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An attempt to determine the resistance of poplars to infection by *Chondroplea populea* (Sacc. Kleb.) = *Dothichiza populea* Sacc. et Briard) on the basis of the composition of epiphytic bacterial microflora

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Abstract

Epiphytic bacteria isolated from the bark of shoot nodes of two year poplar cuttings hydrolyse chitin whereas those isolated from the internodes do not show this property. The epiphytic microflora of the sensitive variety *P.* 'Robusta' embraces coryneform bacteria and *Pseudomonas* which can as a carbon source utilize o-OH benzoic acid, gentisic acid or trans-cinnamic acid in 10^{-2} M concentration. The resistant variety *P.* 'NE-42' is featured by *Pseudomonas* capable of catabolizing only genetisic acid in 10^{-2}M concentration. The ability of the bacteria to grow media containing either water extracts from the bark or phenolic acids (lignin model precursors) is correlated which the aromatic compound composition of the bark and resistance to infection by fungi.

INTRODUCTION

The composition of the epiphytic microflora cannot be ruled out asone of a number of traits anabling the determination of the resistance of plants to infection.

During vegetation the quantitative composition of microflora in plants is known to fluctuate but the dominating microflora, by L e b e n (1965) termed resident, could serve as an index of susceptibility and resistance to infection. The possibility of determining resistance to infection on the basis of studies on the composition of the epiphytic microflora was first considered by P o t t e r (1903, after L e b e n, 1965) and also much later by B i e r (1965).

Farkas and Kiraly (1962) pointed out the effect of phenol compounds on the resistance of a plant to infection. Moustafa and Whittenbury (1970) noticed that during plant infection β-glucosidases are considerably activated and the action of the liberated phenolic aglucones is both bacterio- and fungistatic. These authors found not only phenolic acids but also quinones to be exceptionally toxic and to inhibit the growth of the studied bacteria: *Pseudomonas syringae, P. fluorescens, P. mors-prunorum.*

As an additional factor of the resistance of plants to infection can be regarded the myco- and bacteriolytic enzymes produced by the epiphytic microflora. Whiteside and Corpe (1969) determined the lytic effect of *P. fluorescens* towards gram-negative bacteria. The effect of epiphytic micro-organisms on infection by pathogenic fungi is discussed by Blakeman and Fraser (1971), Blakeman (1972), Sztajnberg and Blakeman (1973) who have found these micro-organisms to inhibit the germination of the spores of the investigated fungus.

The purpose of this study was to establish a correlation, if any, between the composition of the epiphytic microflora on poplar shoots and resistance to infection by *Chondroplea populea.*

MATERIAL AND METHODS

In the investigations were used varieties of poplars both resistant and sensitive to infection by *Chondroplea populea* (Danilewicz and Siwecki, 1969; Bugała, 1973). The poplar cuttings grew in a compact monoculture stand, on black fallow, in nurseries from the same physiotherapeutic region: *P.* 'Robusta', 'Grandis', 'NE-42', from Pniewy nursery, 'Robusta' from Ostromęcko nursery and 'Marilandica' from Chełmno nursery. From the middle of the plot were chosen three two year plants from each variety and from their shoots five about 30 cm long parts taken containing upper, middle and base part of one year shoot and upper, middle and base part of two year shoot. Each part of the shoot was separately protected by polystyrene tubing. All procedures were conducted using rubber gloves sterilized with ethanol. The poplar shoots were collected on 17th. November. Samples of bark for isolation of bacteria and preparation of aqueous extracts were taken from the bark just before the examinations after 3 day storage at $+4°$.

Isolation of bacteria. Bacteria were isolated from the nodes and internodes of each part of the shoot from which with a cork borer circular parts of the bark together with epiderm and cambium were removed. The disks were placed in sterile test tubes containing 5 ml

of No. 1 mineral medium which were then shaken to wash off the bacteria. The test tubes, without removing the disks, were then incubated for 10 hours at 22°. After this time dilutions from 10^{-1} to 10^{-5} in sterile saline were prepared and the number of bacteria in the culture fluid determined by the plate method on No. 2 medium. 20 colonies were chosen at random from plates containing an appropriate number of colonies and the material was transferred to slants of No. 2 medium. The slants, after incubation and microscopic determination of the purity of the cultures, were stored at +4°.

Identification of isolated bacterial strains. The classification of strains giving a negative MR-VP reaction was determined on the basis of morphological and physiological traits according to the identification systems of Shewan, Hobbs, Hodgkiss (1960), Thornley (1960), Billing, Baker (1963), Keddie, Leask, Grainger (1966), Stanier, Palleroni, Doudoroff (1966), Stanier (1968), Veldkamp (1970), Bousfield (1972). Biochemical tests of importance in studies on the resistance of poplars to fungi such as the degradation of chitin were also employed. The products of the hydrolysis of chitin were determined by the method of Morgan-Elsen (Rondle and Morgan, 1965, Bergmayer, 1963).

Media. Composition of No. 1 medium: anhydrous K_2HPO_4 0.1%, $CaCl_2 \times 6\,H_2O$ 0.01%, KCl 0.05%, $MgSO_4 \times 7\,H_2O$ 0.05%, $FeSO_4 \times 7\,H_2O$ 0.001%, $NaNO_3$ 0.3%, distilled water, pH 6.2, Difco yeast extract 0.05% was added from a separately sterilized 10% solution and as a carbon source compounds diffusing to the mineral medium from the non-sterile disk of bark in the medium, which also served as a source of bacteria. Composition of No. 2 medium: Difco peptone proteose 2%, glycerol 1%, K_2HPO_4 anhydrous 0.15%, $MgSO_4 \times 7\,H_2O$ 0.05%, $FeSO_4 \times 7\,H_2O$ 0.001%, $NaNO_3$ 0.03%, distilled water, pH 5.6 and glucose 0.1% as a separately sterilized 20% solution 50 ml of the medium was supplemented with 100 mg chitin which was sterilized together with the medium (Lotar, 1961; Jeuniaux, 1966). Hydrolysis of gelatin was determined at pH 6.2 using mineral medium containing 20% gelatin and 0.03% Bacto peptone. The medium was sterilized by tyndallization. Hydrolysis of pectin was determined at pH 6.2 in No. 1mineral medium containing. 0.5% low-methylated citric pectin. Digalacturonic and galacturonic acids were determined by reaction with TBA (thiobarbituric acid) at 550 nm and 515 nm and during oxidation with periodate for 15 min at 80° (Wojciechowicz, 1972). The ability to oxidize or utilize glucose, sucrose and maltose was determined after Hugh and Laifson (1953) at pH 7.2, 6.85, 6.2 and 5.6 in mineral medium containing 0.5% sugar and indicators (bromothymol blue and bromocresol purple). The ability to utilize phenylalanine and arginine as a source of both carbon and nitrogen was determined in No. 1 mineral medium

containing one of these compounds to the exclusion of all other sources of carbon and nitrogen (Thornley 1960). The ability of the bacterial culture to absorb aniline blue and crystal violet was examined during growth of the bacteria on No. 1 mineral medium with 1% surcose, at pH 6.2 with simultaneous examination of the ability of the cultures to grow at 30, 28, 25, 22, 11 and 4°.

Examination of the effect of bark extract on bacterial growth. Bacterial growth was determined by measurement of extinction at 600 nm in ZAL-KF-5 photocolorimeter in No 1 mineral medium supplemented with standard bark extract. The medium was subjected to the following modifications:

a — bark extract from nodes, separate from each part of the shoot of 'Robusta' variety for cultivation of strains isolated from 'Robusta' nodes.

b — bark extract from internodes, separate from each part of the shoot of 'Robusta' variety for cultivation of strains isolated from 'Robusta' internodes.

c — bark extract from nodes, separate from each part of the shoot of 'NE-42' variety for cultivation of strains isolated from 'NE-42' nodes.

d — bark extract from internodes, separate from each part of the shoot of 'NE-42' variety for cultivation of strainsisolated from 'NE-42' internodes.

The cultures were maintained in 20 ml of the medium.

