The [Fe-S] cluster containing enzymes of the nicotinate fermentation pathway of *Eubacterium barkeri*

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Nicotinate (niacin, vitamin B3) is an important constituent of all living cells in the form of NAD(P). Organisms contain NAD(P) concentrations of 0.1-1 mM which upon cell-death supply nicotinate as a nitrogen, carbon and energy source to dedicated nicotinate-utilizing microorganisms. Regardless of organism and environment catabolism of nicotinate starts with hydroxylation to 6-hydroxynicotinate by the well-characterized molybdopterin enzyme nicotinate dehydrogenase. In aerobic organisms 6-hydroxynicotinate is oxidatively decarboxylated to 2,5-dihydroxypyridine or subjected to a second hydroxylation yielding 2,6-dihydroxynicotinate. Under microaerobic or anaerobic conditions the ferredoxin-dependent reduction to 1,4,5,6-tetrahydro-6-oxonicotinate (THON) is observed [1]. The latter pathway is followed by the anaerobic soil bacterium *Eubacterium barkeri*, which belongs to the Clostridia. *E. barkeri* ferments nicotinate to propionate, acetate, CO_2, NH_4^+ and ATP. Early work by E.R. and T.C. Stadtman outlined the pathway and defined intermediates. Several enzymes were characterized: nicotinate dehydrogenase [2,3], 6-hydroxynicotinate reductase [1], 2-methyleneglutarate mutase and 3-methylitaconate isomerase [4]. Later 2,3-dimethylmalate dehydratase [5] and lyase [6] were identified. We purified and characterized two further 'missing' enzymes: enamidase (THON hydrolase) and 2-(hydromethyl)glutarate dehydrogenase. Recently cloning of the *E. barkeri* nicotinate fermentation gene cluster harbouring genes coding for all enzymes was completed. The poster will summarize results and present data on the [Fe-S] cluster containing enzymes nicotinate dehydrogenase, 6-hydroxynicotinate reductase, 2-(hydroxymethyl)glutarate dehydratase and dimethylmalate dehydratase.

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