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Short Communication: Isolation of a bacterium capable of limited degradation of industrial and labelled, natural and synthetic lignins

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***Pseudomonas putida*, isolated from decomposing plant materials, degraded several lignin-related aromatic compounds. After 30 days of incubation in media containing polymeric Kraft-lignin (PKL), the amount of Klason lignin had decreased by about 13%. When ^{14}C-labelled dehydropolymers of coniferyl alcohol (DHP) lignins and ^{14}C-lignin-lignocelluloses were used as substrates, mineralization to $^{14}CO_2$ by the *P. putida* strain ranged from 1.4% to 2.1%.**

Key words: Bacteria, degradation, Kraft-lignin, lignocellulose, synthetic lignin.

Though white-rot fungi are considered the most efficient lignin degraders, other groups of microorganisms, including bacteria, are also thought to be involved in lignin degradation (Vicuña 1988). However, mineralization of (^{14}C-lignin) lignocellulose to $^{14}CO_2$ by these other groups is low in comparison with that achieved by the white-rot fungus *Phanerochaete chrysosporium* (Vicuña 1988).

Pseudomonads have a high metabolic versatility and can degrade many recalcitrant compounds, including extracted lignins, specifically labelled, synthetic lignins and lignin-related compounds (Vicuña 1988).

Due to the difficulties associated with obtaining a lignin preparation in an unmodified form, model compounds and lignins extracted from pulping liquors (e.g. Kraft-lignin) have been used to screen for lignin-degrading bacteria (Ball *et al.* 1989).

In the present study, a bacterial strain, identified as *Pseudomonas putida*, that was capable of degrading lignin-related compounds was isolated and the ability of this bacterium to degrade Kraft-lignin and radiolabelled lignins was studied.

Materials and Methods

Isolation and Identification of the Bacterium

The bacterium was isolated, by an enrichment-culture technique, from decomposing plant materials, using vanillin (0.1%, w/v) as sole carbon source. It was identified according to routine biochemical tests, including API 20E strips used in duplicate.

Lignin and Lignocellulose Preparations

Kraft pine lignin polymer (PKL) (Indulin AT; Westvaco Co, Charleston, SC), was purified as described by Rodríguez *et al.* (1994). ^{14}C-Lignocelluloses and ^{14}C-DHPs were obtained as described by Perestelo *et al.* (1994).

Degradation of Lignin-related Compounds

The isolated bacterium was grown on a basal salts medium (Perestelo *et al.* 1994) supplemented with an aromatic compound as sole carbon source. Duplicate cultures (each with approx. 10^7 cells/ml) and uninoculated controls (25 ml) were incubated aerobically at 28°C in 250-ml flasks on a reciprocal shaker (125 strokes/min). Ferulic, 4-coumaric, vanillic, 4-hydroxy-benzoic, protocatechuic and benzoic acids, vanillin or vanillyl alcohol, each at 0.1% (w/v), syringic acid, guaiacylglyceryl ether, catechol or 4-benzyloxy-3-methoxybenzyl alcohol, each at 0.05% (w/v), 0.025% (w/v) guaiacol, 0.02% (w/v) veratric acid, *trans*-cinnamic acid, phenol, anisoin, 2-benzyl-benzyl alcohol or 3-(4-methoxyphenoxy) benzaldehyde, each at 0.01% (w/v), or biphenyl-2-ol (0.005%, w/v) was used as substrate.

The bacterial populations were followed turbidimetrically at 630 nm and by viable-cell counts on nutrient agar plates. Aliquots were removed periodically and degradation of substrates was monitored by quantitative u.v.-visible spectrophotometry and by TLC. After growth on the different aromatic substrates, the organisms were tested for cleavage of catechol and protocatechuate by the *ortho* or *meta* pathways (Ottow & Zolg 1974).

Degradation Experiments with Lignin Substrates

Duplicate cultures containing PKL (0.1%, w/v) and uninoculated controls were incubated as described before (Perestelo *et al.* 1994). PKL degradation was estimated by residual acid-insoluble Klason lignin, and spectrophotometrically at 280 nm (Janshekar *et al.* 1981; Rodríguez *et al.* 1994). Specifically-radiolabelled-substrate degradation studies and bacterial counts were carried out as by Perestelo *et al.* (1994). All incubations were performed in triplicate and started with similar cellular concentrations (approx. 10^7 cells/ml).

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Table 1. Labelled and unlabelled substrates used and their degradation by *P. putida*.

