Document No: IRG/WP/97-10196

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Working Group 1 Biology

ENVIRONMENTALLY BENIGN BIOLOGICAL WOOD PRESERVATIVES BY *STREPTOMYCES RIMOSUS*, SC-36

by

Suki Croan, Microbiologist

USDA Forest Service
Forest Products Laboratory

Madison, Wisconsin
U.S.A.

Prepared for the 28th Annual Meeting
Whistler, Canada
25 - 30 May 1997

IRG Secretariat
KTH
Brinellvagen 34
S-100 44 Stockholm
Sweden

Environmentally benign biological wood preservatives by *Streptomyces rimosus*, SC-36

Suki Croan, Microbiologist
U.S. Department of Agriculture, Forest Service
Forest Products Laboratory
One Gifford Pinchot Drive
Madison, Wisconsin 53705-2398 U.S.A.

SUMMARY

Microbiocides obtained from mutant culture *Streptomyces rimosus* SC-36 were evaluated for their abilities to inhibit the growth of brown-rot, soft-rot, white-rot, and sapstain fungi on wood. The test fungi were the brown-rot fungi *Antrodia carbonica*, *Gloeophyllum trabeum*, *Neolentinus lepideus*, and *Postia placenta*; soft-rot fungi *Chaetomium globosum*, *Paccilomyces variolli*, *Phialocephala dimorphospora*, *Phialophola mutabilis* *Scytalidium lignicola*; and white-rot fungi *Flammulina velutipes*, *Phanerocheate chrysosporium*, *Shizophyllum commune*, and *Trametes versicolor*. The SC-36 treatment inhibited basidiospore germination and mycelial growth of test fungi in plate assay, plate bioassy, and wood-block (southern yellow pine and sweetgum) tests. Metabolizes from SC-36 inhibited the growth of all test fungi. In field trials, the metabolizes and living cell treatment of green pine log sections and field wood-blocks (eastern white pine and red maple) inhibited natural spore germination and mycelial growth of all forest-inhabiting fungi, thus preventing wood biodeterioration and biodiscoloration. Our results demonstrated that SC-36 can be used as an alternative to synthetic chemicals and an environmentally benign biological wood preservative.

Key words: Forest-inhabiting fungi, wood-attacking fungi, microbiocides, environmentally benign biological wood preservatives.

INTRODUCTION

Biodeterioration of wood is caused by three major types of wood-destroying fungi: brown-rot, soft-rot, and white-rot fungi. Brown-rot fungi rapidly metabolize the cellulose and hemicellulose components, leaving the lignin and/or chemically modify the lignin. As the lignin is still

present, brown-rotted wood remains brown. Brown-rot fungi usually decay conifers, including economically important timber species. Brown-rotted wood loses much of its strength early in the decaying process; decay causes the wood to crack across the grain, shrink, and finally collapse (Forest Products Laboratory, 1987; Highley and Flournoy, 1994; Kirk, 1975; Kirk and Cowling, 1984). White-rot fungi usually remove lignin, leaving the white cellulose. They primarily attack hardwood species but can also attack living trees, wood, or wood products. The wood retains its outward dimensions and does not shrink or collapse (Forest Products Laboratory, 1987; Kirk and Cowling, 1984). Soft-rot fungi are usually associated with wood that is submerged in water such as wooden docks, piers, or water cooling tower timbers. The mycelia of soft-rot fungi follow the cellulose fibrils of the secondary wall, creating cavities within the wall by metabolizing the cellulose. They usually destroy only the outer layers of wood and are not as serious a threat as white- and brown-rot basidiomycetes (Duncan, 1960; 1965; Francis and Leightley, 1984; Singh, et. al., 1991; 1992). On the other hand, sapstain fungi are generally early colonizers that rapidly and extensively penetrate the sapwood of wood and other wood products and are responsible for the discoloration of sapstained wood. Although biodiscoloration does not reduce the strength of wood or change its structure, it does cause great aesthetic damage and is therefore viewed as a serious problem by wood-using industries.

