Robust Performance and Rapid Construction of Live Bacterial Therapeutics Lacking the Colibactin Gene Cluster

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Introduction

- Synthetic Biotics utilize non-pathogenic probiotic *E. coli* Nissle (EcN) as the chassis organism for engineering strains with drug-like properties designed to perform therapeutic functions in patients.
- Safety concerns have been raised regarding the native *pks* gene cluster in EcN, encoding the genotoxin colibactin, prompting an evaluation of removing the *pks* island from Synthetic Biotics.
- Here, we report that Δ*pks* EcN strains maintain engineered activity and display no growth disadvantage when tested in *in vitro* assays and *in vivo* preclinical mouse and nonhuman primate models. Following this, 24 chassis strains, dubbed the Universal Chassis collection, were assembled to enable to the rapid construction of future Synthetic Biotics.

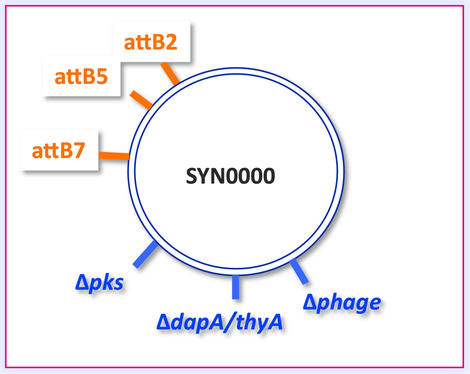

Figure 1. Diagram of Chassis Modifications in genome of Synthetic Biotics. This simplified diagram illustrates the modifications made to the EcN chromosome in each Synthetic Biotic. Three landing pads; attB2, attB5, and attB7; enable quick and simultaneous integration of modality-specific functions. Knockouts of the endogenous phage as well as knockouts of either *dapA* or *thyA*, creating auxotrophy, are necessary for patient safety and biocontainment. Lastly, removal of the ~55kb *pks* island is also included.

No growth disadvantage observed in Δ*pks* EcN compared to Wild Type EcN in *in vitro* assays

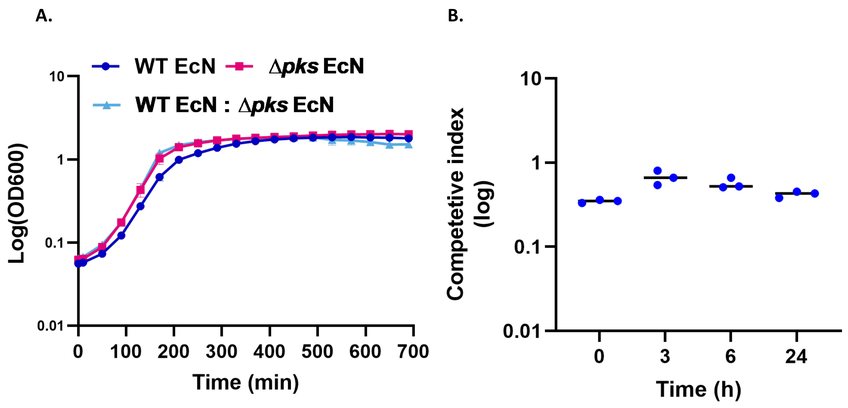

Figure 2. In vitro evaluation of EcN and Δpks EcN fitness. A) Growth curves of WT EcN (dark blue), Δ*pks* EcN (pink) and both strains in mixed culture/competition (inoculated at a 1:1 ratio, light blue). B) Competitive index (CI) for Δpks EcN and WT EcN over a 24h period of growth in LB media. Competitive indices were calculated at each time point by dividing the number of recovered Δ*pks* EcN CFU (kanamycin resistant) by the number of recovered WT EcN CFU (streptomycin resistant). Each dot represents the CI determined from a single culture.

