Oxidation of Short-chain iso-Alkanes by Gaseous Hydrocarbon Assimilating Mold, *Scedosporium* sp. A-4

Masayuki ONODERA, Hiroyuki SAKAI,* Yoshihiko ENDO*
and Nagahiro OGASAWARA*

Department of Biosystem Science, Graduate School of Science and Technology and

*Department of Agricultural Chemistry, Faculty of Agriculture,
Niigata University, Niigata 950-21, Japan

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Oxidation of short-chain iso-alkanes (isobutane, isopentane, 2-methylpentane, and 3-methylpentane) was studied with propane-grown resting mycelia of *Scedosporium* sp. A-4. Isobutane was oxidized to *tert*-butanol, but both isobutane and *tert*-butanol were not used for growth. Isopentane was oxidized to 3-methyl-1-butanol, 2-methyl-2-butanol, and 3-methyl-2-butanol but not to 2-methyl-1-butanol. 2-Methylpentane was oxidized to 4-methyl-1-pentanol, 2-methyl-2-pentanol, and 4-methyl-2-pentanol but not to 2-methyl-1-pentanol or 2-methyl-3-pentanol. 3-Methylpentane was not oxidized. Oxidation of branched alcohols was also studied.

In our previous studies on the oxidation of *n*-alkanes (C_2 to C_5) by resting mycelia of *Scedosporium* sp. A-4 grown on propane or *n*-pentane, it was found that the primary attack on the alkane molecule is on the C_1 and C_2 atom by monooxygenase, which catalyzes the incorporation of one atom of molecular oxygen into *n*-alkane, using an ^{18}O incorporation experiment. However, methane, *n*-hexane, and longer *n*-alkanes (up to C_{10}) were not oxidized.^{1,2)} Subsequently, we found that propane oxidation activity decreases temporarily after it has reached a peak (middle phase, 45–48 hr) and then the observed increase of the activity resumes from the late log- to stationary phase (72–93 hr) in the cultivation using a 2.5-l jar fermentor with propane alone.³⁾ On the other hand, we found that propane-grown resting mycelia oxidize secondary alcohols (C_3 to C_{10}) to the corresponding methylketones, but do not oxidize 3-pentanol and 3-hexanol.⁴⁾

iso-Alkanes seems to be oxidized by comparatively few microorganisms in general.⁵⁾ For example, Thijsse and Zwilling-de Vries reported that *Pseudomonas aeruginosa* 473 can use 2-methylhexane and 2-, 3-, and 4-methylheptane,⁵⁾ and McLee *et al.* isolated

n-butane-using bacteria (*Arthrobacter* sp. and *Brevibacterium* sp.) and molds (*Penicillium nigricans*, *allescheria boydii*, and *Graphium cumeiferum*) which were able to grow on ethane, propane, *n*-butane, and isobutane.⁷⁾ Pirnik *et al.* reported that long-chain iso-alkanes like pristane and 2-methylundecane are degraded as dicarboxylic acids, which subsequently undergo β -oxidation, by *Brevibacterium erythrogenes*.⁸⁾ We also found that *Scedosporium* sp. A-4 used isopentane and 2-methylpentane for growth and that isobutane, 3-methylpentane, 2,2-dimethylbutane, 2,3-dimethylbutane, and isoheptane were not used for growth.⁹⁾

In this study, we have examined the oxidation of short-chain iso-alkanes (isobutane, isopentane, 2-methylpentane, and 3-methylpentane) and branched alcohols by propane-grown mycelia of *Scedosporium* sp. A-4.

Materials and Methods

Organism. *Scedosporium* sp. A-4, which uses gaseous hydrocarbons for growth as sole sources of carbon and energy,⁹⁾ was used throughout.

Culture medium and conditions. The culture medium and culture conditions for this organism in a 2.5-l jar fermentor were described previously.⁹⁾

Chemicals. Isobutane (99.0%) was obtained from Takachiho Kagakukogyo K. K., Tokyo. Isopentane (99.0%), 2-methylpentane (98.0%), 3-methylpentane (98.0%), and other chemicals were obtained from Wako Pure Chemical Ind. Ltd., Tokyo.