Biodeterioration and biodiscoloration caused by wood-attacking fungi constitute a major problem because they cause serious damage to wood and wood products, resulting in major economic and natural resource losses. The primary wood preservatives currently employed to prevent biodeterioration and biodiscoloration are creosote, chromated copper arsenate (CCA), sodium pentachlorophenates (Na-PCP), and inorganic arsenicals. Inorganic arsenicals are effective in softwoods; they perform poorly in hardwoods. In addition, the potential release of naphthalene from creosote-treated heavy timber construction and arsenic or chromium from inorganic arsenical formulations into the environment poses a major hazard. These synthetic chemicals are broad-spectrum toxic chemicals that threaten the environment and human beings. Increasing global concern for the environment necessitates the development of novel methods to prevent the biodeterioration and biodiscoloration of wood. The development of new, environmentally benign biological wood preservatives would provide an attractive alternative to the treatment of wood with toxic, synthetic chemical fungicides.

The use of a mutant culture of Streptomyces rimosus, SC-36, as an environmentally benign biological wood preservative for controlling biodiscoloration by sapstain and mold fungi in wood has been previously reported (Croan and Highley, 1992a, 1994). However, to the best of our knowledge, no one has studied the suitability of the antifungal activities of SC-36 for controlling biodeterioration by brown-rot, soft-rot, and white-rot fungi. The objective of this study was to evaluate the efficacy of the mutant culture SC-36 in the form of metabolizes and living cells for controlling biodeterioration of wood in laboratory tests. Other objectives included the assessment of biodeterioration and biodiscoloration of wood and wood products in field trials against forest-inhabiting fungi. In this study and previous studies on potential biological wood preservativetives, spores rather than mycelia were used in order to mimic natural conditions (Croan, 1994; Croan and

Highley, 1992b). The primary source of the deterioration and discoloration of wood exposed above ground is spores produced by forest-inhabiting fungi in nature, which are usually disseminated by wind, water, or insects.

MATERIALS AND METHODS

Microorganisms

The following wood-rot fungi were selected for the study: the brown-rot fungi Antrodia carbonica (Overh) Ryv. et Gilbn. [FP-105585], Gloeophyllum trabeum (Pers. es Fr.) Murr. [MAD-617], Neolentinus lepideus (Fr. :Fr.) Redhead & Ginns [MAD-534], and Postia placenta (Fr.) M. Lars. et Lomb. [MAD-698]; soft-rot fungi Chaetomium globosum 172D; 6205; 16021, Paccilomyces variolli ATCC 66705, Phialocephala dimorphospora ATCC 66712, Phialophola mutabilis spp., Scytalidium lignicola P-53; white-rot fungi Flammulina velutipes spp., Phanerochaete chrysosporium Bursall in Burds. & Eslyn [BKM-F-1767], Schizophyllum commune Fr. [MAD-619], and Trametes versicolor (L. ex Fr.) Pilate[MAD-697]. The mutant culture was Streptomyces rimosus NRRL 21063, SC-36, derived from S . rimosus Sobin et al.

Maintenance of Fungi and Preparation of Basidiospores

Stock cultures of S . rimosus SC-36 were maintained on nutrient agar (NA) (Difco, Detroit, MI) slants supplemented with 1% peptone (NAP) (Difco) or Hickey-Tresner medium (HIT) agar. Wood-rot fungi were maintained on 2% malt extract agar (MA) plates or slants. Basidiospores of all basidiomycetes were used for the tests (Croan, 1994, 1995) with the exception of one white-rot fungus P . chrysosporium, for which conidia were employed. To obtain conidia, fungal growth on MA or potato dextrose agar (PDA) was scraped from the surface of the plate or slant in sterile water. The conidial suspension was filtered through sterile glass wool to separate conidia from mycelia. The filtrate containing conidia was washed twice by centrifugation in sterile water. The final basidiospore or conidial suspension was prepared in 0.01% Tween 20, and the number of spores was determined by the use of a haemocytometer.

Metabolize Production From SC-36

Cultures were grown in the vegetative growth medium, lx sporulation broth (SB), for the activation and adaptation of inoculum (Croan and Highley, 1994). For vegetative growth, mycelia from SC-36 cultures grown on a NAP slant were scraped from the surface of the slant and cultured in 50 ml of SB medium in 125-ml Erlenmeyer flasks and incubated at 27°C for 2 days with vigorous shaking on a platform shaker at 200 r/min. The settled pellet was transferred into 100 ml of SB medium in 250-ml Erlenmeyer flasks and allowed to grow for an additional 40 h. The settled pellet-inoculum (100%) was then transferred into 50 ml of the production medium (SB at l/2x or l/3x concentration) supplemented with 0.01%-O.02% Tween 20 in 125-ml Erlenmeyer flasks. Flasks were incubated at 30°C and 200 r/min. Crude metabolizes were

harvested after 16 to 24 h incubation with l/3x medium or after 2 to 3 days with l/2x medium (Croan and Highley, 1994). Some crude preparations were concentrated by lyophilization.

