

Microbial Decarboxylation of Dicarboxylic Acid Monomethyl Ester

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Abstract

Plastic is an essential material in modern day society. Most plastics are produced by a chemical process using petroleum. Plastic production thus cannot be said to be either resource- or environmentally friendly. Therefore, in recent years, research has been conducted into bioprocess production of plastics by using a microbial enzyme. In this study, we attempted to identify microorganisms that catalyze the decarboxylation of dicarboxylic acid to produce a carboxylic acid, which is the raw material for plastic production. Our ultimate aim was to identify microorganisms capable of fermentative production of carboxylic acids from biomass.

Although we isolated a large number of bacteria using the dicarboxylate medium, we did not manage to identify any microorganisms that catalyze decarboxylation of dicarboxylic acid. We believe that the microorganisms we isolated may be heterotrophic, and any decarboxylase activity against dicarboxylic acid is very weak. Alternatively, they may be autotrophic and capture carbon dioxide in the air as a carbon source without assimilating the substrates. To distinguish between the two possibilities, it is necessary to screen more samples.

Key words: Dicarboxylic acid, Carboxylic acid, Decarboxylation, Microbial enzyme, Bioprocess, Plastic

Introduction

Bioprocesses, which involve the use of biocatalysts to produce useful compounds, are expected to become a leading player in the field of green chemistry⁽¹⁾. In the coming post-petrochemical era, industrial production processes will be required to save energy and reduce environmental damage. In this sense, biological reactions are now widely recognized as practical alternatives to conventional chemical reactions^(2,3). Carboxylic acids are used to make synthetic resins. Some dicarboxylic acids can be made by bioprocesses. Hence, if it becomes possible to produce carboxylic acids from dicarboxylic acids using an enzymatic catalyst, it will be possible to achieve efficient production of synthetic resins. This study investigates the enzyme-catalyzed decarboxylation of dicarboxylic acid monomethyl ester.

Materials & Methods

Samples were collected in the Kyoto University Botanical Garden (Table 1). Small amounts of each sample were added to test tubes containing 5 ml of an isolation liquid medium comprising 0.10% (w/v) KH_2PO_4 , 0.10% (w/v) K_2HPO_4 , 0.01% (w/v) yeast extract, 0.03% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.10% (w/v) NH_4Cl , and 1.00% (w/v) dicarboxyl-

Table 1. List of samples.

ID	Remarks
1	Soil under nettle tree
2	Mushroom (on the branch)
3	Fruit
4	Mushroom (on the ground)
5	Soil under Trident maple
6	Seed of Aburasugi
7	Fallen leaves under white bark pine
8	Algae
9	Soil under Soapberry
10	Soil of the forest gap

ic acid monomethyl ester, followed by incubation at 20°C with shaking at 300 rpm. Twenty microliters of medium containing observable microbial growth was transferred to another test tube containing the same medium, followed by further cultivation at 20°C with shaking at 300 rpm. The culture medium was streaked on agar plates (pH 7.0) with the same composition as the isolation medium and cultivated at 20°C with shaking at 300 rpm. After cultivation, the colonies that appeared were isolated as dicarboxylic acid monomethyl ester-assimilating microorganisms. Microorganisms obtained from the Kyoto University Botanical Garden were inoculated onto isolation media, and species with ability to assimilate dicarboxylic acid monomethyl ester were selected for by the same methods. These dicarboxylic acid monomethyl ester-assimilating species from samples and the culture collection were used for the subsequent experiments. A qualitative analysis of dicarboxylic acid, dicarboxylic acid monomethyl ester, and carboxylic acid was performed using thin-layer-chromatography (TLC) with silica gel. Samples (100 µg each) were derivatized by processing with ten microliters of reagent A (12 mg/ml phenacyl bromide/acetone) and ten microliters of reagent B (10 mg/ml triethylamine/acetone at 50°C for 2 h). The developing system consisted of hexane and acetone in a 3:1 ratio. After drying, these were irradiated with ultraviolet light.

