Bioconversion of Castor Oil by Microorganisms

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Abstract

Sebacic acid, a monomer of nylon, is a promising carbon-neutral monomer because it is made from castor oil. Castor oil is an inedible vegetable oil that is rich in ricinoleic acid. However, the production of sebacic acid requires a large input of energy due to the need for high pressure and high temperature, as well as highly alkaline reaction conditions. Therefore, we applied microbial technology in an attempt to develop an eco-friendly sebacic acid production process. We collected microbial samples from the Kyoto University Botanical Gardens. These samples were inoculated into medium containing castor oil, following which, we performed subculturing. After isolating pure cultures of several kinds of microorganisms, we performed TLC and GC analysis. According to our TLC analysis, almost all the microbial isolates were able to degrade castor oil. Despite this, our GC analysis indicated that none of the isolated microorganisms were able to produce sebacic acid.

Key words: Castor oil, Sebacic acid, Inedible vegetable oil, Ricinoleic acid, Carbon-neutral

Introduction

Sebacic acid (decanedioic acid) is a representative biomass-derived raw material used to synthesize nylons such as 6,10-nylon. Sebacic acid is made from ricinoleic acid (12-hydroxy-*cis*-9-octadecenoic acid), which is naturally abundant in castor oil and is well-known as a carbon-neutral compound. However, the chemical process for sebacic acid production from ricinoleic acid requires a large input of energy due to the need for high pressure and high temperature, as well as highly alkaline reaction conditions [(1)]. Hence, an eco-friendly sebacic acid production process is highly desirable. The first step in bioprocess development is screening for useful biochemical reactions [(2)]. In this study, we screened microorganisms for the ability to chemically convert castor oil in an attempt to develop a bioprocess for sebacic acid production.

Materials & Methods

Screening of microorganisms

Ten samples were collected from a mushroom, soil around the roots of tree, a wet leaf, a snail shell, wet soils, soil under a moss, an animal nest, a yellow flower, a blue flower, and wet soil under a moss found in the Kyoto University Botanical Gardens. First, the samples were suspended in water to separate the microbes from other components. Next, the microorganisms were inoculated into liquid culture medium containing 0.10% (w/v) KH_2PO_4, 0.10% (w/v) K_2HPO_4, 0.01% (w/v) yeast extract, 0.03% (w/v) $MgSO_4 \cdot 7H_2O$, 0.10% (w/v) NH_4Cl, and several drops of castor oil. The inoculated medium was incubated at 28°C with shaking at 300 rpm. After incubation, these culture medium were streaked and incubated at 28°C on agar medium containing 0.10% (w/v) KH_2PO_4, 0.10% (w/v) K_2HPO_4, 0.01% (w/v) yeast extract, 0.03% (w/v) $MgSO_4 \cdot 7H_2O$, 0.10% (w/v) NH_4Cl, 2.0% (w/v) agar, with several drops of castor oil. Various colonies were observed on agar medium and subsequently picked, restreaked, and incubated at 28°C on fresh agar medium. Isolated microorganisms were inoculated into the liquid medium and incubated at 28°C with shaking at 300 rpm. After culturing the isolates, the lipid composition of their metabolic products was analyzed using thin-layer chromatography (TLC) and gas-liquid chromatography (GC).

Lipid analysis

Lipids were extracted from the culture medium with ethyl acetate containing 10% methanol before being concentrated by evaporation under reduced pressure.

(1) TLC. The resulting lipids were applied on to a silica plate, and separated using solvent containing hexane, diethyl ether, and acetic acid at a ratio of 60:40:1. Lipids were detected using phosphomolybdic acid.

(2) GC. The resulting lipids were saponified at 100°C for 9 min with 0.5 M KOH in methanol. The saponified lipids were methylated with 4% methanolic HCl at 50°C for 20 min. The resulting fatty acid methyl esters were extracted with *n*-hexane and analyzed by (GC) using a Shimadzu (Kyoto, Japan) gas chromatograph equipped with a flame ionization detector and a split injection system and fitted with a capillary column (SPB-1, 30 m x 0.25 mm I.D.; Supelco (Pennsylvania, USA)). The column temperature was initially 180°C for 30 min and subsequently raised to 220°C at a rate of 40°C/min, and maintained at that temperature for 9 min. The injector and detector were operated at 250°C. Helium was used as a carrier gas under 238 kPa. Heptadecanoic acid (17:0) was used as an internal standard for quantification.