Preparation of bark extract. After collection of disks for the isolation of bacteria the shoots were separately barked at the nodes and internodes. 10 g of the bark was extracted with 190 ml boiling distilled water and then the whole sterilized at 3/4 atm. for 15 min. After 2 day storage at +4° the extracts were heated in water bath for 10 min. at 100°. 5 ml of the hot bark extract was added to 20 ml of No. 1 mineral medium thus obtaining a standard extract.

Examination of the effect of phenolic acids on bacterial growth. No 1 mineral medium containing 0.2% Difco yeast extract as carbon source, pH 6.2 was supplemented with one of the following phenolic acids at concentration 10^{-2}M: benzoic acid, o-OH benzoic acid, gentisic acid or trans-cinnamic acid. Cultures were maintained in 5 ml of the medium. Each test tube was inoculated with 0.1 ml suspension of bacteria in saline (extinction 0.01—0.02 at 600 nm). The first measurement of extinction was after 4 hours of growth and was regarded as time 0. All measurement performed in the culture tube the content of which was agitated on micro-shaker before each measurement.

Examination of the effect of bacteria on growth of mycelium. The growth of the mycelium in joint culture of fungus and bacteria was determined after 6 day incubation in No. 1 mineral medium with 0.5%

maltose as a source of carbon. To this end on a Petri dish was prepared a single colony of the fungus with 6—7 cm diameter. 2 cm from the peripheral hyphae of the colony with sterile test tube were cut and agar disks together with mcyelium were placed mycelium down on fresh mineral medium supplemented with maltose. After 5 day incubation material taken from the bacterial cultures was placed with a loop on the mycelium extending outside the disk, taking care to place all the strains a similar distance from the edge of the colony. On each colony of the fungus on Petri dish were inoculated 5 strains of bacteria. After a further 5 days of incubation the mycelium, together with bacterial cultures, was cut out with a test tube, the circle placed growth down on fresh medium. The obtained joint culture was incubated for 5 days at 25°. The effect of the bacteria on the growth and development of the mycelium was estimated on the basis of measurements of the colony and macro- and microscopic observation of lysis of the mycelium. The effect of bacteria on the growth of the mycelium was also examined in liquid mineral medium with 1% bark extract. 20 ml of No 1 mineral medium containing 1% bark extract in 100 ml Erlenkayer flask was inoculated with 0.1 ml suspension of bacteria in saline (extinction 0.01—0.02 at 600 nm) and disk of agar mineral medium with maltose with a 6 day mycelium. After 5 day incubation the culture was filtered through muslin, the agar disk removed and the mylium washed with distilled water. The dry mass of the mycelium was determined after drying in small vessels at 80° for 24 hours.

In the studies a strain of *Chondroplea populea* isolated from *P.* 'Robusta' by K o z ł o w s k a in 1972 was used.

RESULTS

General characterization of epiphytic bacteria isolated from the shoots of poplar varieties both susceptible and resistant to infection by *Chondroplea populea*.

Populus 'Robusta' and other varieties of the *Aigeiros* section and *P.* 'NE-42' of the *Tacamahaca* section differ in the composition of their epiphytic bacteria. *P.* 'Robusta' has both dyphteroids and *Pseudomonas* whereas *P.* 'NE-42', only *Pseudomonas*. Dyphteroids incubated in the dark form grey-white colonies whereas light is found to induce pigmentation, particularly at pH 7.2 when a yellow, brown-orange pigment, insoluble in water, is formed. A limited number of strains hydrolyse gelatin, reduce nitrates to nitrites and degrade arginine with liberation of NH_3. All strains produce cytochrome oxidase, acidify mineral medium with glucose, sucrose or maltose, do not hydrolyse starch and sodium carboxymethylcelluloses and pectins and alkalize mineral

Table 1

Hydrolysis of chitin by strains of bacteria isolated from the surface of the nodes and internodes of shoots of two-year cutitngs of *Populus* 'Robusta' and *P.* 'NE-42'

N-acetyl-D-glucosamine ng/ml

Incubation**	One-year shoot										Two-year shoot									
	upper part				middle part			base			upper part				middle part			base		
	Populus 'Robusta', nodes strain no.																			
	P-1a	D-13a	D-23	D-23a	P-3	D-15	D-25	D-5	D-17	D-27	D-7	D-19	D-28	D-9	D-29			P-11	D-30	
24 hrs	0.5	10.0	35.0	0.5	1.0	0.5	15.0	12.0	12.0	22.0	12.0	12.0	4.00	5.0	30.0			10.0	5.0	
48 hrs	10.0	10.0	65.0	12.0	20.0	25.0	20.0	40.0	25.0	45.0	25.0	40.0	68.0	48.0	65.0			15.0	20.0	
	Populus 'Robusta', internodes strain no.																			
	P-2	P-14	P-24	P-24a	P-4	D-26		P-6	P-18	D-27a	P-8	D-20a	P-28a		P-1a	P-10a	P-29a	D-12		
24 hrs	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0		
48 hrs	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0		
7 days	0.0	0.0	10.0	5.0	15.0	nt		nt	55.0	0.0	2.0	0.0	70.0		0.0	0.0	75.0	70.0		
	Populus 'NE-42', nodes strain no.																			
	P-61a	P-82	P-62		P-62	P-73a		P-63	P-75		P-76	P-85a			P-77	P-87	P-87a	D-69	P-89	
24 hrs	0.0	nt			20.0	35.0		25.0	30.0		20.0	30.0			0.0	20.0	30.0	25.0	35.0	
48 hrs	0.0	nt			20.0	35.0		20.0	10.0		30.0	15.0			0.5	35.0	35.0	30.0	15.0	
	Populus 'NE-42', tnternodes strain no.																			
	P-61a				P-62a			P-64	P-75a		P-76a	P-85a			P-67	P-88	P-88a			
24 hrs	0.0				0.0			0.0	0.0		0.0	0.0			0.0	0.0	0.0			
48 hrs	0.0				0.0			0.0	0.0		0.0	0.0			0 0	0.0	0.0	nt		
7 days	0.0				0.0			0.0	0.0		0.0	0.0			0.0	0.0	0.0			

Explication: P-Pseudomonas strains, D-dyphteroids, ** — no N-acetyl-D-glucosamine detected after 4 hour incubation, nt — not tested.

medium with sodium citrate. All the strains grow well in mineral medium containing 0.5% Difco yeast extract as a source of both carbon and vitamins. *Pseudomonas* isolated from the shoots of *P.* 'Robusta' produces a characteristic yellow-green fluorescent pigment which diffuses to the medium and fluorescens green in UV. The strains differ from each other very little. All strains acidify mineral medium with glucose, sucrose and maltose but do not hydrolyse starch, sodium carboxymethylcellulose, pectins. Arginine is degraded with liberation of NH_3 and CO_2. In medium containing 1% sucrose polysaccharides are formed. The strains do not utilize phenylalanine as a source of both carbon and nitrogen and do not produce indole. They differ in their ability to reduce nitrates. All the strains hydrolyse gelatin and produce cytochrome oxidase. *Pseudomonas* strains isolated from the surface of *P.* 'NE-42' shoots do not produce any pigments. A culture, apart from smooth colonies also contains mucoid colonies showing intensive production of polysaccharides. The strains absorb aqueous solutions of aniline blue and crystal violet tinting the culture pale blue. The optimum for growth of *Pseudomonas* is 25° with minimum at 4° and maximum at 27°. The dyphteroids have been identified as *Corynebacterium* species and several strains of *Arthrobacter globiformis*. Most of the *Pseudomonas* strains have been found to be *Pseudomonas syringae* with single *Ps. fluorescens* strains and a non-fluorescent, obscure *Pseudomonas* species.

The ability of the bacteria isolated from the surface of *P.* 'Robusta' and *P.* 'NE-42' to hydrolyse chitin

Table 1 presents data showing the amounts of N-acetyl-D-glucosamine produced from chitin during growth of the bacteria. The results show a dependence between the origin of the bacteria and the ability to hydrolyse chitin. Most of the species of bacteria from the nodes of *P.* 'Robusta' from 100 mg chitin liberated 10—65 μg/ml N-acetylglucosamine whereas the strains isolated from the the nodes of *P.* 'NE-42' only 10—35 μg/ml. The strains isolated from the internodes of both species do not hydrolyse chitin after 48 hours of growth and only single strains liberate small amounts of N-acetyl-D-glucosamine after 7 days of incubation.

