Transformation of L. *kunkeei*

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Introduction

- *Lactobacillus kunkeei* is a bacterial associate of honey bees and was identified as the most frequent isolate recovered from both the crop and hindgut of the bee, but it was also found in the midgut, honey, floral nectar, and beebread (Anderson et al., 2013).
- Our project analyzed the frequencies at which two strains of *L. kunkeei* were transformed with different plasmids that confer erythromycin and/or chloramphenicol resistance. Transforming *L. kunkeei* cells is essential to allow genetic manipulations to study the roles of specific genes in *L. kunkeei's* potential probiotic functions in honey bees.
- Plasmids are small, circular strands of DNA which often carry genes that can benefit the organism by conferring advantageous traits, such as antibiotic resistance. Plasmids are also useful as vectors for carrying recombinant DNA used to perform genetic modifications. In order to study the role of genes in the probiotic functions of *L. kunkeei*, transformation of the bacterial cells must occur. In this study, we looked at the ability of different vectors to transform two *L. kunkeei* strains, 3L and YH15.

Methods

- For transformation, bacterial strains 3L and YH15 were grown in MRS with 2% glycine at 21°C. After growth, cells were recovered from the medium by centrifugation, washed with sterile water, and stored at -80°C in 30% polyethylene glycol (PEG-8000) until further use.
- For electroporation, 100 µL cells were thawed and mixed with 200 ng vector DNA and transferred to 0.2 cm sterile cuvettes. Electroporation occurred at 25 µF, 200 Ohms, and 2.5 KV, yielding time constants of about 4.0.
- After electroporation, the cells were combined with 900 µL MRS recovery medium and then incubated for 4 hours at 37°C to allow expression of the erythromycin or chloramphenicol resistance gene carried on the vectors.
- Cells were plated on MRS agar containing 2.5 µg/mL erythromycin or 10 µg/mL chloramphenicol and incubated for 2-4 days until colonies appeared.
- Colonies were counted and transformation efficiencies were calculated for each vector.
- Mean transformation efficiencies were determined from the results of two or three transformations for each combination of strain and vector.

