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Degradation of Vinyl Alcohol Oligomers by *Geotrichum* sp. WF9101

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Note

Degradation of Vinyl Alcohol Oligomers by *Geotrichum* sp. WF9101Tatsuma MORI,[†] Michio SAKIMOTO, Takashi KAGI, and Takuo SAKAI^{*,††}

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***Geotrichum* sp. WF9101 isolated from a poly(vinyl alcohol)-degrading mixed culture could utilize vinyl alcohol oligomers, but not the polymers. This strain is proposed to have utilized degradation products of poly(vinyl alcohol) in the mixed culture. Biodegradation of vinyl alcohol oligomers by this strain was discussed using 2,4-pentanediol as a model substrate.**

Key words: biodegradation; vinyl alcohol oligomer; *Geotrichum*; 2,4-pentanediol

Since a symbiotic utilization of poly(vinyl alcohol) (PVA) was first reported by Shimao *et al.*,^{1,2)} there has been great interest in it. We have obtained a PVA-degrading mixed culture that consisted of eight different microorganisms from activated sludge from a textile factory.³⁾ We found that in this mixed culture, PVA was degraded by co-metabolism with a bacterial strain (BX1) that degraded PVA to vinyl alcohol oligomers (OVA), and its symbiotic partner (PN19) and an OVA-utilizing fungus strain (WF9101). To our knowledge, such symbiotic utilization of PVA has not been reported before. In this paper, we describe the characteristics of microbial degradation of OVA by WF9101.

The PVA used (having a polymerization degree of about 500 and a degree of saponification of more than 99 mol%) was purchased from Shin-Etsu Chemical Industries (Tokyo, Japan). Vinyl alcohols with different average polymerization degrees were prepared from the PVA according to the following procedures. Fifty ml of 30% H₂O₂ solution was added to 300 ml of 3% of PVA solution, and the mixture was heated at 90°C for 6 h. After heating, catalase was added to the mixture to decompose excess H₂O₂, and then, the mixture was filtered with an ultra filter UK-10 (ADVANTEC, Japan). The filtrate obtained was evaporated with a rotary evaporator at 50°C under reduced pressure. The dry materials were dissolved in distilled water and then fractionated by gel filtration on a column of Toyopearl HW-40 (2.5 × 80 cm). Poly(ethylene glycol) 6000, 2000, 1000, 600, and 300 were used as size markers. As a dimer of vinyl alcohol, 2,4-pentanediol (PD; purchased from Tokyo Kasei, Japan) was used. The culture conditions and the composition of PVA medium were the same as reported previously.³⁾ The composition of OVA medium (average polymerization degree of about 20), PD medium, and various media containing of vinyl alcohol with different polymerization degrees were the same as the PVA medium except that it contained each of the carbon sources instead of PVA.

To examine OVA-degrading ability, each of the eight different isolates from the mixed culture was cultivated in OVA medium, and changes of chemical oxygen demand (COD) of the medium were measured. The estimation of COD value was done according to the method described in Japan Industrial Standard.⁴⁾

Among the isolates, strain WF9101 showed the most potent

OVA-degradation ability. Strain WF9101 decreased about 60% of the initial COD after 3 days of cultivation. In contrast, the other isolates decreased only by about 10% of the initial COD. BX1 and PN19 could degrade no OVA. Therefore, WF9101 was used for this study. The strain WF9101, which produced white colonies on the malt extract agar and no ascospores, was identified as belonging to the genus *Geotrichum* from the results of a taxonomic study.⁵⁾

Table I shows the cell growth and changes of COD after the cultivation of WF9101 in various media containing 0.1% vinyl alcohol with different polymerization degree. After 3 days of cultivation in the medium containing vinyl alcohol with an average polymerization degree of about 20, the initial COD value decreased by about 60%. In contrast, after the cultivation in the medium containing vinyl alcohol with an average polymerization degree of more than about 50, a trace of the COD value had decreased. From the results, it became clear that WF9101 could utilize OVA,

Table I. Assimilation of Various Vinyl Alcohols with Different Polymerization Degrees by WF9101

Average polymerization degree	Growth (OD ₆₆₀)	COD value decreased (%)
2 (PD)	1.62	62.2
20 ^a	0.85	58.8
50 ^a	<0.03	<1.0
100 ^a	<0.03	<1.0
500 (PVA)	<0.03	<1.0

^a Vinyl alcohols with different average polymerization degree were prepared from PVA by the methods described in the text.

