Enhanced iturin A synthesis in *Bacillus amyloliquefaciens* Through Genetic Modification Targeting Rap Phosphatase Inactivation

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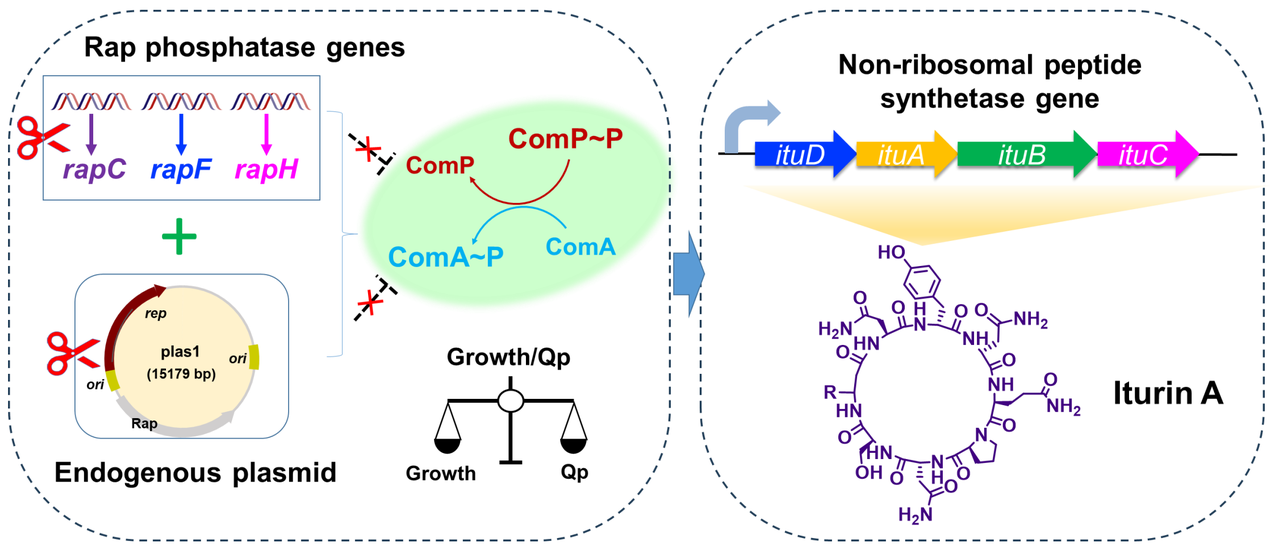
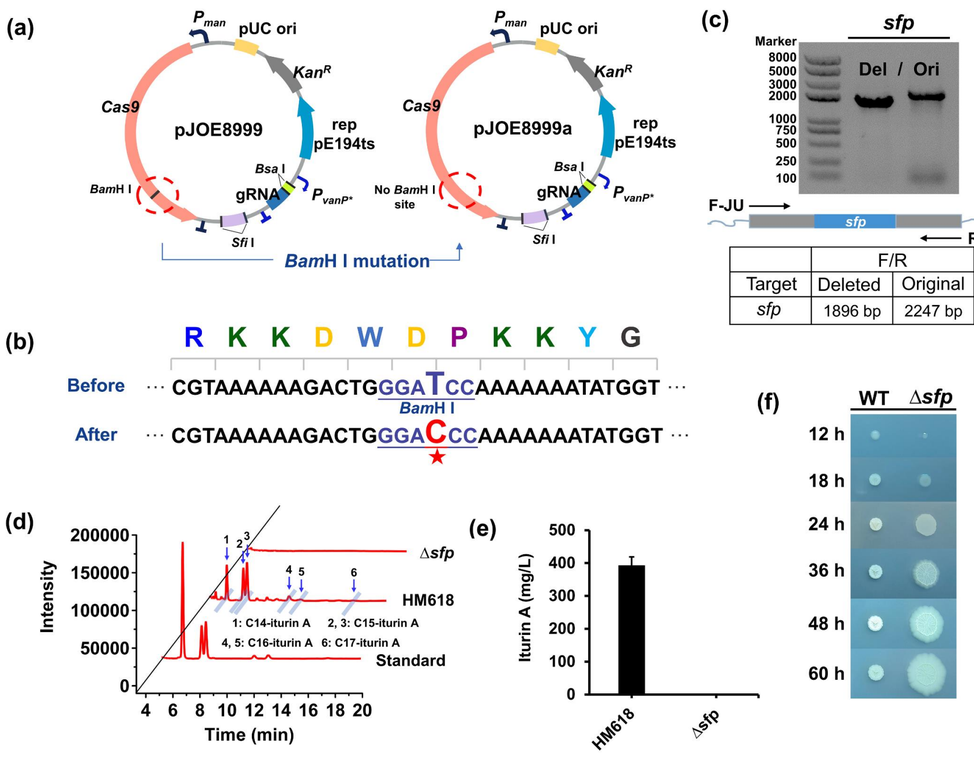
Abstract