Growth test on various branched alcohols. The use of individual branched alcohols was examined under the following conditions. This organism was grown at 30°C in a 0.5-l closed flask containing 100 ml of Z-C medium with 0.1% (v/v) branched alcohol with shaking for 4 days. The mycelia from each flask were separately collected by filtration using preweighed Whatman glass microfibre filters, GF/B, washed several times with deionized water to remove salts and residual substrate. Finally, the mycelia were dried to constant weight in an oven at 105°C. Growth was compared with that of controls in which mycelia were grown in medium without alcohol.

Oxidation of iso-alkanes and branched alcohols by resting mycelia. Preparation of resting mycelia grown on propane was described in our previous paper.¹⁾ Mycelia (0.1 g wet weight) were suspended in 2 ml of 0.1 M potassium phosphate (pH 7.0) in 10-ml vials and then the vials were sealed with double-rubber stoppers. Oxidation of iso-alkanes was assayed in the presence of 10 mM-pyrazole and oxidation of branched alcohols was assayed in the absence of pyrazole. In the case of isobutane, the air in the vials was removed by a vacuum and then replaced with a gas mixture of isobutane and oxygen (7:3, by volume) using a Luer-lock syringe (Precision Sampling Corp., U.S.A.). In the case of liquid iso-alkanes, 4 μ l of the substrate was put into vials. In the case of liquid branched alcohols, 2 μ l of the substrate was put into vials. Vials were then shaken at 120 rpm for 1 hr at 30°C. Then the reaction mixtures were filtered to remove the mycelia. A 2- μ l sample of each filtrate was removed with a syringe and assayed by flame ionized gas-liquid chromatography (GC-8A; Shimadzu, Kyoto) on a glass column (1.5 m by 2.6-mm inner diameter) packed with 10% PEG 1000 on 60/80 Uniport R (Gaskuro Kogyo Inc. Tokyo). The column temperature was maintained isothermally between 60 and 90°C with nitrogen carrier gas flow rates of 65 ml/min. The various products were identified by retention time on gas chromatography with co-chromatography of an appropriate authentic standard. The amount of a product was estimated from the peak area in comparison with a standard curve of the authentic standard.

GC-MS conditions. A Hitachi Model M-60 gas chromatography-mass spectrometry combination system was used under the following conditions: ionizing voltage, 70 eV; accelerating voltage, 3.2 kV; ion source temperature,

180°C; and carrier gas, helium. A computer with a data processing system (002B 8DK; Hitachi) was used to analyze the mass spectral data and to prepare mass chromatograms.

Results and Discussion

Thijsse and van der Linden reported that 2-methylhexane was mainly degraded *via* 5-methylhexanoic acid and iso-valeric acid by *Pseudomonas* sp. grown on 2-methylhexane, although 2-methylhexanoic acid was observed to accumulate, and they did not mention the appearance of dicarboxylic acids.¹⁰⁾ Imai *et al.* reported that isobutane was oxidized to isobutanol, iso-butyraldehyde, and *tert*-butanol by cell suspensions of the methane-oxidizing bacterium, H-2.¹¹⁾ Patel *et al.* reported isobutane was oxidized to isobutanol and *tert*-butanol by a soluble methane monooxygenase from *Methylobacterium* sp. CRL-26.¹²⁾

Scedosporium sp. A-4 grew on isobutanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 4-methyl-1-pentanol, and 2-methyl-1-pentanol,

Fig. 1. Gas Chromatographic Analysis of 2-Methylbutane Oxidation by *Scedosporium* sp. A-4.

A, the product of 2-methylbutane oxidation by propane-grown resting mycelia in the presence of pyrazole; B-1, 2-methyl-2-butanol; B-2, 3-methyl-2-butanol; B-3, 3-methyl-1-butanol and 2-methyl-1-butanol.

but not on *tert*-butanol, 3-methyl-2-butanol, 2-methyl-2-butanol, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 2-methyl-2-pentanol, or 3-methyl-1-pentanol.

Isobutane was oxidized to *tert*-butanol, but not to isobutanol by resting mycelia of *Scedosporium* sp. A-4 grown on propane irrespective of the presence or absence of pyrazole, an inhibitor of alcohol dehydrogenase. The rates of conversion of isobutane to *tert*-butanol in the presence or absence of pyrazole were 0.424 and 0.800 $\mu\text{mol/hr}$ per mg dry mycelia, respectively. Pyrazole inhibited 47% of isobutane oxidation at 10 mM.