Qualitative and quantitative composition of the bacterial microflora on bark disks from *P.* 'Robusta' and *P.* 'NE-42'

Tables 2 and 3 present the results of the number of bacteria on the bark of the nodes and internodes of varieties both susceptible and resistant to infection by fungi. The number of bacteria on the bark of *P.*

Table 2

The number of bacteria on the surface of the bark of nodes and internodes of two-year cuttings of Populus 'Robusta', 'Grandis' and 'Marilandina' (*Aigerois section*) and 'NE-42' (*Tacamahaca section*)

Part of shoots		'Robusta' (Pniewy)	'Robusta' (Ostromęcko)	'Grandis' (Pniewy)	'Marilandica' (Chełmno)	'NE-42' (Pniewy) *
		colony forming units/0,1 ml of culture				
		one-year shoot				
Upper part	A	$3.5 \cdot 10^2$	$2.7 \cdot 10^4$	$5.0 \cdot 10^2$	$2.2 \cdot 10^3$	$1.7 \cdot 10^2$
	B	$3.0 \cdot 10^1$	$1.8 \cdot 10^2$	$1.0 \cdot 10^1$	no bact.	$1.0 \cdot 10^2$
Middle part	A	$1.6 \cdot 10^2$	$7.0 \cdot 10^1$	$2.8 \cdot 10^3$	no bact.	$2.0 \cdot 10^2$
	B	$3.0 \cdot 10^1$	$1.0 \cdot 10^2$	$1.4 \cdot 10^2$	,,	$1.5 \cdot 10^2$
Base	A	$2.0 \cdot 10^3$	$1.6 \cdot 10^3$	$3.1 \cdot 10^3$	$1.0 \cdot 10^2$	$5.4 \cdot 10^2$
	B	$1.5 \cdot 10^2$	$4.0 \cdot 10^3$	$2.0 \cdot 10^2$	$1.5 \cdot 10^2$	$2.5 \cdot 10^2$
		two-year shoot				
Upper part	A	$2.0 \cdot 10^3$	$1.0 \cdot 10^2$	$3.0 \cdot 10^2$	$3.0 \cdot 10^4$	$3.5 \cdot 10^2$
	B	$1.5 \cdot 10^2$	$5.0 \cdot 10^1$	$3.0 \cdot 10^3$	$1.0 \cdot 10^3$	$2.5 \cdot 10^2$
Middle part	A	$1.5 \cdot 10^3$	$1.2 \cdot 10^2$	$4.1 \cdot 10^3$	$2.0 \cdot 10^1$	$6.3 \cdot 10^2$
	B	$1.5 \cdot 10^2$	$1.3 \cdot 10^2$	$4.0 \cdot 10^2$	$1.5 \cdot 10^2$	$4.1 \cdot 10^2$
Base	A	$2.2 \cdot 10^2$	$1.0 \cdot 10^4$	$1.5 \cdot 10^2$	$2.0 \cdot 10^1$	$3.5 \cdot 10^2$
	B	$1.1 \cdot 10^2$	$1.5 \cdot 10^2$	$1.5 \cdot 10^2$	no bact.	$3.2 \cdot 10^2$

* nursery, A — node, B — internode

'Robusta' can be observed to be about 10 times greater than on the bark of the internodes. Such a correlation is not observed in the resistant to infection *P.* 'NE-42'. Pronounced, however, between both varieties are the differences in the qualitative composition of bacteria. On *P.* 'Robusta' are found dyphteroids and also, to a lesser extent, *Pseudomonas*. On *P.* 'NE-42' only *Pseudomonas* is found. Similar dependences between the number of dyphteroids and *Pseudomonas* can be observed in all varieties of the *Aigeiros* section, sensitive to infection.

Growth of bacteria isolated from *P.* 'Robusta' and *P.* 'NE-42' in mineral medium containing bark extracts

Strains of the bacteria isolated from the nodes of *P.* 'Robusta' grow much better in mineral medium with bark extract as a source of carbon than the strains isolated from the internodes. Such differentiation is not observed between strains isolated from the nodes and internodes of *P.* 'NE-42' the growth of all the strains is poor and similar to that of the strains isolated from the internodes of *P.* 'Robusta'. The mentioned dependences are illustrated in Fig. 1.

Table 3

Quantitative occurrence of strains of dyphteroids and *Pseudomonas* on the surface of bark from the nodes and internodes of two-year cuttings of *Populus* 'Robusta'.' Grandis' and 'Marilandica' (*Aigeiros* section) and 'NE-42' (*Tacamahaca* section)

Pact of shoots		'Robusta' (Pniewy)	'Robusta' (Ostomęcka)	'Grandis' (Pniewy)	'Marilandica' (Chełmno)	'NE-42' (Pniewy)*
		dyphteroids : *Pseudomonas* in 20 colonies				
		a : b	a : b	a : b	a : b	a : b
		one-year shoot				
Upper part	A	4 : 1	1 : 1	1 : 1	1 : 3	0 : 2
	B	0 : 4	2 : 1	1 : 0	0 : 0	0 : 3
Middle part	A	2 : 1	2 : 0	2 : 1	0 : 0	0 : 3
	B	1 : 1	1 : 7	1 : 1	0 : 1	0 : 3
Base	A	3 : 0	2 : 1	1 : 2	2 : 0	0 : 3
	B	1 : 0	1 : 2	0 : 2	0 : 2	0 : 3
		two-year shoot				
Upper part	A	2 : 0	2 : 0	1 : 1	2 : 0	0 : 2
	B	1 : 2	1 : 0	0 : 0	1 : 1	0 : 3
Middle part	A	2 : 0	2 : 0	1 : 2	1 : 0	0 : 4
	B	1 : 2	0 : 2	0 : 2	1 : 0	0 : 4
Base	A	2 : 0	1 : 1	1 : 1	1 ; 0	1 : 2
	B	1 : 1	1 : 0	1 : 0	1 : 0	0 : 3
Total		20 : 12	17 : 7	10 : 3	10 : 7	1 : 35

* — nurseries,
a — dypteroids, b — Pseudomonas
A — nodes, B — internodes

The effect of phenolic acids on the growth of bacteria isolated from *P.* 'Robusta' and *P.* 'NE-42'

The effect of o-OH benzoic acid (a), benzoic acid (b), gentisic acid (c) and trans-cinnamic acid (d) in concentration 10^{-2}M on the growth of bacteria in mineral medium with Difco yeast extract as a carbon source is illustrated by Figs 2—13. All strains isolated from the nodes and internodes of *P.* 'Robusta' have been found to tolerate this concentration of phenolic acids. Estimates of turbidity and colour of the culture allow the assumption that these acids can serve as a carbon source. However, the strains isolated from the shoots of *P.* 'NE-42' tolerate und utilize as a carbon source only gentisic acid. No differences have been determined between the utilization of phenolic acids by the dyphteroids and *Pseudomonas.*

