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Elucidating the Regulatory Mechanism of the Global Transcription Factor Cra on Cytidine Synthesis in *Escherichia coli*

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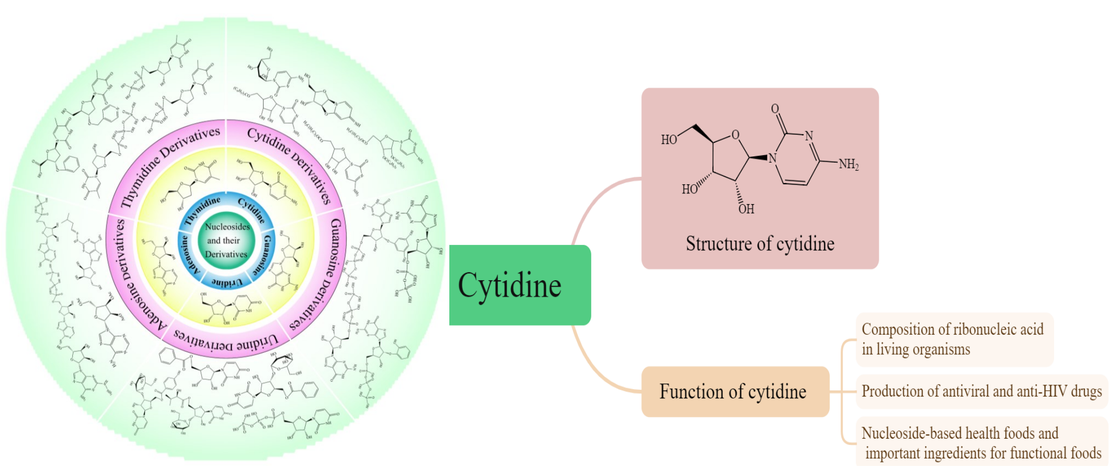
INTRODUCTION & AIM



Obtaining high-yielding cytidine strains with excellent phenotypes is a prerequisite for fermentation.

