Enhanced Coenzyme Q10 Yield by Blocking Hopanoids Pathway

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Abstract

Microbial synthesis of coenzyme Q10 (CoQ10) by fermentation processes has been emerging in recent years. Given that the synthesis of hopanoids occurs as a branched pathway of CoQ10 synthesis, we hypothesized that blocking the hopanoids pathway might improve the CoQ10 yield. The recombinant strain *R. palustris* (Δshc) with blocked hopanoids pathway was employed for studying the accumulation of CoQ10. It was found that the CoQ10 content in *R. palustris* (Δshc) reached 3.71 mg/g DCW, corresponding to 34.7% improvement over the wild type strain.

Moreover, rate-limiting enzymes including endogenous UbiA and Dps from Rhodobacter sphaeroides were co-expressed, and the resulting strain yielded an additional 94% improvement of CoQ10 content over strain *R. palustris* (Δshc). Upon supplementing with NaHSO₃, the CoQ10 content was further increased to 6.51 mg/g DCW and the biomass was enhanced from 1.07 g/l to 1.33 g/l. The effectively strategies represented here will enhance our ability to design the system with metabolic engineering techniques for higher CoQ10 production.

Keywords: Coenzyme Q10; Co-expression; Hopanoids; Pathway blocking; Rhodopseudomonas palustris

Abbreviations: CoQ10: Coenzyme Q10; DCW: Dry Cell weight; DPP: Decaprenyl Diphosphate; FPP: Farnesyl Diphosphate; Phba: Parahydroxybenzoic Acid; UQ: Ubiquinone; PNSB: Purple Non-Sulfur Bacteria

Introduction

Coenzyme Q10 (CoQ10), otherwise known as ubiquinone (UQ), is a valuable bioactive compound used both medically and cosmetically [1]. Many studies focus on enhancing CoQ10 production in *Escherichia coli* (E.coli) by genetic engineering [2-9]. *Agrobacterium tumefaciens* also have been employed as the producers of CoQ10 [10,11]. Typical purple non-sulfur bacteria (PNSB) generally have a relatively high content of CoQ10, as ideal CoQ10 producers [12-16]. *R. Palustris* (*Rhodopseudomonas palustris*) is a PNSB with a high content of inherent CoQ10, whose fermentation processes have been well researched over the years [17,18]. Therefore, *R. Palustris* could be a potential host for CoQ10 production by metabolic engineering.

*R. palustris* TIE-1 contains more than 30 mg/l DCW (dry cell weight) of hopanoids that are not required for growth under normal conditions, although they play a role in membrane integrity and pH homeostasis [19]. The deletion of squalene-hopene cyclase protein (Shc), which cyclizes squalene to the basic hopene structure, can make the strain not produce any hopanoids [19]. As the synthesis of hopanoids occurs as a branched pathway of CoQ10 synthesis in *R. palustris* TIE-1 (Figure 1), it was hypothesized that blocking the hopanoids pathway might direct FPP flux towards CoQ10 pathway.

![Coenzyme Q10 biosynthesis and branched pathways](image)

**Figure1**: Coenzyme Q10 biosynthesis and branched pathways. Relevant abbreviations: DMAPP: Dimethylallyl Diphosphate; DPS: Decaprenyl Diphosphate Synthase Gene; DXP: 1-deoxy-xylulose 5-phosphate; FPP: Farnesyl Diphosphate; GAP: D-glyceraldehyde-3-phosphate; IPP: Isopentenyl Diphosphate; DPP: Decaprenyl Diphosphate; PYR: pyruvate; PHBA: Parahydroxybenzoic Acid; SCL: Squalene Hopene Cyclase Gene; UBI: 4-hydroxy Benzoate Octaprenyl Transferase Gene.

Given that sodium hydrogen sulfite (NaHSO₃) could enhance the growths of plant [20,21] and *Cyanobacterium synechocytis*

Results and Discussion

Increased CoQ10 content in \textit{R. palustris} (Δshc)

For accumulating squalene in \textit{R. palustris}, the shc gene was deleted to block hopanoids pathway in our previous study \cite{26}. Although the titer of squalene in \textit{R. palustris} (Δshc) reached 3.8mg/g DCW, it was much lower than the yield of total hopanoids (about 30mg/g DCW) in the wild strain, suggesting that many FPPs had been directed into other pathways. Here, \textit{R. palustris} (Δshc) was employed to study the accumulation of CoQ10 and carotenoids. Lycopene, a metabolite in the carotenoids pathway, was analyzed to represent the accumulation of carotenoids.

