The Antibiotic Relationships of Some Free-living Bacteria

By J. D. STOUT

Soil Bureau Experimental Station, Eastern Hutt Road, Lower Hutt, New Zealand

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SUMMARY

The sensitivity to antibiotics and the reactions to antibiotic-producing bacteria, streptomycetes and fungi of a range of bacteria isolated from soil, leaves and forest litter showed broad differences between the Gram-positive and Gram-negative organisms, the fermentative and non-fermentative ones, and, to a lesser extent, between those of leaf origin and those of soil origin. These differences are not alone sufficient to explain the differences between the leaf flora and the soil flora. Other factors, including the capacity to produce antibiotics, are also important.

INTRODUCTION

The most distinct boundary separating natural populations of bacteria is that which divides the flora of the growing leaf (epiphytic flora) from the soil flora. Despite the very large populations which may obtain on leaves, such as those of pasture plants, in close proximity to the soil surface there is a rapid depletion of the epiphytic bacteria eluted into the soil so that few survive within the profile (Stout, 1960*b*). The biochemical and other physiological properties of the epiphytic bacteria do not appear to be very different from those of the common soil bacteria and it is difficult to attribute the failure of the epiphytic flora to colonize soil to their nutritional or other physiological shortcomings. One feature of the epiphytic flora, however, is the absence or virtual absence of streptomycetes and the comparatively small numbers of fungi and spore-forming bacteria. These organisms are common in soil and are the most notable sources of antibiotic substances (Spector, 1957). It has therefore been thought possible that the extinction of the epiphytic flora in soil is due to microbial antagonisms and, in particular, to the effects of antibiotic substances. To test this hypothesis a range of bacterial isolates from various habitats (leaf, forest litter, soil) were tested against a selection of antibiotic substances produced by fungi, streptomycetes and bacteria and against living fungi, yeasts, streptomycetes and bacteria.

MATERIALS AND METHODS

The bacteria tested were isolated during recent years in the course of a survey of the grasslands, peats and forests of New Zealand. They have been classified as far as possible according to *Bergey's Manual* (1957). Further taxonomic and ecological details are given elsewhere (Stout, 1958, 1960*a, b*). Altogether 93 strains were used.

The antibiotic substances used are listed in Table 1 which also gives the source, the preparation, the diluent, and the concentrations tested. The bacterial and streptomycete antibiotics were generally readily soluble but some of the fungal antibiotics

Table 1. *List of antibiotics used; source, preparation, diluent, concentrations tested*

