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Degradation of Softwood, Hardwood, and Grass Lignocelluloses by Two *Streptomyces* Strains†

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Two *Streptomyces* strains, *S. viridosporus* T7A and *S. setonii* 75Vi2, were grown on softwood, hardwood, and grass lignocelluloses, and lignocellulose decomposition was followed by monitoring substrate weight loss, lignin loss, and carbohydrate loss over time. Results showed that both *Streptomyces* strains substantially degraded both the lignin and the carbohydrate components of each lignocellulose; however, these actinomycetes were more efficient decomposers of grass lignocelluloses than of hardwood or softwood lignocelluloses. In particular, these *Streptomyces* strains were more efficient decomposers of grass lignins than of hardwood or softwood lignins.

Certain *Streptomyces* strains are known to degrade both the lignin and the carbohydrate components of lignocellulose (2, 5, 6; R. L. Crawford, D. L. Crawford, and G. J. Dizikes, Arch. Microbiol., in press). Lignin degradation by these bacteria includes the oxidation of both aromatic ring and propane side chain lignin carbons to CO_2 (10). *Streptomyces* strains have been shown to destroy the integrity of both lignified and nonlignified plant cell walls within intact woody plant tissues (1, 13). The extent to which lignin and cellulose components of lignocellulose are degraded by *Streptomyces* strains has not been established (4), nor have the relative abilities of specific *Streptomyces* strains to decompose softwood, hardwood, and grass lignocelluloses been examined. In the present work, we compared the abilities of two lignocellulose-degrading *Streptomyces* strains, *S. viridosporus* T7A and *S. setonii* 75Vi2, to decompose softwood (spruce), hardwood (maple), and grass (quack grass) lignocelluloses. Decomposition was monitored by following weight loss of lignocellulose and depletion of lignin and carbohydrate components over time.

S. viridosporus T7A and *S. setonii* 75Vi2 were isolated from soil by D. L. Sinden (M.S. thesis, University of Idaho, Moscow, 1979). Both strains have been shown to oxidize ^{14}C-labeled lignocellulose to $^{14}CO_2$ (Crawford et al., in press; unpublished data), and both readily metabolize a variety of single-ring aromatic compounds (14; A. L. Pometto III, J. B. Sutherland, and D. L. Crawford, Can. J. Microbiol., in press). Stock cultures were maintained at 4°C on soil extract agar.

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The streptomycetes were grown on semisolid media on lignocelluloses prepared from twigs of a softwood, blue spruce (*Picea pungens*), and of a hardwood, Norway maple (*Acer platanoides*), and from whole plants of a grass (*Agropyron repens*). Lignocelluloses were prepared by methods previously described (2, 7; Crawford et al., in press). Samples (500 ± 0.1 mg each) of air-dried lignocelluloses were sterilized in cotton-plugged, 150-ml flasks by autoclaving at 121°C for 2 h. Sterile lignocelluloses were inoculated with 2 ml of an active inoculum of *S. viridosporus* or *S. setonii*. Active inocula were prepared by growing each streptomycete in 1.0% (wt/vol) yeast extract solution for 48 h at 37°C. The 2.0 ml of inoculum was the only liquid added to the cultures. Uninoculated control flasks were dampened with 2.0 ml of sterile yeast extract solution. For a single experiment, 12 replicate flasks, each containing 500 mg of spruce, maple, or grass lignocellulose, were inoculated with a specific streptomycete or with sterile medium in the case of controls. Flasks were incubated in a humid incubator at 37°C. Every 2 weeks, two flasks were harvested for determination of lignocellulose weight loss, carbohydrate loss, and lignin loss. Weight losses were determined gravimetrically as previously described (2). Lignin contents of residues were determined by a modified Klason procedure (8). Carbohydrate contents of residues were determined by a Somogyi-Nelson procedure modified for the present work. Since the acid hydrolysis step of the Klason procedure solubilized the carbohydrate component of the residues as free sugars, carbohydrate assays were carried out on the Klason supernatants. In the assay, a 1.0-ml sample of supernatant (three replicates) from a known volume was placed into a test tube. Three

milliliters of $CuSO_4$ solution (12) was added. The tube was lightly covered and placed into a boiling water bath for 1 h (11). After cooling, 3 ml of Nelson reagent was added, the solution was mixed, and 10 ml of water was added. The absorbance of the solution at 500 nm was then determined (9). Standard curves were prepared using glucose.

