Biological Stereoselective Reduction of 4-*tert*-Butylcyclohexanone by Anthracnose Fungi

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Abstract : The biotransformation of 4-*tert*-butylcyclohexanone was conducted using ten anthracnose fungi as biocatalysts. The 4-*tert*-butylcyclohexanone was reduced to the corresponding *cis*- and *trans*-alcohols. The biotransformation of 4-*tert*-butylcyclohexanone resulted mainly in its transformation to *trans*-4-alcohol, using *Colletotrichum lagenarium*, *C. atramentarium* MAFF 712102, *C. fragariae*, *C. graminicola* MAFF 305460, *C. lindemuthianum*(C-3) and *C. lindemuthianum*(C-13). The *cis*- and *trans*-alcohol products were obtained at a ratio of 1.0 : 6.0 with stereoselectivity by *Colletotrichum lagenarium* after 7 days incubation. The *cis*-alcohol was formed with stereoselectivity by *Glomerella cingulata*, *C. lindemuthianum*(C-1), *C. trifolii* MAFF 305389 and *C. dematium* MAFF 410046.

Key words : biotransformation, 4-*tert*-butylcyclohexanone, biological stereoselective reduction, anthracnose fungi

1 Introduction

We have investigated the microbial transformation of terpenoids and relative cyclic compounds using a plant pathogenic fungus. In our previous papers, we reported the biotransformation of monoterpene ketones (+)-camphorquinone^(1,2), (-)-camphorquinone^(1,2), karahanaenone⁽³⁾ by various fungi. Camphorquinone and karahanaenone were reduced to alcohols with high stereoselectivity by *Aspergillus niger* and *Glomerella cingulata*, respectively. In addition, biological reduction of 4-methylcyclohexanone by *G. cingulata* was formed *trans*-alcohol as main product⁽⁴⁾. From the report of other researches, the biotransformation of 4-*tert*-butylcyclohexanone by *Aspergillus repens* MA 0197⁽⁵⁾ *Chlorella pyrenoidosa*⁽⁶⁾ and Rabbit⁽⁷⁾ were known to give the corresponding *cis*- and/or *trans*-alcohols. Here we report the biological stereoselective reduction of 4-*tert*-butylcyclohexanone by ten kinds of anthracnose fungi.

2 Materials and Methods

2.1 Microorganism

Glomerella cingulata, *Colletotrichum fragariae*,

C. lindemuthianum(C-1), *C. lindemuthianum*(C-3), *C. lindemuthianum*(C-13) and *C. lagenarium* used in this study were obtained from Gifu University, where it was prepared by Prof. Dr M. Hyakumachi. *C. atramentarium* MAFF 712102, *C. trifolii* MAFF 305389, *C. dematium* MAFF 410046 and *C. graminicola* MAFF 305460 were purchased from the Genetic Resources Center National Institute of Agrobiological Resources Ministry of Agriculture, Forestry and Fisheries(MAFF), Japan.

2.2 Culture conditions

Spores of microorganisms which had been preserved at low temp. were inoculated into sterilized culture media : 1.5%(w/v) saccharose, 1.5%(w/v) glucose, 0.5 % (w/v) polypeptone, 0.1%(w/v) K₂HPO₄, 0.05%(w/v) KCl, 0.05%(w/v) MgSO₄·7H₂O, 0.001%(w/v) FeSO₄·7H₂O and distilled water, pH 7.2 in an Erlenmeyer flask which was autoclaved at 121°C for 15 min. and shaken at 27°C for 3 d. The mycelia were transplanted in a 1 L stirred fermenter containing 800 ml of a medium. Cultivation was carried out at 27°C with stirring for 3 d with aeration. After the growth of *G. cingulata*, the 4-*tert*-butylcyclohexanone(200 mg) was added directly into the medium and cultivated for 7 d.

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2.3 Time-course experiment

Everyday, 10 ml of the culture medium was removed and saturated with sodium chloride. Then, the culture medium was extracted with dichloromethane(3 ml). The extract was analyzed by GC. The ratio between the substrate and metabolic products were determined on the basis of the peak area of GC and are shown in Figs.1~3, respectively.

2.4 Isolation of metabolites

After incubation time of 7 d, culture medium and mycelia were separated by filtration, and the filtrate(730 ml) was saturated with sodium chloride and then extracted with dichloromethane(200 ml). The extracts were evaporated and analyzed by GC and GC-MS (see below). The products were identified by comparison of retention times (*cis*-alcohol; 22.5 min, *trans*-alcohol; 23.8 min) and mass spectra⁶⁾ with those of authentic specimens.

