Note

Oxidation of *n*-Pentane by a Gaseous Hydrocarbon Assimilating Mold, *Scedosporium* sp. A-4

Masayuki ONODERA and
Nagahiro OGASAWARA

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

Received April 20, 1989

Scedosporium sp. A-4 can utilize *n*-alkanes (C_2 to C_5) as sole sources of carbon and energy for growth. Previously, we reported that this organism formed acetone from propane and 2-butanone from *n*-butane, and that homologous primary and secondary alcohols, from gaseous *n*-alkanes (C_2 to C_4), were accumulated extracellularly by resting mycelia in the presence of pyrazole, which is an inhibitor of alcohol dehydrogenase.^{1,2)}

Colby *et al.* reported that a soluble methane monooxygenase from the obligate methane-utilizing bacterium, *Methylococcus capsulatus* (Bath), catalyzed the hydroxylation of *n*-alkanes (C_1 to C_8) to the corresponding 1- and 2-alcohols, but not to 3- or 4-alcohols.³⁾ Imai *et al.* reported that *n*-alkanes (C_1 to C_8) were hydroxylated and oxidized to the corresponding alcohols, aldehydes, acids and ketones by whole-cell suspensions of the methane-oxidizing bacterium, H-2.⁴⁾

In this report we describe the oxidation of liquid *n*-alkanes (C_5 to C_{10}) by *Scedosporium* sp. A-4. The Z-C and M-3 media were described in the previous paper.¹⁾ This organism was grown at 30°C in a 0.5 l-closed flask containing 100 ml of Z-C medium and *n*-pentane (0.1%, v/v) under air with shaking for 48 hr (seed culture). The seed culture was inoculated into a 3 l-closed flask containing 500 ml of M-3 medium and *n*-pentane (0.1%, v/v). The culture was grown at 30°C with shaking for 48 hr. Preparation of resting mycelia was described in the previous paper.²⁾ The mycelia (0.1 g wet weight) were suspended in 2 ml of 0.1 M potassium phosphate (pH 7.0, containing 10 mM pyrazole) in 10 ml vials and then the vials were sealed with double-rubber stoppers. 4 μ l of a liquid *n*-alkane (C_5 to C_{10}) was put into the vials. The vials were then incubated at 30°C on a shaking device at 120 rpm. After 1 hr incubation, 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 ionization gas chromatography (GC-8A; Shimadzu, Kyoto) on a glass column (3.0 m by 2.6 mm) packed with 10% PEG1000 on 60/80 Uniport R (Gasukuro Kogyo Inc.,

Tokyo). The column temperature was maintained isothermally at 80°C, at the nitrogen carrier gas flow rate of 37 ml/min. The various products of liquid *n*-alkane oxidation were identified by means of gas chromatography retention time comparison and co-chromatography with authentic standards. The amounts of the various products accumulated were determined from the peak areas using a standard curve which had been constructed with authentic standards. As shown in Fig. 1B, *n*-pentane was hydroxylated, yielding mixtures of 1-pentanol and 2-pentanol; no 3-pentanol was formed. The rates of conversion of *n*-pentane to 1-pentanol and 2-pentanol were 0.018 and 0.024 μ mol/hr per mg dry mycelia, respectively. *n*-Hexane to *n*-decane were not oxidized, like methane.

An aliquot of each culture filtrate was removed with a syringe and assayed with a gas chromatography system and a gas chromatography-mass spectrometry combination system (M-60; Hitachi, Tokyo). The ion source temperature was held at 180°C during GC-MS runs. The mass spectra were obtained at an electron energy level of 70 eV and an accelerating voltage level of 3.2 kV. A computer with a data processing system (002B 8DK; Hitachi) was used to analyze the mass spectral data and to prepare mass chromatograms. As shown in Fig. 1C and 2, this organism accumulated 2-pentanone (0.28 mmol/l) and 2-pentanol (0.11 mmol/l) from *n*-pentane.

These and our previous data²⁾ demonstrated that *Scedosporium* sp. A-4 oxidizes *n*-alkanes (C_2 to C_5) to the

Fig. 1. Gas Chromatographic Analysis of *n*-Pentane Oxidation by *Scedosporium* sp. A-4.

A-1, 2-pentanone and 3-pentanone; A-2, 3-pentanol; A-3, 2-pentanol; A-4, 1-pentanol. B, the products of *n*-pentane oxidation by resting mycelia in the presence of pyrazole. C, accumulated products in the culture filtrate.

Fig. 2. Mass Spectrometric Analysis of the Culture Filtrate.
 a, peak 1 material in Fig. 1C; b, 2-pentanone; c, 3-pentanone.

corresponding 1- and 2-alcohols, but not 3-alcohols, and that methane, *n*-hexane and longer hydrocarbons (up to C₁₀) are not oxidized.

Acknowledgments. We are grateful to Dr. T. Uchiyama of our laboratory for the kind advice as to GC-MS analysis. We also wish to thank Mr. H. Sakai for his technical assistance.

References

- 1) M. Onodera, Y. Endo and N. Ogasawara, *Agric. Biol. Chem.*, **53**, 1431 (1989).
- 2) M. Onodera, Y. Endo and N. Ogasawara, *Agric. Biol. Chem.*, **53**, 1947 (1989).
- 3) J. Colby, D. I. Stirling and H. Dalton, *Biochem. J.*, **165**, 395 (1977).
- 4) T. Imai, H. Takigawa, S. Nakagawa, G.-J. Shen, T. Kodama and Y. Minoda, *Appl. Environ. Microbiol.*, **52**, 1403 (1986).