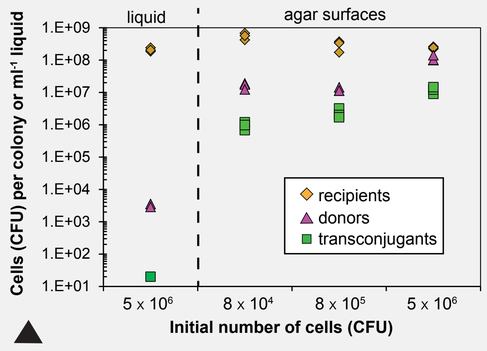


Fig. 4. *Transfer efficiency on 0.1×TSB agar plates and in 0.1×TSB after 20 h of incubation. Initial R:D ratio was 10:1. Cells were resuspended and plated on selective agar media followed by cell counts (Tecon et al., 2018)*

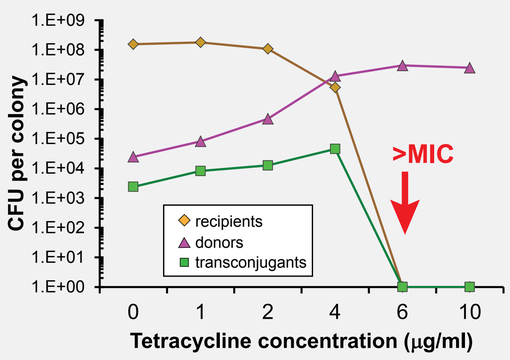


Fig. 5. *Transfer efficiency on separate 0.1×TSB agar plates with increasing Tet concentration. Initial R:D ratio was 10:1. Cells were resuspended and plated after 20 h of incubation*

Fragmentation and plasmid transfer in micromodel

Initial tests with the micromodels demonstrate feasibility of aqueous fragmentation and the creation of disconnected microhabitats (Fig. 6), as well as plasmid transfer detection at local and system scales (Fig. 7).

Fig. 6. *Aqueous distributionin hierarchical and random micromodels before and after fragmentation imposed by air entry through the central square. Close-up shows fragmentation paths (red arrows) driven by channel size*

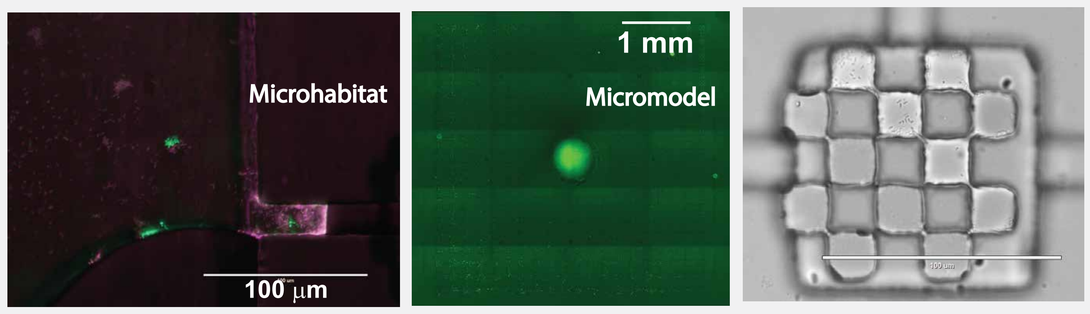


Fig. 7. *Plasmid transfer can be detected at the scale of microhabitats by visualization of transconjugants (green cells), as well as at the scale of the whole micromodel (364 microhabitats). Grayscale image shows roughness elements (20 X 20 X 10 μm) within a microhabitat that can retain water and bacteria*

4. Conclusions

- ★ Cell density and selective pressure (antibiotics) control the spread of plasmids in bacterial populations colonizing surfaces
- ★ New micromodels permit the fragmentation of the aqueous habitats. Further testing of the importance of spatial heterogeneity and habitat fragmentation on plasmid spread are pending

References:

- Klümper U., Riber L., Dechesne A., Sannazzarro A., Hansen L. H., Sorensen S. J., and B. F. Smets (2015) Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. *The ISME Journal* **9**:934-945.
- Tecon R., Ebrahimi A., Kleyer H., Erev Levi S., and D. Or (2018) Cell-to-cell bacterial interactions promoted by drier conditions on soil surfaces. *PNAS* **115**(39):9791-9796.

Acknowledgements

Financial support from the European Research Council (ERC-3200499-'SoilLife') and from the Swiss Initiative SystemsX.ch. We thank Arnaud Dechesne for the gift of the conjugative strains, Peter Lehmann for the lattices modeling, and Dirk van Swaay (www.wunderlichips.ch) for the manufacturing of the PDMS chips.

European Research Council

MicroscapesX
www.microscapesx.ethz.ch