THE CONJUGATION FREQUENCY OF THE pOX38:Cm CONJUGATIVE PLASMID IN *IN VITRO* MODELS OF HUMAN INTESTINE AND IN AN ANAEROBIC CHAMBER

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Bacterial antimicrobial resistance (AMR) has emerged as one of the leading public health threats of the 21st century [1]. AMR can occur by mutations or by acquisition of antibiotic resistance genes (ARGs) via horizontal gene transfer (HGT), the latter is considered to be the most important factor in the AMR spread. Of the three canonical mechanisms of HGT, conjugation is thought to have the greatest influence on the dissemination of ARGs [2]. The human gut microbiome is a known 'melting pot' for conjugation, with ARG transfer in this environment widely documented [3].

The aim of this study was to determine the conjugation frequency of the plasmid pOX38:Cm from the *Escherichia coli* N4i strain, the probiotic Nissle 1917 strain with gentamicin resistance, (=MSE259) [4] to the DL82, a uropathogenic *E. coli*, under different conditions in TNO intestinal models (TIM-1 and TIM-2) and in anaerobic atmosphere. The TIM-1 is a multi-compartment model of the upper gastro-intestinal tract with four compartments mimicking conditions in the stomach and three parts of the small intestine, while the TIM-2 mimics the colon containing the human microbiota. The effect of MSE259 and DL82 on human microbiota present in TIM-2 was also studied by sequencing. In addition, similar conjugation experiments were performed in an anaerobic chamber. The obtained conjugation frequencies were compared with the those obtained in mating assays performed aerobically in LB liquid medium. In TIM-1 pOX38:Cm conjugation was successful, albeit the conjugation frequency was lower compared to the one obtained aerobically in LB liquid medium. From assays in TIM-2 conjugation frequency could not be calculated, as no transconjugants were detected, which could be due to lower conjugation frequency under anaerobic conditions, lower viability of the MSE259 or DL82 in TIM-2 and presence of other microbiota. Addition of MSE259 in TIM-2 provoked a change in the structure of microbiota: a significant increase of 5 taxa and a decrease of 10 taxa was observed. In order to exclude an effect of the peristaltic movement, which is simulated in the TNO intestinal models, mating assays with mixing of the mating mixture were performed. The mixing did not have any significant effect on conjugation frequency. Further, to exclude the effect of lower pH, mating assays in liquid LB with lower pH were performed. A decrease in conjugation frequency in such medium

was observed, but not as much as in the TIM-1 model. Mating assays performed in TIM-1 medium (a special solution with salts and no nutrients) aerobically in test tube, did not affect conjugation frequency. From mating assays performed in liquid LB in anaerobic chamber conjugation frequencies were calculated, a significant decrease in conjugation frequency was observed, when compared to the conjugation frequency obtained aerobically in LB liquid medium.

References:

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