Antibiotic	Source	Preparation	Diluent	Concentrations tested
Polymyxin B sulphate	<i>Bacillus polymyxa</i>	Burroughs, Wellcome & Co. London, England	Water	100,000, 10,000, 1,000, 100 units/ml.
Tyrothricin (Gramicidin and Tyrocidine)	<i>Bacillus brevis</i>	Parke Davis & Co., Detroit, Michigan, U.S.A.	Ethanol	20,000, 10,000, 1,000, 100 μ g./ml.
Bacitracin	<i>Bacillus subtilis</i> <i>Bacillus licheniformis</i>	Chas. Pfizer & Co. Inc., New York, U.S.A.	Water	500, 50, 5, 0.5 units/ml.
Streptomycin sulphate	<i>Streptomyces griseus</i> <i>S. bikiniensis</i> <i>S. mashuensis</i>	Glaxo Labs. (N.Z.) Ltd. Palmerston North, N.Z.	Water	10,000, 1,000, 100, 10 units/ml.
Neomycin sulphate	<i>Streptomyces fradiae</i>	Glaxo Labs. (N.Z.) Ltd.	Water	1, 0.1, 0.01, 0.001 % (w/v)
	<i>Streptomyces</i> spp.	Palmerston North, N.Z.		
Spiramycin	<i>Streptomyces ambofaciens</i>	May & Baker, Ltd., Dagenham, England	Methanol	10,000, 1,000, 100, 10 units/ml.
Oleandomycin phosphate	<i>Streptomyces antibioticus</i>	Chas. Pfizer & Co. Inc., New York, U.S.A.	Water	1,000, 100, 10, 1 units/ml.
Tetracycline hydrochloride	<i>Streptomyces</i> spp.	Chas. Pfizer & Co. Inc., New York, U.S.A.	Water	1,000, 100, 10, 1 units/ml.
Erythromycin lactobionate	<i>Streptomyces erythreus</i>	Abbott Labs., North Chicago, Illinois, U.S.A.	Water	5, 1, 0.1, 0.01 % (w/v)
Penicillin G (sodium salt)	<i>Penicillium notatum</i> <i>Penicillium</i> spp. <i>Aspergillus</i> spp.	Glaxo Labs. (N.Z.) Ltd., Palmerston North, N.Z.	Water	100,000, 10,000, 1,000, 100 units/ml.
Patulin	<i>Penicillium</i> spp. <i>Aspergillus</i> spp.	Wellcome Research Laboratories London, England	Water	2, 1, 0.1, 0.01 % (w/v)
Griseofulvin	<i>Penicillium griseofulvum</i> <i>Penicillium</i> spp.	Development Dept., Glaxo Labs. (N.Z.) Ltd., Palmerston North, N.Z.	Ethanol	2, 1, 0.1, 0.01 % (w/v)
Gliotoxin	<i>Aspergillus fumigatus</i>	Dr P. W. Brian, I.C.I. Ltd., Welwyn, Herts., England	Acetone and ethanol	2, 1, 0.1, 0.01 % (w/v)
Viridin	<i>Trichoderma viride</i>	Dr P. W. Brian, I.C.I. Ltd., Welwyn, Herts., England	Acetone and ethanol	2, 1, 0.1, 0.01 % (w/v)
Frequentin	<i>Penicillium frequentans</i>	Dr P. W. Brian, I.C.I. Ltd., Welwyn, Herts., England	Acetone	2, 1, 0.1, 0.01 % (w/v)
Mycophenolic acid	<i>Penicillium brevi-compactum</i> <i>Penicillium</i> spp.	Dr P. W. Brian, I.C.I. Ltd., Welwyn, Herts., England	Ethanol	2, 1, 0.1, 0.01 % (w/v)
Mixed	<i>Gliocladium roseum</i>	Dr P. W. Brian, I.C.I. Ltd., Welwyn, Herts., England	Ethanol	2, 1, 0.1, 0.01 % (w/v)

were difficult to dissolve. Tests with acetone and ethanol showed that these solvents did not themselves inhibit growth in the concentrations used.

Broth cultures of the bacteria were prepared and used to inoculate agar plates which for most of the experiments consisted of tryptone (0.1 %), Difco yeast extract (0.1 %), agar (1.5 %), made up in distilled water. Potato carrot agar was used in experiments with the streptomycete plugs. This medium consisted of diced potatoes (20 g.) and carrots (20 g.), steamed in 1 l. distilled water for 20 min.; this was then filtered, made up to 1 l., agar added to 1.5 % (w/v) and autoclaved. Four filter paper disks (1 cm.) impregnated with the appropriate dilutions of the selected antibiotic were used for each plate. The total diameter of the zone of inhibition was measured after incubation for 24 hr. at 25° and summed for each plate.

The reproducibility of the technique was tested on several occasions with reasonably satisfactory results (Table 2). Consequently only a single plate was poured for each isolate tested. Strain variation (Table 3) did not appear to be important with

Table 2. *Results of duplicate plating*

Sum of the diameters of the four zones of inhibition for each plate in mm.

Antibiotic	Species and strain tested			
	<i>P. fluorescens</i> (C45)		<i>Aerobacter cloacae</i> (E6)	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
	Inhibition zone diam. (mm.)			
Polymyxin	49	47	49	58
Streptomycin	81	95	88	88
Tetracycline	31	40	21	33
Oleandomycin	0	0	15	23

Table 3. *Strain variation*

Sum of the diameters of the four zones of inhibition for each plate in mm. n.d. = not done.