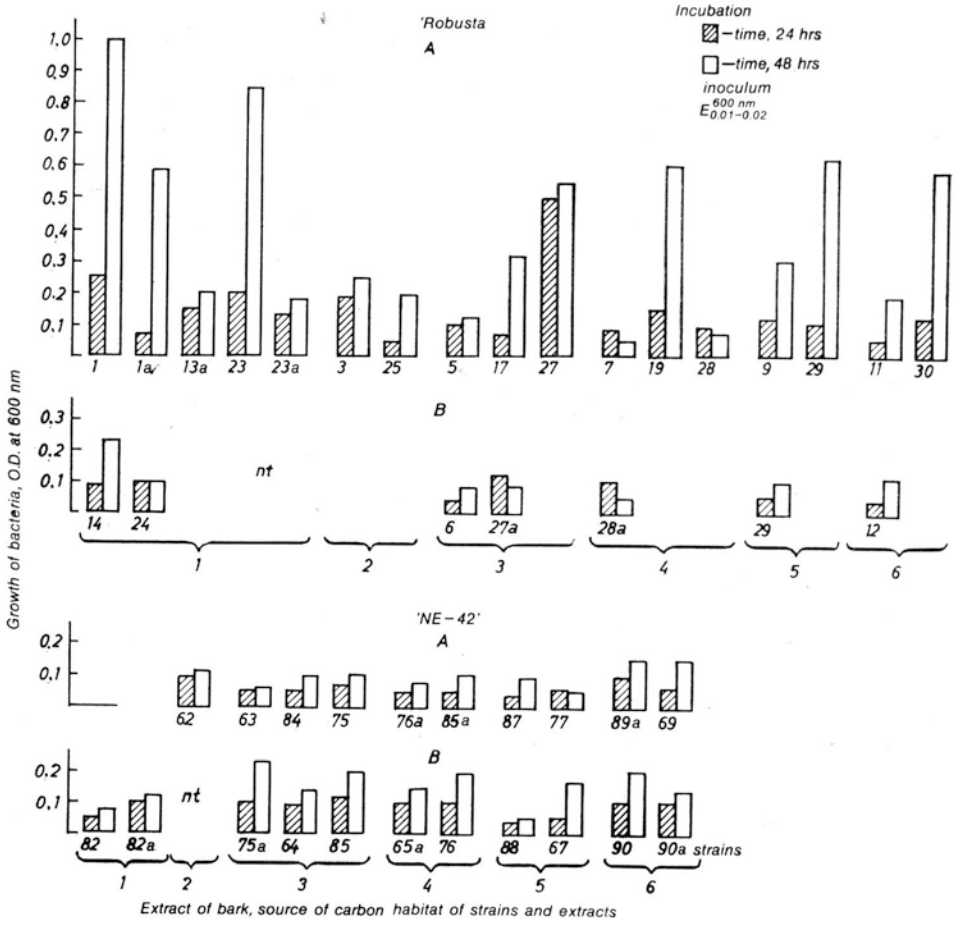


Fig. 1. Growth of bacteria isolated from the surface of the bark of nodes (*A*) and internodes (*B*) of *P.* 'Robusta' and *P.* 'NE-42' varieties on mineral medium with 1% aqueous bark extract from the nodes (*A*) and internodes (*B*) of these plants

Incubation: 24 and 48 horus. Bacterial isolates and extract from bark of one-year (1. 2. 3) and two-year (4. 5. 6) shoots; 1 and 4 — upper part of shoot, 2 and 5 — middle part of shoot, 3 and 6 — base of shoot

The effect of bacteria on growth of mycelium in agar medium with maltose

In mineral medium with maltose a majority of the strains from *P.* 'Robusta' and *P.* 'NE-42' has been found to inhibit in part or in full the growth of the mycelium (Fig. 14). In a culture with *P. syringae* lysis of the mycelium is observed but also sectors resistant to lytic enzymes (Photo 1).

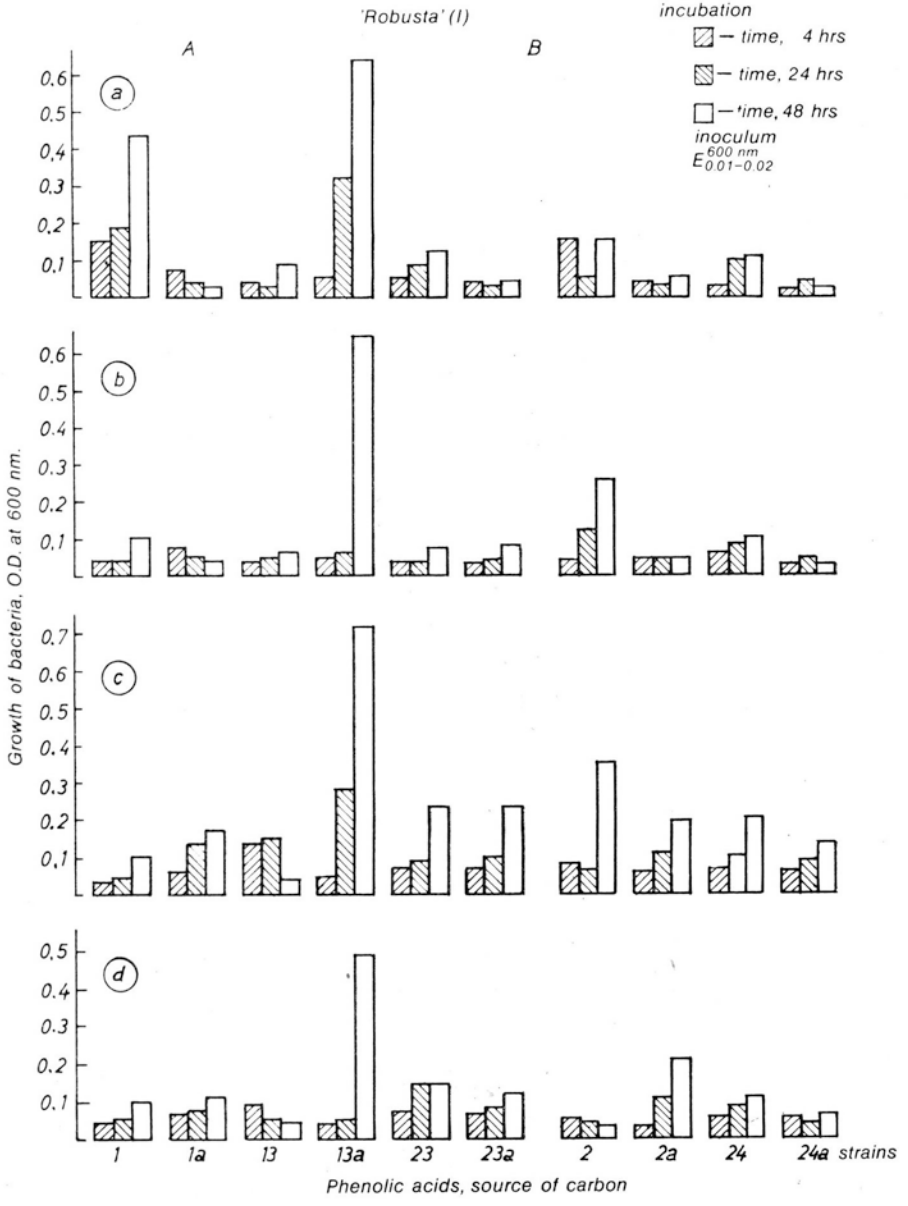


Fig. 2. Growth of bacteria on minimal medium with phenolic acids as carbon source. Bacteria were isolated from the surface of the bark of the nodes and internodes of three plants each of the varieties *Populus* 'Robusta' and *P.* 'NE-42'. Incubation after 4, 24, and 48 hours

a — o-OH benzoic acid, *b* — benzoic acid, *c* — gentisic acid, *d* — trans-cinnamic acid. Concentration 10^{-2}M
A — nodes, *B* — internodes

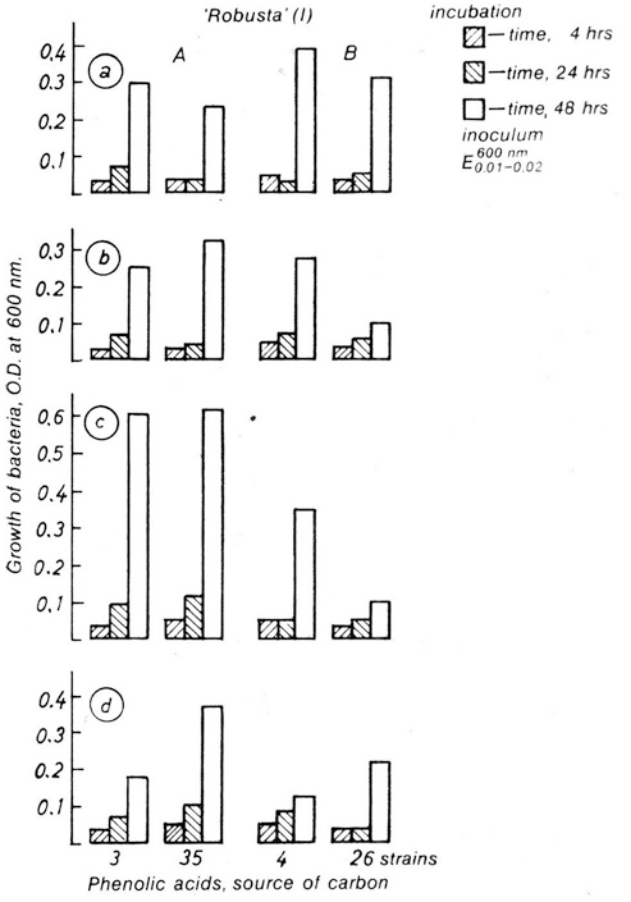


Fig. 3. Bacteria isolated from middle part. Explanations as in page 175.

