Promoter engineering for improved B_{12} production in *Pseudomonas denitrificans* ATCC 13867

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Abstract

- Coenzyme B_{12} is a cofactor for glycerol dehydratase, an important enzyme for 3-HP production.
- The synthesis of coenzyme B_{12} by *P. denitrificans* is transcriptional/ translational regulated by riboswitches (RSs) (Fig. 1).
- Promoters were engineered to improve B_{12} production.

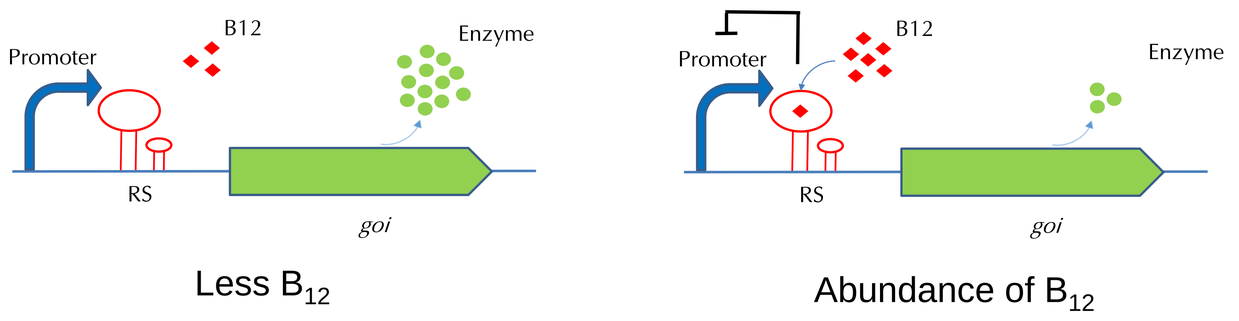

Fig. 1 Regulation of B_{12} biosynthetic genes by riboswitch.