Synthetic Biotics engineered for phenylalanine and oxalate degradation show no activity loss with removal of *pks in vitro*

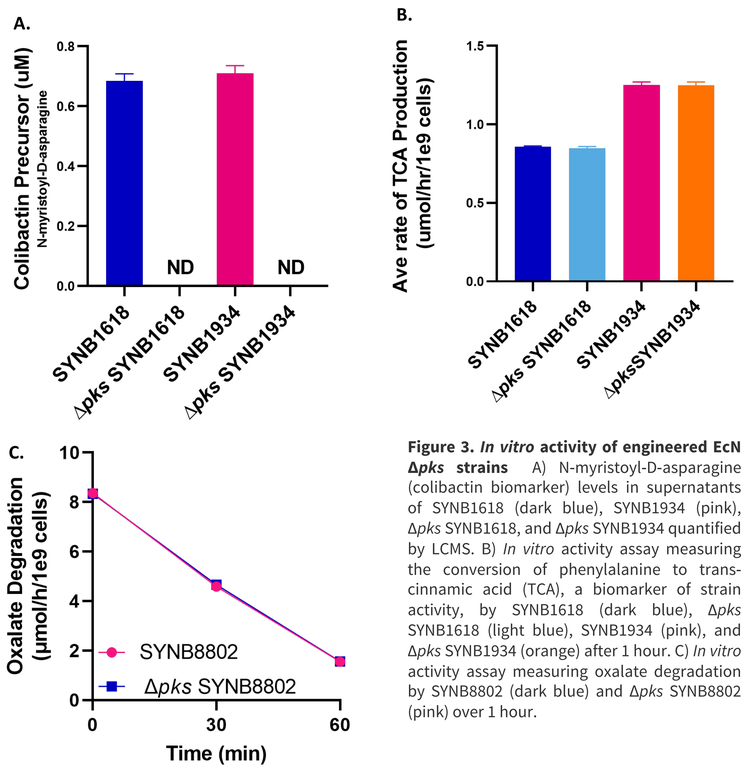

Figure 3. *In vitro* activity of engineered EcN Δ*pks* strains A) N-myristoyl-D-asparagine (colibactin biomarker) levels in supernatants of SYNB1618 (dark blue), SYNB1934 (pink), Δ*pks* SYNB1618, and Δ*pks* SYNB1934 quantified by LCMS. B) *In vitro* activity assay measuring the conversion of phenylalanine to trans-cinnamic acid (TCA), a biomarker of strain activity, by SYNB1618 (dark blue), Δ*pks* SYNB1618 (light blue), SYNB1934 (pink), and Δ*pks* SYNB1934 (orange) after 1 hour. C) *In vitro* activity assay measuring oxalate degradation by SYNB8802 (dark blue) and Δ*pks* SYNB8802 (pink) over 1 hour.

WT EcN and Δ*pks* EcN show similar transit kinetics in naïve (left) and streptomycin-treated mouse models (right)

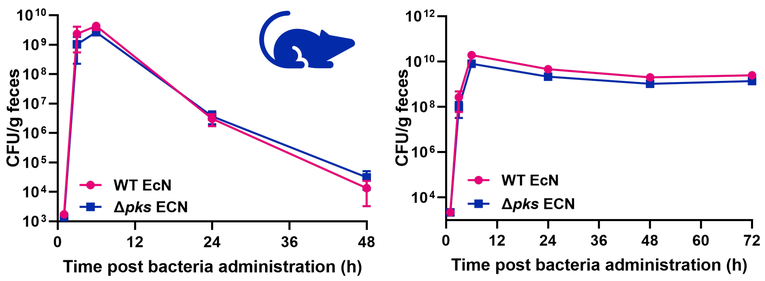

Figure 4. *In vivo* kinetics of WT EcN and Δ*pks* EcN strains in mice. (A) Fecal excretion of EcN strains in C57BL/6J mice (n = 5 per group) following oral administration of a single dose ($1x10^{10}$ CFU) of WT EcN (pink) or Δ*pks* EcN (dark blue) over 48h. (B) Fecal excretion of EcN strains in streptomycin-treated C57BL/6J mice (n = 10 per group) following oral administration of a combined dose ($5x10^{9}$ CFU of each bacterial strain) of WT EcN (pink) and Δ*pks* EcN (dark blue) over 72h. In both cases, bacterial abundance was determined by CFU enumeration at each time point.

