Antimicrobial target identification by Genome Scale Metabolic Modelling

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- Identify metabolic responses of resistant bacteria to antibiotic challenge.
- Develop methods to integrate TraDIS data into metabolic model.
- Identify potential drug targets i.e., ezymes involved in identified metabolic pathways.

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- Diarrhea in the postweaning period due to ETEC is an economically relevant disease in pig production worldwide.
- Macrolides are widely used in pig farming but are ineffective against ETEC due to outer membrane impermeability.
- Macrolides inhibit bacterial protein synthesis by binding to 50S subunit of ribosome, thereby inhibiting protein synthesis.
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TraDIS : **Tra**nsposon **D**irected **I**nsertion Site **S**equencing

- Input libraries were constructed with >1 mn mutants.
- Each mutant in library had atleast 1 random transposon insertion in it rendering the gene non-functional.
- Mutant libraries were grown in 2 conditions:
  - Control - without antibiotic
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- Libraries were sequenced and sequence reads analysed by Biotradis software pipeline.
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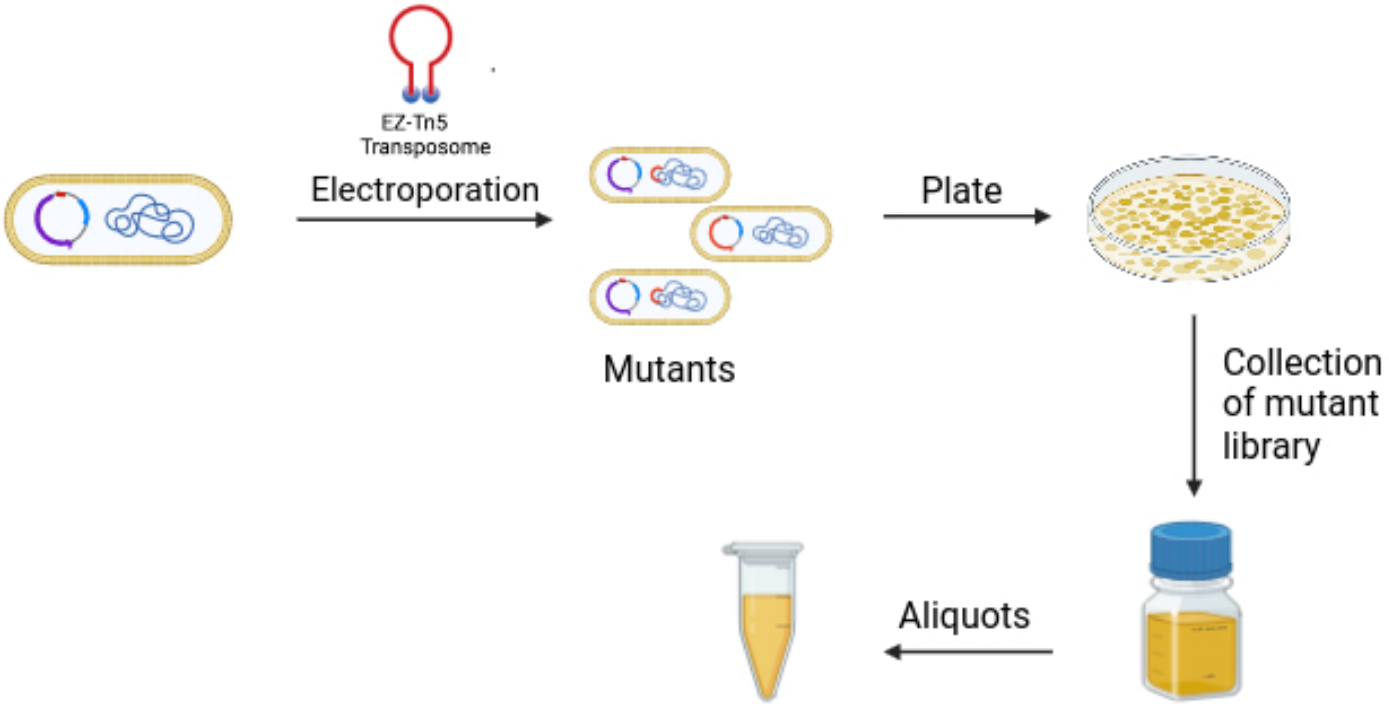
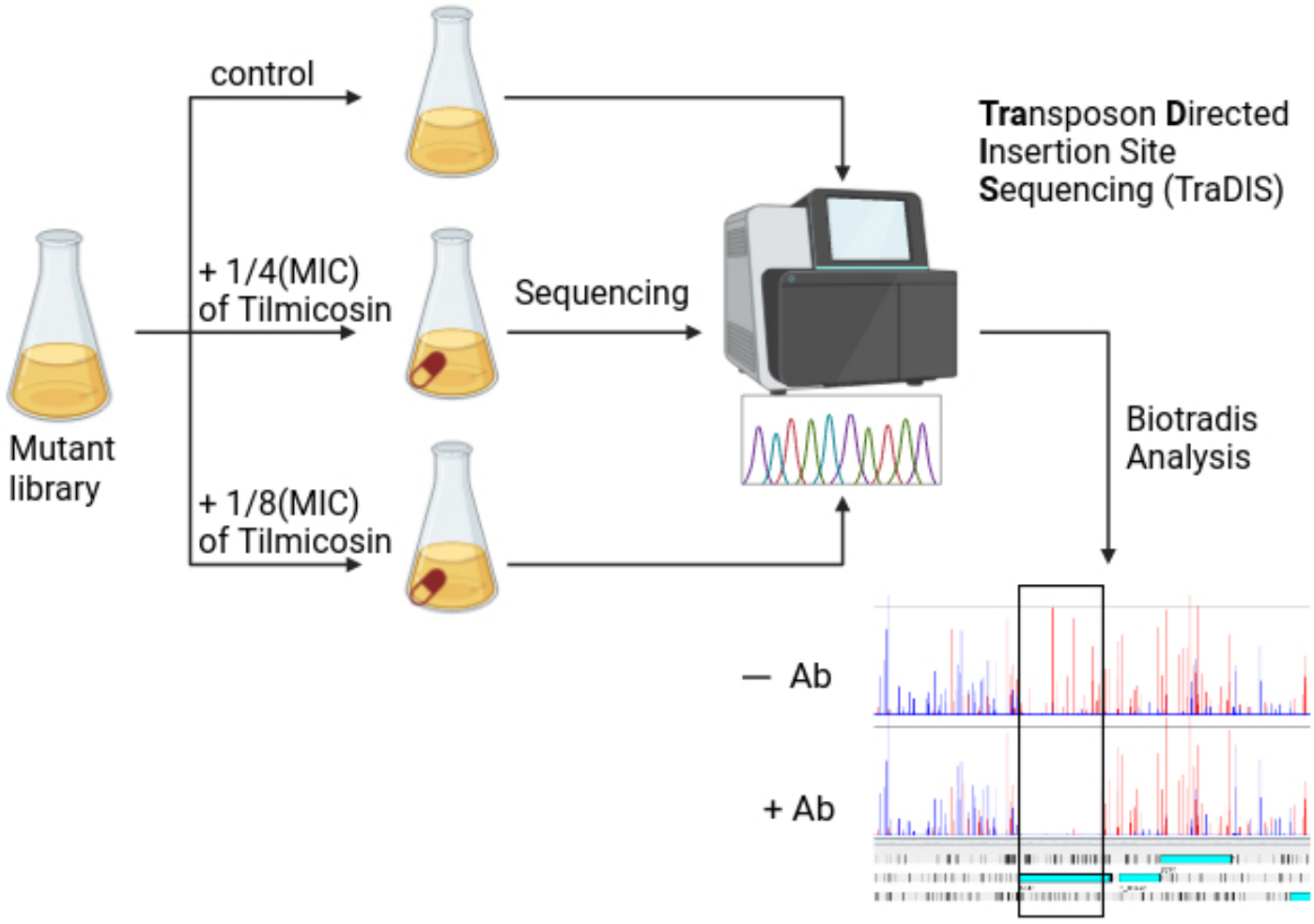
Experiment - Tn Mutant Library construction