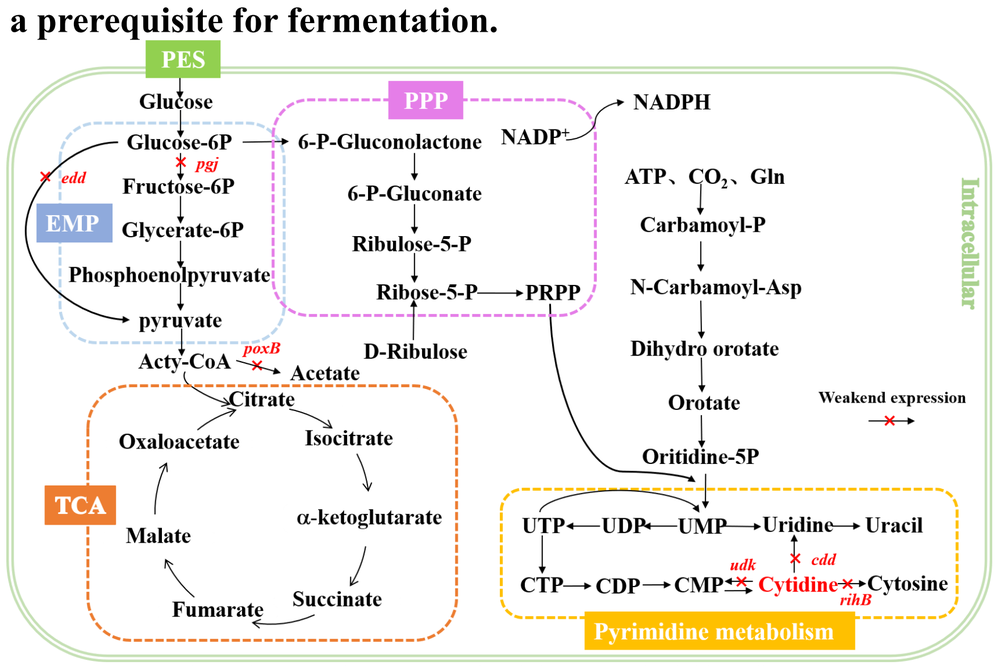


Fig.1 Biosynthetic pathway of cytidine in *Escherichia coli*

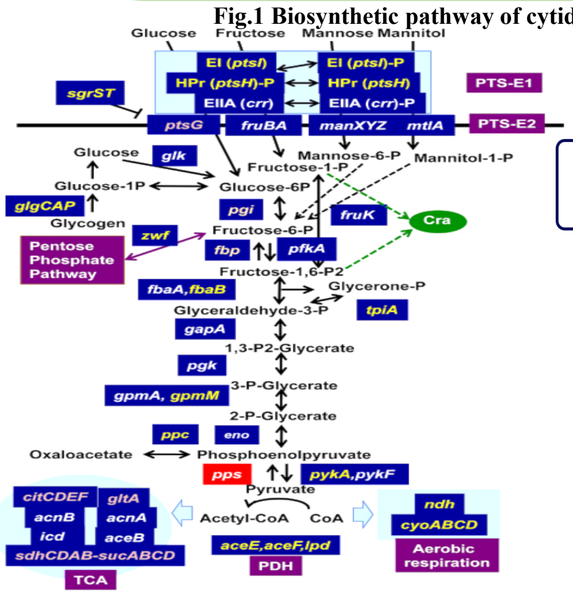


Fig.2 Genes regulated by the global transcription factor Cra

Global transcription factor Cra

- Catabolic in hibition/activation protein encoded by Cra
- A multifunctional DNA-binding transcription co-regulator
- Transcription levels of most enzymes in the carbon metabolism pathway of the regulatory center