Results & Discussion

Various kinds of microorganisms were observed on the agar medium (Figure 1). Following TLC, spots of castor oil were detected in S10, but castor oil was not detected in the other samples (Figure 2). The spots of ricinoleic acid derived from castor oil degradation were detected in S1, S3, S4, S5, S6, S8, S9, S10, S11, S12, S14, and S15 (Figure 2). According to GC analysis, a decrease in ricinoleic acid was observed in S2, S4, S5, S7, S9, S11, S12, S13, and S16 (Figure 3). These results are consistent with the results of the TLC analysis. A representative GC chromatogram is shown in Figure 4. Sebacic acid

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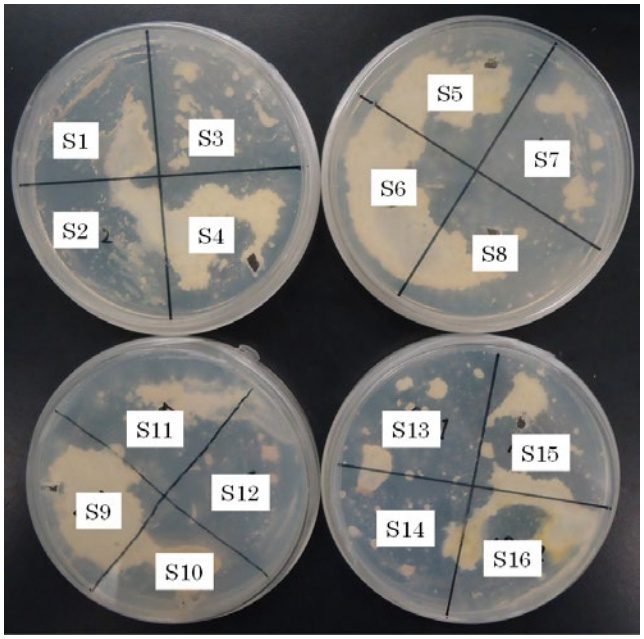

Fig. 1. Microorganisms isolated by the enrichment culture method using castor oil as the sole carbon source.

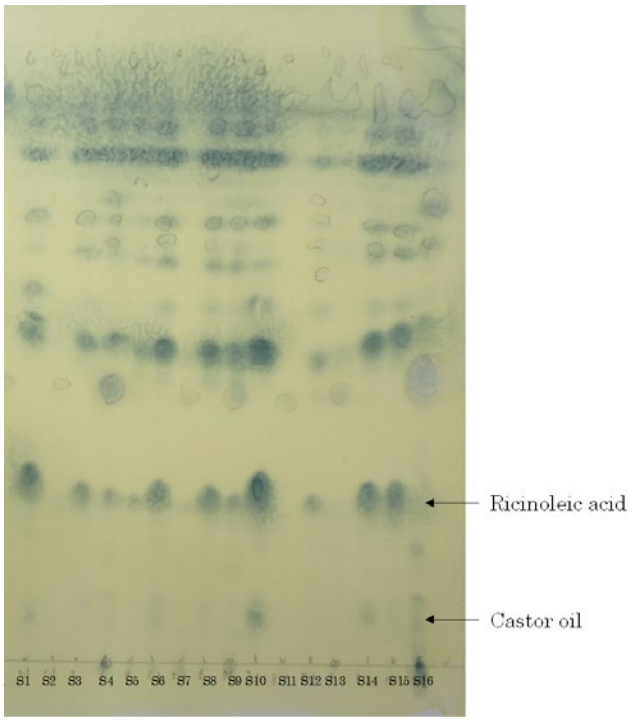

Fig. 2. TLC analysis of lipid composition in culture medium containing microorganisms isolated by the enrichment culture method. TLC methods were described in Materials and Methods.

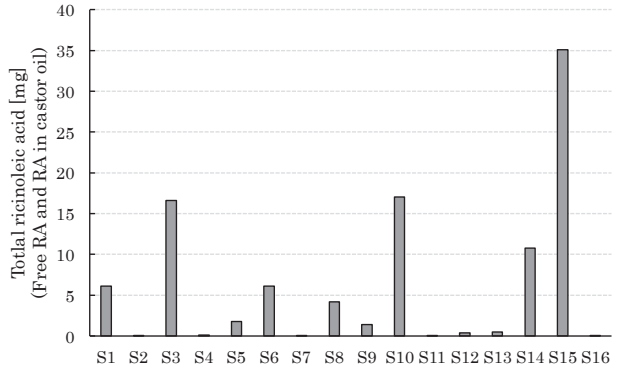

Fig. 3. Total amount of ricinoleic acid in culture medium of microorganisms isolated by the enrichment culture method. Ricinoleic acid was quantified by GC using an internal standard.

