FOREST PRODUCTS LABORATORY (Madison, Wis. 53705)
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WOOD PRESERVATIVE DEGRADATION BY MARINE BACTERIA

WALLACE E. ESLYN

Forest Service, USDA, Forest Products Laboratory,
Madison, Wisconsin 53705, U.S.A.

Summary

Various bacteria, including species of *Bacillus*, *Pseudomonas*, and *Micrococcus*, were isolated from wood treated with creosote, copper chrome aersenate (CCA), ammoniacal copper arsenate (ACA), or combinations; all the treated wood had been exposed in the sea for varying times. To determine the significance of the bacteria in preservative detoxification, small wood blocks impregnated with these preservatives were- incubated for one year in liquid cultures containing one or more species of the isolated bacteria. Blocks were then exposed to the action of wood decay fungi. Amount of decay wrought by the fungi indicated the effect of bacterial action upon the preservative involved. All tested bacteria substantially reduced toxicity in ACA-treated blocks and, to a much lesser extent, in blocks treated with creosote and creosote + CCA. However, CCA-treated blocks exposed to bacterial action decayed less than the controls.

To determine effectiveness and permanence of several wood preservatives in the marine environment, the Forest Products Laboratory initiated a study in 1964 in cooperation with the U.S. Navy and the Wm. F. Clapp Laboratories, Inc., Duxbury, Mass. Wood samples treated with various preservatives were exposed in the ocean for periods ranging from 6 months to 5 years. The wood samples were then examined for presence of marine borers and chemically analysed for preservative content (Baechler *et al.*, 1970b).

Additionally, isolations were attempted from the fresh samples to determine the microorganisms associated with the different chemical treatments. The present work describes the results of these isolations and considers the possible effects of selected bacterial isolates upon durability of preservative-treated wood.

MATERIALS AND METHODS

Samples of wood from southern pine (*Pinus* sp.) and Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco], 102 by 76 by 19 mm (4 by 3 by 3/4 inches), were treated at several concentrations with creosote, creosote-coal tar solution, ammoniacal copper arsenate (ACA), chromated copper arsenate (CCA, Type 1), or with a mixture of creosote plus ACA or CCA. In all, 27 treatments were applied—13 to southern pine and 14 to Douglas fir wood samples. After treatment, half of the samples receiving treatments (1-18) were immersed about 0·9 m (3 ft) below the water surface in the harbour at Wrightsville Beach, N.C., and half about 0·6 m (2 ft) above bottom in the harbour at Boston, Mass., for periods up to 5 years. Treatments 19-27 received the highest preservative retentions, and these were exposed only in the Wrightsville Beach harbour. Preservative applications, immersion in seawater, and

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the schedule for sample removal for examination and testing are described by Baechler *et al.* (1970b).

When samples were removed from the sea, a cross section about 3·2 mm ($^1/_8$ in) thick was cut from one sample in each treatment group. The surface of each sample was flamed, split apart, and small pieces asceptically removed from their interiors. These pieces were placed on media of casamino acid (3·0 g casamino acids, 3·0 g yeast extract, 1·0 g dextrose, and 2·0 g agar per litre of distilled water) and artificial seawater-yeast-peptone (24·7 g NaCl, 0·7 g KCl, 6·3 g $MgSO_4 \cdot 7H_2O$, 4·6 g $MgCl_2 \cdot 6H_2O$, 1·0 g anhy. $CaCl_2$, 0·2 $NaHCO_3$, 5·0 g yeast extract, and 5·0 g peptone per litre of distilled water), and on malt agar. Isolations were made by Dr C. G. Duncan on the samples removed every 6 months during the first three and a half years' immersion in seawater. After her death, the author continued the isolations with material immersed in the sea for periods of 4 to 5 years.

Many isolates obtained by Duncan were lost. Those that survived and those isolated by the author were screened against concentrations of preservatives to determine the more preservative-tolerant and preservative-degrading isolates. Thus, isolates from wood treated with creosote, creosote + CCA, or creosote + ACA were inoculated into flasks of casamino acid medium (withoutagar)plus 10,20, 40, 80, or 95% of the water soluble components of creosote; from CCA or creosote + CCA treatments into similar media containing 1, 3, 5, or 10% CCA; and from ACA and creosote + ACA treatments into media containing similar concentrations of ACA instead of CCA.

