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Studies on the novel menaquinone biosynthetic pathway and its application to anti-*Helicobacter pylori* drugs

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In prokaryotes, menaquinone (MK) is used for respiration. In *Escherichia coli*, MK is biosynthesized from chorismate by eight enzymes. However, we recently identified an alternative pathway (the futalosine pathway), which operates in bacteria, including some pathogens, such as *Helicobacter pylori* and *Campylobacter jejuni*[1,2]. However, the details of each of the biosynthetic steps in the futalosine pathway remain unclear. In this study, we investigated the second step of the futalosine pathway, which is the conversion of futalosine (FL) into dehypoxanthinyl futalosine (DHFL) by MqnB (futalosine hydrolase, EC number, EC3.2.2.26), in *Acidothermus cellulolyticus*, *H. pylori* and *Streptomyces coelicolor*. Although the *mqn* genes were scattered throughout the genomes in most of microorganisms, the *mqn* genes and other menaquinone-related genes such as prenylation and methylation genes were clustered in two loci on the genome of *A. cellulolyticus*, a thermophilic actinobacteria. Interestingly, a gene for an adenosine deaminase ortholog (Acel_0264) exists in one of the clusters. Considering that biosynthetically related genes are usually clustered in microorganism genomes, we can assume that aminodeoxyfutalosine (AFL) might be an intermediate in the futalosine pathway operating in this strain. To examine this possibility, we prepared the recombinant Acel_0264 gene product and incubated it with chemically synthesized AFL. As expected, the protein converted AFL into FL. We also examined whether the *A. cellulolyticus* MqnB protein, the gene product of Acel_0106, could directly use AFL as a substrate. However, we did not detect the formation of DHFL, despite the fact that AFL and FL have very similar structures. On the other hand, the recombinant MqnB protein from *Thermus thermophilus* (the product of TTHA0556) only reacted on FL. Judging from the genome database of *T. thermophilus*, this strain may not have any adenosine deaminases. Therefore, it was quite reasonable that the *T. thermophilus* MqnB only accepted FL as a substrate.

We next searched for the MqnB ortholog in the genome database of *H. pylori* strain 26695 using the *A. cellulolyticus* MqnB protein as a query. However, we did not find a typical MqnB ortholog with a significant estimated value (e-value), although the strain had an MqnC ortholog

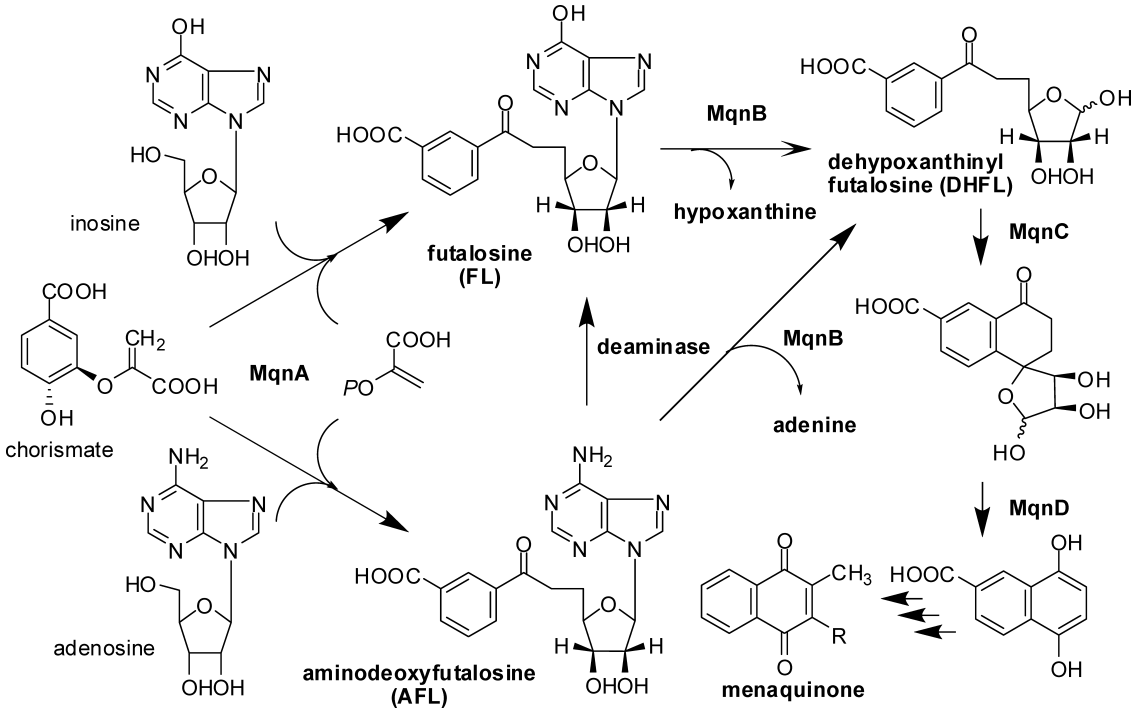

Fig. 1. An alternative menaquinone biosynthetic pathway (the futalosine pathway).

(HP0656, e-value $4e^{-81}$) and an MqnD ortholog (HP0152, e-value $3e^{-16}$). The gene from strain 26695 encoding the most similar protein to the *A. cellulolyticus* MqnB protein was HP0089 (e-value, 0.18), which was annotated as a gene encoding a 5′-methylthioadenosine/S-adenosylhomocysteine nucleosidase (EC: 3.2.2.9). Therefore, we prepared a recombinant protein encoded by HP0089 and incubated it with FL; however, the protein did not act on FL. Therefore, we assumed that AFL may be an intermediate in the futalosine pathway operating in *H. pylori* strain 26695. Therefore, the recombinant HP0089 gene product was incubated with AFL and the reaction product was analyzed by HPLC. As expected, AFL was consumed and two products were detected. The products were confirmed to be adenine and DHFL by LC-MS analysis. This result showed that AFL was directly converted into DHFL. This direct conversion without deamination was also in agreement with the observation that strain 26695 did not have an ortholog of Acel_0264, which encodes an AFL deaminase.

In summary, three routes to the formation of DHFL in the futalosine pathway were suggested to exist (Fig. 1)[3]. In *T. thermophilus*, FL might be directly formed from chorismate by MqnA and converted into DHFL. On the other hand, AFL is likely formed from chorismate by MqnA in *A. cellulolyticus*, *S. coelicolor*, and *H. pylori*. In the case of the former two bacteria, AFL is thought to be converted to FL by deaminases, which is subsequently metabolized into DHFL. In contrast, AFL is most likely directly converted to DHFL in *H. pylori*.

References

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