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Bioconversion Of Pinrnes By Newly Isolated Bacteria *Pseudomonas* Sp. Strain Pin

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Pinenes (α- and -β) are major components of turpentine, a byproduct of pulp making industry. Of turpentine produced in the United States, about 90% is mostly derived from the pulp and paper industry via chemical or thermomechanical processes[1]. The main components of turpentine from the South-eastern United States are α- pinene (70%) and β-pinene (25%)[2]. Of the available turpentine oil, which amounts to about 13, 761 metrc tons produced in the United States, 20 to 25% is used by the flavor and fragrance industry [3]. Recently, the application of terpenes have been extended to substitute for chloroflurocarbons and halogenated solvents. Thus, a better understanding of the fate and biotransformation of these compounds in biological treatment process would be necessitated[4].

Terpenes, α-pinene, ß-pinene, p-cymene, α-terpinolene, limonene, α-terpinene, α-pinene γ- terpinene, verbenol, α-terpineol, endo-borneol, fenchyl alcohol, camphor, camphene, iso-borneol, mytenol, and carveol were obtained from Aldrich Chemical Company (Milwaukee, WI, USA). *Pseudomonas* sp. strain PIN isolated from terpene soaked soil was maintained on sterile S-2 mineral medium [5]. Bioconversion was performed with resting cells. Transformation treatments were prepared using test tubes (25 × 150 mm) containing 5 μl of cell suspension and 50 μ1 of pinenes. All test tubes were extracted with 2 ml of diethyl ether containing tetradecane as an internal standard. The ether layer was dried over anhydrous sodium sulfate and concentrated under a stream of nitrogen to about 1 ml. Concentrated extracts were transferred into 2 ml amber vials equipped with teflon-lined caps and stored at- 20°C until analysis. For the identification of monoterpenes metabolites, two μl of each extract was injected into a fused silica open tubular column (Supelcowax 10; 0.25 mm i.d .× 60 m length × 0.25 μm d_f; Supelco, Inc., Bellefonte, PA, USA) installed in a Hewlett-Packard (Palo Alto, CA, USA) 5790A gas chromatograph (GC). Helium with a linear velocity of 25 cm/s was used as GC carrier gas. The injector temperature was set at 200°C. Column oven temperature programmed as follows: 40°C for 10 min, and then a rise of 6°C/min to 200°C where it was held for 20 min. The GC was coupled to an HP 5970B mass selective detector (MSD, Hewlett-Packard Co., USA). Mass selective detector was operated in the electron ionization (EI) mode with the ion source temperature at 200°C, ionization energy at 70 eV, mass range at m/z 33-300, electron multiplier voltage at 1800 V, and scan rate at 1.6 scans/sec. The MS interface was set at 200°C.

In the present study to elucidate products by the side reaction, we established bioconversion of pinenes in three ways: fermentation with substrate and bacteria, bacterial control (without substrate), and substrate control (without bacteria). Names, RI values, and identification methods of each identified bioconversion product and other products are presented in Table 1. Typical total ion chromatograms of fermentation, bacterial control, and substrate control are presented in Fig 1. Compounds identified included 7 hydrocarbons such as α-pinene (1), β-fenchene (2), camphene (3), ß-pinene (4), limonene (5), *p*-cymene (6), α-terpinolene (7), camphor (9), and 5 alcohols such as fenchyl alcohol (10), α-

Table 1. Products and related compounds from bioconversion of α-and β-pinene by *Pseudomonas* sp. strain P

Peak No.	Compound	RI[a]	Indentification[b]
1	α-Pinene	1029	IR, MS, RI
2	α-Fenchene	1058	MS
3	Camphene	1066	IR, MS, RI
4	β-pinene	1080	IR, MS, IR
5	Limonene	1197	IR, MS, RI
6	*p*-Cymene	1273	IR, MS, RI
7	α-Terpinolene	1285	IR, MS, RI
8	Tetradecane	1400[c]	
9	Camphor	1526	IR, MS, RI
10	Fenchyl alcohol	1580	IR, MS
11	α-Terpinene-4-ol	1622	IR, MS
12	α-Terpineol	1686	IR, MS, RI
13	Borneol	1697	MS, RI
14	*p*-Cymene-8-ol	1833	MS

[a]RI = retention index. [b]Basis for identification: IR = infrared spectrum, MS = mass sepctrum, RI = retention index. [c]Internal Standard

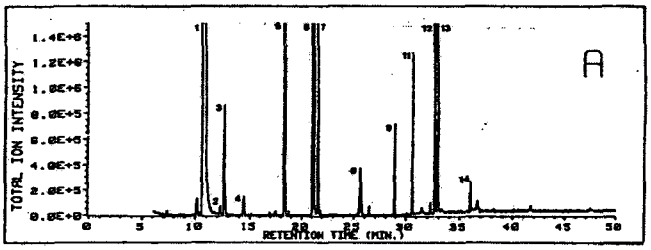

Fig. 1. Total ion chromatogram of the bioconversion of alpha-pinene by Pseudomonas sp. strain PIN after 24 h growth

terpinen-4-ol (11), α-terpineol (12), borneol (13), and *p*-cymene-8-ol (14). All metabolites were either monocyclic or bicyclic terpenes. No acyclic compounds were identified. Monoterpenes with esters and aldehydes linkages were not detected. The five major bioconversion products from α-pinene were limonene, *p*-cymene, α-terpinolene, α-terpineol, and endo-borneol. Minor compounds were camphor, α-terpinen-4-ol, and *p*-cymene-8-ol, as well as several *p*-menthene derivatives. The five major metabolites from ß-pinene were the same as those from α-pinene. The minor bioconversion products from ß-pinene also were similar to those derived from α-pinene except for the presence of camphor and absence of fenchyl alcohol.

References

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