Tests of Antifungal Activity

Petri Plate Assay. The modified dual-culture techniques were similar to those described by Croan and Highley (1991). The SC-36 culture was tested for its antagonistic activities against wood-decay fungi on MA. Agar plates were inoculated at the center and spread over the 20-mm-diameter surface with SC-36 or with an agar plug (8-mm diameter) of SC-36 grown on HIT agar and incubated for 2 to 3 days prior to inoculating test fungi. Mycelial agar plugs (6-mm diameter) of brown-rot and white-rot fungi were then inoculated along the perimeters of the plate. Four brown-rot, four soft-rot, and three white-rot fungi were inoculated per plate. The plates were incubated for 14 days in the dark at 27°C and 70% relative humidity. The radial growth of the fungal colony was measured. Three replications were examined for each experiment. Three plates inoculated with each test fungus without SC-36 were used as controls.

Plate Bioassay. The plate bioassay was used to determine the antifungal activity of metabolizes obtained from SC-36. A basidiospore or conidial suspension of wood-decay fungi (2×10^5 conidia or 1×10^6 basidiospores per 200 ul in 0.005% Tween 20) was spread-plated onto 2% MA plates. Three to six 6-mm diameter wells were cut with a cork cutter. Twenty-five microliters of lx metabolizes was pipetted into the wells. The clear-zone diameter around the wells was measured after 2 to 3 days incubation at 27°C and 70% relative humidity. The clear-zone diameter around the wells was compared for different treatments.

Soil-Wood-Block Tests

Blocks of two wood species, *Pinus* sp. (southern yellow pine) and *Liquidambar styraciflua* L. (sweetgum), were used. The size of the wood blocks was 3 by 6 by 6 mm in tangential, radial, and longitudinal directions, respectively. The wood blocks were soaked overnight to replicate the moisture content of green wood. They were then steam-sterilized for 30 min at normal atmospheric pressure for 3 consecutive days. The steam-sterilized blocks were pressure treated with metabolizes (1/4x and 1x) for 60 min; the control blocks were treated with water. All blocks were placed into sterile soil bottles, inoculated with 2×10^5 conidia or 1×10^6 basidiospores, and incubated 12 to 16 weeks at 27 and 70% relative humidity using the modified soil-block method of ASTM 1413 (ASTM, 1986a) and 2017 (ASTM, 1986b).

Fungal growth on the treated wood was rated using the following scale:

0 Clean: no fungal growth
1 Minor: fungal growth covering less than 5% of upper surface
2 Light: fungal growth covering 5%-20% of upper surface
3 Moderate: fungal growth covering 20%-40% of upper surface
4 Heavy: fungal growth covering more than 40% of upper surface

Field Exposure

Green Pine Log Sections. Green southern pine (Pinus sp.) sapwood log sections, 170-mm-diameter by 12-mm, were cut from a freshly felled tree and immediately frozen. The thawed sections were dipped into water, metabolites (lx and 10x), or metabolizes (lx) with Tim-Bor-disodium octaborate, 4% boric acid equivalent (BAE) as a cobiocide for 60 s. The living cells were brushed onto the surface of the sections. The control was dipped in water; Tim-Bor (4% BAE), 0.1% sodium pentachlorophenate (penta), or 1% penta was used as a positive control. The treated sections were exposed at the Valley View Test Site near Madison, Wisconsin, for 8 to 10 weeks during the summer. The sections were hung on a fence under trees to avoid direct exposure to the sun. The treated wood was rated for fungal growth or sapstain using the same scale used in the soil-wood block tests.