The extracts were subjected to chromatography on Si-60 columns with a hexane / diethyl ether gradient(from 99 : 1 to 8 : 2) to give the products : *C. atramentarium* MAFF 712102(1-1; 21 mg, 1-2; 112 mg), *C. dematium* MAFF 410046 (1-1; 103 mg, 1-2; 60 mg), *C. fragariae* (1-1; 27 mg, 1-2; 135 mg), *C. graminicola* MAFF 305460 (1-1; 28 mg, 1-2; 112 mg), *C. lagenarium* (1-1; 20 mg, 1-2; 120 mg), *C. lindemuthianum* (C-1) (1-1; 120 mg, 1-2; 40 mg), *C. lindemuthianum* (C-3) (1-1; 60 mg, 1-2; 121 mg), *C. lindemuthianum* (C-13) (1-1; 24 mg, 1-2; 72 mg), *C. trifolii* MAFF 305389 (1-1; 56 mg, 1-2; 28 mg), *G. cingulata* (1-1; 54 mg, 1-2; 27 mg) respectively.

2.5 GC conditions

GC was carried out using a Hewlett Packard 5890 equipped with a flame ionization detector on a capillary column (TC-WAX FFS Fused Silica 0.25 mm i.d. X 60 m). The column temperature was programmed from 80°C to 210°C at the rate of 4°C/min and then held at 210°C. The injector and detector temperatures were 240°C. The flow rate of carrier gas(He) was 0.6 ml/min.

2.6 GC-MS conditions

GC-MS was carried out with a Hewlett Packard 5972. GC conditions were the same as previously described(2.5 GC conditions). The flow rate of the carrier gas(He) was 0.6 ml/min. The detector interface temperature was set 280°C, with the actual temperature in the MS source reaching approximately 180°C and the ionization voltage 70

eV.

2.7 Chemicals

4-*tert*-butylcyclohexanone(1) and a mixture of *cis*- and *trans*-4-*tert*-butylcyclohexanol were purchased from Tokyo Kasei Kogyo Co., Ltd.

3 Results and Discussion

To clarify the time course of the microbial transformation of 4-*tert*-butylcyclohexanone(1) by ten fungi the analytical amount of 1 was incubated with fungi for 7 d, respectively. 4-*tert*-Butylcyclohexanone(1) was used as starting material, and the medium was analyzed at one day intervals for 7 d. After one day of cultivating the biological reduction was detectable. The mass and GC data of the products were identical to those of authentic 4-*tert*-butylcyclohexanols, the predominant isomer being *trans*-4-*tert*-butylcyclohexanol(1-1). The compound 1 was reduced to the corresponding *cis*- and *trans*-alcohol (Scheme 1). Other product was not detected by GC analysis.

Scheme 1 The Reduction of 4-*tert*-butylcyclohexanone (1).

Fig.1 Time Course of Metabolic Products of 4-*tert*-butylcyclohexanone (1) by *C. lindemuthianum* (C-3).

□ : 4-*tert*-butylcyclohexanone(1); ● : *cis*-4-*tert*-butylcyclohexanol(1-1); ○ : *trans*-4-*tert*-butylcyclohexanol (1-2).

The time course of 4-*tert*-butylcyclohexanone by *C. lindemuthianum* (C-3) were shown **Fig.1**. The compound **1** had completely disappeared after 7 d incubation period and the corresponding *cis*-alcohol(33.3%) and *trans*-alcohol(66.7%) were generated. The ratio of *cis*- and *trans*-alcohol was 1.0 : 2.0. The compound **1** was transformed to *trans*-4-*tert*-butylcyclohexanol as the major product at the end of 7 d. In the case of *C. lagenarium*, the ratio of *cis*- and *trans*-alcohol was 1.0 : 6.0 after 7 d incubation period (**Fig.2**). On the other hand,

Fig.2 Time Course of Metabolic Products of 4-*tert*-butylcyclohexanone (**1**) by *C. lagenarium*.

□ : 4-*tert*-butylcyclohexanone(**1**) ; ● : *cis*-4-*tert*-butylcyclohexanol(**1-1**) ; ○ : *trans*-4-*tert*-butylcyclohexanol (**1-2**).

in the case of *Glomerella cingulata*, *C. lindemuthianum*(C-1), *C. trifolii* MAFF 305389 and *C. dematium* MAFF 410046, the microbiological reduction of **1** was metabolized mainly *cis*-alcohol. As shown in **Fig.3**, the main product was *cis*-alcohol(56.8%) by using *C. dematium* MAFF 410046. In the case of four fungi (*G. cingulata*, *C. lindemuthianum*(C-1), *C. trifolii* MAFF 305389 and *C. dematium* MAFF 410046), the thermodynamically unstable isomer (*cis*-form) was produced rather than the stable isomer (*trans*-form).

Fig.3 Time Courses of Metabolic Products of 4-*tert*-butylcyclohexanone (**1**) by *C. dematium* MAFF 712102.

□ : 4-*tert*-butylcyclohexanone(**1**) ; ● : *cis*-4-*tert*-butylcyclohexanol(**1-1**) ; ○ : *trans*-4-*tert*-butylcyclohexanol (**1-2**).