From these inoculations, isolates most tolerant to the preservative treatments were selected for experiments to determine effects of marine bacteria upon preservative toxicity. Cubes (19 mm, or $^3/_4$ in) of southern pine wood were treated with marine grade creosote (128 kg^3 or 8 pcf, and 256 kg^3 or 16 pcf retentions), CCA (56 kg^3 or 3.5 pcf), ACA (32 kg^3 or 2·0 pcf), creosote + CCA (208 kg^3 + 32 kg^3 or 13 pcf + 2 pcf), or creosote + ACA (208 kg^3 + 16 kg^3 or 13 pcf + 1 pcf) according to the standard soil-block test method (ASTM, 1961). Each treated block was then placed into a bottle containing peptone-yeast-seawater medium which had been previously inoculated (except for controls) with one or two bacteria tolerant to the type of preservative with which the block was treated. Hence, creosoted blocks were inserted into cultures of bacteria tolerant to creosote, creosote + CCA, and creosote + ACA. The remainder were inserted into cultures in this fashion:

Wood block treatment	Bacteria tolerant to
Creosote + CCA	Creosote + CCA
Creosote + ACA	Creosote + ACA
CCA	CCA and Creosote + CCA
ACA	ACA and Creosote + ACA

Treated controls were placed in bottles containing sterile medium. Culture media were changed and, where necessary, reinoculated bi-weekly during the first 6 months of incubation. Thereafter, media were changed about once a month. At the end of 1 year's incubation the blocks were removed, conditioned at 80°F and 70% relative humidity, and weighed.

To determine whether bacteria had or had not degraded preservatives, the

blocks were then autoclaved and subjected to degradation by wood decay fungi according to the soil-block procedure (ASTM, 1961). Exceptions were that the incubation periods were increased and *Lentinus lepideus* Fr. (Mad 534), a creosote-tolerant fungus, was used to test creosote-treated blocks, while *Poria vaillantii* (DC. ex Fr.) Cke. (MD 497), a fungus tolerant of CCA (DaCosta, 1959), was used to test remaining toxicity of ACA- and CCA-treated blocks. Test blocks treated with a combination of creosote + salt were inoculated with each of the above fungi.

After this exposure, weight losses were calculated and comparisons made between losses occurring in blocks exposed to bacterial action and losses occurring in control blocks. Although fungi toxicity was used to determine extent of detoxification of marine preservatives, the results are thought to apply to wood-destroying marine animals as well.

RESULTS

Isolations from wood samples exposed in the sea

The percentage of samples in each treatment from which bacteria were isolated from both locations after 48, 54, or 60 months' immersion in the sea are presented in Table I. The values are based upon wood samples treated

Table I

PERCENTAGE OF PRESERVATIVE-TREATED WOOD SAMPLES FROM WHICH BACTERIA WERE ISOLATED AFTER STORAGE IN SEAWATER AT TWO HARBOURS FOR 48-60 MONTHS

Wood treatment	*Percentage of samples bacterially infected*[a]	
	Wrightsville Beach	*Boston*
Creosote	67 (60)	21
CCA	80 (87)	58
ACA	62 (45)	33
Creosote + CCA	83 (50)	0
Creosote + ACA	33 (17)	0

[a] Values are for low retention only; numbers in parentheses represent combined high and low retentions.

with low retentions of preservatives (treatments 1–18), permitting a direct comparison of results obtained at both harbours which similarly treated wood samples. Bacteria were isolated much more. frequently from wood immersed in Wrightsville Beach harbour than from that in Boston harbour, regardless of type of preservative treatment.

Bacteria were isolated most frequently from CCA-treated wood and least frequently from creosote + ACA-treated wood (Table I). Average number of isolations from creosote- and ACA-treated woods were similar and second in frequency to CCA-treated wood.

No fungi were isolated from the wood samples. In addition, no signs of fungal soft-rot attack were evident in slides made from each of the samples from which isolations were made.

Bacterial identifications

Twenty-three bacterial cultures containing strains of one or more species shown to be tolerant to one or more test preservatives were identified (Table 11) and included in further tests. Among the eight species in the genus

Table II
IDENTITY AND PRESERVATIVE-TOLERANCE OF TEST BACTERIA

Bacterial species	*Isolate no.*	*Preservative tolerance*
Bacillus brevis Migula	1241	ACA
B. cereus Frankland & Frankland	1178	Creosote
B. circulans Jordan	866-1	Creosote + CCA
B. lentus Gibson	226	Creosote
	485,491-1	Creosote + CCA
	287-2	ACA
B. megaterium de Bary	425	CCA
B. pumilus Meyer & Gottheil	1148	Creosote
	392, 1328	CCA
	488-2, 491-2	Creosote + CCA
B. sphaericus Meyer & Neide	326	ACA
	548	Creosote + ACA
B. subtilis (Ehrenberg) Cohn	359	CCA
	488-1	Creosote + CCA
Micrococcus colpogens Campbell & Williams or	293	ACA
M. varians Migula	544,557	Creosote + ACA
	866-3	Creosote + CCA
Pseudomonas creosotensis O'Neill, Drisko & Hochman	ATCC-14582	Creosote
P. membranoformis? (ZoBell & Allen) ZoBell	386, 1331	CCA
P. sp. A	644	Creosote
P. sp. B	287-1	ACA
P. sp. c	1265	ACA