Field-Wood Block Tests. Blocks of two wood species, Pinus sp. (eastern white pine) and Acer rubrum (red maple), were used in 7 replications. The size of the wood blocks was 50 by 153 by 19 mm in tangential, radial, and longitudinal directions, respectively. The field wood blocks were soaked overnight to replicate the moisture content of green wood. They were dipped into water (control), Tim-Bor (4% BAE), metabolizes (lx and 5x), and metabolizes (lx) with Tim-Bor (4% BAE) as a cobiocide for 60 s. Living cells, both freshly harvested and lyophilized cells (reconstituted cells with sterile distilled H_2O), were brushed onto the surface of wood blocks. The treated wood blocks were stacked, covered with clear plastic, and exposed above ground at the Valley View Test Site for 3 years. The stacked wood blocks were covered with plastic so as to avoid direct exposure to rain. The treated wood was rated for fungal growth or sapstain using the same scale used for the green pine log sections.

RESULTS AND DISCUSSION

The antagonistic effect of SC-36 against various brown-rot, soft-fot, and white-rot fungi is shown in Table 1 and Figure 1. After 2 weeks of incubation, fungal growth on 2% malt extract agar (MA) was inhibited at a considerable distance from the inoculation point, leaving a clear zone of inhibition between the test fungi and SC-36. SC-36 inhibited 78% to 100% of fungal radial growth in MA plates inoculated with an agar plug of SC-36 grown on HIT agar at the center of the plates (Table 1, Fig. lC and D). SC-36 grew poorly on MA, compared to its growth on nutrient agar (NA) or HIT agar, but the culture was nevertheless effective in producing an inhibition zone between the host and test fungi. In fact, no growth by brown-rot or white-rot basidiomycetes was observed. Mycelial growth was inhibited by 100% in plate assay (Fig. 1A and B). The composition of the medium evidently plays an

important role with respect to the production of antifungal secondary metabolizes by the wild type but not by the mutant culture.

Table 2 shows the results of the interaction between fungi and metabolizes on 2% MA in the plate bioassay. Basidiospore germination of all brown-rot and white-rot fungi was inhibited by 25 ul of l-day-old crude metabolizes from l/3x production medium or 3-day-old metabolizes from l/2x production medium introduced in each well. The basidiospore germination of test fungi was completely inhibited, leaving a clear zone around the wells. The diameter of this clear-zone area varied from 22 to 31.5 mm per 25 ul of crude metabolizes or from 53,240 to 109,840 mm^3 per milliliter of culture fluid.

The results of the interaction between test brown-rot and white-rot basidiomycetes and metabolizes on wood blocks in soil-wood block tests were compared with the interaction between test fungi and diluted or undiluted (1/4x or 1x) metabolizes (Table 3 and Fig. 2). The undiluted metabolizes from SC-36 were effective against all fungi tested; the metabolizes protected the wood blocks against basidiospore or conidia germination after a 16-week incubation. The only fungal growth observed occurred on wood blocks treated with l/4x metabolizes (Table 3 and Fig. 2). However, diluted metabolizes prevented weight loss by 95% to 100%, indicating that basidiomycetes gain nourishment from feeder blocks or from soil, but not from treated wood blocks. The metabolizes, either undiluted or diluted, effectively protect wood blocks from biodeterioration.

Table 4 and Figure 3 compare the results of field exposures of concentrated metabolizes (1OX), metabolizes (lx) with diluted boron as a cobiocide, and living cells to the results of tests with diluted boron, 0.1% penta, or 1% penta. The concentrated metabolites, unconcentrated metabolizes with diluted boron, and living cells inhibited conidia or basidiospore germination of all forest-inhabiting fungi. Treatment with unconcentrated metabolites, diluted boron, 0.1% penta, or 1% penta did not protect the wood from wood-attacking fungal growth and sapstain for 8 to 10 weeks during the summer. However, the concentrated metabolites and living cells inhibited basidiospore, conidia, spore, or oidia germination of all natural wood-attacking fungi.

Table 5 represents the results of pine and maple blocks of field exposures for 3 years at the Valley View Test Sites. Treatments of concentrated metabolites (5x), metabolites (lx) with diluted boron as a cobiocide, living cells, both freshly harvested and lyophilized, were compared to those of unconcentrated metabolites with diluted boron (4% BAE). These metabolites were unable to protect field wood blocks from biodeterioration by brown-rot, soft-rot, and white-rot fungi and biodiscoloration by blue-stain or sapstain and mold fungi. However, living cells, both freshly harvested and lyophilized, concentrated metabolites, and metabolites with diluted Tim-Bor inhibited reproductive spore germination of all wood-attacking fungi. As a result, wood blocks in the field exposures were protected from biodeterioration and biodiscoloration.