Inuring A biosynthesis bottlenecks persist in its low productivity in wild strains and the ability to engineer *Bacillus amyloliquefaciens* producers. This study reveals deleting the endogenous plasmid, plas1, from the wild-type *B. amyloliquefaciens* HM618 notably enhances iturin A synthesis. Further, inactivating Rap phosphatase-related genes (*rapC*, *rapF,* and *rapH)* in the genome of the strain also improved iturin A level and specific productivity while reducing cell growth. Strategic rap genes and plasmid elimination achieved a synergistic balance between cell growth and iturin A production. Engineered strain HM-DR13 exhibited an increase in iturin A level to 849.9 mg/L within 48 h, significantly shortening the production period. These insights underscore the critical roles of endogenous plasmids and Rap phosphatases in iturin A biosynthesis.

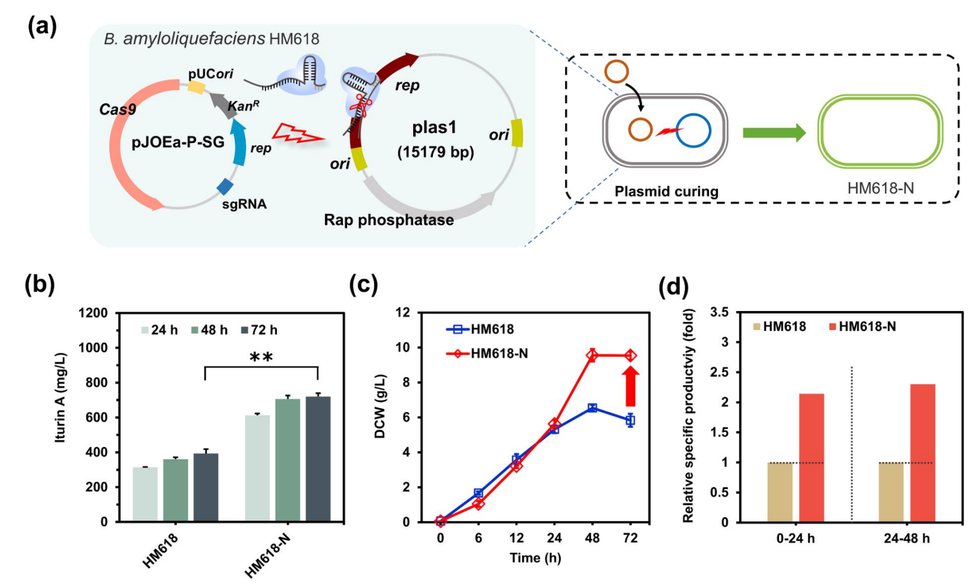
Design