Antibiotic	Species and strain tested				
	<i>Chromobacterium lividum</i>		<i>Bacillus cereus</i>		
	45/1/3	45/1/25	60/2/79	60/2/121	60/21/2
Polymyxin	35	29	61	n.d.	63
Tyrothricin	0	0	0	n.d.	30
Bacitracin	0	0	0	n.d.	20
Streptomycin	72	45	102	n.d.	102
Neomycin	81	25	47	45	52
Spiramycin	0	0	25	40	26
Oleandomycin	30	37	77	n.d.	65
Tetracycline	45	16	58	n.d.	51
Erythromycin	85	61	0	109	111
Penicillin	88	0	0	40	70
Patulin	65	50	10	58	70
Griseofulvin	0	0	0	0	10
Gliotoxin	0	0	87	142	111
Viridin	20	23	108	142	130
Frequentin	0	10	0	37	23
Mycophenolic acid	0	0	10	10	10
Mixed (Gliocladium)	25	25	46	53	30

the Gram-negative bacteria, e.g. *Chromobacterium*, but was more significant with the bacilli, particularly *Bacillus cereus*. The strains of *B. cereus* used were, however, as diverse as possible, one having been isolated as a smooth mucoid form, another as a pigmented form, and a third the more typical rough cream form.

For testing the inhibition of bacterial growth by other organisms two techniques were used, the plug method and the streak method. In the plug method the test organisms (fungi, yeasts, streptomycetes) were grown on the soya bean agar plates (Smith, Gordon & Clark, 1952) for 14 days. Plugs of agar (0.6 mm. diameter) were then cut and placed on the bacterial plates (four to a plate) and the zone of inhibition measured in the same way as with the antibiotic disks. In the streak method soya bean agar plates were streaked with the test organisms (bacteria, yeasts) in four parallel lines and the plates incubated for 7 days. Loops of broth cultures of the different bacteria were then streaked between these initial inocula and the degree of growth arbitrarily assessed. Four degrees were recognized: no growth, slight growth, moderate growth, and good growth, which were scored, respectively, 0, 1, 2, 3. Neither of these techniques was wholly satisfactory, the plug method being superior to the streak method. The latter was used, however, principally for the bacteria for which the plug method was clearly unsuitable because of their rapid and spreading growth on the tryptone yeast-extract agar. In the event, both methods provided a better index of antibiotic-producing organisms rather than of antibiotic-sensitive organisms. This was particularly so with the streak method, for it was observed with the control plates that certain of the bacterial strains tested grew very poorly on the soya bean agar plates while others grew very well.

RESULTS

Inhibition of bacterial growth by antibiotic substances

Comparison of the efficacy of the different antibiotic substances is qualified by their differences in purity, solubility, and the concentrations tested. Of the seventeen antibiotics tested only griseofulvin proved virtually ineffective. The results are given in Table 4. These show:

(a) The Gram-positive bacteria (*Micrococcus*, *Sarcina*, *Bacillus*, *Nocardia*) were more sensitive than the Gram-negative bacteria (*Pseudomonas*, *Xanthomonas*, *Aeromonas*, *Flavobacterium*, *Achromobacterium*, *Chromobacterium*, *Aerobacter*, *Escherichia* and *Serratia*).

(b) The aerogenic fermenters (*Aeromonas*, *Flavobacterium* sp. Type IV, *Serratia*, *Escherichia* and *Bacillus polymyxa*) tended to be relatively insensitive, but *Escherichia coli* appeared to be more sensitive than the other Gram-negative aerogenic fermenters.

(c) The Gram-negative bacteria of leaf origin (*Xanthomonas*, *Flavobacterium*) were generally more sensitive than those commonly found in soil (*Pseudomonas*) but the dominant leaf taxon *Flavobacterium* Type I was no more sensitive than the pseudomonads or the aerogenic fermenters.