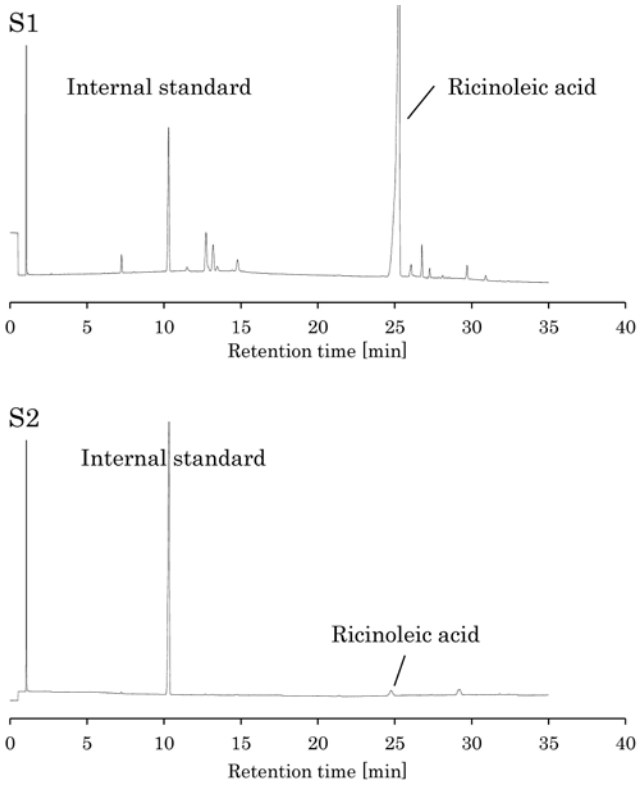

Fig. 4. GC chromatograms of methyl esterified lipids in culture medium of S1 and S2. GC methods were described in Materials and Methods.

was not observed in any samples as a result of GC analysis.

Strains S2, S4, S5, S7, S9, S11, S12, and S13 could degrade castor oil and ricinoleic acid. They should hydrolyze castor oil by lipase and degrade the resulting ricinoleic acid via β-oxidation pathway. However, the metabolites generated from ricinoleic acid in these samples were not detected (Figure 4). The GC analysis method used may not be suitable for detecting metabolites from ricinoleic acid.

Instead of ricinoleic acid, 10-hydroxy and 10-oxo fatty acids could be converted into sebacic acid using laccase[3]. Laccase can oxidize not only phenol compounds but also many kinds of chemicals via a mediator. Sebacic acid (0.58 mM) could be produced from 10-oxo-*cis*-12,*cis*-15-octadecadienoic acid (1.6 mM) under best conditions. The substrates, hydroxy and oxo fatty acids, are intermediates of saturation metabolism in *Lactobacillus plantarum* AKU 1009a, which can saturate linoleic acid into oleic acid and *trans*-10-octadecenoic acid[4]. The saturation metabolism consists of CLA-HY (hydratase), CLA-DH (dehydrogenase), CLA-DC (isomerase), and CLA-ER (enone-reductase). The 10-hydroxy and 10-oxo fatty acids could be produced using these four enzymes.

I could not detect any microorganisms that produced sebacic acid from castor oil. More samples must be collected, cultured under suitable conditions such as inhibition of castor oil hydrolyzation and β-oxidation of ricinoleic acid, and analyzed in order to screen for useful biological reactions. It is important to identify useful species among the immense number of microorganisms in existence given their seemingly infinite diversity and versatility[2].

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微生物によるヒマシ油の変換

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要旨

ナイロン原料のセバシン酸は非可食性植物油であり，リシノール酸を豊富に含むヒマシ油から作られている．しかしそのプロセスは高アルカリ条件下で高温・高圧を必要とするため，環境負荷が高いという問題がある．そこで微生物のバイオテクノロジーを利用し，温和な条件でリシノール酸からセバシン酸を生産することを目的に研究を行った．京都大学理学部植物園でサンプリングを行い，ヒマシ油を基質とした最少栄養培地で植菌を行った．その後植え継ぎを行い，画線による分離，単離後，TLC と GC による脂質組成の分析を行った．単離した結果，16 種類のバクテリアやカビを得た．TLC 分析の結果，ほとんどのサンプルにおいてヒマシ油が分解されていることが分かった．GC 分析の結果，セバシン酸を生産している微生物は得られなかった．

重要語句：ヒマシ油，セバシン酸，非可食性植物油，リシノール酸，カーボンニュートラル

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