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LIMITED DEGRADATION OF INDUSTRIAL, SYNTHETIC AND NATURAL LIGNINS BY *Serratia marcescens*

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SUMMARY

Serratia marcescens was found to degrade kraft lignin by only 15%. When ^{14}C-radiolabelled lignocelluloses and DHP lignins were used as substrates the bacterium mineralized to $^{14}CO_2$ only 1.1-1.9% and 0.4-0.8% of the lignins respectively. However, some 44.4% of the ^{14}C-ß-DHP lignin was recovered as soluble radiolabelled products.

INTRODUCTION

The degradation of lignin by bacteria was reported many years ago but more recently attention has been given to the role of the bacteria in this process (Zimmermann, 1990). The ability of filamentous and non-filamentous bacteria strains, including *Streptomyces* (McCarthy, 1987), *Nocardia* (Trojanowski et al., 1977), *Pseudomonas* (Kaplan and Hartenstein, 1980), *Arthrobacter* (Kerr et al., 1983), *Bacillus* (Robinson and Crawford, 1978), *Xanthomonas* (Kern, 1984), and *Acinetobacter* (Vasudevan and Mahadevan, 1991), to degrade radiolabelled natural or synthetic lignins, has been established.

Different enzymes have been described in relation to lignin degradation. Particularly, laccase, a phenoloxidase enzyme, have been involved in ligninolysis by causing demethoxylation of lignin and formation of phenoxy radicals as the initial step in C-C bond cleavage (Shimada and Higuchi, 1991).

In this work, we have studied the ability of a *Serratia marcescens* strain to attack kraft lignin and natural and synthetic radiolabelled lignins.

MATERIALS AND METHODS

Isolation and specification of the bacterium.- The bacterium was isolated from compost heaps (Perestelo et al. 1989a).

Media and culture conditions.- The basal medium was prepared according to Odier and Monties (1978). When kraft lignin degradation was studied, the medium was dispensed into 1L Erlenmeyer flasks (300 ml/flask), the pH adjusted to 7 with phosphoric acid, and autoclaved at 121°C for 20 min. Kraft lignin sterilized by

filtration, was added to basal medium to a final concentration of 1g/l, and glucose was added at 1 g/l and 5 g/l, a third flask being without glucose. Two controls without lignin, and containing glucose to the above mentioned concentrations, were incubated in the same conditions (125 strokes/min and 28°C). A third control was carried out by incubating cell suspensions in phosphate buffer (100 mM, pH 7) in order to prove that cellular lysis was absent. The same media with 2% agar were used for counts of viable cells.

When specifically radiolabelled substrates were used, cultures and bacterial counts were carried out as indicated by Benner et al. (1984). All incubations were done in duplicate.

Lignin preparation.- Kraft pine lignin polymer (Indulin AT, Westvaco Co., Charleston, S.C., USA) was used.

Radiolabelled substrates.- Different radiolabelled substrates have been used (Table 1). (^{14}C-U-lignin) lignocellulose and (^{14}C-3-side chain lignin) lignocellulose were provided by Dr. Hodson, Department of Microbiology, University of Georgia, Athens, GA, USA. (^{14}C-2-side chain lignin) lignocellulose, ^{14}C-Cellulose, and (^{14}C-U-ring) or (^{14}C-2-side chain) dehydropolymers of coniferyl alcohol (DHP), were provided by Dr. Trojanowski, Forstbotanisches Institut der Universität Göttingen, FRG.

Decolourization assay.- Decolourization activity of the bacterium on the polymeric dye Poly B-411 (polyvinalamine sulfonate-anthroquinone) was determined as indicated by Chet el al., (1985).

Measurement of lignin and cellulose degradation.- The extent of kraft lignin degradation after 7 days incubation was estimated by UV-spectroscopy (Ulmer et al., 1983) using uninoculated cultures as controls. Mineralization rates of the radiolabelled substrates were monitored as described by Benner et al.(1984).

Assay for laccase.- Laccase activity was determined as indicated by Law and Timberlake (1980).

RESULTS AND DISCUSSION

This paper reports for the first time, the ability of *S. marcescens* to degrade kraft lignin and natural and synthetic radiolabelled lignins. Before inoculation onto lignin substrates, the bacterium was tested to decolourize Poly B-411 (Fig. 1). A good correlation between lignocellulose degradation and the ability to decolourize Poly B-411 by filamentous bacteria, has been reported (Pasti and Crawford, 1991). Results show that this *Serratia* strain is more effective at decolourizing the dye than the *Streptomyces* strains tested by Pasti and Crawford (1991) (Fig. 1).

When *S. marcescens* was grown in the presence of 0.1% kraft lignin, as sole carbon and energy source, the bacterial population declined until total kill after 4 days incubation (Fig. 2). This inhibitory effect was not present when medium was supplemented with 1 and 5 g/l glucose, in which the viable cell counts at the end of the incubation period (44×10^6 cfu/ml and 69.4×10^6 cfu/ml, respectively), were slightly lower than those in the absence of kraft lignin (22.5×10^7 cfu/ml and 63.1×10^7 cfu/ml, respectively) (Fig. 2). This same inhibitory effect of kraft lignin has been observed previously (Perestelo et al., 1989b). It may be due to some low molecular weight fragments of lignin (Zemek, 1979; Perestelo et al., 1989b).

Quantitative determination of acid-precipitable polymeric lignin, showed 15% degradation when *Serratia marcescens* was incubated with 1 g/l glucose, while no appreciable lignin loss was observed when the bacterium was incubated with 5 g/l glucose nor in its absence (Data not shown).The lower extent of lignin degradation in the medium with 5 g/l glucose may be due to the delay in the appearance of ligninolytic activity at this time, ors to catabolite repression (Forney and Reddy, 1979).