Inhibition of bacterial growth by streptomycete and fungal plugs

The pattern of bacterial sensitivity to these inhibitory moulds and streptomycetes was similar to that of the antibiotics. Of the Gram-negative bacteria, only the flavobacteria showed inhibition, the pseudomonads and aerogenic fermenters were

Table 4. *Inhibition of bacterial growth by antibiotics using 1 cm. filter paper disks*

Medium: peptone yeast extract agar. Figures given are the sum of the diameters of the four zones of inhibition for each plate in mm. Where more than one strain was used the average for the taxon is given.

Bacteria tested. Taxon and type	No. of strains	Ecological habitat	Bacterial antibiotics				Streptomycete antibiotics								Fungal antibiotics								Total Av.	
			1	3	3	Av.	4	5	6	7	8	9	Av.	10	11	12	13	14	15	16	17			
Pseudomonadaceae																								
<i>Pseudomonas aeruginosa</i>	1	Parasite	41	0	0	14	30	60	40	10	37	21	33	20	30	10	10	10	10	10	10	14	21	
<i>P. fluorescens</i> I	1	Soil	48	0	0	16	83	64	0	0	29	32	35	17	30	10	59	25	10	0	21	22	25	
<i>P. fluorescens</i> II	2	Soil	46	0	0	15	90	67	10	0	52	33	42	15	25	0	0	10	0	0	10	8	21	
<i>Pseudomonas</i> sp. III	6	Soil, litter	38	0	0	13	67	65	10	0	68	40	42	46	40	0	38	40	5	0	26	24	28	
<i>Pseudomonas</i> sp. IV	3	Soil, litter	34	0	10	15	37	44	22	3	74	21	34	69	54	5	11	15	5	0	11	21	24	
<i>P. chlororaphis</i> V	1	Soil	58	10	10	26	62	45	0	0	12	45	21	0	25	10	0	10	0	0	0	6	17	
<i>Pseudomonas</i> sp. VI	1	Soil	39	0	10	16	40	60	40	40	57	22	43	10	23	10	0	10	0	0	0	7	21	
<i>Xanthomonas</i> sp.	2	Leaf	38	31	37	35	45	45	39	61	131	35	59	114	60	0	42	34	23	16	16	38	45	
<i>Aeromonas</i> sp.	1	Soil	38	0	0	13	58	60	0	10	25	23	20	10	26	0	38	24	10	0	0	14	18	
Rhizobiaceae																								
<i>Chromobacterium lividum</i>	2	Soil, leaf	42	0	0	14	59	53	0	38	73	30	42	88	58	0	0	22	5	0	25	25	29	
Achromobacteriaceae																								
<i>Achromobacter</i> sp.	6	Soil	18	15	40	24	37	48	21	27	125	46	51	74	61	2	46	50	20	15	38	38	40	
<i>Flavobacterium</i> sp. I	2	Leaf	10	10	0	7	34	42	13	0	64	14	28	47	49	0	10	10	27	0	30	22	21	
<i>Flavobacterium</i> sp. II	4	Leaf	44	30	42	39	62	44	32	47	126	40	59	142	35	0	38	35	20	0	13	35	44	
<i>Flavobacterium</i> sp. III	5	Leaf	22	68	0	30	105	49	21	10	106	59	58	107	55	0	48	38	10	25	26	38	44	
<i>Flavobacterium</i> sp. IV	1	Leaf	63	0	0	21	73	58	0	17	90	56	49	45	39	0	47	30	10	0	10	23	32	
Enterobacteriaceae																								
<i>Escherichia coli</i>	1	Parasite	61	0	0	20	85	57	0	10	64	46	44	43	102	0	37	34	10	0	30	32	34	
<i>Aerobacter cloacae</i>	5	Soil, leaf, litter	43	0	0	14	66	46	13	20	63	22	38	45	42	0	34	27	3	0	10	20	26	
<i>Serratia</i> sp.	2	Soil	54	0	0	20	85	57	0	5	58	21	34	20	52	0	28	20	0	0	10	16	23	
Micrococaceae																								
<i>Micrococcus</i> spp.	8	Leaf	51	17	38	35	86	52	29	34	130	42	62	107	38	3	94	97	21	3	34	50	52	
<i>Sarcina</i> sp.	1	Litter	13	0	89	34	103	40	61	112	145	57	86	142	61	0	94	102	11	0	25	54	62	
Bacillaceae																								
<i>Bacillus megaterium</i>	3	Soil	29	30	33	31	60	70	65	77	134	64	78	109	54	0	116	85	22	0	42	54	58	
<i>B. cereus</i>	5	Soil, leaf, litter	49	20	17	29	65	30	30	71	110	56	60	55	46	3	113	127	20	10	43	52	51	
<i>B. mycoides</i>	2	Soil, leaf, litter	75	30	25	43	82	40	32	117	150	109	88	81	103	0	96	162	41	10	53	63	71	
<i>B. polymyxa</i>	3	Soil	38	13	0	17	50	49	25	55	115	58	59	118	55	0	66	47	22	0	42	44	44	
<i>B. circulans</i>	2	Soil	27	30	120	59	75	51	10	52	142	65	66	145	131	0	180	168	43	90	99	107	84	
<i>B. pantothenicus</i>	1	Soil	27	30	20	26	60	46	37	58	112	41	59	40	65	0	138	118	42	10	32	56	52	
<i>B. sphaericus</i>	3	Soil	15	30	24	23	0	50	64	43	115	31	51	114	52	0	81	88	35	0	44	52	46	
Actinomycetaceae																								
<i>Nocardia</i> sp. I	3	Soil, litter	22	30	41	31	45	68	40	34	127	44	60	128	63	0	62	20	37	29	26	46	48	
<i>Nocardia</i> sp. II	3	Soil	25	30	52	32	39	41	27	22	135	37	50	132	51	0	95	58	22	24	33	52	48	
Myxococcaceae																								
<i>Myxococcus</i> sp.	1	Soil, litter	10	30	98	46	30	120	95	50	139	70	84	120	17	0	56	60	20	10	69	44	58	