Bacillus, B. lentus and *B. pumilus* occurred most frequently. Collectively, the eight *Bacillus* species exhibited tolerance to all preservatives included in present tests. A *Micrococcus* species, either *colpogens* or *varians,* was associated with ACA, Creosote + ACA-, and creosote + CCA-treated woods. Four *Pseudomonas* species, one of which may be *P. membranoformis,* were tolerant to CCA, creosote, or ACA (Table II). *Pseudomonas creosotensis* was not isolated, but it has been associated with creosoted marine piling (O'Neill *et al.,* 1961). Therefore, a culture (ATCC-14582) was obtained from the American Type Culture Collections for comparison with tolerant or detoxifying bacteria isolated in these studies.

Effect of wood decay fungi upon preservative-treated blocks

The amounts of decay caused by *Lentinus lepideus* in wood blocks treated with 128 and 256 kg^3 of creosote and by *L. lepideus* + *Poria vaillantii* in blocks treated with 208 kg^3 creosote + 32 kg^3 CCA are given in Table III. Blocks exposed first to bacterial action underwent greater decay, except for blocks treated with 256 kg^3 creosote and exposed to *B. lentus* isolate 226. The largest average weight loss due to prior bacterial action occurred in blocks treated with 256 kg^3 creosote and exposed to a bacterial mixture of *B. lentus* + *B. pumilus.* Here, weight losses averaged 13·9% compared to 6.7 % for the control.

Decay brought about by *P. vaillantii* in ACA-treated control blocks was

Table III
DECAY LOSSES IN CREOSOTE- AND CREOSOTE + CCA-TREATED WOOD PREVIOUSLY EXPOSED TO PRESERVATIVE-TOLERANT BACTERIA

Bacterial isolate	*Average weight loss*[a] (%)		
	8 *pcf Creosote*[b]	16 *pcf Creosote*[b]	*Creosote+CCA*[c]
Control	7·6	6·7	7·1
B. cereus (1178)	11·6	11·7	
B. circulans + *M. colpogens* (866-1, -2, -3)	9·8	12·7	9·6
B. lentus (226)	9·7	6·1	
B. lentus (485)	10·5	9·5	11·5
B. lentus + *B. pumilus* (491-1, -2)	11·2	13·9	10·9
B. pumilus (1148)	11·2	13·1	
B. pumilus + *B. subtilis* (488-1, -2)	8·9	11·1	11·0
B. sphaericus (548)	11·5	10·3	
M. colpogens (554)	8·8	10·7	
M. colpogens (557)	11·8	11·0	
P. creosotensis (ATCC 14582)	10·7	11·8	
Pseudomonas sp. A (644)	10·9	10·4	

[a] Average of four replications.
[b] Weight losses as caused by *L. lepideus.*
[c] Weight losses as caused by *L. lepideus* + *P. vaillantii.*

Table IV
DECAY LOSSES IN ACA-TREATED WOOD PREVIOUSLY EXPOSED TO PRESERVATIVE-TOLERANT BACTERIA

Bacterial isolate	*Average weight loss*[a] (%)
Control	21·7
B. brevis (1241)	33·9
B. lentus + *Pseudomonas sp.* B. (287-1, -2)	41·4
B. sphaericus (326)	41·8
B. sphaericus (548)	37·0
M. colpogens (293)	37·7
M. colpogens (554)	35·8
M. colpogens (557)	39·1
Pseudomonas sp. C (1265)	44·6

[a] Average of eight replications.

Table V
DECAY LOSSES IN CCA-TREATED WOOD PREVIOUSLY EXPOSED TO PRESERVATIVE-TOLERANT BACTERIA

Bacterial isolate	*Average weight loss*[a] (%)
Control	36·5
B. lentus (485)	24·3
B. megaterium (425)	16·7
B. pumilus (392)	11·1
B. pumilus (1328)	14·9
B. subtilis (359)	19·8
P. membranoformis? (386)	16·1
P. membranoformis? (1331)	19·1

[a] Average of eight replications.

high (21·7%), but similarly treated blocks that had been previously exposed to bacteria were decayed to a considerably greater degree (Table IV). The greatest weight loss was 44·6% and occurred in blocks first exposed to the action of *Pseudomonas* sp. C (isolate 1265).

