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NEW INSIGHTS INTO THE METABOLISM OF *n*-ALKENES AND *n*-ALKANES IN ANAEROBIC BACTERIA

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A review of the actual knowledge of the anaerobic oxidation of *n*-alkenes and *n*-alkanes in anaerobic bacteria will be expanded with recent results giving new insights into the catabolism and the anabolism of these hydrocarbons in sulphate-reducing (SRB) and denitrifying bacteria.

The mechanism of the activation of hydrocarbons in the absence of oxygen is of particular environmental and geochemical interest. Different works during the past decade have demonstrated the utilization of hydrocarbons under anoxic conditions, but still many gaps remain in the understanding of the anaerobic oxidation of these apolar molecules. In some early studies of alkane-degrading microorganisms, an oxygen-independent initial metabolism of alkanes via dehydrogenation to 1-alkenes and hydration to primary alcohols was suggested. In recent investigations, however, alkane dehydrogenation to 1-alkenes was viewed critically and two alternative pathways for the initial oxidation of alkanes could be described: addition of fumarate at C-2 or carboxylation at C-3 (e.g. Callaghan et al., 2006). Despite few indications that alkanes and alkenes are degraded differently in certain SRB, the initial reactions of *n*-alkenes activation in anaerobic bacteria still remain enigmatic.

We investigated the anaerobic biodegradation of C_{14}-C_{18} *n*-alk-1-enes in a marine SRB (*Desulfatibacillum aliphaticivorans* strain CV2803^T) recently isolated from marine sediments and known to degrade *n*-alkanes by addition of fumarate (Cravo-Laureau et al., 2005). This strain predominantly transformed C-odd and C-even *n*-alk-1-enes to C-odd and C-even fatty acids, respectively. In addition to classical bacterial fatty acids, unusual 2- and 4-ethyl-branched fatty acids and saturated and mono-unsaturated 4-methyl-branched fatty acids with carbon chain-length related to that of the growth substrate were systematically identified. Except for saturated 2-Me- and 4-Me-branched fatty acids, specific metabolites produced during the metabolism of *n*-alkanes by addition of fumarate (i.e. alkyl-succinates and 6-Me-

and 8-Me-branched fatty-acids) were never detected during growth on *n*-alkenes. Cultures of strain CV2803^T using synthetic (per)deuterated *n*-alkenes allowed to unambiguously demonstrate that the initial activation of these unsaturated hydrocarbons proceeds mainly by hydration of the double bond (C-1) and to a lesser extent by carbon addition at C-2 and C-3, yielding linear fatty acids and branched fatty acids, respectively. Further experiments performed with antibiotics suggested that enzymes required for *n*-alkene oxidation by strain CV2803^T are inducible.

Our results clearly show that *n*-alkanes and *n*-alkenes are degraded by distinct pathways in some anaerobic bacteria and that diverse initial reactions of *n*-alkenes activation can occur simultaneously in the same strain (Figure 1). Besides describing the first metabolic pathways of *n*-alkenes in an anaerobic bacterial isolate, our work also proposes some (unsaturated) methyl- and ethyl-branched fatty acids as potential diagnostic biomarkers of *n*-alkenes anaerobic biodegradation.

On the other hand, a survey of the cellular fatty acid composition of different bacteria able to oxidize *n*-alkanes in the absence of oxygen showed that a marine denitrifying strain can transform *n*-alkanes to fatty acids by a (yet undetermined) mechanism clearly different from the two already known to occur in anaerobic bacteria (see above). This indicates that (at least) a third possibility for the initial oxidation of *n*-alkanes in anaerobic bacteria is likely to exist and highlights the need for further investigation in this research area.

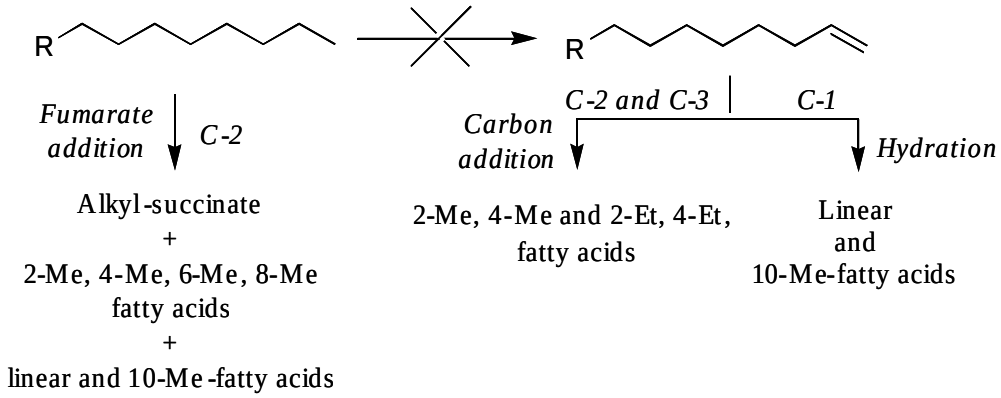

Figure 1. Initial reactions of *n*-alkane and *n*-alkene activation in the sulfate-reducing bacteria *D. aliphaticivorans* strain CV2803^T.

REFERENCES

Callaghan A., Gieg L., Kropp K., Suflita J. and Young L. (2006) Comparison of mechanisms of alkane metabolism under sulfate-reducing conditions among two bacterial isolates and a bacterial consortium. *Applied and Environmental Microbiology* **72**, 4274-4282.

Cravo-Laureau C., Grossi V., Raphel D., Matheron R. and Hirschler-Réa A. (2005) Anaerobic *n*-alkane metabolism by a sulfate-reducing bacterium, *Desulfatibacillum aliphaticivorans* strain CV2803^T. *Applied and Environmental Microbiology* **71**, 3458-3467.