Substrate	Source	Specific activity (d.p.m./mg)	Degradation* (%)
Natural lignins			
(^{14}C-U-Lignin) lignocellulose	Pine	12,160	2 (14)†
(^{14}C-3-side-chain-lignin) lignocellulose	Sedge	1424	2.1 (14)†
(^{14}C-methoxyl-lignin) lignocellulose	Spruce	4525	2 (18)†
Synthetic lignins			
(^{14}C-U.ring) DHP		61,512	1.4 (14)†
(^{14}C-2-side-chain) DHP		370,729	1.5 (16)†
Polymeric Kraft-lignin	Pine		8.7 (30)‡ or 13 (30)§

* Total degradation observed less the (abiotic) degradation (0.1% to 0.2%) seen in uninoculated controls. Values in parenthesis are the days of incubation.
† Mineralization measured as $^{14}CO_2$-evolved.
‡ Determined by spectrophotometry.
§ Determined by Klason-lignin content.

Results and Discussion

Cells of the isolated bacterium were short rods, motile, Gram-negative and non-endospore forming. They were not susceptible to ampicillin or to chloramphenicol but were susceptible to tetracycline. Based on these characteristics and on the biochemical test results, the isolate was classified as *Pseudomonas putida*.

The bacterium metabolized vanillin and protocatechuic, vanillic, benzoic, ferulic, 4-coumaric and 4-hydroxybenzoic acids within 12 h, reaching concentrations of about 10^9 cells/ml. Catechol and vanillyl alcohol were metabolized more slowly and cellular yields were lower on these substrates (10^7 to 10^8 cells/ml). Benzoic acid was an inducible substrate for catechol 1,2-dioxygenase, whereas other assimilated substrates induced protocatechuate 3,4-dioxygenase. The presence of these enzymes indicated that catabolism proceeded with an *ortho*-ring fission mechanism. Glucose-grown cells did not produce the dioxygenases.

When *P. putida* was incubated with PKL, a 8.7% decrease in A_{280} was observed after 30 days of incubation. In this case, the decrease in the amount of Klason lignin was estimated to be 13% (Table 1). Utilization of high-molecular-weight Kraft-lignin has also been described in actinomycetes (Ball *et al.* 1989).

The bacterium mineralized between 2.0% and 2.1% of pine, sedge and spruce lignocelluloses, specifically labelled in the lignin component (Table 1). When ring- and side-chain labelled DHP were used, *P. putida* mineralized between 1.4% and 1.5% of the label (Table 1). Degradation percentages are similar to those reported for other *Pseudomonas* strains (Rüttimann *et al.* 1991). In both PKL and radiolabelled-substrate cultures, final cellular concentrations were about 10^8 cells/ml.

The isolated *P. putida* strain therefore seems capable of degrading polymeric Kraft-lignin and producing limited mineralization of natural and synthetic lignins. Optimisation of the culture parameters could lead to further improvements in the ligninolytic activity of the isolated strain.

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References

Ball, A.S., Betts, W.B. & McCarthy, A.J. 1989 Degradation of lignin-related compounds by actinomycetes. *Applied and Environmental Microbiology* **55**, 1642–1644.

Janshekar, H., Brown, C. & Fiechter, A. 1981 Determination of biodegraded lignin by ultraviolet spectrophotometry. *Analytica Chimica Acta* **139**, 81–91.

Ottow, J.C.G. & Zolg, W. 1974 Improved procedure and colorimetric test for the detection of ortho and meta cleavage of protocatechuate by *Pseudomonas* isolates. *Canadian Journal of Microbiology* **20**, 1059–1061.

Perestelo, F., Falcón, M.A., Carnicero, A., Rodríguez, A. & De La Fuente, G. 1994 Limited degradation of industrial, synthetic and natural lignins by *Serratia marcescens*. *Biotechnology Letters* **16**, 299–302.

Rodríguez, A., Carnicero, A., Perestelo, F., De La Fuente, G., Milstein, O. & Falcón, M.A. 1994 Effect of *Penicillium chrysogenum* on lignin transformation. *Applied and Environmental Microbiology* **60**, 2971–2976.

Rüttimann, C., Vicuña, R., Mozuch, M.D. & Kirk, T.K. 1991 Limited bacterial mineralization of fungal degradation intermediates from synthetic lignin. *Applied and Environmental Microbiology* **57**, 3652–3655.

Vicuña, R. 1988 Bacterial degradation of lignin. *Enzyme and Microbial Technology* **10**, 646–655.

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