Enzymatic production of lipid A using synthetic biology approaches

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Introduction

Lipid A is the essential component of Gram-negative bacterial lipopolysaccharide and its biosynthesis follow the Raetz pathway.[1] Lipid A triggers proinflammatory response through TLR4-MD2 signaling and thus has great immunomodulatory potential. Monophosphoryl lipid A (MPLA), a detoxified lipid A derivative, has been approved by the FDA and it is being used as a vaccine adjuvant. However, lipid A and its derivatives are currently synthesized chemically, which is expensive and labor intensive.[2]

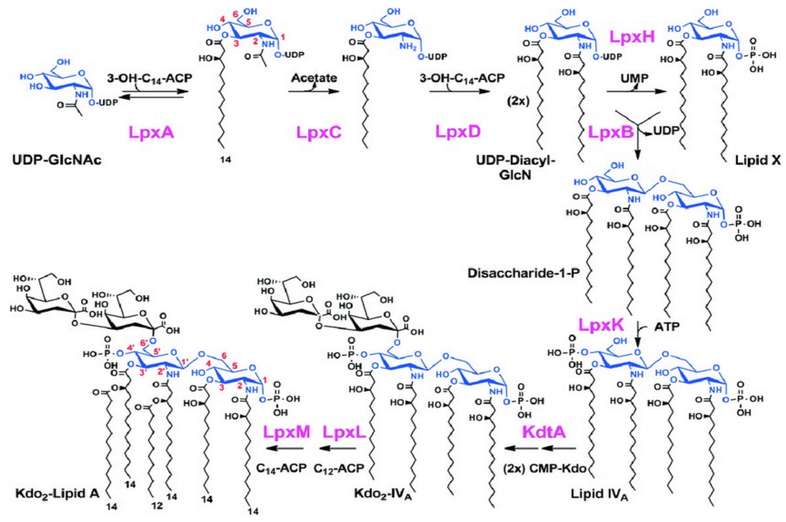


Figure 1. The Raetz pathway[1]

Objectives

- Create nine *Escherichia coli* K12 strains deficient in the production of nine Raetz pathway enzymes
- Identify the rate limiting enzyme of the pathway using the mutant strains
- Optimize lipid A production and synthesize lipid A enzymatically using synthetic biology approaches

JUMP Modular Assembly

The genes encoding for the enzymes of the Raetz pathway will be complemented from plasmids before they are knocked out. JUMP Modular Assembly toolkit is used for plasmid construction:

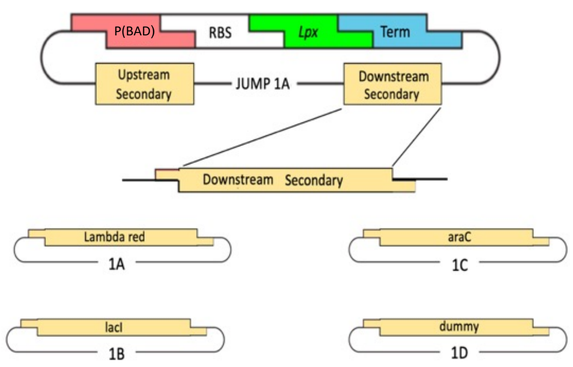


Figure 2. Illustration of the designed JUMP assemblies

Verification of constructs:

- Antibiotic and color selection
- Colony PCR
- Sanger sequencing

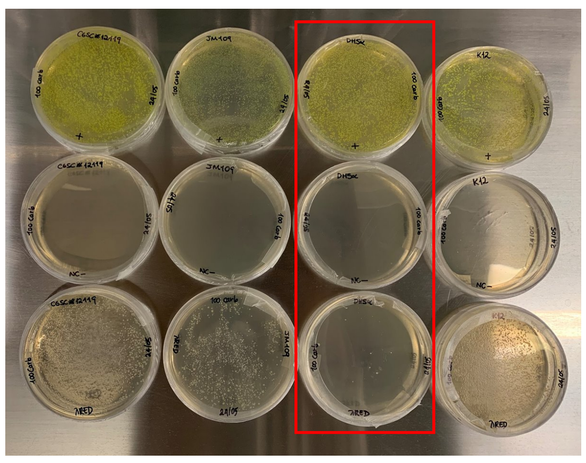


Figure 3. Result of the strain-plasmid instability test

- DH5α cells don't grow with the ƛ-Red basic part plasmid but grow with empty basic part plasmid (pUC ori)
- Four strains were tested: CGSC#12119, JM109, DH5α, K12 WT
- Combination of *phoA8(del)*, *deoR481*, *rfC1* mutations in DH5α, with ƛ-Red genes in the basic part plasmid is unstable

