Plasmid host range (permisiveness) in microbial communities of activated sludge in wastewater treatment plant.

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appears that *V. anguillarum* isolate PF430-3 can employ two different anti-phage strategies, and the choice of strategy depends on QS.

To gain insight on how QS regulates *ompK* at the single cell level, we measure here the expression of a biotin-tagged OmpK receptor on individual cells. As predicted, OmpK levels of wild-type and Δ*vvanT* strains were higher than the Δ*vvanO* strain. To circumvent QS-regulation, we then expressed *ompK* from an IPTG-inducible plasmid-borne construct in the QS-mutant strains. Surprisingly, the Δ*vvanT* ΔompK *ompK* mutant was still more susceptible to phage KVP40, whereas Δ*vvanO* ΔompK *ompK* mutant was more resistant to phage KVP40, compared to the Δ*ompK* *ompK* mutant. Using the biotin-tagged OmpK, we confirmed that single cells of the QS-mutants expressing *ompK* from the plasmid contains similar numbers of OmpK receptors per cell. Thus, abolishing QS-regulation of the *ompK* gene is not sufficient to explain the different phage susceptibility of the QS-mutants.

Taken together, our preliminary results indicate that the prevalence of abundant anti-phage defense strategies in the investigated *V. anguillarum*, which allowing flexible and dynamic co-existences of phage and host.

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**[P98] PLASMID HOST RANGE (PERMISSEVENESS) IN MICROBIAL COMMUNITIES OF ACTIVATED SLUDGE IN WASTEWATER TREATMENT PLANT**

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Horizontal gene transfer (HGT), especially conjugal plasmid transfer, is one of the key drivers in global antibiotic resistance transmission. To predict the fate of antibiotic resistance gene (ARG), the transfer and host range of ARG carrying plasmids in relevant microbial communities needs to be understood. Wastewater treatment plants (WWTPs) are a potential conduit of ARG transfer between human intestinal and environmental bacteria, and WWTPs are being examined as potential hot spot of antibiotic resistance dissemination. In this study, a comprehensive assessment of antibiotic resistance transmission was performed in activated sludge (AS) of WWTP. Utilizing the well-established fluorescent reporter system, plasmid permissiveness in AS microbial communities were evaluated by transfer frequency using microscopic image analysis and by host range identification through combining flow-cytometry sorting and 16S rRNA gene amplicon sequencing. Under mimic sewer conditions (e.g., synthetic wastewater as growth medium), we challenged the sampled AS communities (Danish WWTP Mølleaværket, Lyngby-Taarbæk) with model plasmids from three subclades in IncP-1 compatibility group (*pKJK5* (α), *pB10* (β-1) and *RP4* (α)) which were harbored by two different host strains - *Escherichia coli* MG1655 and *Pseudomonas putida* KT2440. The results showed that different donor-plasmid combinations had distinct transfer frequencies in the AS microbial communities, ranging from 3.39 x 10⁻⁵ to 5.05 x 10⁻⁴ T/R (transconjugant/recipient) (0.3 to 5 T per 10,000 R), with the most efficient transfer realized in *E. coli* (*pKJK5*). Unexpected broad host range across plasmid-host pairs was revealed in phylogenetic profile of transconjugant communities with total 308 exact sequence variants distributed over 13 phyla, including major
group *Proteobacteria* (mainly by *Enterobacteriales* and *Pseudomonadales* in *Gammaproteobacteria*) and a few rare phyla in Gram-positive groups (e.g., *Actinobacteria* and *Firmicutes*), indicating that ‘long-distance’ transfer across phylogenies and Gram-positive/negative might be frequent under environmental conditions.

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