**Metabolic flux analysis by GC/TOF-MS**

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***Introduction and Results***

Lignocellulosic biomass is one of the most abundant renewable resources and composed of carbohydrate polymers (cellulose, hemicellulose). Among the component, hemicellulose may contain mannan, galactan, and acetic acid as well as xylose. Therefore, it is important to find microbes utilizing a wide range of carbohydrates for using lignocellulose as a carbon source. In this study, we isolated a butyric acid- producing bacterium, *Clostridium* sp. S1, which was capable of utilizing various carbohydrates. Interestingly, *Clostridium* sp. S1 converted acetic acid which was generated during pretreatment to butyric acid. To confirmation of acetate conversion to butyric acid, isotope 13C-acetate was used to track carbon flow and the result confirmed acetate was converted to butyric acid by GC/TOF-MS. Fermented broth with 13C-labeled isotope was extracted two times with ethyl acetate and injected into the GC/TOF-MS. Comparison of the mass spectrum of butyric acid standard and samples, we have confirmed that 13C-labeled butyric acid was detected in the samples.

Bio-ethanol is one of the well known bio-fuel but has reported the problem such as corrosion of internal combustion engine. Bio-butanol can resolve the problem because butanol has less corroded an internal combustion engine and has higher heat capacity than ethanol. Hexanol is emerging as a biofuel than ethanol and butanol but, it cannot be produced using microorganism for toxicity. Therefore, we were trying to produce the hexanol using the hexanoic acid which is less toxic than hexanol. Some of the microorganisms to produce the hexanoic acid function to metabolize by using the inserted organic acid. In this study, we analyze the production mechanism using the 13C-butyric acid. Also, we tried to find how external butyric acid was metabolized, and what direction was being taken during the condensation by the GC/TOF-MS. Fermented broth was extracted two times with diethyl ether and injected into the GC/TOF-MS. Comparison of the mass spectrum, we detected the 13C-hexanoic acid (substituted C-3,4,5,6 position) in the sample and the result confirmed external butyric acid was converted to hexanoic acid and produced hexanoic acid has same direction.

Keywords: metabolic flux, isotope, GC/TOF-MS

Reference

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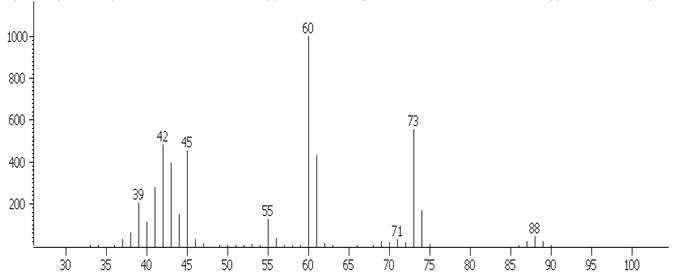
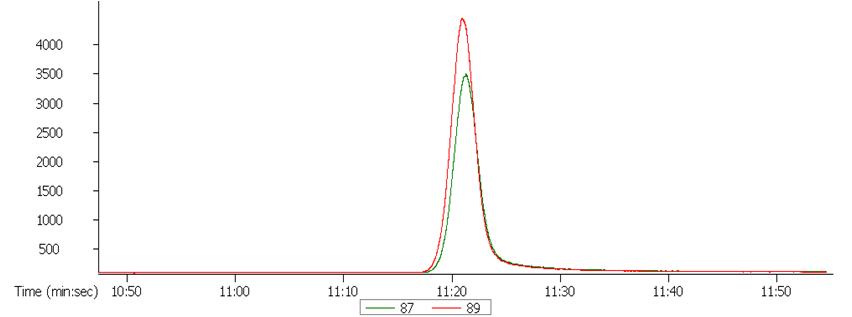


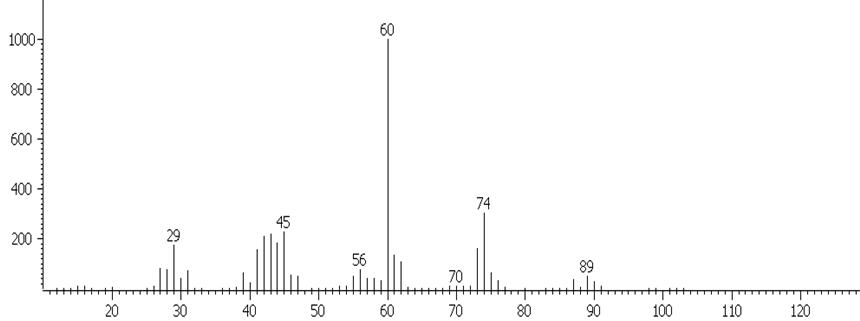
Figure 1. Mass spectrum of 13C-butyric acid and butyric acid in the sample.



hexanoic acid (12C) (green)

3,4,5,6-13C hexanoic acid (red)

Figure 2. GC chromatogram of 13C-hexanoic acid and hexanoic acid in the sample





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Figure 3. Mass spectrum of 13C-hexanoic acid and hexanoic acid in the sample.