Inhibition by the microbiocides SC-36 in the form of metabolites or living cells is due to the synthesis of antifungal compound(s) produced during the secondary metabolic phase of SC-36. The use of antifungal metabolites is advantageous to a biological wood preservative system because the metabolites can easily be obtained. This is achieved through single-step daily product fermentation by inducing microscopic pellet-inoculum, using a very diluted medium as the production medium to limit primary growth phase and to promote only the secondary metabolic phase of SC-36, and then recycling the microscopic pellet-inoculum continuously. These metabolites can be used to treat wood and wood products. The diluted boron can be used as a cobiocide with metabolites, which can be as effective as or occasionally more effective than concentrated metabolites. The metabolites can also be easily concentrated by lyophilization. Living cells are sometimes even more effective than concentrated metabolites in protecting wood from biodeterioration and biodiscoloration. Pine log sections and field wood-blocks treated with living cells continued to be protected from wood-attacking fungi for 2 or 3 years, even after the cells had dried or were washed out by rain (unpublished data). In contrast, many of the commercial preservatives we tried did not protect wood against wood-attacking fungi (data not shown). These results suggest that nondecaying living cells, concentrated metabolites, or unconcentrated metabolites with diluted boron as a cobiocide can be used for long-term protection of wood against wood-attacking fungi.

CONCLUDING REMARKS

The results of our study show that a mutant culture, SC-36, of *Streptomyces rimosus* is an effective, environmentally benign biological wood preservative against wood-attacking fungi. Microbiocides from mutant SC-36 are effective against biodeterioration by brown-rot, soft-rot, and white-rot fungi, and biodiscoloration by blue-stain or sapstain and mold fungi. This technology presents an alternative to synthetic chemical preservatives and represents a significant advance that addresses both environmental and health concerns associated with current treatments.

REFERENCES

ASTM. 1986a. Standard Method of Testing Wood Preservatives by Laboratory Soilblock Cultures, ASTM D 1413 - 76, American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1986b. Standard Method of Accelerated Laboratory Test of Natural Decay Resistance of Woods, ASTM D 2017 - 81, American Society for Testing and Materials, Philadelphia, PA.

Croan, S.C. and Highley, T.L. (1991). Biological Control of the Blue Stain Fungus Ceratocystis Coerulescens With Fungal Antagonists. Mater. Org. 25(4), 255-266.

Croan, S.C. and Highley, T.L. 1992a. Biological control of sapwood-inhabiting fungi by living bacterial cells of Streptomyces rimosus as a bioprotectant. International Research Group on Wood Preservation Doc. No: IRG/WP/1564-92.

Croan, S.C. and Highley, T.L. 1992b. Conditions for carpogenesis and basidiosporogenesis by the brown-rot basidiomycete Gloeophylyllum trabeum. Material und Organismen 27(1): 1-10.

Croan, S.C. 1994. Basidiosporogenesis by white-rot basidiomycetes in vitro. International Research Group on Wood Preservation Doc. No: IRG/WP 94-10081.

Croan, S.C. and Highley, T.L. 1994. Biological control of sapwood-inhabiting fungi by metabolites from Streptomyces rimosus. In: Biodeteroration Reseaarch 4: 243-255. (Llewellyn, G.C., Dashek, W.V., and O'Rear, C.E. eds.) Plenum Press, NY.

Croan, S.C. 1995. Basidiosporogenesis by Brown-rot basidiomycetes in vitro. International Research Group on Wood Preservation Doc. No: IRG/WP 95-10126.

Duncan, C.G. 1960. Wood-attacking capacities and physiology of soft-rot fungi. Forest Products Laboratory report No. 2173.

Duncan, C.G. 1965. Determining resistance to soft-rot fungi. Forest Products Laboratory report No. 48.

Forest Products Laboratory. 1987. Wood Handbook: Wood as an Engineering Material. USDA Agric. Handb. 72. U.S. Department of Agriculture, Forest Service, Washington, D.C., 466 pp.

Francis, D.M. and Leightley L.E. 1984. An unusual soft-rot decay pattern caused by the Ascomycete Hypoxlan mediterraneum (de Not.) J. Miller. International Research Group on Wood Preservation Doc. No: IRG/WP/1222-84.