Antibiotics tested: Bacterial, polymyxin (1), bacitracin (2), tyrothricin (3); Streptomycete, streptomycin (4), neomycin (5), spiramycin (6), oleandomycin (7), erythromycin (8), tetracycline (9); Fungal, penicillin (10), patulin (11), griseofulvin (12), gliotoxin (13), viridin (14), frequentin (15), mycophenolic acid (16), mixed Gliocladium (17). Description of the bacterial types is given in Stout (1958, 1960*a, b*).

insensitive. Inhibition occurred most commonly with the Gram-positive bacteria although one strain of *Micrococcus* proved insensitive to the streptomycete plugs, and a strain of *Sarcina* and the majority of the *Bacillus* species were unaffected by the moulds. The results are given in Tables 5 and 6. Most of the streptomycetes tested showed inhibitory activity against some bacterial strains although the two strains of *Streptomyces hygroscopicus* (Table 5, nos. 4 and 5) were appreciably more effective than the others. The pattern of bacterial sensitivity resembled that shown by the streptomycete antibiotics.

Table 5. *Inhibition of bacterial growth in potato carrot agar by 8 streptomycete plugs grown on soya bean agar*

All the *Pseudomonodaceae* (nine strains), *Rhizobiaceae* (one strain) and *Enterobacteriaceae* (four strains) tested showed no inhibition. Figures given are the sum of the diameters of the four zones of inhibition for each plate in mm. Where more than one strain was used the average for the taxon is given.

