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Short Communications

On the Bacterial Degradation of Lignin

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Summary

Several bacteria which can degrade numerous phenols with structural relationships to lignin were tested for their ability to degrade lignin. The biodegradation with all the tested bacteria was poor. The method of lignin extraction, presence of glucose as co-substrate and changes in the nitrogen source of the medium did not affect the extent of lignin degradation. The poor degradation does not seem to be influenced by medium composition and culture condition but is more probably due to the inability of the tested bacteria to degrade lignin to any considerable extent.

Introduction

Lignins are generally classified into the three major groups of gymnosperm, angiosperm and grass lignins. The structural units of all of them consist of different proportions of coniferyl, sinapyl, and p-coumaryl alcohol polymers (Higuchi,1980). For this reason many authors have used different phenols with structural relationships to the lignin building blocks for isolation, selection, and degradation studies of lignin decomposing bacteria (Muranaka et al., 1976; Fukuzumi and Katayama, 1977; Haider et al., 1978; Ohta et al., 1979)

In this short communication, several bacteria which had been isolated according to their ability to degrade phenolic compounds (Haider et al., 1978) were tested for their ability to degrade differently extracted lignins.

Materials and Methods

Microorganisms and inoculum: The bacteria studied are listed in Table 1. The origin of the bacteria obtained from the DSM and the Inst. Pasteur was lignin containing lake water.

Pseudomonas aeruginosa and Corynebacterium sp. were isolated from phenol containing waste water. The Pseudomonas sp. were maintained on agar slants of medium No. 1 and the remainder on medium No. 65 of the DSM catalogue (1977). The same media were used for pre-cultures.

Lignins: Four differently extracted lignins were used:

a) cold dioxane-hydrochloric acid lignin (Odier and Monties, 1978)
b) hot dioxane-hydrochloric acid lignin (Odier and Monties, 1977)
c) alkali lignin isolated with 1 % sodium hydroxide at 121 °C for 1 h
d) commercially available Kraft pine lignin polymer (Indulin AT) manufactured by Westvaco Co., Charleston, South Carolina USA

The air-dried straw used for lignin extraction was ground to pass a screen of about 24 mesh. The free lignin was determined spectrophotometrically in the whole culture. Details are reported elsewhere (Janshekar et al., 1981).

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Basal Medium: Medium (M1) contained the following per litre of distilled water: K_2HPO_4, 1.60 g; KH_2PO_4, 0.50 g; $(NH_4)_2SO_4$, 1.25 g; NH_4NO_3, 1.00 g; $MgSO_4 \cdot 7H_2O$, 0.50 g; NaCl, 0.25 g; $FeCl_3 \cdot 6H_2O$, 25 mg; $CaCl_2$, 10 mg; Yeast extract (Difco), 0.10g.

For media (M2), (M3), and (M4) the nitrogen salts were substituted for with 2.67 g $NaNO_3$, 2.70 g oat meal, and 0.94 g urea respectively. For medium (M5) the nitrogen salts were omitted. Medium (M6) had the same constituents as (M1) but 10 times less concentrated. The medium was adjusted to pH 7.2 and sterilized by autoclaving. Avicel, xylan and aromatic compounds (benzoic, p-OH-benzoic, vanillic, veratric, syringic, p-coumaric, and sinapinic acids; coniferyl alcohol) were added to the basal medium (M1) befor autoclaving. Autoclaving did not affect the aromatic compounds. Lignins were sterilized with ether, added as a dry powder to the sterilized basal medium (1 gl^{-1}; for (M6), 0.1 gl^{-1})mixed for several hours, aseptically dispensed into test tubes (5 ml/tube) and closed with foam plugs. Each tube was inoculated with 10% (v/v) of a pre-culture. Some of the tubes were inoculated with sterile pre-cultures to serve as controls. The tubes were incubated on a rollerdrum at 30°C for 14 days.

Results and Discussion

Growth on cellulose and himcellulose: Since lignin in woody materials is accompanied by cellulose and hemicellulose, initially the bacteria were tested for their ability to grow on avicel and xylan. Plate and shake flask tests were negative (Pseudomonas aeruginosa and Corynebacterium sp. were not tested) which should be considered as a positive factor in favour of these bacteria. Having bacteria which can remove the lignin from lignocellulose without also decomposing cellulose is in fact desirable.

