**Anaerobic degradation of butane by marine sulfate-reducing bacteria: Metabolic insights from ultra-high resolution mass spectrometry.**

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Anaerobic degradation of the non-methane, short chain alkanes (ethane, propane, *n*-butane and *iso*-butane) by marine sulfate reducing bacteria was proposed to explain the apparent differences between rates of anaerobic oxidation of methane (AOM) and sulfate reduction (SRR) in the vicinity of deep sea hydrocarbon seeps. In situ and cultivation studies have revealed cold-adapted, meso and thermophilic bacterial strains and enriched cultures that are able to degrade one or several of the short chain alkanes. Among them, a pure culture of a sulfate-reducing bacterium, strain BuS5, afﬁliated with the *Desulfosarcina–Desulfococcus* cluster of the *Deltaproteobacteria*, has been shown to degrade both propane and *n*-butane.

Medium and long chain alkanes are typically activated at the secondary (subterminal) carbon atom, followed by addition to fumarate yielding methylalkylsuccinate derivatives. Metabolite analysis of the *n*-butane degrading strain BuS5 by GC-MS revealed the presense of (1-methylpropyl)succinate. This indicated a similar mechanism of activation for butane, via a radical-initiated C-H cleavage at the subtermial C atom. Further degradation is assumed to proceed by ligation to coenzyme A (CoASH), yielding (1-methylpropyl)succinyl-CoA thioester, C-skeleton re-arrangement and -oxidation to acetyl-CoA.

Here we investigated the intracellular metabolome of strain BuS5 growing with *n*-butane under sulfate-reducing conditions. The biomass (from 150 mL) was centrifuged, washed with isoosmotic buffer and lysed via sonication in methanol. The supernatant was flash frozen and used for direct infusion positive and negative electrospray ionization coupled to a 12T Fourier transform ion cyclotron resonance mass spectrometer (Bruker SolariX XR). The resolution of the mass spectrometer (1,000,000 at m/z 400) is sufficient to distinguish metabolites that differ in their molecular mass by just the mass of an electron, providing a comprehensive overview and annotation of the intracellular metabolites of strain BuS5. Main focus was on the identification of the (1-methylpropyl)succinyl-CoA thioester, and of further downstream CoA thioesters, which may serve as a fingerprint for the detection of anaerobic biodegradation processes is various samples. The metabolite information will be correlated with genomic and proteomic analysis of strain BuS5, broadening our understanding of short chain alkane degradation by marine sulfate reducing bacteria.