Abstract

Vitamin B12, also known as cobalamin, is a vital nutrient required across all branches of life, but the ability to synthesize this complex molecule de novo is limited to only a few archaea and bacteria. De novo synthesis begins with glutamate and utilizes over 30 gene products to produce an active cobalamin [1]. Previous studies suggest that of the available bacterial genomes, only half utilizing cobalamin can synthesize it [2]. The other half either take up complex cobalamin from the environment via an ABC transporter, or scavenge incomplete corrinoids (partial cobalamin molecules) as precursors to synthesize active cobalamin [3, 4].

The evolutionary histories and identities of multiple genes within these B12 pathways are unknown, leaving gaping holes in our understanding of the only source of biosynthesis of the vitamin so essential to human survival. Genes of particular interest to this investigator are those responsible for producing reductases that act upon the central cobalt atom of B12. Three reductases with unknown gene identities are located within the B12 biosynthetic pathway and it is the aim of this research to identify those genes responsible.

Background

Vitamin B12

- Most complex cofactor
- Essential micronutrient required by life
- Only made by certain bacteria and archaea.
- Structure contains modified tetrapyrrole ring bearing resemblance to heme, chlorophyll, siroheme and cyanochrome Fe3+. 
- Each organism’s B12 pathway unique

Fig. 1. Vitamin B12 (cobalamin). The central cobalt atom is the key to the collector’s biological activity and versatility.

Ways to get Vitamin B12

1. De novo synthesis
2. Cobinamide salvaging
3. Transport of external B12

Fig. 2. Bacterial vitamin B12 biosynthetic pathway. Organisms can utilize one of three major ways to get vitamin B12: 1. De novo synthesis, 2. Cobinamide salvaging, 3. Transport of external B12.

Identifying reductases in the B12 pathway

- Parts of the B12 pathways poorly understood
- Specifically reductases that act upon the central cobalt atom of the B12
- Reduction of the cobalt is the driving force behind B12
- Few reductases have been identified
- Salmonella enterica and Escherichia coli [6]
- Pseudomonas denitrificants [7, 8]

Fig. 5. B12 production experimentally verified in Thermosiphon. Cell extracts of Ts. Africanus H17 and TCF tested for B12 production by UPLC/MS (shown above) and Lactobacilli assay. This was verified by qRT-PCR analysis of gene expression.

Fig. 6. Gene neighborhoods of reductase candidates. Genes of interest (top: Tmel_0728 and 0733) are located within the vicinity of genes related to B12 biosynthesis or B12 dependent reactions.

Table 1. Gene candidate sequence analysis as done by Pfam and CofSPP. [9, 10]

- These three candidates are currently being expressed for purification and characterization
- Candidates will be compared to known reductases from Salmonella enterica, Escherichia coli and Pseudomonas denitrificants

Conclusion

The main goal of this work is to identify the genes responsible for the catalytic reductases that act within the B12 cofactor of the Thermosiphon pathway. These three reductase candidates have been made in certain bacteria and are currently being expressed and characterized for further experiments.

References


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Investigating the origins of B12 biosynthesis in the most ancient roots of the tree of life

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