Results with CCA-treated blocks were opposite to those obtained with creosote- and ACA-treated blocks. *P. vaillantii* reduced weight 36·5 % in the control blocks and considerably less in blocks exposed first to bacteria, the greatest loss being 24·3 % and least, 11·1 % (Table V).

DISCUSSION

Bacillus, Pseudomonas, and *Micrococcus* species tolerant to creosote, CCA, and ACA were isolated from preservative-treated wood exposed in the sea. *Pseudomonas creosotensis* was not found among these isolates although it had previously predominated in isolations made from creosoted pilings located on both east and west coasts of the United States (O'Neill *et al.,* 1961). However, the species may have been among those isolated earlier by Duncan, and subsequently lost, as she had reported (unpublished) the frequent isolation of *Pseudomonas* species from Wrightsville Beach samples.

On the basis of isolations made on wood samples immersed in the sea for 48–60 months (Table I), greater populations and more species of bacteria were present in Wrightsville Beach harbour than in Boston harbour. For example, bacteria tolerant to dual treatments (creosote + salt) were apparently present in Wrightsville Beach but not in Boston waters (Table I). This may reflect differences in temperature or pollution between the two harbours, or differences in the depths to which the samples were immersed in the sea at the two locales.

Creosoted piling has been noted, at times, to lose resistance to marine borer attack although considerable quantities of preservative still remained in the wood (Drisko and O'Neill, 1966; Hochman, 1967). Mixed populations of unidentified marine bacteria were found to metabolize and chemically alter creosote; however, a culture of *P. creosotensis* alone was unable to metabolize whole creosote although it was capable of utilizing a number of neutral constituents of the creosote (Drisko and O'Neill, 1966).

In the present study, pure cultures of *P. creosotensis, Bacillus* spp., and *Micrococcus colpogens* (*varians* ?) brought about consistent small decreases in toxicity to decay fungi of creosote- and creosote + CCA-treated wood. Considerably more bacteria may be capable of degrading creosote, as ZoBell (1946) reported wide distribution of hydrocarbon-oxidizing microorganisms, including bacteria, in soil and water.

To deter biodegradation of creosoted piling, Hochman (1967) suggested three alternatives: (a) incorporation of a bactericidal and *Limnora*-toxic additive to creosote; (b) double treatment of piling when chemicals not dissolvable in creosote are to be added; or (c) use of a barrier wrap about piling. Double- or dual-treatment of piling using inorganic water-borne salts in combination with creosote was suggested many years ago and has received renewed interest during recent years (Baechler *et al.,* 1970a). Recent field tests of wood dually treated with high retentions of creosote and copper-arsenic compounds indicate such treatments protect against marine organisms more effectively than does creosote alone (Baechler *et al.,* 1970b; Baechler *et al.,* 1970a; Johnson *et al.,* 1973).

In present tests, however, wood blocks treated to retentions of 208 kg³ of creosote plus 32 kg³ of CCA lost fungitoxicity at a rate similar to that occurring in blocks treated solely with creosote. Apparently, the marine environment contains bacteria capable of metabolizing or altering creosote and a combination of creosote and CCA at similar rates. Perhaps incorporation of a bactericide would prevent biological detoxification and even permit use of lower retentions of these preservatives. Because tests of wood treated with creosote + ACA have not yet been completed, it is not known whether bacterial detoxification of this preservative combination occurs.

In these trials, blocks treated with 32 kg³ of ACA or with 56 kg³ of CCA were subject to considerable decay whether exposed to bacteria and leaching or solely to leaching in sterile media. CCA controls, in fact, experienced substantially more decay than did bacterially exposed test blocks. The isolate of *P. vaillantii* used in these tests was subsequently found to be abnormally tolerant to CCA-treated wood, but some isolates of this species have been reported capable of degrading wood loaded to a retention of 77 kg³ (Wallace, 1968) and sawdust at a retention of about 102 kg³ of CCA (DaCosta, 1959). (Assuming that these loadings are not based on the current oxide method of calculating CCA retentions, conversion would still result in calculated retentions of at least 58% of the values shown by Wallace and DaCosta—retentions still sufficient for protection of wood.) Performance of CCA in field and service has, however, been much better than has been indicated by laboratory tests (Wallace, 1968). Scheffer (1964) has recommended that fungus strains showing median, rather than high or low, preservative tolerance be used in laboratory assays of wood preservatives. Presumably, this would help guard against occurrence of large differences in performance of preservatives in laboratory and field.

In view of the high CCA tolerance of the *P. vaillantii* strain used, and the resultant poor performance of the copper-arsenates undergoing test herein, new tests have been set up to determine the capability of marine bacteria to detoxify these preservatives. A different test fungus, less tolerant to copper-arsenate, will be used for this work with the results reported later.

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