Experiment - TraDIS



TraDIS - Results

26 genes were implicated in survival under antibiotic treatment :

- 8 genes : metabolic
- 2 genes : efflux pumps
- 9 genes : outer membrane assembly
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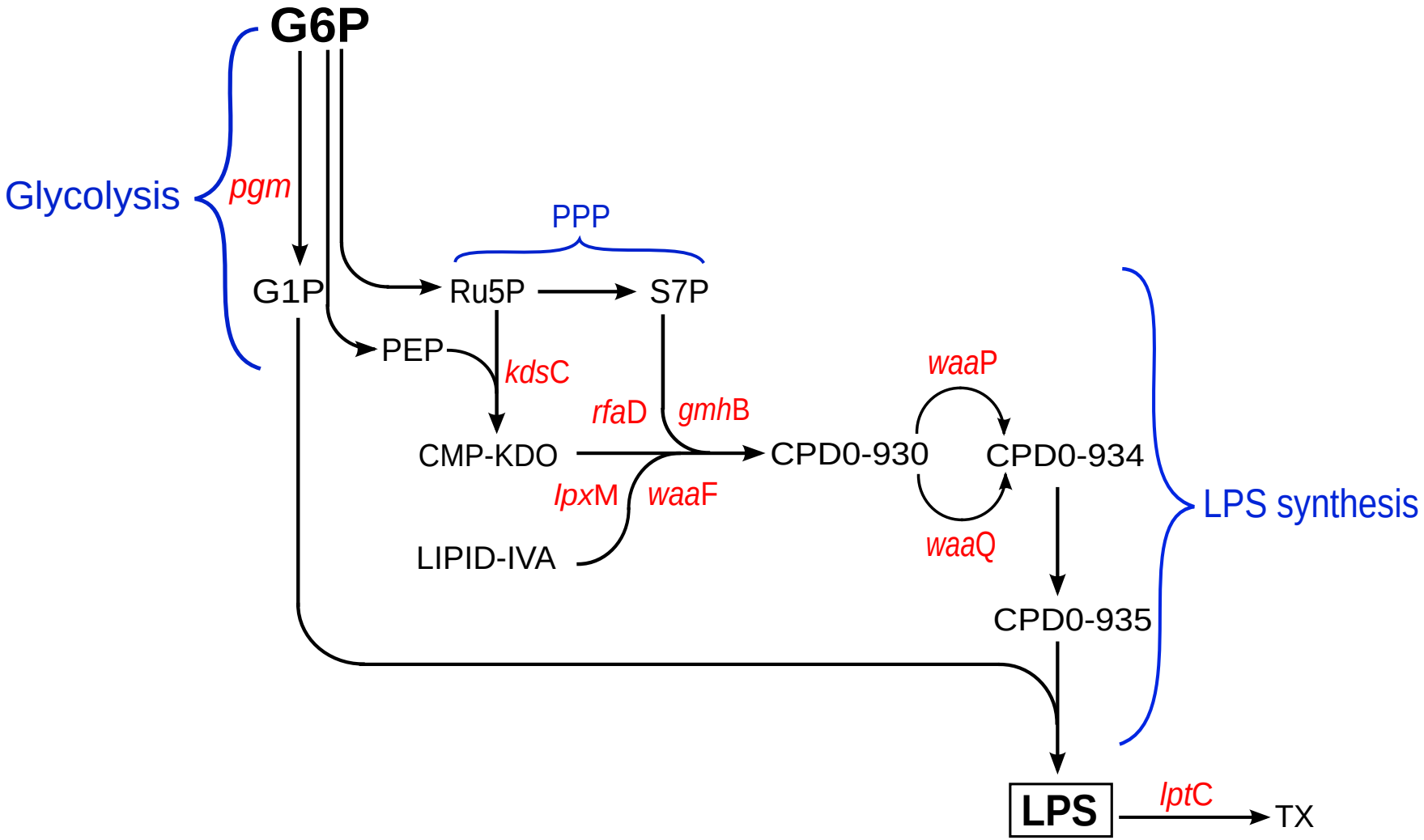
Model properties

Model property	***E. coli* MG1655**
Reactions	1542
Metabolites	1443
External metabolites	92
Biomass transporters	44
Reactions involved in biomass production	256
C source	GLC
S source	SO_4
N source	NH_3

TraDIS genes <> Metabolic reactions

Gene	Enzyme	Reaction
*gmh*B	β-HBP	RXN0-4361
*waa*F	HepII	RXN0-5061
*waa*P	LPS core hepI kinase	RXN-22461
		RXN0-5121
*lpx*M	myr-transferase	MYRISTOYLACYLTRAN-RXN
*rfa*D	AGME	5.1.3.20-RXN
pgm	Phosphoglucomutase	PHOSPHOGLUCMUT-RXN
*kds*C	KDO-8P phosphatase	KDO-8PPHOSPHAT-RXN
*waa*Q	HepIII	RXN-22462
		RXN0-5122

Pathway involved



Knockout analysis - LP

$$\begin{aligned} \min \quad & \sum_{i=1}^{n} |v_i| \\ \text{subject to} \quad & \begin{cases} \mathbf{Nv} = \mathbf{0}\,, \\ v_{LPS} = 1, \\ v_{gene} = 0 \end{cases} \end{aligned} \tag{1}$$