Results and Discussion

Oxygen dependent pathway for B_{12} biosynthesis includes 26 steps

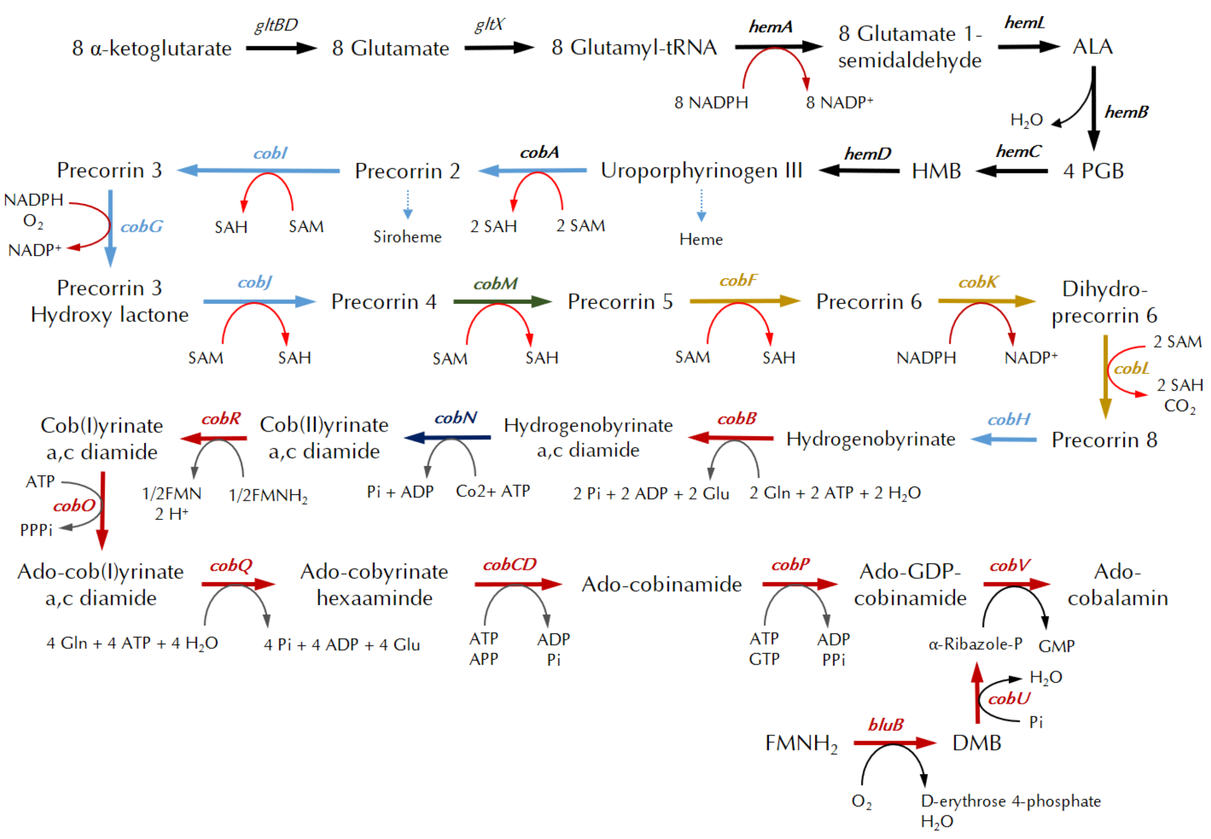

Fig. 2 B_{12} biosynthetic pathway.

Locations of 4 B_{12} riboswitches in cluster I and II

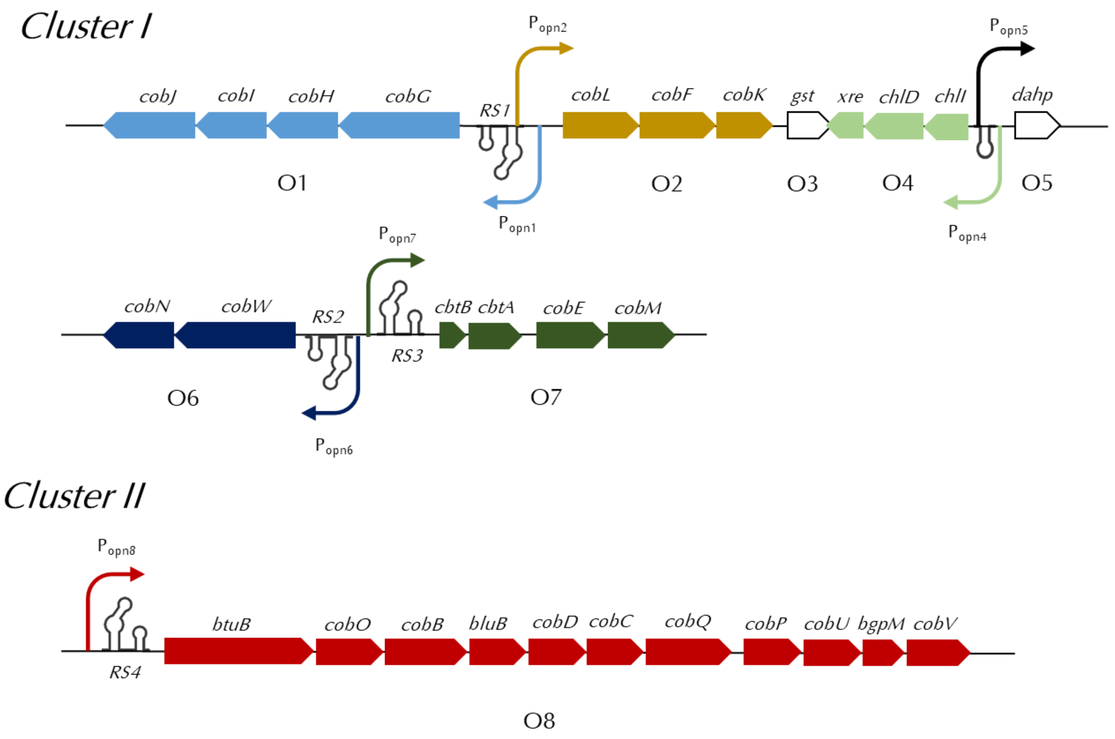

Fig. 3 Arrangement of B_{12} biosynthetic genes in cluster I and cluster II.

