Modular engineering of *Mycobacteria* for high-titer production of 9α-hydroxyandrostene 4-ene-3,17-dione from phytosetrols

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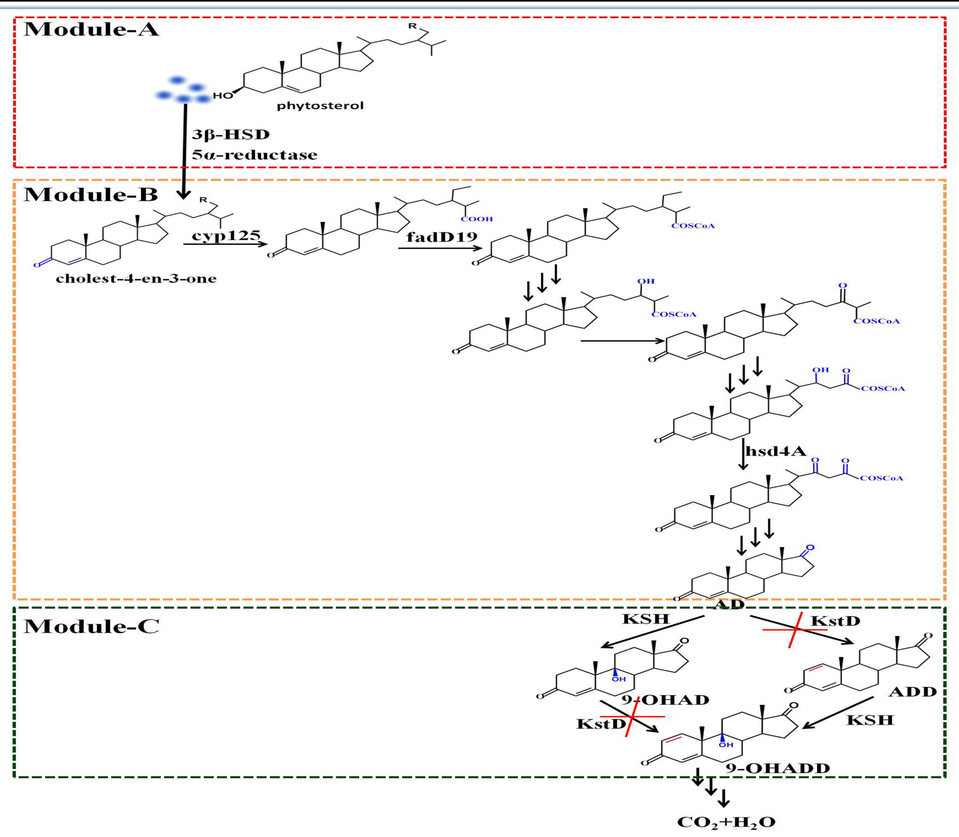
Highlights:

- We used modular synthetic biology approaches to engineer an industrial mycobacterium strain, enabling a high titer production of 9-OHAD (6.8 g/L), 1.4-fold of that of the parental strain (4.8 g/L). Such a 42% increase in the 9-OHAD titer by the engineered industrial Mycobacterial strain showed great promises in the industrial production of 9-OHAD.

Introduction:

- 9α-hydroxyandrostene 4-ene-3,17-dione (9-OHAD) is an important precursor of a large number of steroid drugs, such as hydrocortisone series and prednisone series glucocorticoids. Mycobacteria could use phytosterols as the carbon source for the production of 9-OHAD. To enhance the conversion ratio from phytosterols to 9-OHAD, modular synthetic biology approaches could be used to engineer Mycobacteria. Previous studies mainly focused on the modules of nuclear open cycle and degradation of steroids.
- Three catabolic modules were involved in the microbial production of 9-OHAD from phytosterols in an industrial *Mycobacterium* sp. strain MS136, namely sterol uptake (Module-A), sterol side-chain degradation (Module-B), and steroid nucleus open cycle and degradation (Module-C). Here, we systematically analyzed the important genes in the three modules and constructed *Mycobacterium* recombinant strains using modular synthetic biology approaches.

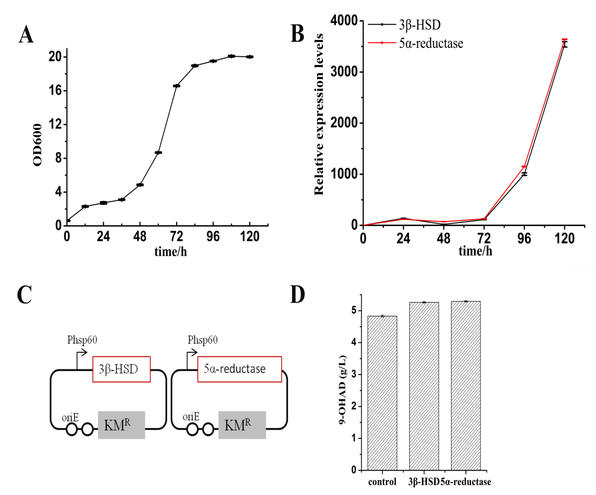
Modular pathway engineering:



Three modules of the phytosterol catabolism in *Mycobacterium* MS136 for the bioconversion of phytosterols.