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Knockout analysis

Gene	Reaction/BioCyc-ID	Knockout impact (LP Solution)
*gmh*B	RXN0-4361	No Solution
*waa*F	RXN0-5061	No Solution
*waa*P	RXN-22461	Solution
	RXN0-5121	Solution
*lpx*M	MYRISTOYLACYLTRAN-RXN	No Solution
*rfa*D	5.1.3.20-RXN	No Solution
pgm	PHOSPHOGLUCMUT-RXN	No Solution
*kds*C	KDO-8PPHOSPHAT-RXN	No Solution
*waa*Q	RXN-22462	Solution
	RXN0-5122	Solution

Elementary Mode analysis - Recap

- Minimal set of reactions which cannot be further decomposed into simpler pathways in a metabolic network at steady-state.
- Set of reactions in an EM is unique.
- Net metabolic behaviour of a system can always be expressed as a linear combination of its EMs.

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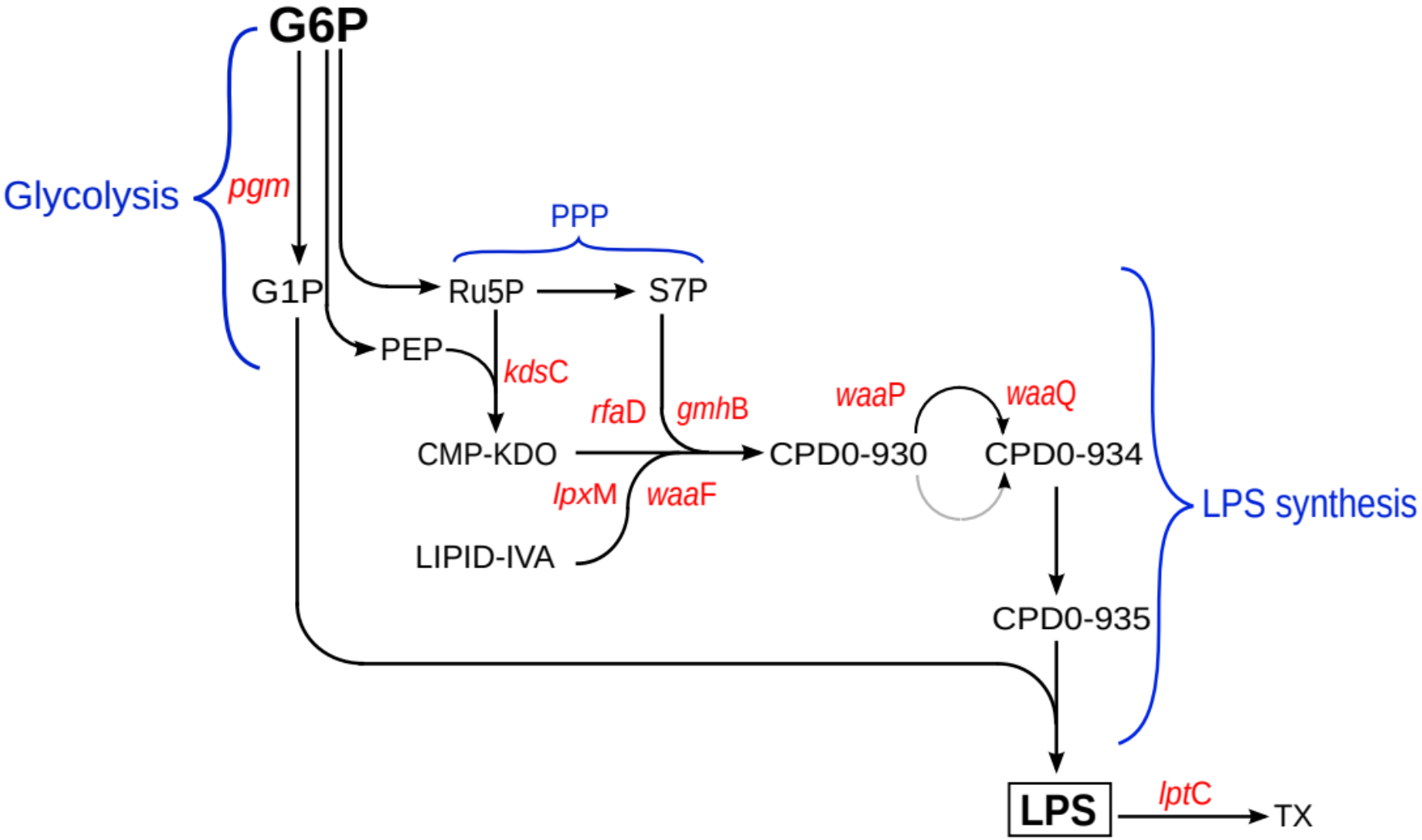