Highley, T.L. and Flournoy D.S. 1994. Recent Advances in Biodeterioration and Biodegradation: Decomposion of Cellulose by Brown-rot fungi. K.L.Garg, et al., Eds. Vol.II: 191-221.

Kirk, T.K. 1975. Chemistry of lignin degradation by wood-destroying fungi. In: Liese, W., ed. Biological transformation of wood by microorganisms. Proceedings of the sessions on wood products pathology at the 2nd international congress of plant pathology.

Kirk, t.K. and Cowling E.B. 1984. Biological decomposition of solid wood. In: Rowell, R.M., ed. The chemistry of solid wood. Advances in chemistry series 207. Washington, DC: A.C.S.; chapter 12.

Singh, A.P., Hedley, M.E., Page, D.R., Han C.S. and Atisongkroh, K. 1991. Fungal and bacterial attack of CCA-treated Pinus radiata timbers from a water cooing tower. International Research Group on Wood Preservation Doc. No: IRG/WP/1488.

Singh, A.P., Hedley, M.E., Page, D.R., Han C.S. and Atisongkroh, K. 1992. Microbial degradation of CCA-treated cooing tower timbera. IAWA Bulletin n.s., 13(2); 215-231.

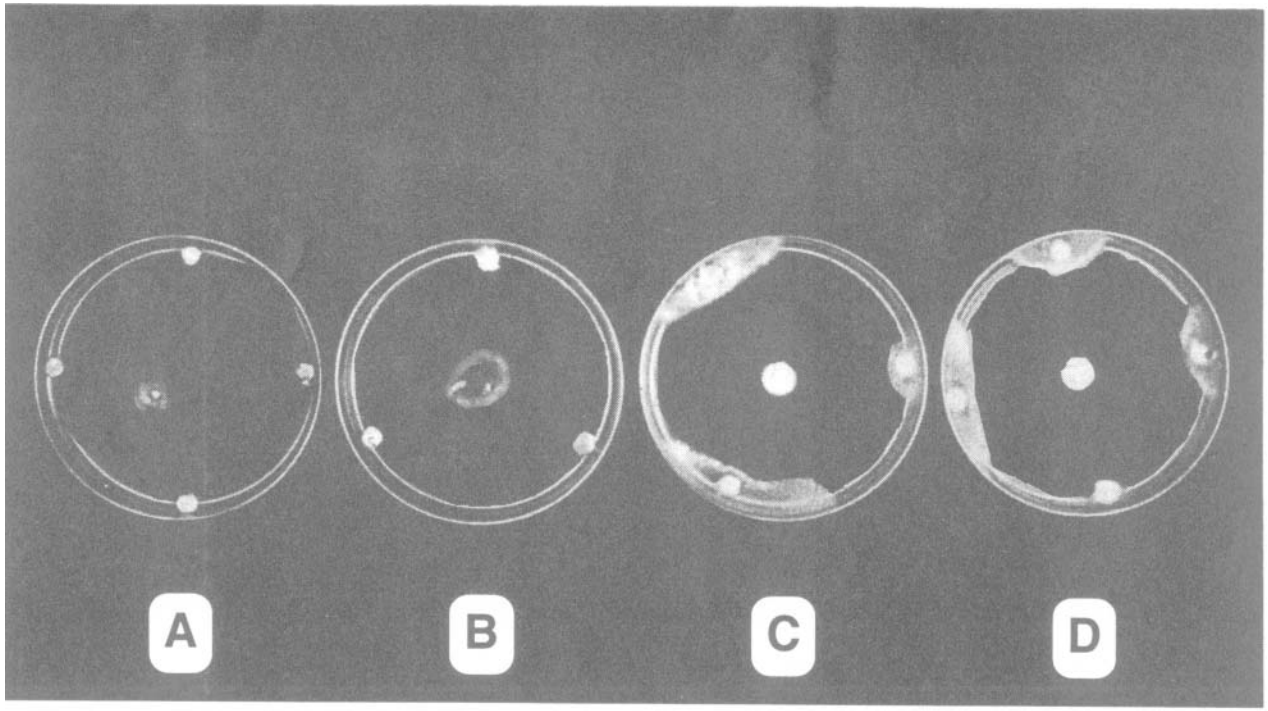


Figure 1. Inhibition of Radial Growth of Wood-decay Fungi in Petri plate Assay; Plate A,D–*Antrodia carbonica*, *Gloeophyllum trabeum*, *Neolentinus lepideus*, and *Postia placenta*: Plate B,C–*Flammulina velutipes*, *Schizophyllum commune*, and *Trametes versicolor*.