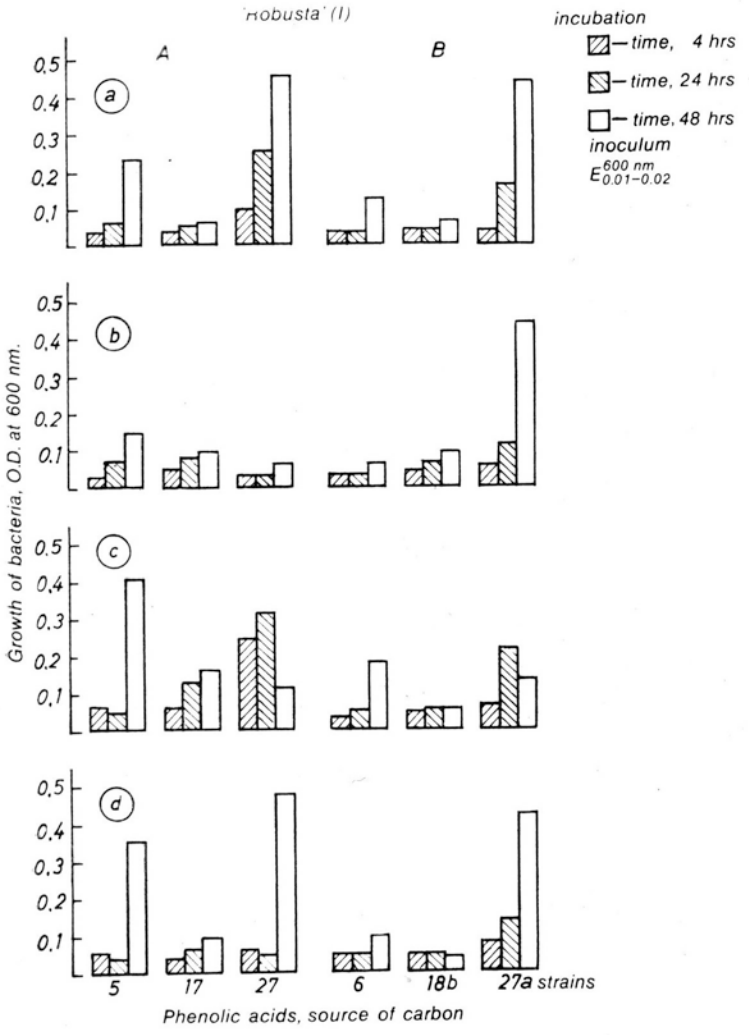


Fig. 4. Bacteria isolated from base. Explanations as in page 175.

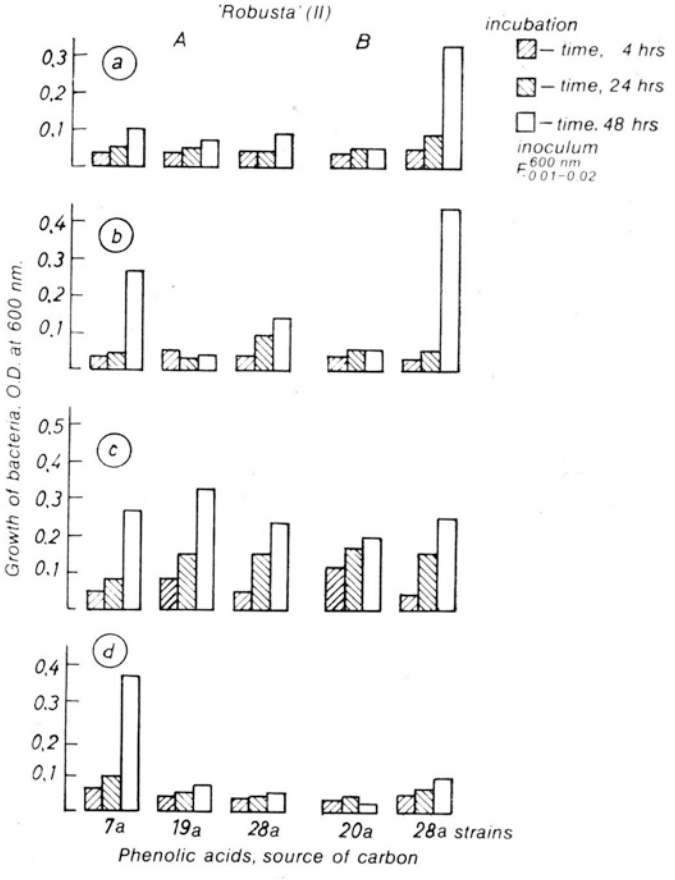


Fig. 5. Bacteria isolated from upper part. Explanations as in page 175.

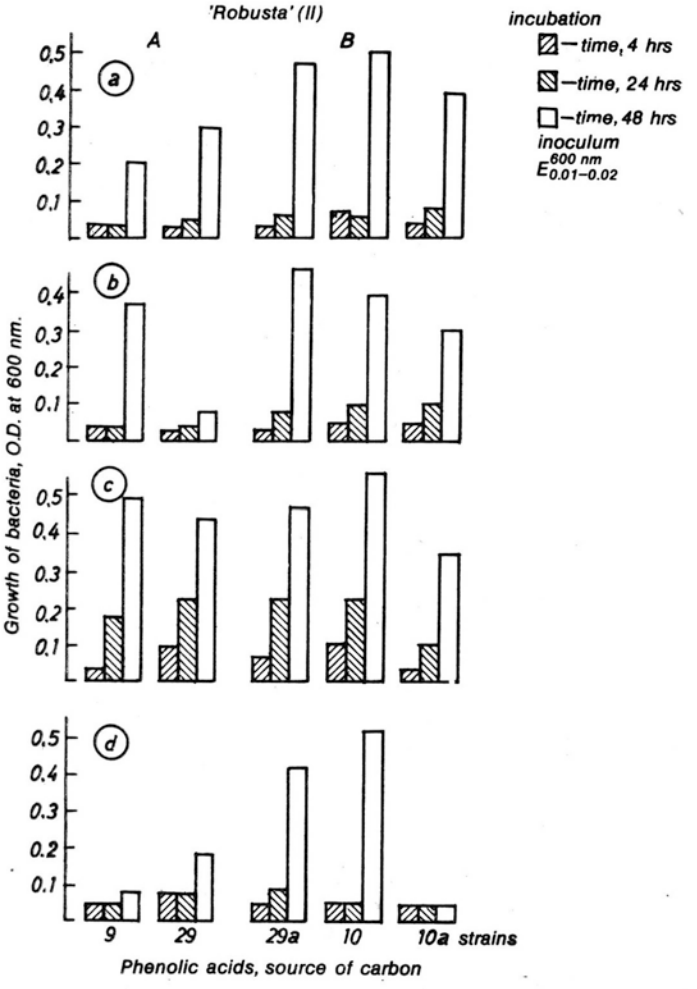


Fig. 6. Bacteria isolated from middle part. Explanations as in page 175

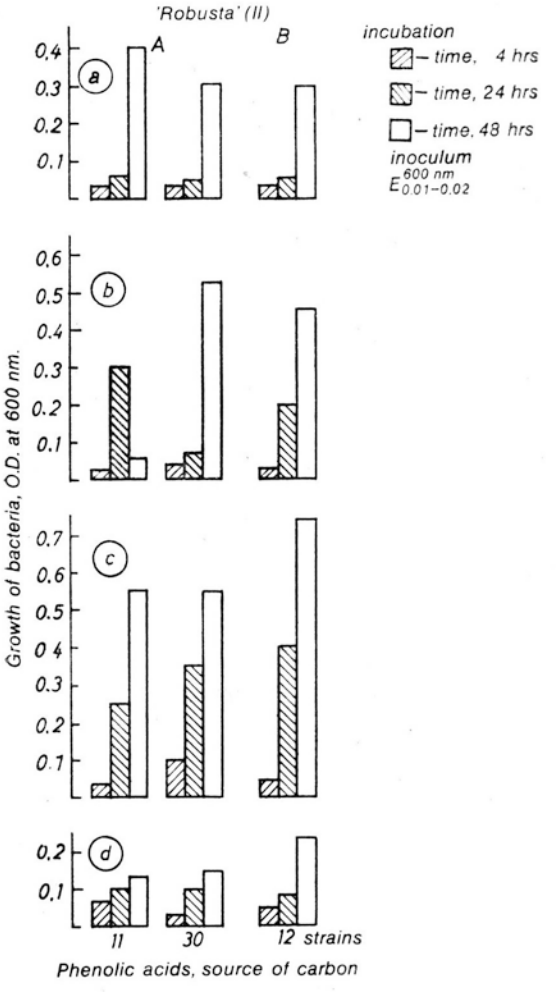


Fig. 7. Bacteria isolated from base. Explanations as in page 175.

