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Genetic Engineering to Enhance Surfactin Production in *Bacillus subtilis* via Nitrogen Metabolism and Membrane Transport Pathways

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INTRODUCTION & AIM

Highlights

- Nitrate enhances surfactin production in *Bacillus subtilis* ATCC 21332.
- Nitrate-induced surfactin overproduction mechanisms in *B. subtilis* were unveiled.
- Comprehensive systems-level analysis identified *secA*, *ftsY*, and *ftsE* genes in surfactin transport.
- Combinatorial genetic engineering achieved a 41.4-fold increase in surfactin production.

Background

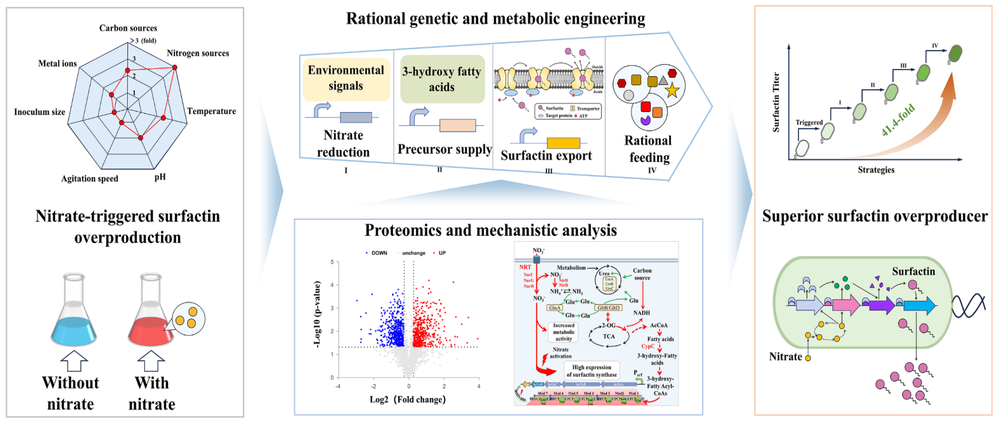
- Surfactin biosynthesis in *Bacillus subtilis* is orchestrated by intricate genetic networks responsive to environmental stimuli. [1]
- Low productivity of surfactin remains a significant constraint in its industrial applications. [2,3]

Aim Of The Study

- Explore the genetic determinants involved in the surfactin production pathway responding to different nitrogen sources.
- Provide valuable strategies for further genetic improvement and enhancement of surfactin-producing strains.

METHOD

1. The growth performance and surfactin production of *B. subtilis* ATCC 21332 were optimized.
2. The mechanism of nitrate-induced surfactin overproduction was investigated using comprehensive systems omics.
3. Strategies for combinatorial genetic engineering are outlined, including enhanced nitrate reduction metabolism, fatty acid substrate hydroxylation, membrane transporter engineering.



RESULTS & DISCUSSION

Identifying key differential genes involved in surfactin metabolism via comprehensive systems omics analysis.

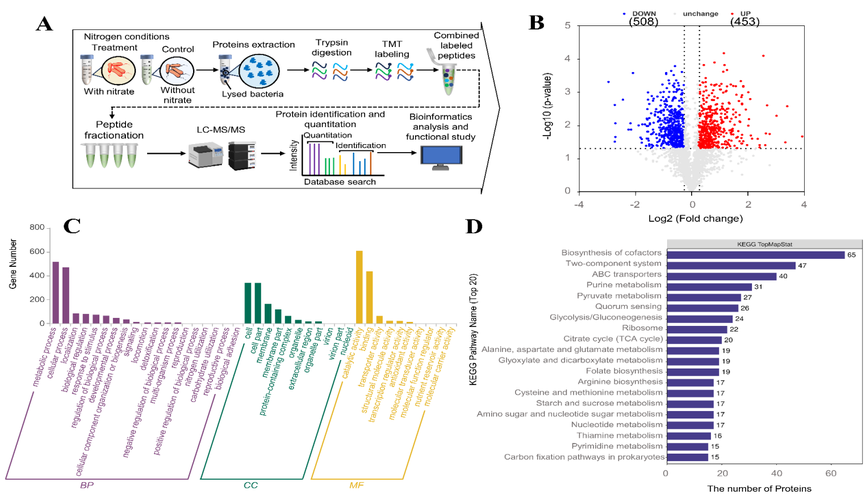


Fig 1. Comparative proteomic analysis in the presence and absence of sodium nitrate. (A) Flowchart of comprehensive systems omics analysis with and without sodium nitrate; (B) Volcano plot depicting differential gene expression with and without sodium nitrate. The horizontal axis represents Log_2 (FC) values, whereas the vertical axis represents $-Log_{10}$ (p value). Red dots indicate upregulated genes (FC ≥ 1.2), blue dots indicate downregulated genes (FC ≤ 0.8), and gray dots represent proteins with no differential changes; (C) GO function annotation plot; (D) KEGG pathway enrichment map (Top 20).