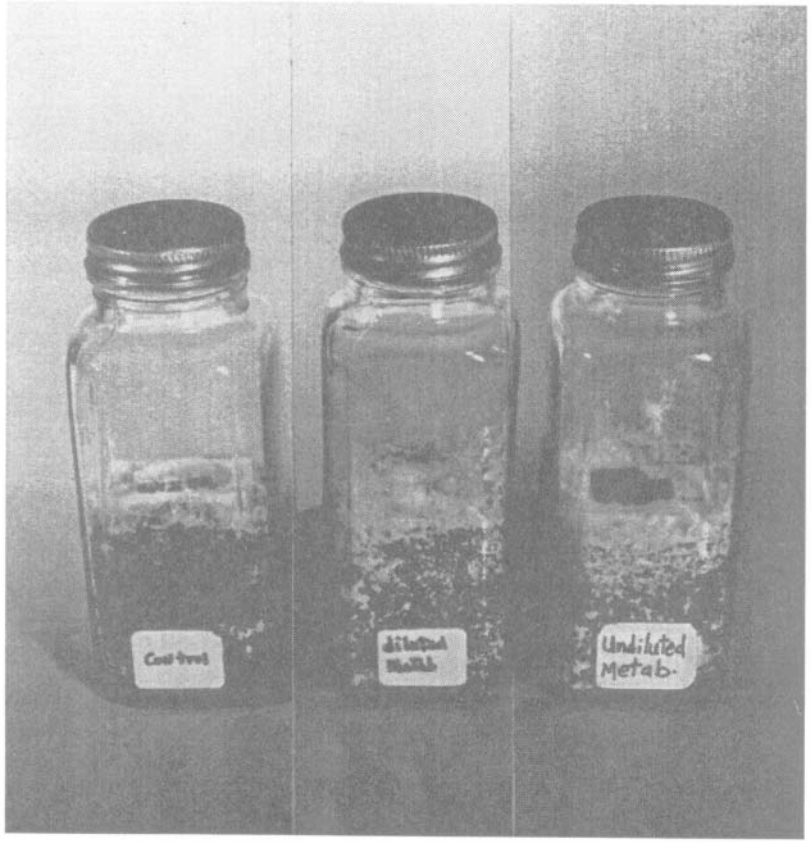
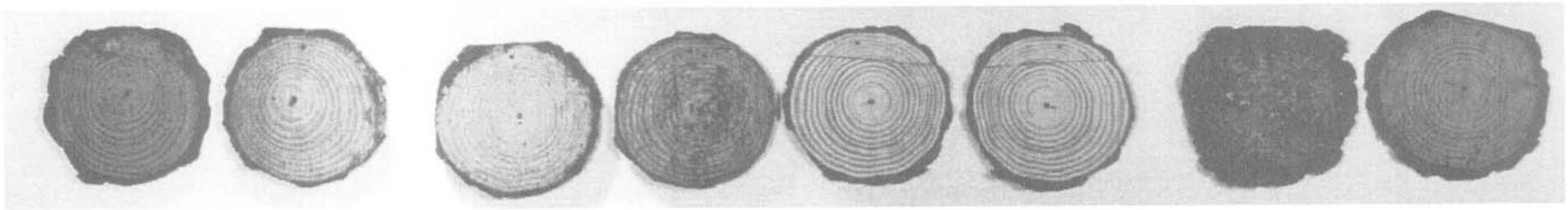


Figure 2. Inhibition of Basidiospore Germination of Phanerochaete chrysosporium on Soil-wood-blocks test by diluted and undiluted Metabolites from SC-36.

Figure 3. Inhibition of wood-attacking fungi on Green pine log sections treated with unconcentrated or concentrated metabolites, and living cells of SC-36 exposed in the field.



1x 10x Living Cells Control 1% Penta

Table 1--Inhibition of radial growth of brown-rot and white-rot fungi by SC-36 in petri plate assay on 2 percent malt extract agar.

