

Isolation of *Megasphaera* nov. sp. BS-4 producing hexanoic acid and characterization of biosynthesis of hexanoic acid based on exogenous isotopic butyrate

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An unidentified strictly anaerobe, highly producing pentanoate and hexanoate, was isolated and characterized. Based on 16S rRNA gene sequence analyses, the strain was closely related to the species of the *Megasphaera* group (96% similarity) and now designated *Megasphaera* nov. sp. BS-4. Main metabolic products of the strain were acetate, butyrate, isobutyrate, hexanoate, heptanoate and octanoate. Interestingly, the profile of those products was significantly influenced by the supplementary acids that can be used for an electron acceptor during fructose fermentation. When propionate was added, pentanoate was produced up to 5.74 g/L. When additional acetate or butyrate was added, hexanoic acid was produced up to 9.72 g/L. Subsequently, the pathway of butyrate utilization to hexanoate production in the strain was investigated by GC-MS using isotope labeled [1, 2, 3, 4-<sup>13</sup>C] butyrate. The isotopic hexanoate was the form of [3, 4, 5, 6-<sup>13</sup>C] hexanoate, implicating that exogenous butyrate was directly incorporated in hexanoate synthesis without degradation.