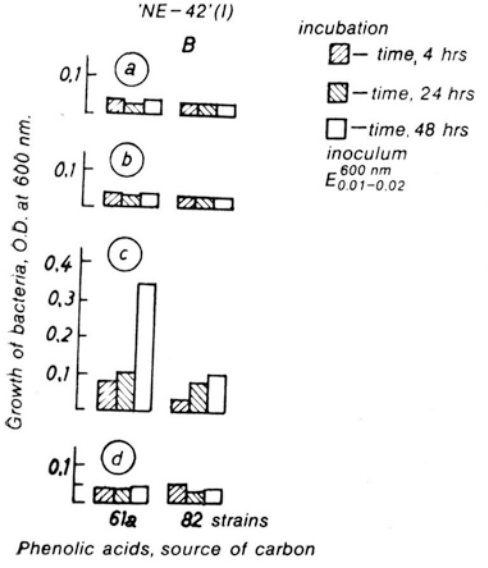


Fig. 8

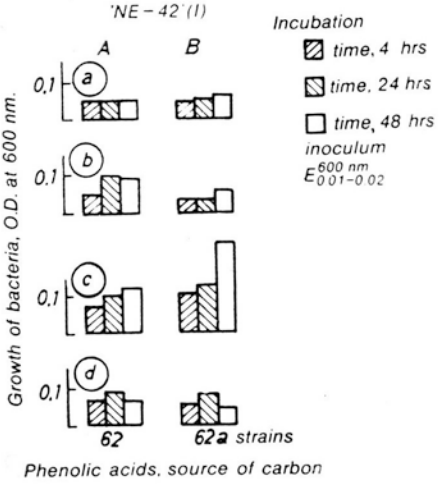


Fig. 9

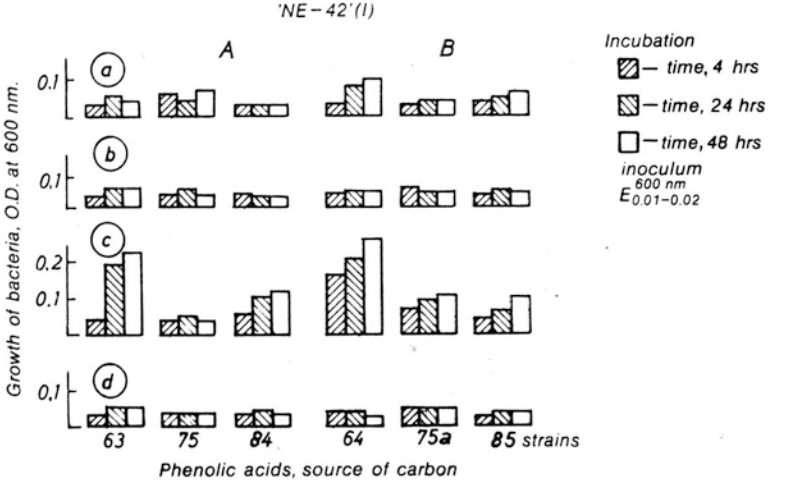


Fig. 10

Figs. 8—10. Bacteria isolated from the surface of the nodes (A) and internodes (B) of a one-year shoot of *P.* 'NE-42'

8 — from upper part, 9 — from middle part, 10 — from base

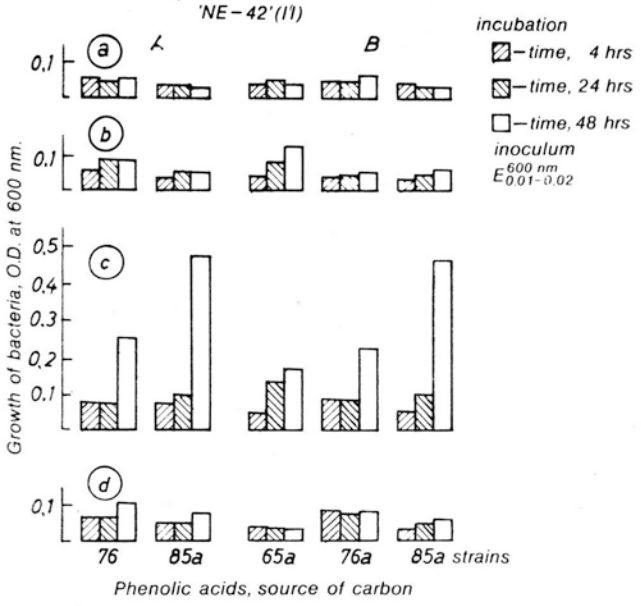


Fig. 11

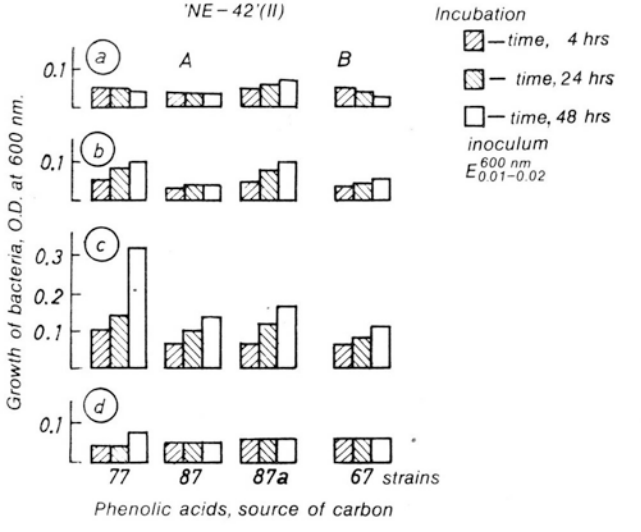


Fig. 12

Figs. 11 and 12. Bacteria isolated from upper part (11) and middle part (12). Explanations as in page 175.

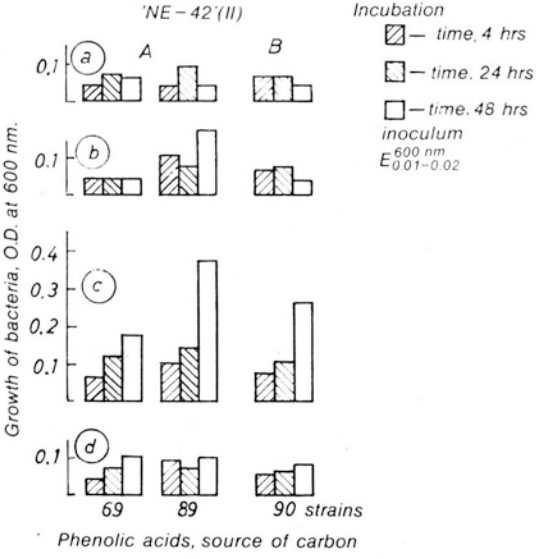


Fig. 13. Bacteria isolated from base. Explanations as in page 175.