As shown in Fig. 1, three peaks were observed in the products of isopentane oxidation by resting mycelia of *Scedosporium* sp. A-4 grown on propane in the presence of pyrazole. The corresponding compounds of peak 1 and peak 2 were identified as 2-methyl-2-butanol and as 3-methyl-2-butanol, respectively, by co-chromatography of authentic compounds, while we could not identify peak 3. To identify peak 3, the sample was analyzed by GC-MS. The reaction mixture of 2-methylbutane oxidation (200 ml) was fully saturated with sodium chloride, extracted by diethylether, and dried over anhydrous sodium sulfate. A sample of concentrated diethylether extract was removed with a syringe and assayed with GC-MS, and peak 3 was identified as 3-methyl-1-butanol. The rates of conversion of isopentane to 3-methyl-1-butanol, 3-methyl-2-butanol, and 2-methyl-2-butanol were 0.068, 0.004, and 0.007 $\mu\text{mol/hr}$ per mg dry mycelia, respectively.

2-Methylpentane was oxidized to 4-methyl-1-pentanol, 4-methyl-2-pentanol, and 2-methyl-2-pentanol, but not to 2-methyl-1-pentanol or 2-methyl-3-pentanol by resting mycelia in the presence of pyrazole. 3-Methylpentane was not oxidized. The rates of conversion of 2-methylpentane to 4-methyl-1-pentanol, 4-methyl-2-pentanol, and 2-methyl-2-pentanol were 0.017, 0.005, and 0.004 $\mu\text{mol/hr}$ per mg dry mycelia, respectively.

As shown in Table I, 3-methyl-2-butanol and 4-methyl-2-pentanol were oxidized to 3-methyl-2-butanone and 4-methyl-2-pentanone

respectively by resting mycelia of *Scedosporium* sp. A-4, but *tert*-alcohols and 4-methyl-3-pentanol were not oxidized.

The oxidation of isobutane to *tert*-butanol by resting mycelia of *Scedosporium* sp. A-4 was inhibited in the presence of propane as propylene inhibited the propane oxidation,¹⁾ and these suggested that oxidation of isobutane, propane, and propylene may be catalyzed by the same or a similar enzyme (Fig. 2).

From these results, the growth of *Scedosporium* sp. A-4 on iso-alkanes (isopentane and 2-methylpentane) was only conducted by the

Table I. OXIDATION OF *tert*- AND ISO-ALCOHOLS BY RESTING MYCELIA OF *Scedosporium* sp. A-4 GROWN ON PROPANE

Substrate	Product	Conversion rate ^a
<i>tert</i> -Butanol	N.R. ^b	—
3-Methyl-2-butanol	Isopropylmethylketone	0.31
2-Methyl-2-butanol	N.R. ^b	—
4-Methyl-2-pentanol	Isobutylmethylketone	0.27
2-Methyl-3-pentanol	N.R. ^b	—
2-Methyl-2-pentanol	N.R. ^b	—

^a $\mu\text{mol/hr}$ per mg of dry mycelia.

^b No reaction.

Fig. 2. Effects of Propane on the Oxidation of Isobutane by Resting Mycelia of *Scedosporium* sp. A-4.

A, isobutane:propane:O₂ = 6:0:2; B, isobutane:propane:O₂ = 5.5:0.5:2; C, isobutane:propane:O₂ = 5:1:2; D, isobutane:propane:O₂ = 4:2:2; E, isobutane:propane:O₂ = 3:3:2; F, isobutane:propane:O₂ = 2:4:2; G, isobutane:propane:O₂ = 1:5:2; H, isobutane:propane:O₂ = 0:6:2.

The reactions were done as described in Materials and Methods. The products were assayed by gas chromatography after 1 hr of incubation at 30°C.

▲—▲, isobutane oxidation; ■—■, propane oxidation.

Fig. 3. Oxidation of Short-chain iso-Alkanes by *Scedosporium* sp. A-4.

initial oxidative attack on the terminal methyl group at a distance from a branched position in the molecule, and the oxidation of short-chain iso-alkanes by *Scedosporium* sp. A-4 is summarized in Fig. 3.

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