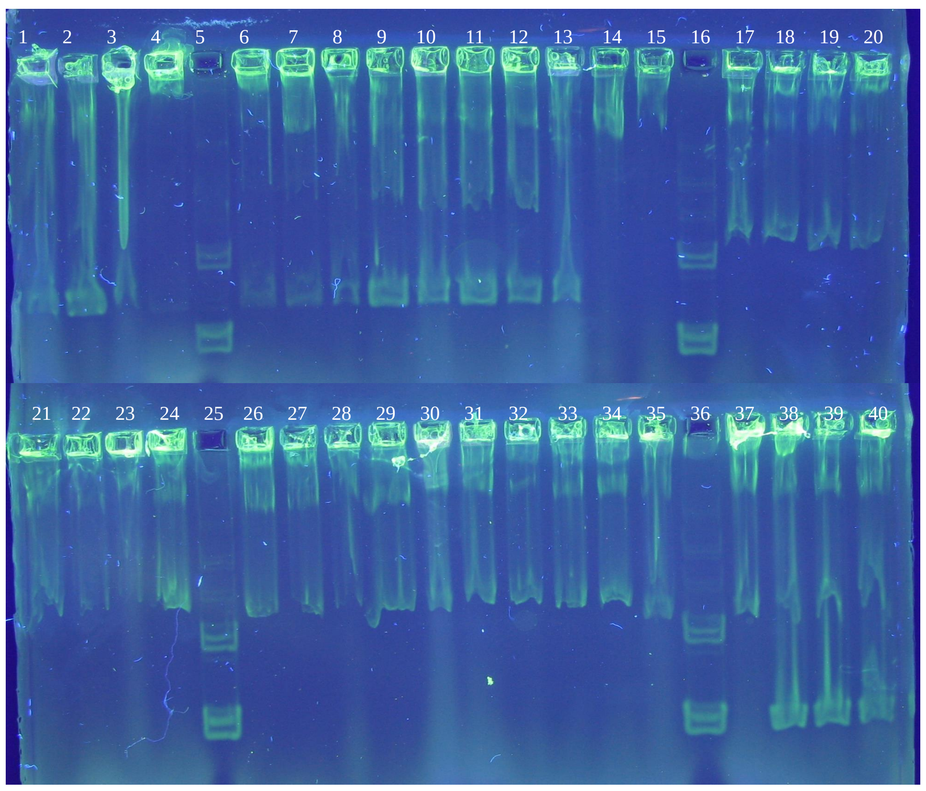

Figure 1: Example of a miniscreen gel that was used to verify vector transformation. DNA was recovered from 3L transformants and used to transform *E. coli* DH5α cells to allow visualization of the vector DNA. Lanes 1-4 and 6-13 show bands ~4.2 kb which corresponds to the vector pTW8 in the DH5α cells. Lanes 17-20, 21-24, 26-35, and 37 show bands at ~6.7 kb, which corresponds to vector pTRKH2. Lanes 38-40 show bands at ~3 kb, which corresponds to vector pTRK687. Lanes 5, 16, 25 and 36 contain supercoiled and open circle pGEM5Z marker at 3 kb.

Reference

Anderson K.E., T. Sheehan, B.M. Mott, P. Maes, L. Snyder, M. Schwan, A. Walton, B. Jones, and V. Corby-Harris. 2013. Microbial ecology of the hive and pollination landscape: Bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). *PLoS ONE*. doi:10.1371/journal.pone.0083125

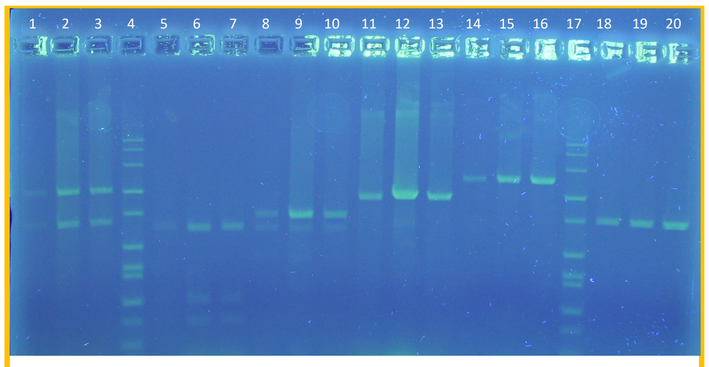

Figure 2: Gel electrophoresis of vectors recovered from *L. kunkeei* 3L transformants. DNA was recovered from the 3L transformants and used to transform *E. coli* DH5α cells to allow recovery of the vector DNA. Plasmid DNA from the *E. coli* cells was digested with restriction enzymes to show that the recovered vectors yielded restriction fragments with sizes similar to those of the starting vectors. Lanes 1-3, pTRKH2 and two transformants, *Hind*III; lanes 5-7, pTW8 and two transformants, *Hind*III and *Sal*I; lanes 8-10, pTRK687, and two transformants, *Pst*I; lanes 11-13, pGH9 and two transformants, *Hind*III; lanes 14-16, pGK13 and two transformants, *Hind*III; lanes 18-20, pNZ8048 and two transformants, *Hind*III; lanes 4 and 17 contain HiLo marker DNA fragments.