Results & Discussion

Some microorganisms were observed on the agar plates streaked with samples from the Kyoto University Botanical Garden (Fig. 1). There are no microorganisms that can produce carboxylic acid from dicarboxylic acid monomethyl ester. Strains no. 1, 4, 5, 7, 8, 9, and 10 can hydrolyze dicarboxylic acid monomethyl ester into dicarboxylic acid (Fig. 2). These strains should utilize the products of hydrolyzation, dicarboxylic acid or methanol. Further experiments of whether they prefer dicarboxylic acid to methanol should be done with medium

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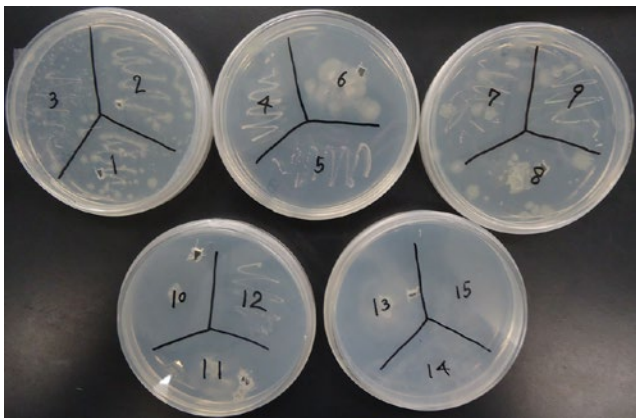


Fig. 1. Culture results.

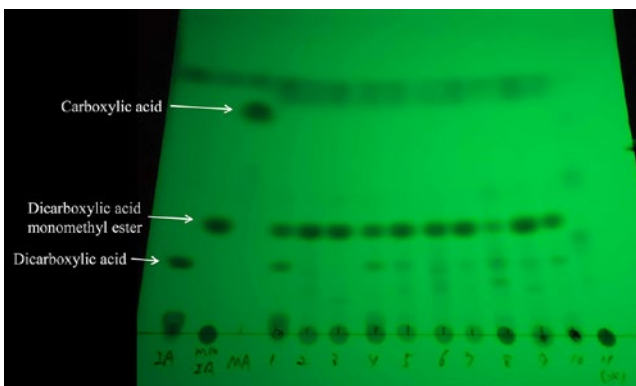


Fig. 2. TLC results.

containing dicarboxylic acid or methanol as a sole carbon source.

It may be important to synthesize acyl-CoA for decarboxylation of dicarboxylic acid. For example, succinic acid (C4 dicarboxylic acid) is converted into propionic acid (C3 carboxylic acid) by decarboxylation. In detail, succinyl-CoA is converted into methylmalonyl-CoA by methylmalonyl-CoA mutase and the resulting methylmalonyl-CoA is decarboxylated to propionyl-CoA by methylmalonyl-CoA decarboxylase⁽⁴⁾. Succinyl-CoA could be prepared by the technique of Simon and Shemin⁽⁵⁾. Coenzyme A thioester of dicarboxylic acid may be a good substrate for decarboxylation. Most importantly, immense number of microorganisms should be screened in order to get useful microorganisms with a high activity of a target reaction⁽¹⁾.

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微生物によるジカルボン酸モノメチルエステルの脱炭酸反応

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要旨

プラスチックは現代社会を支える重要な素材であるが、これらの多くは石油を使用した化学プロセスによって生産されている。しかし、このプロセスは資源環境の面で好ましい生産法とは言えない。そのため、近年では、微生物酵素を用いたバイオプロセスによる生産が進められている。そこで、バイオマスによる発酵生産が可能なジカルボン酸を脱炭酸反応させ、プラスチックの原料となるカルボン酸を生成する反応を触媒する微生物の発見を最終目標として研究を行った。結果は、培地上には多数の菌が見られたが、TLC分析では、今回サンプリングしたサンプル中にはジカルボン酸の脱炭酸反応を触媒する微生物は見つからなかった。ここから、得られた菌が従属栄養生物であり、基質に対する活性が非常に弱いこと、または独立栄養生物であり、基質を資化せずに空気中の二酸化炭素を炭素源として取り込んでいることなどが考えられる。

重要語句: ジカルボン酸, カルボン酸, 脱炭酸反応, 微生物酵素, バイオプロセス, プラスチック

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