No change in transit kinetics or phenylalanine degradation activity between WT EcN and Δ*pks* EcN in NHP models

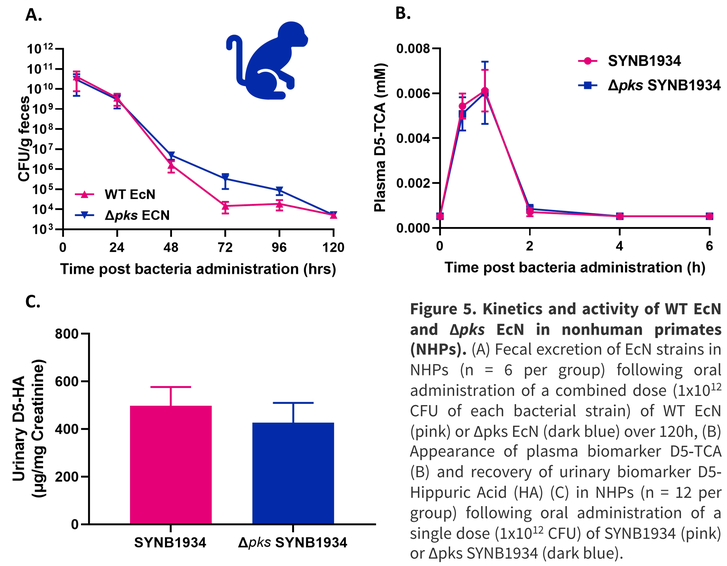

Figure 5. Kinetics and activity of WT EcN and Δ*pks* EcN in nonhuman primates (NHPs). (A) Fecal excretion of EcN strains in NHPs (n = 6 per group) following oral administration of a combined dose ($1x10^{12}$ CFU of each bacterial strain) of WT EcN (pink) or Δpks EcN (dark blue) over 120h, (B) Appearance of plasma biomarker D5-TCA (B) and recovery of urinary biomarker D5-Hippuric Acid (HA) (C) in NHPs (n = 12 per group) following oral administration of a single dose ($1x10^{12}$ CFU) of SYNB1934 (pink) or Δpks SYNB1934 (dark blue).

Universal Chassis collection enables rapid construction of Synthetic Biotics

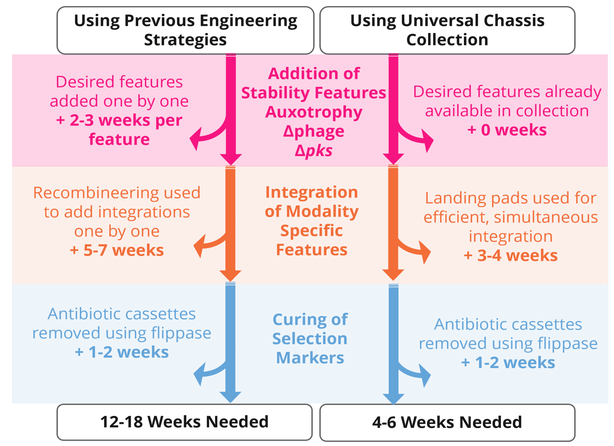

Figure 6. Diagram of Synthetic Biotic Construction. This simplified diagram illustrates the engineering workflow used to make each Synthetic Biotic. With addition of the Universal Chassis collection, containing 24 chassis strains with all desired combinations of necessary stability features, and efficient landing pad integration, the time needed for strain construction is significantly decreased.

Conclusions

- Δ*pks* is a well-tolerated chassis modification and will be included in all Synthetic Biotics
- The Universal Chassis Collection enables construction of clinic-ready prototypes in as little as 4-6 weeks.

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Kalantari A, James MJ, Renaud LA, Perreault M, Monahan CE, McDonald MN, et al. (2023) Robust performance of a live bacterial therapeutic chassis lacking the colibactin gene cluster. PLoS ONE 18(2): e0280499.