

Substrate specificity of an acetyl-CoA acetyltransferase from newly isolated *Megasphaera* species

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An acetyl-CoA acetyltransferase is the enzyme that condenses two unit of acyl CoA or cleavages 3 keto acyl CoA molecules into two units of acyl CoA molecules. In this study, acetyl-CoA acetyltransferase related to the condensation of acyl CoA was selected and designated as a *Megasphaera* acetyl CoA acetyl transferase (MACAT). So as to evaluate substrate specificity, MACAT was expressed in *Escherichia coli* BL21 (DE2) and purified by Ni-NTA column. The activity of expressed MACAT was measured using spectrophotometer (at 340 nm). 3-hydroxy-butyryl-CoA dehydrogenase and nicotinamide adenine dinucleotide (NADH) was used as an auxiliary enzyme. Acetyl CoA, propionyl CoA, and butyryl CoA molecules were used as substrates. Decrease of absorbance due to oxidation of NADH was observed and the activities of MACATs were compared according to substrates. The products were analyzed by HPLC-UV and MS. When acetyl CoA was added as a substrate, 3-hydroxy-butyryl-CoA (the product condensed two unit of acetyl CoA) was produced. When acetyl CoA and butyryl CoA were added as substrates, 3-hydroxy-hexanoyl-CoA (the product condensed acetyl CoA and butyryl CoA) was produced.