The maximum level of laccase activity (25 U/ml) was recorded after 24 h of growth, and no laccase activity was detected in the presence of 2 mM thioglycollate (Evans, 1985). Results showed that when laccase activity was not present in the culture medium, neither acid precipitable lignin loss nor changes in total lignin spectrum were observed. Thus, a positive correlation between laccase production and lignin degradation was found.

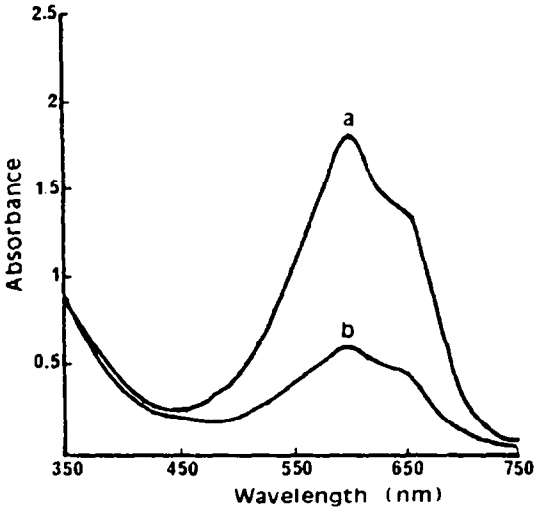

Figure 1.- Scanning spectroscopy of Poly-B411 (a) before and (b) after 16 days incubation, in presence of the bacterium.

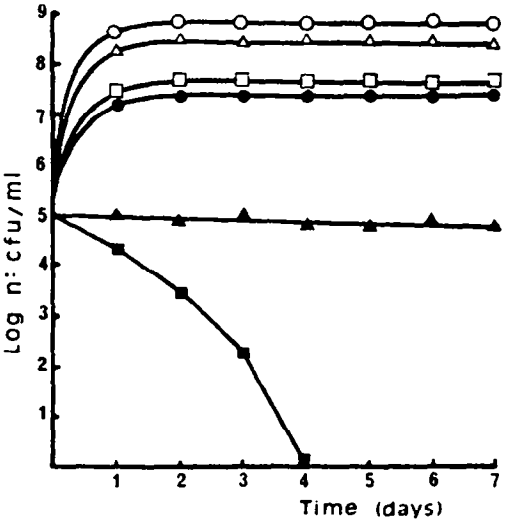

Figure 2.- Viable cell counts in different media containing 0.5% glucose (○); 0.5% glucose and 0.1% kraft lignin (△); 0.1% glucose (□); 0.1% glucose and 0.1% kraft lignin (●); 0.1%kraft lignin (■). Each value is the mean of four determinations.

Table 1.- Radiolabelled substrates and degradation produced by *Serratia marcescens.*

Substrates	Source	Specific Activity (dpm/mg)	Lignin Degradation[a]	
			Sol. Prod.	$^{14}CO_2$
Natural Lignins:				
(^{14}C-U-lignin)-lignocellulose	Pine	12,160	N.T.[b]	1.9 (14)
(^{14}C-2-side chain lignin) lignocellulose	Spruce	4,525	N.S.	1.1 (25)
(^{14}C-3-side chain lignin) lignocellulose	Sedge	1,424	N.S.	1.2 (14)
Synthetic lignins:				
(^{14}C-U-ring) DHP		61,512	N.S.	0.4 (25)
(^{14}C-2-side chain) DHP		370,729	44.4	0.8 (25)
^{14}Cellulose	Spruce	9,000	N.S.	6.7 (25)

[a] Values are the percentages of the initial radioactivity recovered as either soluble products (Sol. Prod), or as $^{14}CO_2$. Incubation periods (days) are shown in parenthesis.
[b] Values found were
N.T. = Not tested
N.S. = Not significantly different from controls

Serratia marcescens mineralized between 1.1-1.9% and between 0.4-0.8% from (^{14}C-lignin) lignocelluloses and ^{14}C-DHPs, respectively (Table 1). When (^{14}C-2-side chain) DHP was used as a substrate, an additional 44.4% of the initial radioactivity was recovered as soluble products in the culture medium. Furthermore, the bacterium mineralized 6,7% from ^{14}C-cellulose (Table 1).

As far as is known, only Kern (1984) and Vasudevan and Mahadevan (1991), have reported high rates of lignin degradation by bacteria, using radiolabelled DHPs. Degradation percentages reported for *Serratia marcescens*, are similar to those reported by Kaplan and Hartenstein (1980) for some *Pseudomonas* strains, using ^{14}C-DHPs. More recently (Rüttimann et al., 1991) have reported higher mineralization rates of radiolabelled DHPs in two *Pseudomonas* strains. However, water soluble radiolabelled products have not been reported for these bacteria, while this *Serratia marcescens* strain solubilized 44.4% of the (^{14}C-2-side chain) DHP lignin. As far as we know, similar solubilization rates of (^{14}C-2-side chain) DHP lignin have only been reported in fungi by Trojanowski and Hüttermann (1987). This bacterium has a greater capacity to solubilize (^{14}C-2-side chain) DHP compared to (^{14}C-2-side chain lignin) lignocellulose. Thus (^{14}C-2-side chain) DHP appears to have characteristics which facilitate biotransformation by *Serratia marcescens*, probably due to a lower degree of polymerization. In conclusion, this *Serratia marcescens* strain shows a limited mineralization rate of natural lignins and high solubilization rate of (^{14}C-2 side chain) DHP.

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