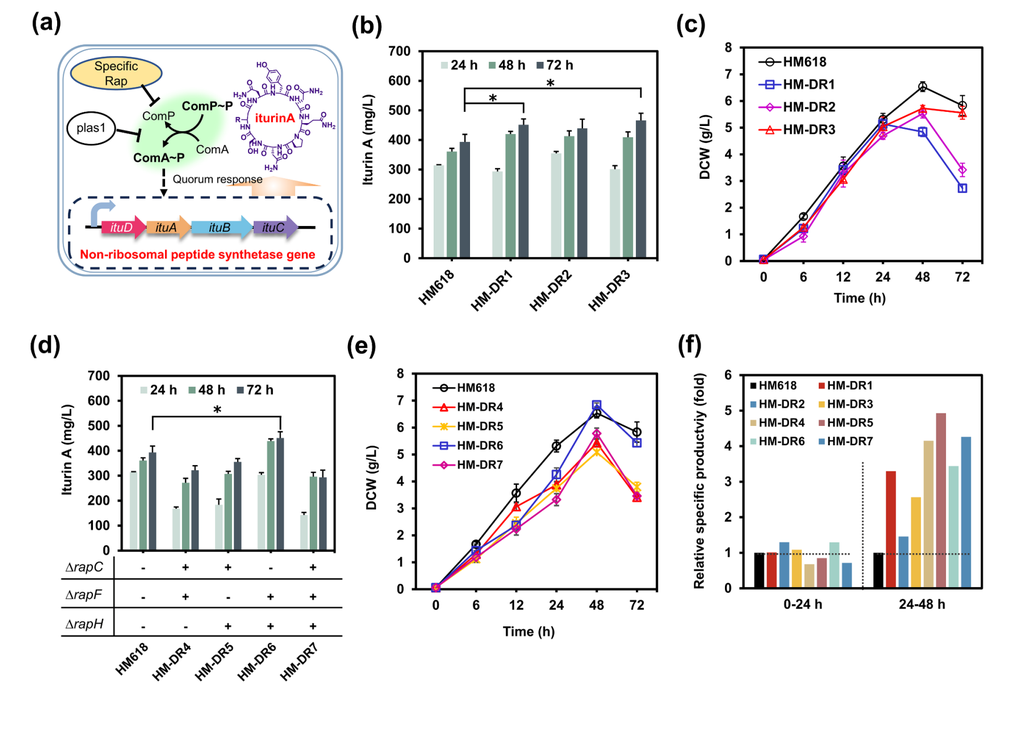
Result



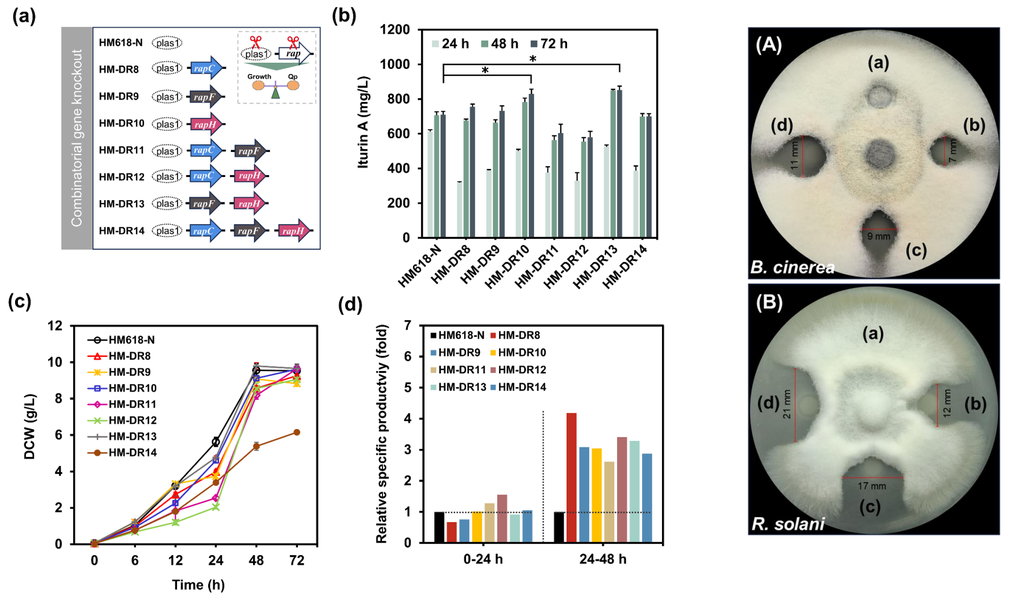
- **Genome Editing of *B. amyloliquefaciens* by Updated Gene-Editing Tool.**



- **Targeted Elimination of Endogenous Plasmid from *B. amyloliquefaciens* HM618.**



- **Elimination of Specific Rap Phosphatase Genes in the Genome.**



- **Coordinated Removal of Rap Genes and Plas1.**

- **Antifungal Activities.**

Conclusions

- Knocking out the endogenous plasmid increased iturinA production by 83.4%, and the biomass of strain HM618-N showed a remarkable 63.5% increase.
- Knocking out *rapC*, *rapF* and *rapH* increased the specific productivity of iturinA synthesis.
- Synergistic elimination of endogenous plasmids and Rap phosphatases balances cell growth and product synthesis.
- Underscore the critical roles of endogenous plasmids and Rap phosphatases in iturin A biosynthesis.

Acknowledgment

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