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## Measuring community-wide conjugative plasmid permissiveness

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## Measuring community-wide conjugative plasmid permissiveness

Smets, B.F.<sup>1</sup>, Klümper, U.<sup>1,2</sup>, Dechesne, A.<sup>1</sup>, Riber, L.<sup>3</sup>, Brandt, K.K.<sup>4</sup>, Gülay, A.<sup>1</sup>, Sørensen, S.J.<sup>3</sup>

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To predict the fate of conjugative plasmids, their transfer and host range in entire complex microbial communities needs to be ascertained and understood: this involves identifying the fraction as well as the identity of the microbial community members that can serve, at least transiently, as recipients of the considered plasmid. We have called this the permissiveness of a microbial community towards a specific plasmid (Musovic et al. 2009). We have developed a cultivation-minimal assay to measure community-wide permissiveness of conjugative plasmids (Klümper et al., 2014): the assay relies on challenging a microbial community with an *mCherry* red-fluorescently tagged donor strain which carries a target plasmid that, in turn, is tagged with a zygotically-expressed *gfp*. Conjugation events are subsequently detected as green fluorescent signals via fluorescence microscopy (e.g. CSLM) and transconjugants can be isolated by fluorescence activated cell sorting (FACS).

We investigated the transfer range of IncP-type broad host range plasmids to a soil bacterial community. Conjugation events were detected at approx. 1 in 10<sup>4</sup> to 10<sup>5</sup> of the initial soil recipient cells and transconjugants belonged to 11 different bacterial phyla. We were able to modify the assay further to assess whether exposure to metals (Cu, Cd, Ni, Zn) at concentrations causing partial growth inhibition, modulates community permissiveness (Klümper et al. 2016). For certain Operational Taxonomic Units (OTUs), stress increased or decreased plasmid permissiveness by more than 1000-fold and this response was typically correlated across different metals and doses. The response to some stresses was, in addition, phylogenetically conserved.

Our approaches can be applied to further our understanding of the ecology of broad-host range plasmids in various microbial communities and examine the effect of environmental conditions.

Musovic, S., A. Dechesne, J. Sørensen, and B. F. Smets. (2010) A novel assay to measure the permissivity of microbial communities towards horizontal receipt of exogenous mobile elements. *Appl Environ. Microbiol.* 76: 4813-4818

Klümper, U.; Riber, L.; Dechesne, A.; Sannazzarro, A.; Hansen, L. H.; Sørensen, S. J.; Smets, B. F. (2014) Broad Host Range Plasmids Can Invade an Unexpectedly Diverse Fraction of a Soil Bacterial Community. *ISME Journal* 9: 934–945.

Klümper, U., A. Dechesne, L. Riber, K.K. Brandt, A. Gülay, S.J. Sørensen, B.F. Smets. (2016) Different metal stressors consistently modulate bacterial conjugal plasmid uptake potential in a phylogenetically conserved manner. *ISME Journal. In Press*