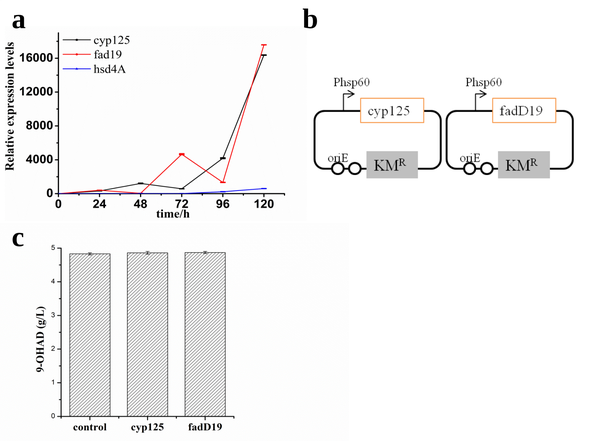
Results and Discussion:

Transcriptional analysis of the genes and engineering of Module-A.



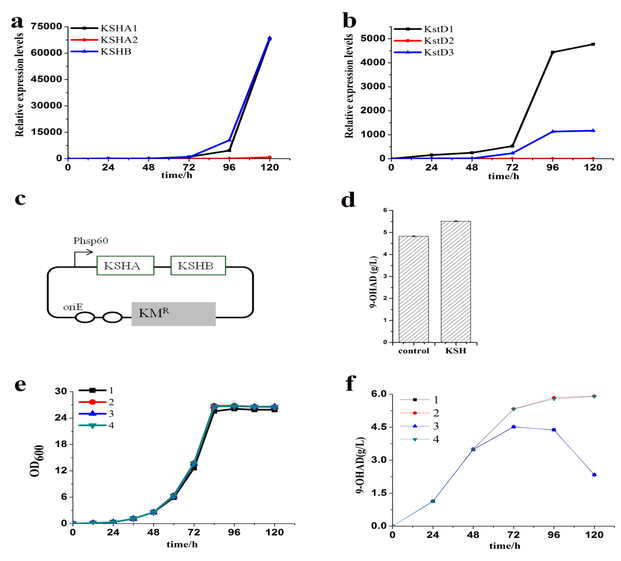
(A) Growth curve of *Mycobacterium* MS136. (B) The transcriptional expression level of the genes *3β-hsd* and *5α-reductase* in the sterol ingestion module (Module-A) in response to phytosterols. (C) The target gene (*3β-hsd* or *5α-reductase*) was ligated into the pMV261 plasmid to overexpress the individual gene, forming two recombinant plasmids. (D) The production of 9-OHAD upon overexpression of *3β-hsd* or *5α-reductase*. Phytosterol (13 g/L) were used as the substrate.

Transcriptional analysis of the genes and engineering of Module-B.



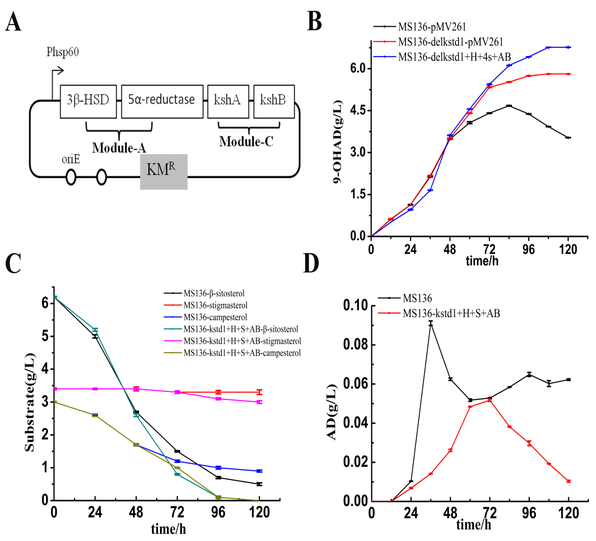
(A) Transcriptional expression level of the genes *cyp125*, *fadD19* and *hsd4A* in the side-chain degradation module (Module-B) in response to phytosterols. (B) Each of the target genes (*cyp125* or *fadD19*) was ligated into the pMV261 plasmid, forming three recombinant plasmids. Just to overexpress individual genes. (C) The production of 9-OHAD of each genes was overexpressed.

Transcriptional analysis of the genes and engineering of Module-C.



(A) Transcriptional expression level of the genes *KSH* in the sterol nucleus open cycle module (Module-C) in response to phytosterol. (B) Transcriptional expression level of the genes *KstD* in the sterol nucleus open cycle module (Module-C) in response to phytosterol. (C) The target gene (*KSH*) was ligated into the pMV261 plasmid, forming one recombinant plasmid. (D) The production of 9-OHAD of *KSH* gene was overexpressed. (E) Phenotypic analyses of *Mycobacterium sp.* MS136 mutant cells. (F) Real-time production of 9-OHAD throughout the aerobic fermentation.

Construction of the recombinant strain *Mycobacterium* sp. MS136-delkstd1+H+4s+AB for the enhanced production of 9-OHAD.



(A) The construction of the recombinant plasmids. The selected genes 3β-hsd (in Module-A), 5α-reductase (in Module-A) and ksh (in Module-C) were ligated into the pMV261 plasmid. (B) The time profile of the 9-OHAD titer of each recombinant strain in the aerobic fermentation. (C) The consumption of individual components in the phytosterols (feed-in 13g/L) during the120 hours' fermentation. (D) The production of AD by the wild-type (MS136) and the recombinant Mycobacteria (MS136-kstd1+H+S+AB).

Reference:

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