Construction of strains

CGSC#12119: *E. coli* K12 strain with mutations to allow homogenous expression from the arabinose-inducible promoter P(BAD), purchased from the Yale Coli Genetic Stock Centre
Two additional gene deletions in CGSC#12119:

- *rfaD*, to promote KDO_2-lipid A accumulation[3]
- *cdh*, to prevent precursor hydrolysis upon cell lysis[4]

Scarless Cas9 Assisted Recombineering (no-SCAR) system will be used (designed, but not completed)[5]

Identification using mass spectrometry

- Matrix Assisted Laser Desorption Ionization (MALDI-MS) and Electrospray
- Ionization (ESI-MS) were done
- KDO_2-lipid A standard fragmentation patterns is in line with literature[6] and an additional fragment (m/z= 1413.14) was observed in negative mode

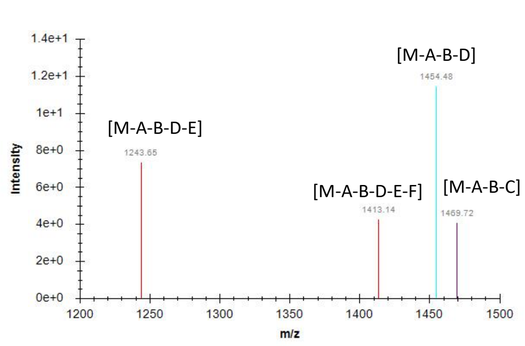
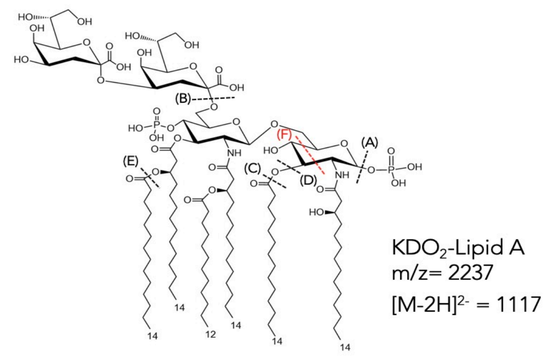


Figure 4. MS^4 spectra obtained by ESI-MS LTQ Orbitrap XL Ions tracked (m/z): 1117 → 1796 → 1698



KDO_2-Lipid A
m/z= 2237
$[M-2H]^{2-}$ = 1117

Figure 5. Fragmentation map of KDO_2-lipid A [M]

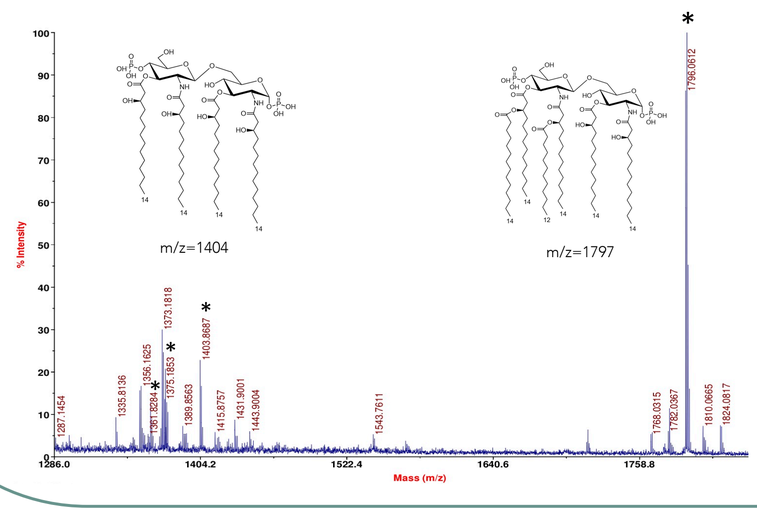


Figure 6. MALDI-TOF/MS spectra of lipid A purified from *E. coli K12*. 9-aminoacridine matrix was used in negative mode.

Conclusion

The first aim of this project is to identify the rate-limiting step of the Raetz pathway. The pathway will then be optimized using synthetic biology approaches, which will be determined depending on the nature of the rate-limiting enzyme. Up to this date, modular plasmids have been constructed (except 1A-Lambda Red and JUMP-1A), gene knock-out experiments have been designed and mass spectrometry measurements have been performed on *E. coli K12* extracts and lipid standards.

Acknowledgements

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