Tables 1 to 3 summarize the weight losses and the carbohydrate and lignin losses from each lignocellulose after degradation by the two streptomycetes. The *Streptomyces* strains significantly decomposed all three lignocelluloses. However, both actinomycetes decomposed the grass lignocellulose to a final weight loss significantly greater than the weight losses of the softwood and hardwood substrates. The softwood and hardwood lignocelluloses were decomposed to average weight losses of 18.8 to 23.0%. In contrast, the grass lignocellulose was decomposed to weight losses of 56.7 and 49.2%, respectively, by *S. viridosporus* and *S. setonii*. These values are uncorrected for biomass present and therefore represent minimal decomposition values. A comparison of weight loss and of carbohydrate and lignin losses from the lignocelluloses over time shows that the grass lignocellulose was still being decomposed by both *Streptomyces* strains even after 12 weeks of incubation, whereas decomposition of the spruce and maple lignocelluloses had almost ceased after 10 weeks.

Both *S. viridosporus* and *S. setonii* depleted substantial amounts of lignin and carbohydrate from the lignocelluloses. Both strains depleted carbohydrate more extensively from the grass and softwood lignocelluloses than from the hardwood lignocellulose. *S. viridosporus* removed over two-thirds of the carbohydrate from the grass and softwood substrates (Tables 1 and 3). In contrast, the culture depleted slightly less than half of the carbohydrate from the hardwood substrate (Table 2). Even though *S. viridosporus* decomposed approximately equal amounts of carbohydrate from the softwood and grass lignocelluloses, the initial degradation rate was more rapid with the grass substrate. *S.*

TABLE 1. *Weight losses and lignin and carbohydrate losses from the softwood spruce lignocellulose resulting from growth of S. viridosporus T7A and S. setonii 75Vi2*

Incubation time (wk)	Wt loss of lignocellulose (%)[a]		Lignin loss from lignocellulose (%)[b]		Carbohydrate loss from lignocellulose (%)[c]	
	S. viridosporus	*S. setonii*	*S. viridosporus*	*S. setonii*	*S. viridosporus*	*S. setonii*
2	9.2 (±0.2)	5.7 (±1.1)	10.1 (±2.2)	9.8 (±0.8)	23.6 (±3.2)	5.4 (±1.3)
4	10.8 (±0.8)	7.5 (±0.9)	11.0 (±0.8)	13.1 (±1.3)	41.1 (±0.8)	12.7 (±1.9)
6	11.6 (±0.3)	13.3 (±1.3)	16.5 (±1.6)	26.5 (±1.2)	46.4 (±0.6)	23.7 (±1.1)
8	17.7 (±1.8)	18.5 (±3.0)	24.7 (±1.4)	33.4 (±1.8)	60.2 (±3.7)	35.7 (±1.3)
10	18.1 (±0.2)	20.7 (±2.8)	28.2 (±2.8)	34.4 (±1.9)	64.8 (±1.4)	46.9 (±1.9)
12	18.8 (±0.6)	20.8 (±2.6)	30.9 (±2.4)	34.1 (±2.5)	65.1 (±2.5)	47.7 (±1.5)

[a] Based upon 500 mg of initial lignocellulose. Weight losses in uninoculated controls averaged 5.2% after 12 weeks of incubation.

[b] Lignin losses in uninoculated controls averaged 5.6% after 12 weeks of incubation. The Klason lignin content of the initial lignocellulose was 34.5%.

[c] Carbohydrate losses in uninoculated controls averaged 12.4% after 12 weeks of incubation. The carbohydrate content of the initial lignocellulose was 61.0%.

TABLE 2. *Weight losses and lignin and carbohydrate losses from the hardwood maple lignocellulose resulting from growth of S. viridosporus T7A and S. setonii 75Vi2*

Incubation time (wk)	Wt loss of lignocellulose (%)[a]		Lignin loss from lignocellulose (%)[b]		Carbohydrate loss from lignocellulose (%)[c]	
	S. viridosporus	*S. setonii*	*S. viridosporus*	*S. setonii*	*S. viridosporus*	*S. setonii*
2	2.8 (±0.1)	2.2 (±0.2)	3.5 (±1.2)	1.6 (±0.8)	1.0 (±0.2)	4.9 (±0.9)
4	3.1 (±0.1)	9.1 (±1.8)	13.2 (±0.3)	14.8 (±1.6)	11.9 (±2.4)	18.9 (±3.7)
6	12.2 (±0.8)	10.0 (±0.3)	25.3 (±1.9)	21.6 (±2.3)	24.5 (±2.2)	20.4 (±2.1)
8	20.8 (±0.9)	12.9 (±1.4)	28.9 (±0.5)	24.7 (±1.2)	45.1 (±1.3)	27.0 (±1.2)
10	23.0 (±0.9)	16.2 (±1.3)	31.0 (±1.4)	28.7 (±1.9)	45.5 (±2.1)	34.6 (±2.6)
12	23.0 (±0.8)	19.3 (±0.1)	32.0 (±2.0)	29.5 (±2.3)	45.9 (±1.7)	37.1 (±1.0)