The effect of bacteria on the growth of mycelium in mineral medium with aqueous bark extract from *P.* 'Robusta' and *P.* 'NE-42'

The bark extracts have generally been found to inhibit the growth of the mycelium. An exception is only the extract from the middle part of a one year shoot which has been found to stimulate growth of the mycelium. In joint culture of the fungus and a number of bacterial strains the inhibitory effect of the bacteria on the growth of the mycelium is observed. Some strains have, however, been found to stimulate the growth of mycelium during joint cultivation in medium with extract from the bark of *P.* 'Robusta', and to a lesser degree from *P.* 'NE-42'. The stimulating effect of bacteria isolated from *P.* 'Robusta' and *P.* 'NE-42' is about 22—27% of the increase in dry mass of the mycelium (Fig. 15).

DISCUSSION AND CONCLUSIONS

Studies on the epiphytic bacteria of poplar shoots demonstrate the purposefulness of attempts to determine the resistance of poplars to infection by *Chondroplea populea* and possibly also by other pathogens

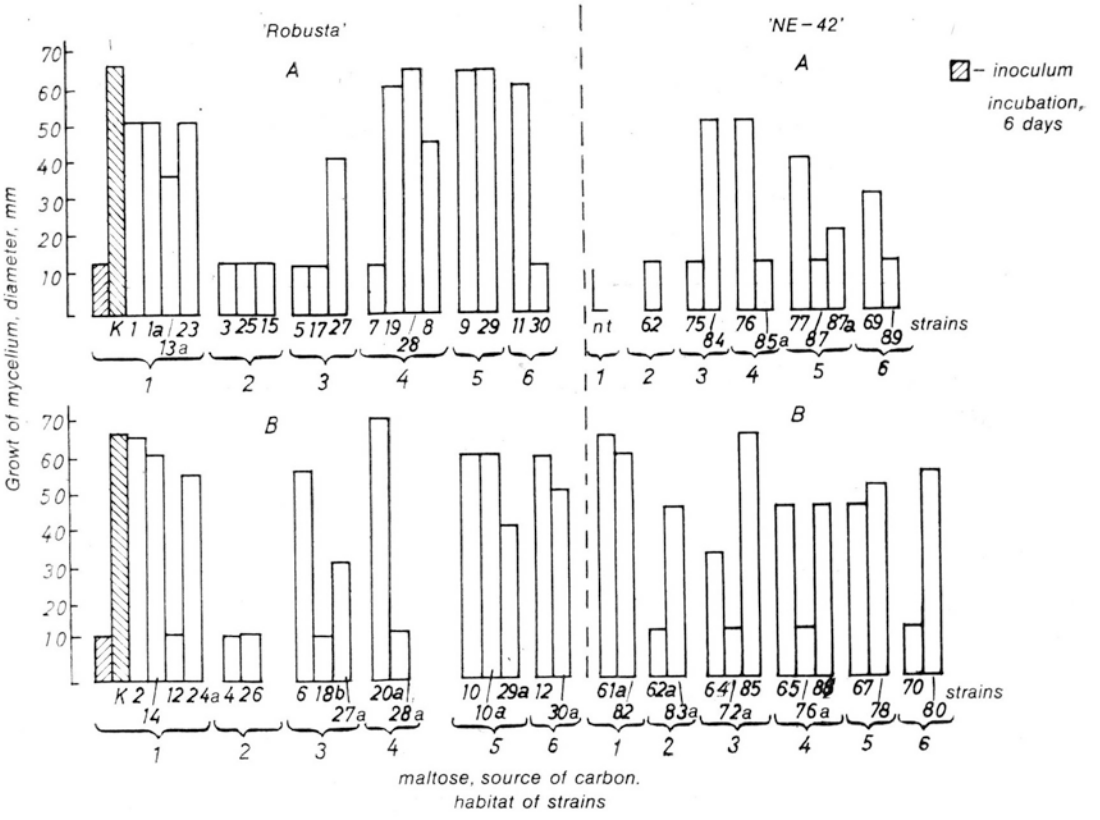
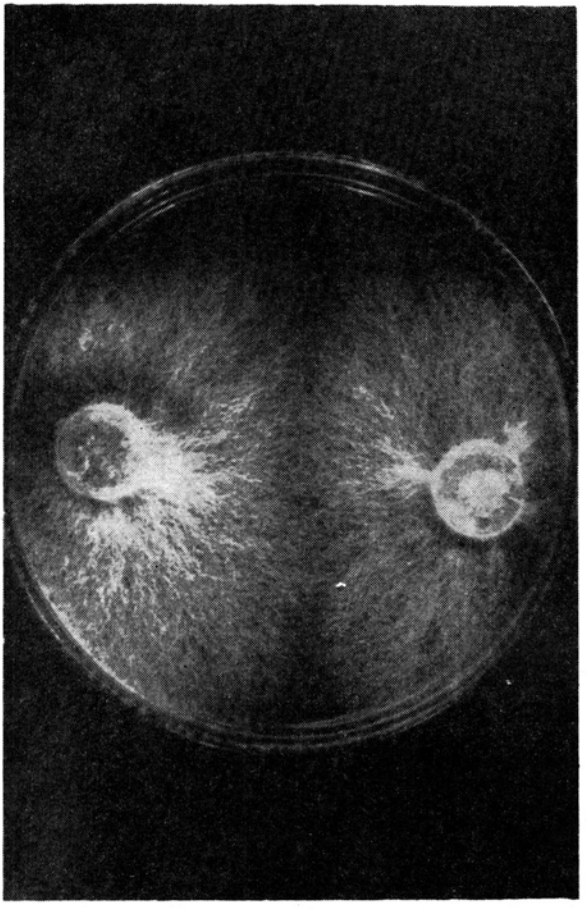


Fig. 14. The effect of bacteria isolated from the surface of the bark of nodes and internodes of two-year cuttings of *P.* 'Robusta' and *P.* 'NE-42' varieties on the growth of *Ch. populea* mycelium (s.m.) (agar mineral medium with maltose)

a — control, *b* — inoculum-mycelium, *c* — growth of mycelium with bacteria

infecting the bark on the basis of qualitative composition of epiphytic bacteria and their biochemical traits. In this regard, of the epiphytic bacteria on the bark of two year poplar cuttings, the most important are *Pseudomonas* strains. These bacteria are able to catalyze one of the precursor of the lignin of poplars, p-OH benzoic acid by splitting the ring at either ortho-OH or metha-OH. Another model precursor of lignin in poplars is gentisic acid glycosidically bound in the trichocarpine, the concentration of which in resistant poplars from the *Tacamahaca* section is 300 times higher than in the sensitive varieties of the *Aigeiros* section (T o m a s z e w s k i, 1972—1973). As a result of oxidation by *Pseudomonas* gentisic acid is converted to p-1,4-methoxyquinone which is more toxic towards fungi than either the glycoside of the trichocarpine or its aglycone gentisic acid. *Pseudomonas* is also able to utilize other aromatic



Phot. 1. Lysis of the mycelium of *Chondroplea populea* by *Pseudomonas syringae* after 5 day incubation at 25°

models lignin precursors such as sinapic acid, syringic acid, ferrullic acid, vanillic acid, coniferyllic acid, and cinnamic acid (D a n i l e w i c z, T o m a s z e w s k i, 1972).

The epiphytic microflora of the sensitive *P.* 'Robusta' variety embraces dyphteroids and *Pseudomonas* which as a carbon source utilize o-OH benzoic acid, benzoic acid, gentisic acid and trans-cinnamic acid in concentration 10^{-2}M. The resistant *P.* 'NE-42' variety characterized by *Pseudomonas* strains able to catabolize only gentisic acid in concentration 10^{-2}M.

The ability of bacteria to grow on media with bark extracts or phenolic acids (lignin model precursors) is correlated with the aromatic compound composition of the bark and resistance of the varieties from which the bacteria were isolated to infection by fungi.

