D5.1 *E. coli* strains demonstrated to be suitable for tranformation

Workpackage 5

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GENERAL INFORMATION

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D-JRP15-FED-AMR-WP5.1: *E. coli* strains demonstrated to be suitable for transformation

Preliminary Remarks

The aim of the project is to demonstrate the role of extracellular DNA in the transfer of antimicrobial resistance (AMR) through transformation and conjugation using *E. coli* and *C. difficile* as model organisms. We are employing and modifiying our *in vitro* gut fermentation model as a representative environment for the pig gut.

Description of deliverable

E. coli is generally known to be naturally transformable; however, standard laboratory strains are also known to be naturally competent in rapidly acquiring new genetic material at a relatively high rate. Thus, *E. coli* is commonly selected and used a model bacterial species to study bacterial transformation. *E. coli* K12 is a standard transformation strain that is often used in studying antimicrobial resistance gene transfer. *E. coli* K12-J53 is a derivative strain of K12 that is sodium azide resistant which is often used as a selection marker. Thus, *E. coli* K12-J53 has been obtained from AGES and is used as a reference strain in the transformation experiments in both the *in vitro* and in the gut fermentation model where sodium azide is used as the first selection marker.

E. coli 912, a strain that was isolated from healthy pigs and carries multiple resistance genes on a conjugative plasmid and has been demonstrated to colonise uninfected pigs [1]. Therefore, this strain will also be used in the pig gut model in future conjugation experiments.

Under antibiotic stress, *E. coli* can undergo spontaneous mutation in the RNA polymerase β subunit (*rpoB*) gene conferring resistance to rifampicin. Using the spontaneous mutant generation method, *E. coli* J53 was used to generate rifampicin resistant mutants so rifampicin can be used as a second selection marker for transformation conjugates. Six rifampicin resistant mutants were generated and named *E. coli* J53-Rif$^{R1\text{-}6}$ and a growth curve was performed for both rifampicin resistant strains and the parental J53 strain to confirm the growth rate of mutant strains and ensures the lack of intrinsic fitness burden associated with the mutations. All rifampicin mutant strains grew successfully with minimum or no effect on the growth rate. *E. coli* J53-Rif^{R1} was chosen, cultured and its DNA was extracted to be used as a donor DNA. PCR primers were designed targeting part of the *rpoB* gene (~2250 bp) including all RNA polymerase β subunit clusters where mutation frequently occur. Targeted *rpoB* sequence of both *E. coli* J53 and *E. coli* J53-Rif^{R1} were successfully amplified, purified and quantified.

Preliminary natural transformation experiments were performed using *E. coli* J53 strain as the recipient strain (rifampicin sensitive) and the *rpoB* DNA amplicon (0.2-0.5 µg) from *E. coli* J53-Rif^{R1} as the donor DNA (rifampicin resistant) in LB broth according to [2]. Our results showed the successful recovery of *E. coli* J53 that is both sodium azide and rifampicin resistant with controls showing colonies only on sodium azide/MacConkey no.3 agar plates. The transformation experiment was also showen to be dependent on time and DNA concentration as demonstrated in the literature. The transformation frequency was calculated and showed to be about 10^{-6}-10^{-7}. This demonstrates the success of the generation of the rifampicin mutant strain, PCR primer design, amplification of the target sequence and transformation of the *E. coli* J53-Rif^{S} with rifampicin resistance contraining amplicon, which confirms the suitability of the strains used to be utilised for transformation experiments in the *in vitro* gut model.

In the future, we will setup the *in vitro* fermentation pig gut model and inoculate it with *E. coli* J53 as the recipient strain (rifampicin sensitive) and the *rpoB* DNA amplicon from *E. coli* J53-Rif^{R1} as the donor

DNA (rifampicin resistant) to assess the transformation frequency at different time points and experimental conditions.

References

[1] Herrero-Fresno, A., Zachariasen, C., Hansen, M.H., Nielsen, A., Hendriksen, R.S., Nielsen, S.S. and Olsen, J.E., 2016. Apramycin treatment affects selection and spread of a multidrug-resistant Escherichia coli strain able to colonize the human gut in the intestinal microbiota of pigs. *Veterinary research*, *47*(1), pp.1-10.

[2] Ray, J.L. and Nielsen, K.M., 2005. Experimental methods for assaying natural transformation and inferring horizontal gene transfer. *Methods in enzymology*, *395*, pp.491-520.