Replacement of native promoters by constitutive promoters

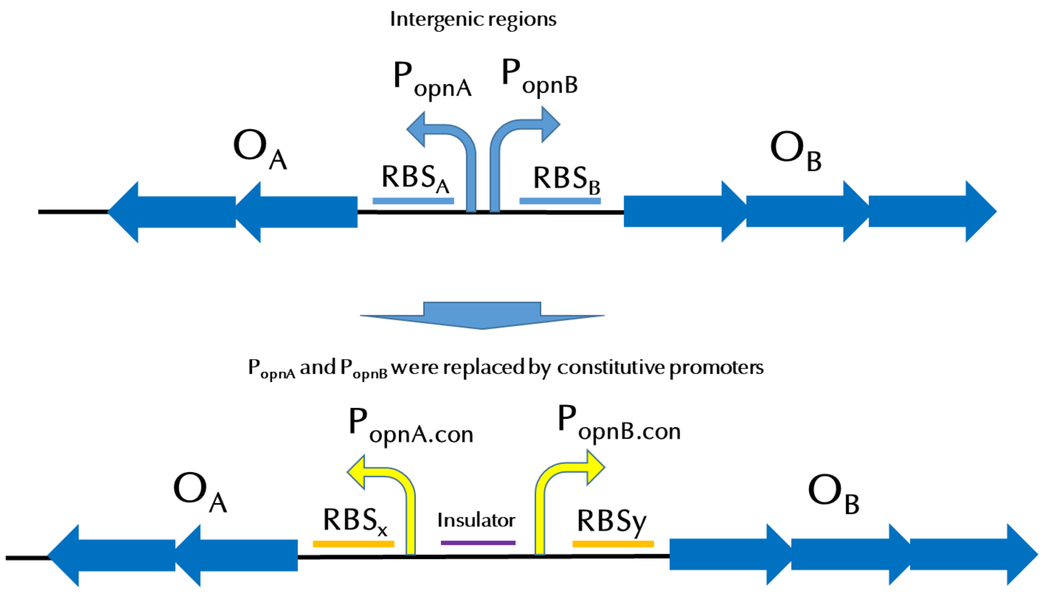

Fig. 4 Strategy for replacement of native promoters by constitutive promoters.

Enhanced B_{12} production

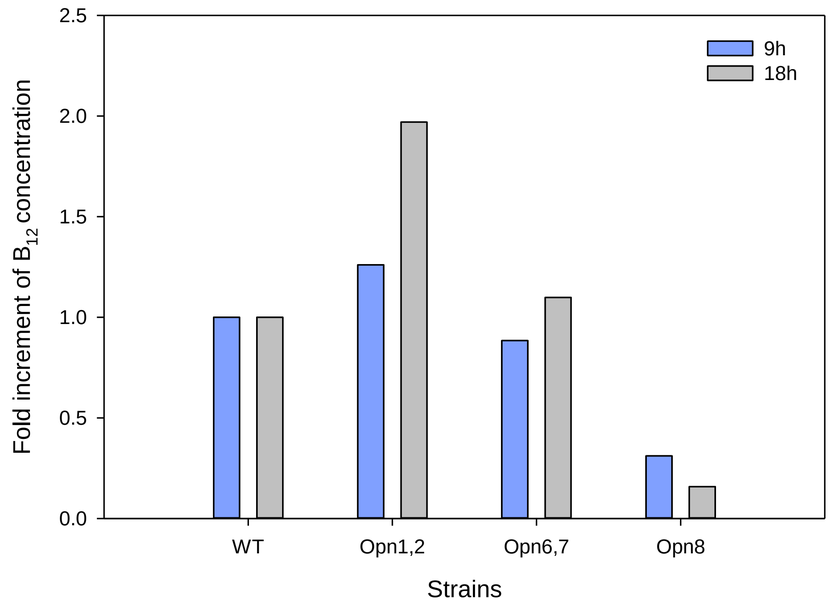

Fig. 5 Fold increasing of B_{12} concentration. "Opn" stands for "Operon", the numbers indicated the operons whose promoters has been changed.

Conclusion

- Riboswitch regulation was removed.
- Constitutive promoters were developed and were used to improved B_{12} production.
- Interestingly, replacement of promoter for operon 8 reduced B_{12} production.

ACKNOWLEDGEMENT

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