Growth on lignin-related aromatic compounds: Benzoic, p-OH-benzoic, vanillic, veratric, syringic, and p-coumaric acids were utilized by most of the bacteria as carbon source. Coniferyl alcohol was either metabolized or oxidized to aldehyde and acid. Sinapinic acid, however, remained unchanged. Studies with ^{14}C labelled aromatic compounds and the dehydropolymer of coniferyl alcohol (DHP) labelled on the ring, the chain or the methoxyl group have also shown $^{14}CO_2$-release by several of these bacteria (Haider et al., 1978).

Growth on lignin: The percentage of lignin degradation after 14 days of incubation is shown in Table 1. Most of the data are within the deviations of control test tubes and can not be considered to show significant degradation. However, hot dioxane-acid extracted lignin (b) seemed to be more degraded than the cold dioxane-acid extracted lignin (a). This could have been due to the degradation of low molecular weight fractions of lignin as their formation is more probable under the conditions used for extraction of the former lignin. Similar explanations may be given for the comparatively higher degradation of Indulin. Kraft lignin from pine wood is polydispersed and no attempt was made to remove the low molecular weight components. Because of the low extent of lignin degradation and the possible deviation in the values obtained, it is not possible to predict which individual bacterium or which group of bacteria have higher ligninolytic activity. The solubilization of lignin in alkaline solution befor its introduction into basal medium did not give a better result.
It is reported that some low-molecular weight fragments of lignin may have inhibitory effects on the growth of certain microorganisms (Zemek et al., 1979). It may be assumed that such compounds could have been produced after the initiation of lignin depolymerization and that their presence could have hindered further growth. However, cultivation on a 10 times less concentrated medium which would lead to a reduction in this effect, did not improve the extent of lignin degradation. Nor was it better when a mixture of bacteria were used.

Effect of glucose and nitrogen additions: Addition of 1 gl^{-1} glucose to the medium did not appear to have any stimulating effect on lignin degradation (Table 1). Additionally changes in the type and amount of exogenous nitrogen source did not affect the results.

Table 1: Lignin degradation by different bacteria. The bacteria were grown on different media for 14 days at 30°C with shaking. Initial lignin concentration was 1 gl^{-1}; for medium (M6), 0.1 gl^{-1}. Degraded lignin is the percentage of decrease in absorbance of culture dissolved in dioxane at 281 nm compared with an uninoculated control. (-) means no change.

Lignins*	Co-substrate	Basal* medium	Degraded lignin, % of control												Dev. in control %
			N. autotrophica DSM 43089	N. autotrophica DSM 43099	N. autotrophica DSM 43088	N. corallina DSM 43001	N. opaca DSM 43002	N. asteroides DSM 43003	N. globerula DSM 43253	P. testosteroni DSM 50244	P. putida DSM 50906	P. putida Inst. Pasteur 6323	P. aeruginosa sp. (phenol)	Corynebacterium sp. (phenol)	
a	-	M1	2	2	-	3	-	-	-	3	1	-	5	-	3
a	glucose	M1	-	-	2	-	-	-	-	-	-	-	-	-	3
b	-	M1	7	13	10	2	6	-	-	9	-	2	7	1	4
c	-	M1	6	3	3	1	-	2	-	4	-	2	1	-	2
d	-	M1	4	6	3	5	1	1	6	6	4	5	10	4	4
a	-	M2	6	3	1	3	-	1	-	3	3	5	6	1	5
a	-	M3	1	-	1	-	-	-	-	1	-	-	-	-	5
a	-	M4	4	2	-	2	-	-	-	4	3	4	5	-	2
a	-	M5	2	3	2	1	-	-	-	2	-	2	4	2	4
a	-	M6	3	4	5	1	-	-	-	2	-	-	4	-	2
a	-	M6	← Mixed: 2 →												2

* See materials and methods.

This study shows that in spite of the ability of the above mentioned bacteria to degrade monomers, they cannot effect any considerable degradation of the lignin polymer. This may not be attributed to the medium composition and culture conditions since these were the same as for growth on lignin-related aromatic compounds. Therefore, the ability of bacteria to degrade monomer phenols with a structural relationship to lignin may not be an important criterion for lignin degradation. However, the importance of Nocardia and Pseudomonas sp. in the biotransformation of lignin fragments cannot be excluded (Crawford et al,. 1973; Cox and Goldsmith, 1979; Daumy et al., 1980).

For example these bacteria may be used together with microorganisms able to attack the lignin polymer. Or the lignin may first be broken down into fragments chemically e.g. by ozonolysis before being introduced to microbial transformation. But before that, there

are many investigations to be undertaken in order to elucidate the behaviour of bacteria in a complex medium consisting of a variety of aromatic compounds which are left after the chemical or biological depolymerization of lignin. The most difficult task is to control the bioconversion process of lignin fragments toward a desirable pure product.

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