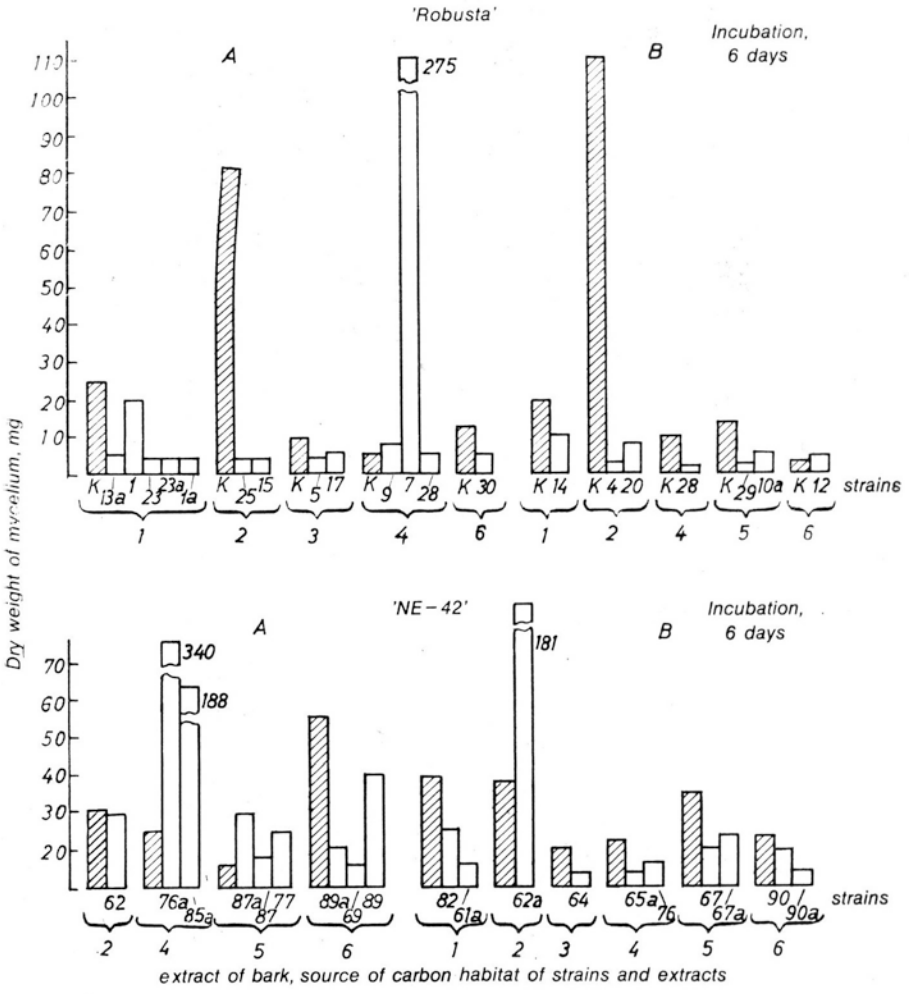


Fig. 15. The effect of bacteria isolated from the surface of the bark of the nodes (*A*) and internodes (*B*) of two-year cuttings of *P.* 'Robusta' and *P.* 'NE-42' varieties on the growth of *Ch. populea* mycelium. Liquid mineral medium with 1% aqueous extract from bark of one-year and two-year shoots.

1 and 4 — from upper parts, 2 and 5 — from middle parts, 3 — and 6 — from base *a* — control, *b* — growth of mycelium with bacteria
(A5, A3, B1 and B3 not tested)

REFERENCES

Bugała Wł., 1973, Systematyka i zmienność. Topole (*Populus* L.), PWN, Warszawa, Poznań.

Bier J. E., 1966, The possibility of microbiological types with different degrees of disease resistance within a tree species or clones. Proc. of NATO and NSF symposium held at the Pensylvania State University, August to September, 11, 1964.

Billing E., Baker A. E., 1963, Characteristic of *Erwinia* like organisms found in plant material. J. Appl. Bact. 26:58.

Blakeman J. P., Fraser A. K., 1971, Inhibition of *Botrytis conerea* spores by bacteria on the surface *Chrysanthemum* leaves. Physiol. Pl. Path. 1:45.

Blakeman J. P., 1972, Effect of plant age on inhibition of *Botrytis cinerea* spores by bacteria on beet root leaves. Physiol. Pl. Path. 2:143.

Bousfield I. J., 1972, A taxonomic study of some Coryneform bacteria. J. Gen. Microbiol. 7:441.

Danilewicz K., Siwecki R., 1969, A new method of standardized inoculation of rooted poplar cutting by the fungus *Chondroplea populea* (Sacc.) Kleb. (= *Dothichiza populea* Sacc. et Briard). Acta Soc. Bot. Pol. 16:73.

Danilewicz K., Tomaszewski M., 1972, Degradation of lignin by *Pseudomonas* Migula isolated from intestinal contents of *Paranthrene tabaniformis* Rott. Acta Microbiol. Pol. Ser. B, 4:37.

Farkas G. L., Kiraly Z., 1962, Role of phenolic compounds in the physiology of plant disease resistance. Phytopath. Z. 44:105.

Hugh R., Leifson E., 1973, The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bact. 66:24.

Jeuniaux Ch., 1966, Chitinase. Methods of Enzymology 8:644.

Keddie R. M., Leask B. G. S., Grainger J. M., 1966, A comparison of coryneform bacteria from soil and herbage:cell wall composition and nutrition. J. Appl. Bact. 29:17.

King E. O., Ward M. K., Raney D. E., 1954, Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. clin. Med. 44:101.

Leben C., 1965, Epiphitic microorganisms in relation to plant disease. Annual Rev. of Phytopath. 3:209.

Lotar C., 1971, Untersuchungen über die Chitinasewirkung des Insektentötenden Pilzes *Beaveria bassiana* (Bals.) Vuill. Arch. f. Mikrobiol. 40:17.

Moustafa F. A. (Darwiish), Whittenbury R., 1970, Properties which appear to allow phytopathogenic *Pseudomonas* to counteract plant defence mechanisms. Phytopath. Z. 76:214.

Shewan J. M., Hobbs G., Hodgkiss M., 1960, A scheme for the identification of certain genera of Gram-negative bacteria with special reference to the *Pseudomonadaceae*. J. Appl. Bact. 23:279.

Stanier R. Y., Palleroni N. J., Doudoroff M., 1966, The aerobic *Pseudomonas* nads:a taxonomy study. J. Microbiol. 43:159.

Stanier R. Y., 1969, Chemotaxonomy and serotaxonomy. Ed. J. G. Hawker. Acad. Press, London.

Sztajnberg A., Blakeman J. P., 1973, Studies on leaching of *Botrytis sinerea* conidia and dye absorption by bacteria in relation to competition for nutrients on leaves. J. Gen. Microbiol. 78:15.

Thornley M. I., 1960, The determination of *Pseudomonas* from other Gram--negative bacteria on the basis of arginine metabolism. J. Appl. Bact. 23:373.

Tomaszewski M., 1972—1973, Resistance to fungal infection by *Dothichiza populea* in fast growing poplar Hybrids. Second Tech. Report, Inst. of Dendrology, Polish Academy of Sciences.

Whiteside Th. L., Corpe W. A., 1969, Extracellular enzymes produced by *Pseudomonas* sp. and their effect on cell envelopes of *Chromatium violaceum*. J. Gen. Microbiol. 15:81.

Wojciechowicz M., 1971, Liaza pektynianowa. Post Biochem. 17:437.
Veldkamp H., 1970, Saprophitic coryneform bacteria. Annual Rev. of Microbiol. 24:209.

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Próba określenia oporności topoli na zakażenie przez Chondroplea populea (Sacc. Kleb.) = Dothichiza populea (Sacc. et Briard) na podstawie składu epifitycznej mikroflory bakteryjnej

Streszczenie

Epifityczne bakterie, wyodrębnione z kory węzłów pędów dwuletnich sadzonek topoli, hydrolizują chitynę; wyodrębnione z międzywęźli nie wykazują tej właściwości. Mikroflora epifityczna wrażliwej odmiany *P.* ‚Robusta' obejmuje dyfteroidy i *Pseudomonas*, wykorzystujące jako źródło węgla kwas o-OH benzoesowy, genetyzynowy, transcynamonowy w stężeniu 10^{-2} M. Odmiana oporna *P.* ‚NE-42' charakteryzuje się szczepami *Pseudomonas*, zdolnymi do katabolizowania tylko kwasu gentyzynowego w stężeniu 10^{-2}M. Zdolność do wzrostu bakterii na pożywkach z wodnymi ekstraktami kory lub kwasami fenolowymi (modelowymi prekursorami ligniny) jest skorelowana ze składem związków aromatycznych w korze i opornością na infekcje grzybowe.