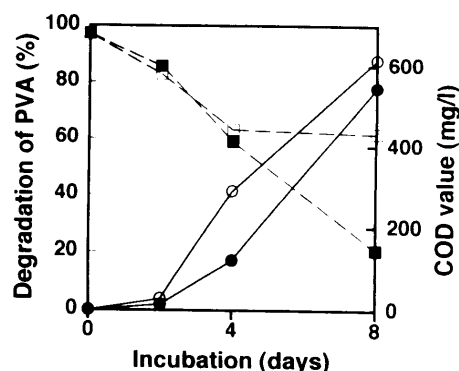


Fig. Changes of COD Value during Cocultivation of BX1 and PN19 with or without WF9101.

■, COD value (with WF9101); □, COD value (without WF9101); ●, degradation of PVA (with WF9101); ○, degradation of PVA (without WF9101).

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Abbreviations: OVA, vinyl alcohol oligomer; PVA, poly(vinyl alcohol); PD, 2,4-pentanediol; COD, chemical oxygen demand.

Table II. Stoichiometry of Reaction of Cell-free Extract of WF9101 on PD

Time (h)	PD decomposed (nmol)	Degradation products (nmol)				Decomposed PD/A + B + 1/2(C + D)
		4-Hydroxy-2-pentanone (A)	2,4-Pentanedione (B)	Acetone (C)	Acetic acid (D)	
0.5	13.1	10.5	2.2	0	0	1.03
1	20.8	15.2	6.3	1.0	1.0	0.97
2	39.5	23.4	10.5	2.5	2.5	1.09
3	51.8	28.5	18.5	4.8	5.0	1.00
4	58.0	25.6	19.4	10.0	10.4	1.05

Reaction mixture (2 ml) contained 10 mM NAD^+ , 10 mM PD, and cell-free extract of WF9101 in 50 mM Tris-HCl buffer (pH 8.0). The reaction was done at 30 °C for 4 h.

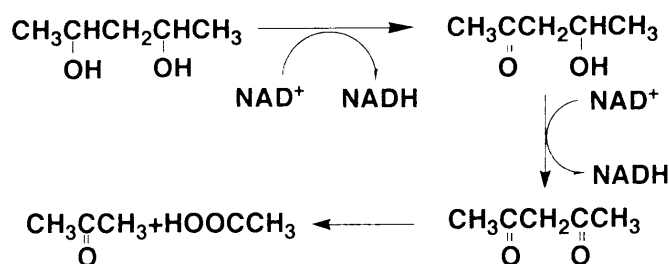
but not PVA. A limitation to biodegradation by molecular weight has also been reported on microbial degradation of acrylate oligomer.⁶⁾

The figure shows changes of COD of the medium after 4 days of cocultivation of BX1 and PN19 with or without WF9101 in PVA medium. In the inoculation of BX1 and PN19 without WF9101, the initial COD value decreased by only 34.1%, nevertheless 90% of the initial amount of PVA in the medium detected with iodometry⁷⁾ was decomposed. It might indicate that PVA was degraded to OVA (which does not react with iodine), but not degraded completely. In contrast, 76.5% of the initial COD value of the medium decreased and 70% of the initial amount of PVA was decomposed in the cocultivation of BX1 and PN19 with WF9101. These results indicate that PVA is decomposed by the combination of BX1 and PN19 and the PVA decomposed is utilized by WF9101. A similar phenomenon has been reported for PVA-degrading *Pseudomonas vesicularis* var. isolated by Hashimoto and Fujita.⁸⁾ In their case, most of the organic carbon in the medium remained after the 5-day cultivation of *P. vesicularis* var. They suggests that this happens because *P. vesicularis* var. can degrade PVA but not OVA.