As shown in Figure 2, the CoQ10 content of \textit{R. palustris} (Δshc) reached 3.71mg/g DCW, which corresponds to a 34.7\% improvement over the parental strain \textit{R. palustris} TIE-1. Moreover, \textit{R. palustris} (Δshc) yielded a 46.2\% improvement of the lycopene content over \textit{R. palustris} TIE-1. These results suggested that shc deletion diverted the FPP flux from the production of hopanoids to the biosyntheses of both CoQ10 and carotenoids. It is noteworthy that there was not a noticeable decrease in biomass of the recombinant strain, demonstrating the hopanoids pathway blocking was an efficient strategy for improving CoQ10 production. However, carotenoids content increased more significantly, means that the increase in CoQ10 production might be restricted by the increased carotenoids yield because it consumed more FPP (Figure 2).

CoQ10 production improvement by the co-expression of dps and ubiA

To divert more FPP to the biosynthesis of CoQ10, decaprenyl diphosphate synthase (Dps) from Rhodobacter sphaeroides 2.4.1 which catalyzes FPP to form DPP was expressed. The investigation revealed that the CoQ10 content of \textit{R. palustris} (Δshc)/pMGD was increased to 4.38mg/g DCW but the CoQ10 yield because it consumed more FPP (Figure 2).
*palustris* (Δshc)/pMGD was higher than that in *R. palustris* TIE-1/pMGD. In the meantime the lycopene content in *R. palustris* (Δshc)/pMGD was decreased from 5.06mg/g DCW to 4.67mg/g DCW (Table 1). Results suggested that the Dps expression enhanced FPP flux to the biosynthesis of CoQ10 and decreased the FPP flux to carotenoids pathway. However, the increment of CoQ10 content was not as high as expected. It was hypothesized that the limited pools of pHBA and UbiA restricted the utilization of DPP for CoQ10 biosynthesis, although more DPP had been formed by Dps expression.

UbiA which catalyzes pHBA and DPP to form decaprenylpHBA is one of the rate-limiting enzymes in the synthetic pathway of CoQ10. And its overexpression has been shown to increase CoQ10 content, especially when accompanied with the supplementation of pHBA [2,6]. To increase the utilization of DPP for CoQ10 biosynthesis, endogenous UbiA was over expressed along with the pHBA supplementation (100mg/l). As can be seen in Table 1, the CoQ10 content was increased to 6.16mg/g DCW in *R. palustris* (Δshc)/pMGDI, while that was 4.42mg/g DCW for *R. palustris* TIE-1/pMGDI. Notably, the lycopene content was further decreased to 3.92 mg/g DCW. Obviously, the expression of UbiA and supplementation with pHBA enhanced the competitiveness of CoQ10 pathway for FPP. However, the cell mass was slightly decreased, which may caused by metabolic burden due to protein overexpression (Table 1).

| Strategies          | Coq10 Content (Mg/G DCW) | Lycopene Content (Mg/G DCW) | Biomass (G/L DCW) |
|---------------------|--------------------------|-----------------------------|------------------|
|                     | WT          | Δshc       | WT            | Δshc       | WT      | Δshc            |
| CK                  | 2.75±0.12  | 3.71±0.17  | 3.46±0.16  | 5.06±0.25  | 1.17±0.07 | 1.15±0.09       |
| dps+                | 3.15±0.15  | 4.38±0.22  | 3.24±0.14  | 4.67±0.27  | 1.05±0.06 | 1.08±0.05       |
| dps+ ubiA+pHBA+     | 4.42±0.18  | 6.16±0.26  | 2.73±0.13  | 3.92±0.19  | 1.09±0.09 | 1.06±0.08       |

Table 1: Content of CoQ10 under the co-expression of genes.

NaHSO₃ enhanced both CoQ10 content and biomass

To study the influence of NaHSO₃ on the cell growth and CoQ10 production of *R. palustris* TIE-1, NaHSO₂ at different levels was supplemented into the culture medium. As shown in Figure 3, both the biomass and the CoQ₁₀ content were increased in the presence of NaHSO₂. The supplementation with 0.5mM NaHSO₂ resulted in 6.51mg/g DCW CoQ₁₀ and 1.33g/l biomass, generating the highest CoQ₁₀ production of 8.65mg/g, corresponding to a 33.2% improvement over the control (Figure 3). The increases in biomass and CoQ₁₀ content indicated that NaHSO₂ increased the photo-phosphorylation with intensified electron transport efficiency, and thus the accumulation of CoQ₁₀ was enhanced as one of the electron transfer agents. In future, more studies are needed to explore the functional mechanism of NaHSO₃ (Figure 3).

Conclusion

In this study, we report several strategies, including hopanoids pathway blocking, genes co-expression and NaHSO₃ supplementation, for enhancing CoQ₁₀ production. Based on the combination of these strategies, the content of CoQ₁₀ reached 6.51mg/g DCW, which was 1.3-times higher than that for wild-type strain. This work enriched the strategy for metabolic engineering and showed the potential of producing coenzyme Q₁₀ by *Rhodopseudomonas palustris* TIE-1.

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