Annotation of key differential genes involved in central carbon metabolism, nitrogen metabolism, and surfactin pathways.

Central carbon metabolism
Fatty acid metabolism
Nitrogen metabolism
Surfactin metabolism
Cellular respiration

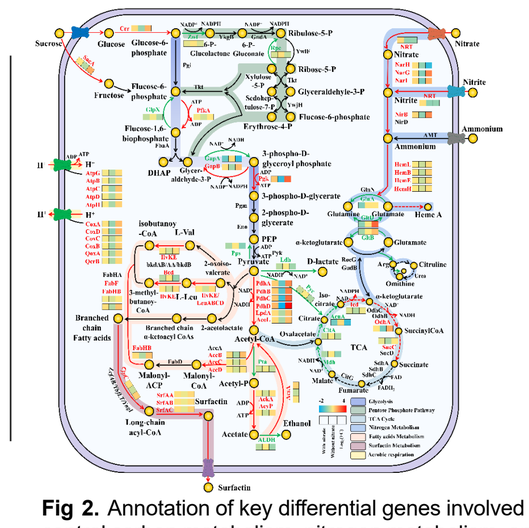


Fig 2. Annotation of key differential genes involved in central carbon metabolism, nitrogen metabolism, and surfactin pathways.

Enhanced nitrate-reducing metabolism and fatty acid substrate hydroxylation.

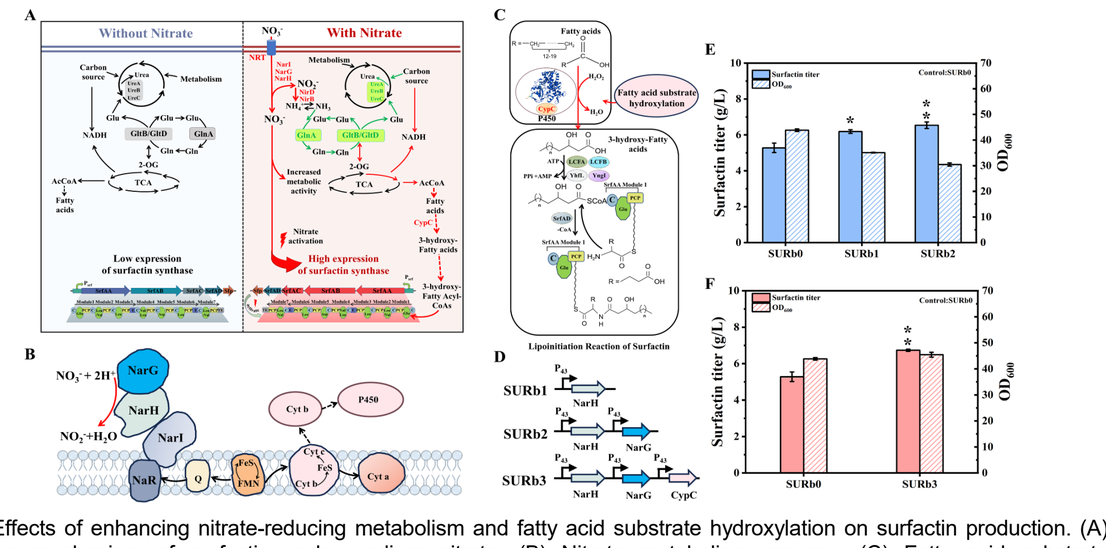


Fig 3. Effects of enhancing nitrate-reducing metabolism and fatty acid substrate hydroxylation on surfactin production. (A) Activation mechanism of surfactin under sodium nitrate; (B) Nitrate metabolism process; (C) Fatty acid substrate hydroxylation process; (D) Engineered strains designed for nitrogen metabolism and fatty acid substrate hydroxylation; (E) Analysis of the effects of *narH* and *narG* modification on surfactin production; (F) Examination of the influence of *cypC* modification on surfactin production.