[a] Based upon 500 mg of initial lignocellulose. Weight losses in uninoculated controls averaged 1.9% after 12 weeks of incubation.

[b] Lignin losses in uninoculated controls averaged 4.1% after 12 weeks of incubation. The Klason lignin content of the initial lignocellulose was 29.3%.

[c] Carbohydrate losses in uninoculated controls averaged 5.9% after 12 weeks of incubation. The carbohydrate content of the initial lignocellulose was 65.7%.

TABLE 3. *Weight losses and lignin and carbohydrate losses from the grass lignocellulose resulting from growth of S. viridosporus T7A and S. Setonii 75Vi2*

Incubation time (wk)	Wt loss of lignocellulose (%)[a]		Lignin loss from lignocellulose (%)[b]		Carbohydrate loss from lignocellulose (%)[c]	
	S. viridosporus	*S. setonii*	*S. viridosporus*	*S. setonii*	*S. viridosporus*	*S. setonii*
2	20.5 (±0.6)	13.2 (±1.9)	5.0 (±0.8)	4.0 (±1.4)	32.2 (±1.4)	22.7 (±3.7)
4	44.9 (±3.6)	26.9 (±1.3)	31.9 (±2.7)	28.6 (±2.6)	53.0 (±2.1)	38.9 (±2.7)
6	52.0 (±7.9)	39.7 (±5.1)	37.6 (±1.4)	31.1 (±0.7)	63.3 (±2.3)	51.0 (±3.7)
8	52.5 (±1.9)	41.9 (±1.8)	40.7 (±2.2)	34.0 (±2.0)	63.9 (±2.3)	52.0 (±3.0)
10	54.6 (±0.9)	45.8 (±3.3)	43.9 (±1.8)	35.1 (±1.8)	64.3 (±1.6)	52.4 (±1.6)
12	56.7 (±1.6)	49.2 (±0.6)	44.2 (±1.6)	39.0 (±1.5)	68.8 (±4.0)	58.7 (±0.6)

[a] Based upon 500 mg of initial lignocellulose. Weight losses in uninoculated controls averaged 5.1% after 12 weeks of incubation.

[b] Lignin losses in uninoculated controls averaged 5.7% after 12 weeks of incubation. The Klason lignin content of the initial lignocellulose was 24.4%.

[c] Carbohydrate losses in uninoculated controls averaged 5.1% after 12 weeks of incubation. The carbohydrate content of the initial lignocellulose was 70.6%.

setonii, although not as efficient a carbohydrate degrader, exhibited generally a similar decomposition pattern, but depleted grass carbohydrate somewhat more than softwood carbohydrate.

Lignin components of the softwood and hardwood lignocelluloses were depleted to about the same degree by both *Streptomyces* strains (Tables 1 and 2). About one-third of the original lignin was decomposed after 12 weeks, irrespective of the specific streptomycete or lignocellulose. In comparison, the grass lignin component was depleted more completely (Table 3). *S. viridosporus* and *S. setonii* degraded about 44 and 39%, respectively, of the initial lignin after 12 weeks of incubation.

S. viridosporus removed approximately 68% of the carbohydrate and 44% of the lignin component from the grass lignocellulose. The respective values for *S. setonii* were approximately 59 and 39%. When compared with the other substrates, the results show that these *Streptomyces* strains exhibited a preference for the grass substrate, particularly in terms of their lignin-degrading capabilities. We have confirmed this pattern (unpublished data) by using another combination of lignocelluloses, including lignocelluloses from Douglas fir (*Pseudotsuga menziesii*, a softwood), white oak (*Quercus alba*, a hardwood), and corn (*Zea mays*, a grass).

A major research objective of this laboratory is the development of microbial bioconversions for the production of useful chemicals from lignin (3). We conclude from the present work that grass lignocelluloses are the preferred substrates for such bioconversions when these two lignin-decomposing *Streptomyces* strains are utilized.

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