Fungus	Inhibition of radial growth[a] (percent)
Brown-rot	
Antrodia carbonica	89.5 (2.1)
Gloeophyllum trabeum	86.6 (0.8)
Neolentinus lepideus	85.0 (1.4)
Postia placenta	78.5 (0.7)
Soft-rot	
Chaetomium globosum 172D	100.0 (0.0)
Chaetomium globosum 6205	100.0 (0.0)
Chaetomium globosum 16021	100.0 (0.0)
Paccilomyces variolli	87.9 (0.5)
Phialocephala dimorphospora	88.9 (6.9)
Phialophola mutabilis	96.0 (1.8)
Scytalidium lignicola	93.9 (1.7)
White-rot	
Flammulina velutipes	86.0 (2.1)
Schizophyllum commune	86.0 (2.1)
Trametes versicolor	78.0 (9.2)

[a]Standard deviation in parentheses.

Table 2--Inhibition of brown-rot and white-rot fungi by metabolites from SC-36 in plate bioassay on 2 percent malt extract agar.

Wood-attacking fungi	Inhibition zone	
	Diameter (mm/25 ul)	Area (mm^3/25-ul)
Brown-rot fungi		
Gloeophyllum trabeum	22.0 (2.8)	1,331 (22)
Neolentinus lepideus	26.5 (2.1)	1,946 (12)
White-rot fungi		
Phanerochaete chrysosporium	31.0 (1.4)	2,643 (5)
Schizophyllum commune	31.5 (2.1)	2,746 (12)
Trametes versicolor.	31.0 (1.4)	2,643 (5)

[a] Standard deviation in parentheses.

Table 3--Inhibition of basidiospore germination on wood blocks of Southern Yellow Pine for brown-rot fungi and wood blocks of sweetgum (*Liquidambar styraciflue* L.) for white-rot fungi treated with the diluted or undiluted metabolites from *Streptomyces rimosus* SC-36 for controlling wood-rotting fungi using soil-block procedure.

	Basidiospore germination[a] and weight loss[b]							
	Brown-rot Fungi				White-rot Fungi			
	Antrodia carbonica		*Gloeophyllum trabeum*		*Phanerochaete chrysosporium*		*Trametes versicolor*	
Treatment	Growth	% wt loss	Growth	% wt loss	Growth	% wt loss	Growth	% wt loss
Control	4	1.3 (0.1)	4	37.5 (2.8)	4	25.9 (8.2)	4	27.7 (8.9)
Metabolites (1/4 X)	3	0	3	0	4	1.3 (1.3)	2	0
Undiluted metabolite	0	0	0	0	0	0	0	0

[a]The blocks were rated for the fungal growth after 16 weeks exposure at 27° C. using the following scale:

0. Clean: fungal growth
1. Minor: fungal growth covering less than 5% of upper surface
2. Light: fungal growth covering 5-20% of upper surface
3. Moderate: fungal growth covering 20-40% of upper surface
4. Heavy: fungal growth covering more than 40% of upper surface

[b]Standard deviation in parenthesis (%).

Table 4 Inhibition of wood-attacking fungi on pine log sections treated with living cells, metabolites (1x or 10x), and metabolites from SC-36 with diluted boron as a co-biocide and exposed in the field

Treatment	Rating for fungal growth or stain[a]
Control	4
0.1% PCP	4
1.0% PCP	2
Boron (4% BAE)	4
metabolites (1x)	1 - 2
metabolites (10x)	0 - 1
metabolites (1x) + boron (4% BAE)	0
Living cells	0

[a]Sapstain or fungal growth on wood after being treated with various form of microbiocides was rated using the following scale:

0. Clean: no stain or fungal growth
1. Minor: stain or fungal growth covering less than 5% of upper surface
2. Light: stain or fungal growth covering 5-20% of upper surface
3. Moderate: stain or fungal growth covering 20-40% of upper surface
4. Heavy: stain or fungal growth covering more than 40% of upper surface

Table 5 Inhibition of wood-attacking fungi on field pine and maple blocks treated with living cells, metabolites (1x or 10x), and metabolites from SC-36 with diluted boron as a co-biocide and exposed in the field for 3 years

Treatment	Rating for fungal growth or stain[a]	
	Maple	Pine
Control	4	4
Boron (4% BAE)	4	4
metabolites (1x)	1	4
metabolites (10x)	0	0
metabolites (1x) + boron (4% BAE)	1	1
Freshly harvested cells	0	0
Lyophilized cells	0	0

[a]See footnote to Table 4 for rating scale.