To clarify the degradation mechanism of OVA by this strain, the degradation of PD, which was assumed to be the smallest unit of PVA degradation, was studied. PD and its degradation products were measured using a gas-liquid chromatography coupled with a flame ionization detector (Shimadzu GC-6A, Japan). A glass column packed with TENAX GC was used (3 mm × 1.1 m, Enkanv Arnhem, Holland, the column temperatures was 150 °C, the carrier gas was N_2 at 60 ml/min).

When WF9101 was cultured in medium containing 0.1% PD, three different PD degradation products were found in the culture broth by gas-liquid chromatography. Their retention times were 10.06 min, 7.16 min, and 4.16 min, corresponding to those of 4-hydroxy-2-pentanone (product 1), 2,4-pentanedione (product 2), and 1,2-propanediol (product 3), respectively. These identifications were confirmed by mass spectroscopy. Mass spectra were taken with a Shimadzu GCMS-QP2000A mass spectrometer coupled with a Shimadzu GC-14A gas-liquid chromatograph with a CDX-B column (2.5 mm × 30 m, J & W Scientific, U.S.A.). Product 1 did not showed the expected molecular ion of 4-hydroxy-2-pentanone, however, it showed a diagnostic fragment ion at m/z of 84, 58, and 43. Product 2 had a molecular ion at m/z of 100 and there were the following diagnostic fragment ions; 85, 72, 58, and 43. Product 3 had a molecular ion at m/z of 76 and there were the following diagnostic fragment ions; 45 and 43. From the results, Products 1, 2, and 3 were identified as 4-hydroxy-2-pentanone, 2,4-pentanedione, and 1,2-propanediol, respectively.

In the cell-free extract of WF9101 grown on PD, NAD^+ -

**Scheme.** Tentative Degradation Pathway of PD in Strain WF9101.

linked alcohol dehydrogenase catalyzing the oxidation of vinyl alcohols with various sizes was detected, but PVA oxidase activity was not. The oxidation rates (relative activity to PD) towards OVA and PVA were 20.4% and 5.3%, respectively. Nevertheless, in the culture broth, the enzyme activity was not detected.

Table II shows the stoichiometry of reaction of the cell-free extract on PD examined by gas-liquid chromatography. The cell-free extract used (in 50 mM Tris-HCl buffer, pH 8.0, containing 0.01% 2-mercaptoethanol) was prepared by the mechanical disruption with a bead beater (Biospec Products, U.S.A.). The reaction mixture that contained 10 mM PD and 10 mM NAD^+ in the cell-free extract was incubated for 4 h at 30 °C.

4-Hydroxy-2-pentanone, 2,4-pentanedione, acetic acid, and acetone were identified as degradation products of PD (identification was done by GC-mass spectra). The number of moles of PD degraded was almost equal to the number of moles of the degradation products 4-hydroxy-2-pentanone, and 2,4-pentanedione plus one half of the number of moles of acetic acid and acetone. Based on the results obtained and knowledge of microbial degradation of PVA,⁹⁾ we propose that strain WF9101 degraded OVA through the same metabolic pathway as that of PD (Scheme).

In the pathway, hydroxy groups of OVA are oxidized to carbonyl groups by the action of dehydrogenase, and then the C-C linkage in the oxidized OVA may be cleaved by the action of β -diketone hydrolase. Consequently, acetic acid may be produced as the end product, and utilized immediately by the strain. Acetone produced from PD is probably reduced to 1,2-propanediol *via* enol-acetone. Such a metabolic fate was reported in hydrocarbon-utilizing *Mycobacteria*.¹⁰⁾

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