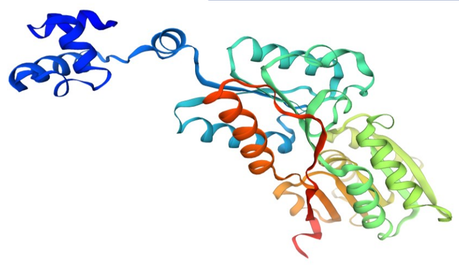


Fig.3 Structure of the Cra protein

METHOD

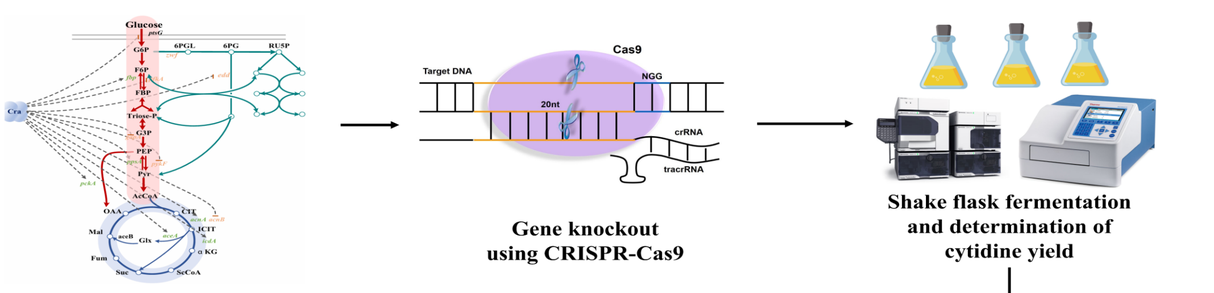


Fig.4 Technology road map

RESULTS & DISCUSSION

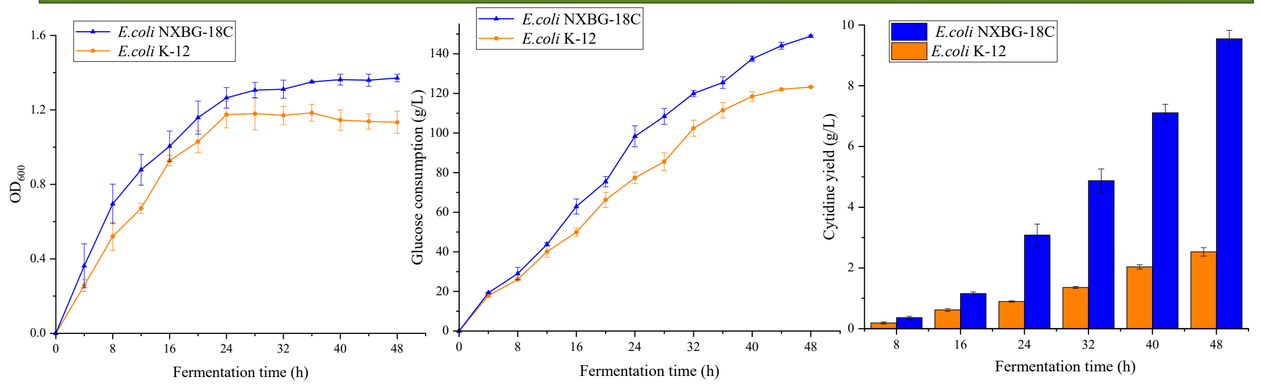


Fig.5 Shaker fermentation results of genetically engineered E. coli strains

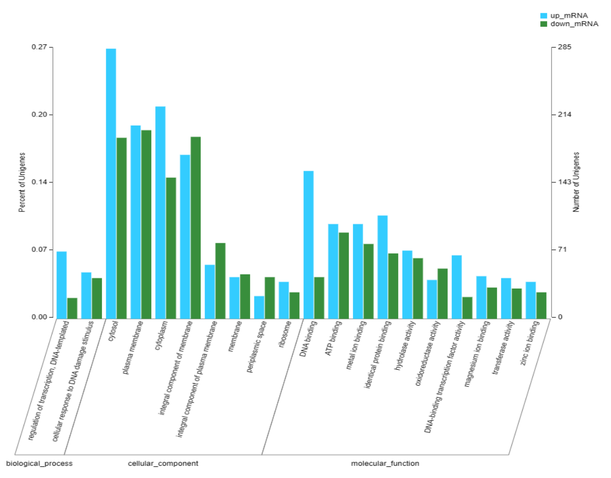


Fig. 6 DEGs GO Functions Annotated Bar Chart

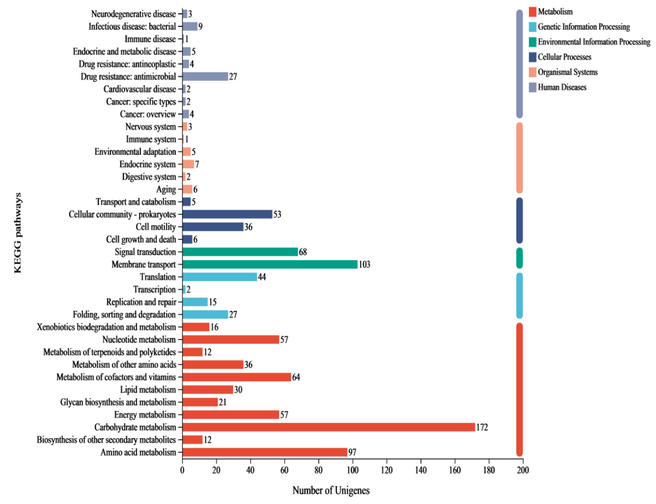


Fig. 7 DEGs KEGG Functions Annotated Bar Chart

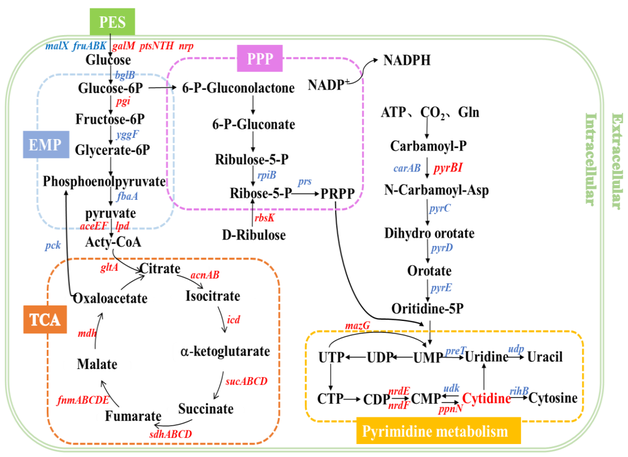


Fig. 8 Significantly altered genes in the cytidine synthesis pathway

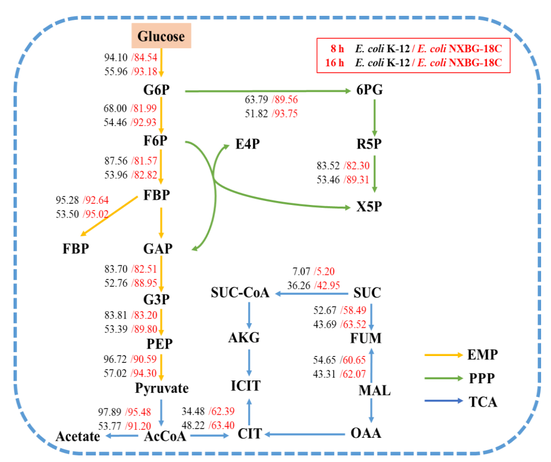


Fig. 9 Distribution of metabolic flows in control and experimental groups

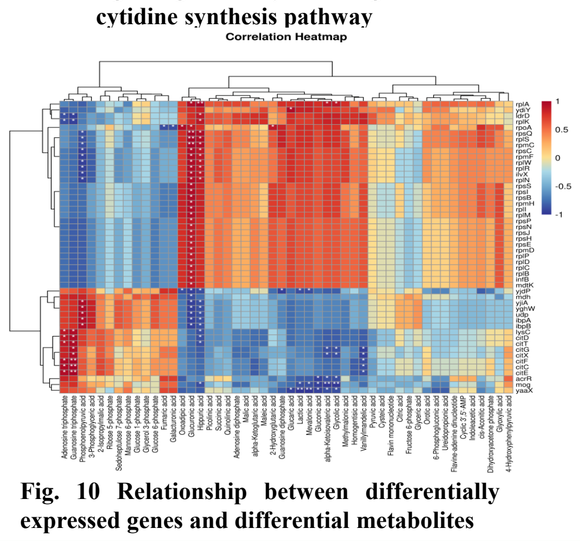


Fig. 10 Relationship between differentially expressed genes and differential metabolites

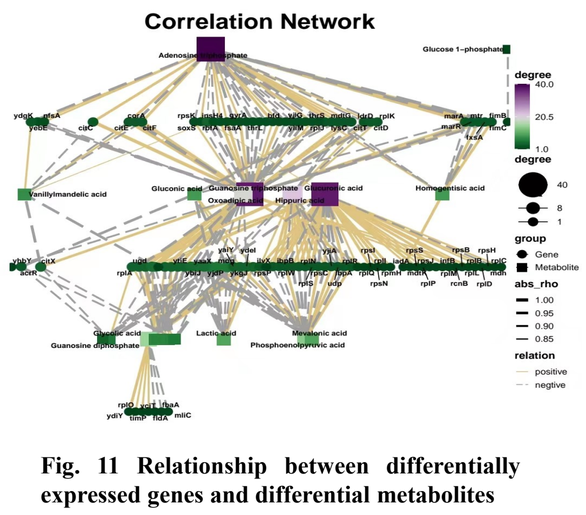


Fig. 11 Relationship between differentially expressed genes and differential metabolites

CONCLUSION

By deleting the global transcription factor Cra in *Escherichia coli* K-12 using CRISPR-Cas9, we constructed the mutant strain *E. coli* NXBG-18C, which produced 9.55 ± 0.29 g/L cytidine, a 3.77-fold increase over the wild-type. Transcriptomics and ^{13}C-metabolic flux analysis showed that Cra deletion redistributes carbon metabolism by upregulating phosphotransferase system (PTS) genes to enhance glucose uptake, reducing flux through the EMP and PPP pathways while reinforcing the TCA cycle. This metabolic shift increased NADPH and PRPP availability, suppressed cytidine degradation (*cdd*) and uridine synthesis pathways, and improved energy allocation, ultimately boosting cytidine production. Our results demonstrate Cra's central role in coordinating carbon metabolism for efficient cytidine biosynthesis in *E. coli*.

FUTURE WORK

- Using metabolic network models
- Development of efficient metabolic engineering strategies
- Optimising the theoretical yield of cytidine synthesis