Table 1 The Ratio of *cis*/*trans*-cyclohexanols after 7 Days Cultivation of Cyclohexanols with 10 Fungi.

microorganisms	yield (%)	4- <i>tert</i> -butylcyclohexanone

		(1-1)	(1-2)
<i>Colletotrichum atramentarium</i> MAFF 712102	74.2	1.0	5.3
<i>Colletotrichum dematium</i> MAFF 410046	90.2	1.7	1.0
<i>Colletotrichum fragariae</i>	91.5	1.0	5.0
<i>Colletotrichum graminicola</i> MAFF 305460	78.4	1.0	4.0
<i>Colletotrichum lagenarium</i>	76.9	1.0	6.0
<i>Colletotrichum lindemuthianum</i> (C-1)	66.0	2.0	1.0
<i>Colletotrichum lindemuthianum</i> (C-3)	100	1.0	2.0
<i>Colletotrichum lindemuthianum</i> (C-13)	53.8	1.0	3.0
<i>Colletotrichum trifolii</i> MAFF 305389	46.8	2.0	1.0
<i>Glomerella cingulata</i>	45.6	2.0	1.0

The ketone:alcohol ratio after 7 d microbiological reduction of 4-*tert*-butylcyclohexanone also the *cis*:*trans* ratios of the 4-*tert*-butylcyclohexanols with ten fungi are shown in **Table 1**. The cyclic ketone was reduced by all of microorganisms, the ratio of *cis*:*trans*-alcohol was different by each fungus. The results(**Table 1**) indicate that the reduction of **1** by *C. lagenarium* produced *trans*-alcohol(**1-2**) with stereoselectivity, and compares favorably with previously reported biotransformation of **1** by algal; *Chlorella pyrenoidosa*⁶⁾. Our findings are different from those of Doi *et al*⁵⁾, in which *A. repens* MA 0197 produced almost exclusively *cis*-alcohol isomer from 4-*tert*-butylcyclohexanone.

Thus, the biotransformation of **1** was formed mainly *trans*-form(**1-2**) by *C. lagenarium*, *C. atramentarium* MAFF 712102, *C. fragariae*, *C. graminicola* MAFF 305460, *C. lindemuthianum*(C-3) and *C. lindemuthianum*(C-13). A noteworthy feature of stereoselective reductions were the produce of *cis*-alcohol(**1-1**) as main product by *G.*

cingulata, *C. lindemuthianum*(C-1), *C. trifolii* MAFF 305389 and *C. dematium* MAFF 410046 at the end of 7 days. All of the above results suggested that ten species of anthracnose fungi were classified two groups of *cis*-formation type and *trans*-formation type.

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日本油化学会誌本号掲載 論文要旨

[総説] リン脂質ヒドロペルオキシドグルタチオンペル
オキシダーゼ (PHGPx) の構造と機能

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リン脂質ヒドロペルオキシドグルタチオンペルオキシダーゼ (PHGPx) は生体膜に生じた過酸化反応の一次生成物であるリン脂質ヒドロペルオキシドを直接還元する細胞内の唯一の抗酸化酵素である。本酵素は活性中心に特殊アミノ酸であるセレノシステインを有するユニークなセレノタンパク質である。本酵素にはミトコンドリアへのターゲットシグナルを有するミトコンドリア型 PHGPx と核や細胞質などに存在する非ミトコンドリア型 PHGPx が存在し、この二つの PHGPx の転写開始点が異なることから、転写レベルで制御されている。PHGPx 高発現株を用いた解析から、PHGPx は生体膜に生成する過酸化脂質を消去するほか、核においては炎症性脂質メディエーターであるロイコトリエン産生の調節に関与し、ミトコンドリアにおいてはミトコンドリア電子伝達系から発生する活性酸素、過酸化脂質を消去し、細胞死の抑制に重要な役割を担っていることが明らかとなってきた。また最近、活性酸素 (ROS) が転写因子 NFκB 活性化などのシグナル伝達経路に関与していることが報告されたが、PHGPx は血管内皮細胞においてインターロイキン 1 による NFκB の活性化を抑制することが報告された。本稿では新たに明らかになってきた PHGPx の細胞内における生理機能を解説する。

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[報文] 炭疽病菌による 4-*tert*-butylcyclohexanone
の立体選択的還元

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テルペノイドおよびそれらの関連化合物の衛生物変換に関する研究の一環として、10 種の植物病原菌 (炭疽病) を生体触媒として用い、4-*tert*-butylcyclohexanone の変換を行った。その結果、基質はケトン基の還元反応が進行し、それぞれ対応する *cis* および *trans*-alcohol 体に変換され、菌種により主要変換物は異なっていた。*Colletotrichum lagenarium*, *C. atramentarium* MAFF 712102, *C. fragariae*, *C. graminicola* MAFF 305460, *C. lindemuthianum* (C-3) および *C. lindemuthianum* (C-13) では *trans*-4-*tert*-butylcyclohexanol を主要変換物として生成するのに対し、*Glomerella cingulata*, *C. lindemuthianum* (C-1), *C. trifolii* MAFF 305389 および *C. dematium* MAFF 410046 では *cis*-alcohol 体を主要変換物として立体選択的に生成することを明らかにした。

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