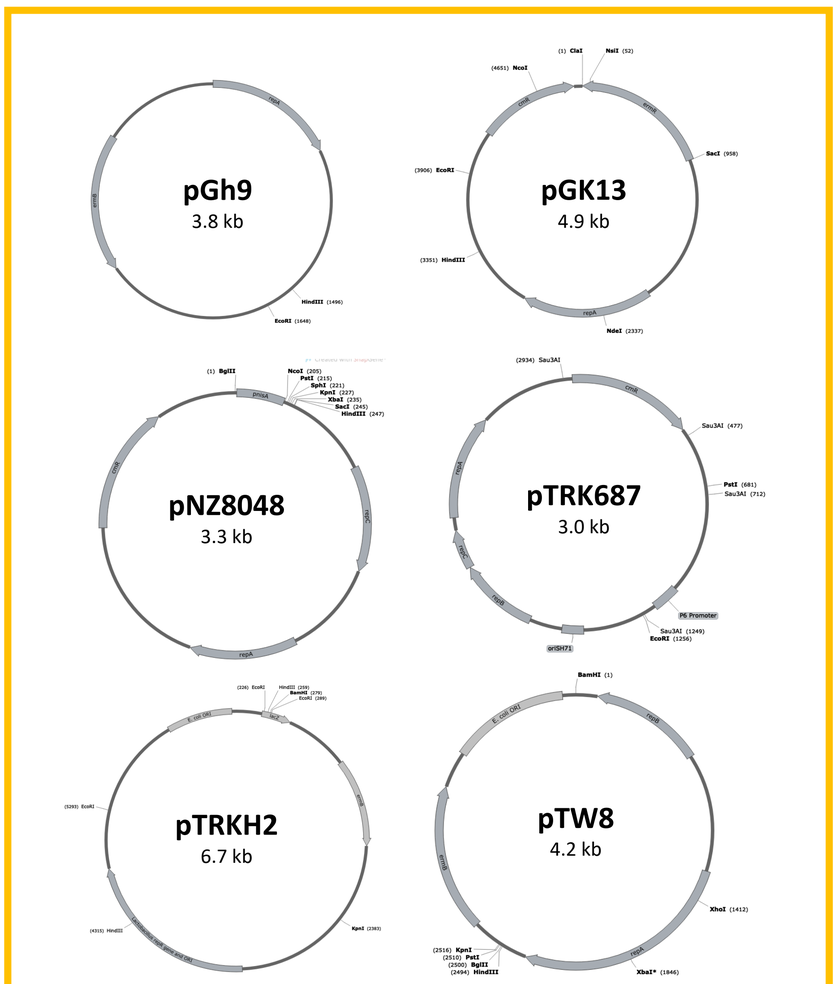

Figure 3: Vectors pGH9, pTRKH2, and pTW8 contain the *erm*B gene, which encodes resistance to erythromycin. Vectors pNZ8048 and pTRK687 contain the *cm*R gene, which encodes resistance to chloramphenicol. Vector pGK13 contains genes for both erythromycin and chloramphenicol resistance. Successfully transformed cells grew on MRS agar containing erythromycin or chloramphenicol and the colonies that formed were used to calculate the transformation efficiency of each vector. Vectors are not drawn to scale. (Figure prepared by B. Crowley)

Transformation Efficiencies of 3L and YH15 strains		
Vector	3L	YH15
pTRKH2	1.6 x 10^4 CFU/µg	1.5 x 10^2 CFU/µg
pTW8	1.4 x 10^3 CFU/µg	4.3 x 10^3 CFU/µg
pGK13	2.4 x 10^3 CFU/µg	no transformants
pGH9	3.7 x 10^2 CFU/µg	1.1 x 10^4 CFU/µg
pTRK687	2.2 x 10^3 CFU/µg	7.1 x 10^3 CFU/µg
pNZ8048	1.0 x 10^2 CFU/µg	no transformants

Table 1:

Strain 3L – All vectors transformed *L. kunkeei* strain 3L, with pTRKH2 having the highest transformation efficiency.

Strain YH15 –Vector pGH9 had the highest transformation efficiency for strain YH15, but vectors pGK13 and pNZ8048 did not transform this strain. Of the vectors that did transform YH15, pTRKH2 had the lowest number of transformants.

Conclusions

The vectors used varied in their ability to transform *L. kunkeei* strains 3L and YH15. Vectors pTRKH2, pTW8, pGH9 and pTRK687 transformed both strains but pGK13 and pNZ8048 were only able to transform strain 3L. The inability to transform YH15 is not due to an inability of the DNA to enter cells since the other vectors were able to transform this strain. Therefore the lack of transformation must relate either to a restriction system that targets pGK13 and pNZ8048 DNA but not the other vectors or to an inability of the pGK13 and pNZ8048 vectors to establish and replicate in YH15.