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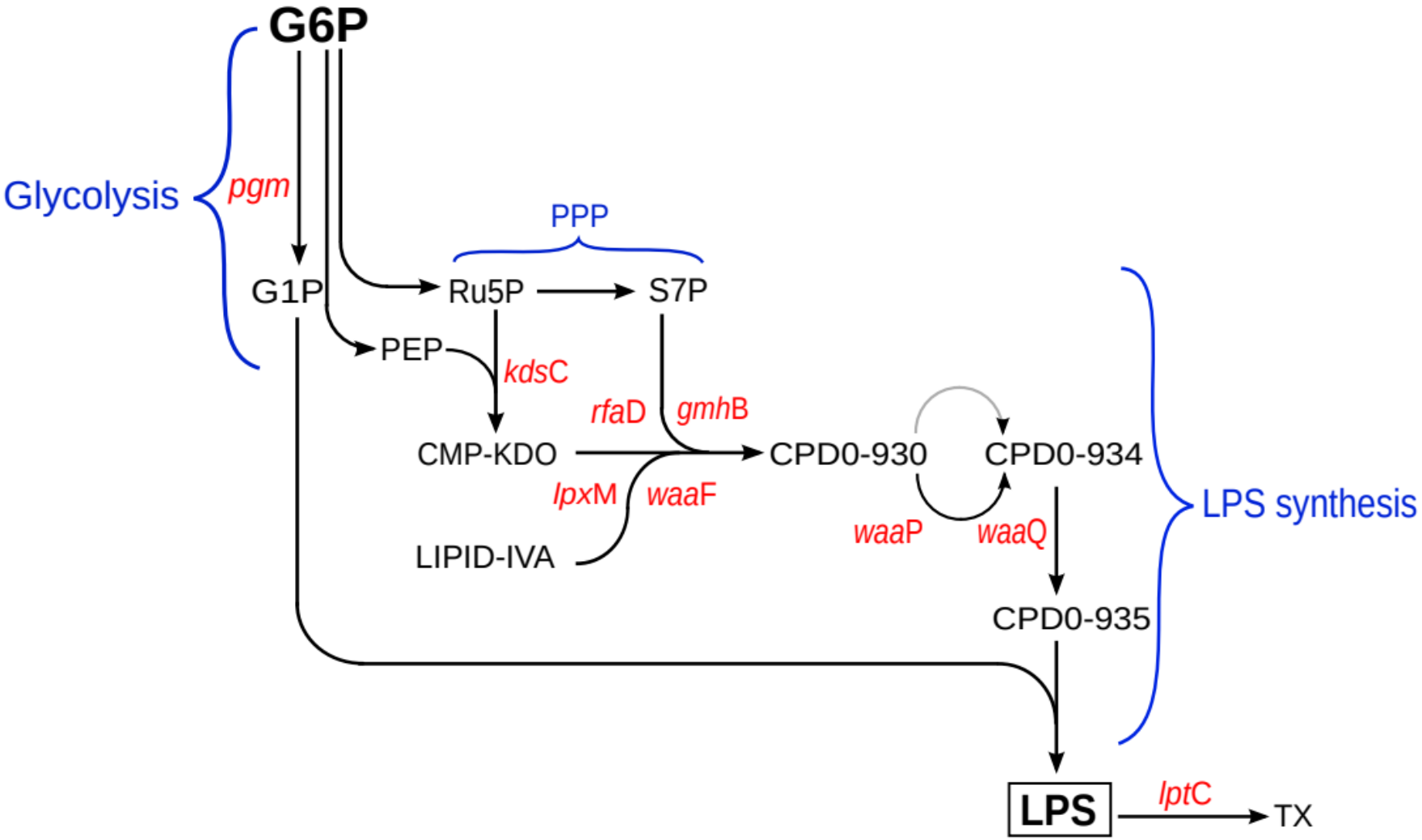

Conclusions

- A metabolic network was identified in response to macrolide treatment in resistant *E. coli* .
- It is mainly associated with lipopolysaccharide (LPS, component of cell wall) synthesis.
- The enzymes present in the network can be potential drug targets.

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