Membrane transporter modification to increase the production of surfactin.

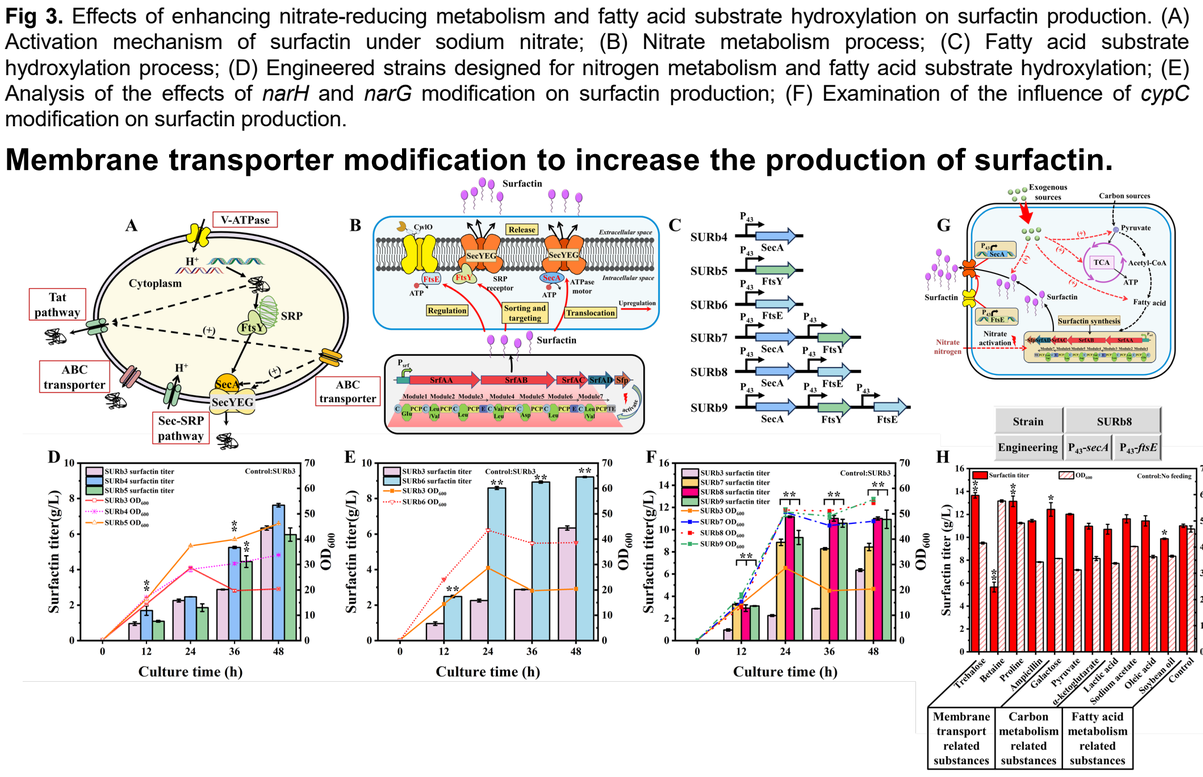


Fig 4. Effects of transporter modification on surfactin production. (A) Transport system of *B. subtilis*; (B) Representation of the Sec pathway and surfactin transport mechanism; (C) Engineered transporter-modified strains; (D) Effects of *secA* and *ftsY* modification on surfactin production; (E) Effects of *ftsE* modification on surfactin production; (F) Combined modification of *secA*, *ftsY*, and *ftsE* on surfactin production; (G) Rational feeding strategy targeting cellular pathways; (H) Effects of rational feeding (cell membrane translocators, carbon metabolizers, and fatty acid metabolizers) on surfactin production in SURb8 strain.

CONCLUSION

- This study demonstrated that surfactin overproduction could be induced under nitrate conditions.
- Comprehensive systems omics analyses unveiling the mechanism by which nitrate regulates surfactin overproduction.
- Modification of *narG*, *narH*, *cypC*, *secA*, and *ftsE* was found to significantly enhance surfactin production.
- Rational supplementation of cellular pathway substances effectively promoted surfactin production.

REFERENCES

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