Bacteria tested. Taxa	No. of strains	Streptomycete plugs used								Total inhibition
		1	2	3	4	5	6	7	8	
Achromobacteriaceae										
<i>Flavobacterium</i> sp. I	3	0	0	0	0	0	0	0	0	0
<i>Flavobacterium</i> sp. II	1	9	9	8	18	13	8	9	0	74
<i>Flavobacterium</i> sp. III	1	8	9	8	20	26	0	8	0	79
<i>Flavobacterium</i> sp. IV	1	0	0	0	0	0	0	0	0	0
<i>Flavobacterium</i> sp.	3	7	3	2	15	15	0	2	0	44
<i>Flavobacterium</i> sp.	1	0	0	0	0	0	0	0	0	0
<i>Flavobacterium</i> sp.	2	0	0	0	0	0	6	0	0	6
<i>Flavobacterium</i> sp.	1	10	11	0	0	26	8	10	6	71
Micrococcaceae										
<i>Micrococcus</i> sp.	1	17	8	9	22	19	0	6	6	87
<i>Micrococcus</i> sp.	1	0	0	0	0	0	0	0	0	0
Bacillaceae										
<i>Bacillus cereus</i>	2	0	3	0	14	18	0	0	3	33
<i>B. mycoides</i>	1	0	0	0	18	18	0	0	0	36
Actinomycetaceae										
<i>Nocardia</i> sp.	1	12	0	0	18	22	0	0	0	52
<i>Nocardia</i> sp.	1	0	0	0	26	16	0	8	0	50
Total inhibition		63	43	27	151	168	22	43	15	532
No. taxa inhibited		6	6	4	8	9	3	6	3	10

The few fungi which showed inhibitory activity included the most commonly recorded antibiotic-producing genera, namely *Aspergillus*, *Penicillium*, *Gliocladium*, *Fusarium* and particularly *Trichoderma* (see Spector, 1957). *Trichoderma viride* was easily the most effective. *Aspergillus fumigatus*, *Gliocladium roseum*, *Penicillium frequentans*, *Fusarium culmorum* and *Cylindrocarpon radicicola* were effective against one or two bacterial strains. The dominant soil fungi, such as *Rhizoctonia* (Thornton, 1958), and the fungi found on leaves (*Pithomyces chartarum* syn. *Sporidesmium bakeri* and *Stemphylium*) were ineffective.

Inhibition by *Trichoderma viride* generally resembled the pattern of sensitivity to viridin but was not absolutely identical. Thus *Bacillus mycoides* and *B. circulans*, the two organisms most sensitive to viridin, were also sensitive to *T. viride*, but other viridin-sensitive bacilli, such as *B. cereus* and *B. megaterium*, were not.

Table 6. *Inhibition of bacterial growth in tryptone yeast extract agar by plugs of fungi grown on soya bean agar*

All the Pseudomonadaceae (5 strains), Rhizobiaceae (1 strain) and Enterobacteriaceae (3 strains) tested showed no inhibition. Figures given are the sum of the diameters of the four zones of inhibition for each plate in mm.

Bacteria tested. Taxa	No. of strains	Fungal plugs used																Total inhibition
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Achromobacteriaceae																		
<i>Flavobacterium</i> sp. I	1	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	14
<i>Flavobacterium</i> sp. II	1	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	14
<i>Achromobacter</i> sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Micrococaceae																		
<i>Micrococcus</i> sp.	1	0	0	0	20	0	45	0	0	0	0	0	0	0	0	0	0	65
<i>Micrococcus</i> sp.	1	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	20
<i>Sarcina</i> sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillaceae																		
<i>Bacillus megaterium</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cereus</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. mycoides</i>	1	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	18
<i>B. polymyxa</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. circulans</i>	1	0	0	0	14	0	45	0	0	0	0	0	20	24	0	0	0	103
<i>B. sphaericus</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinomycetaceae																		
<i>Nocardia</i> sp. I	1	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	12
<i>Nocardia</i> sp. III	1	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0	26
Myxobacteriaceae																		
<i>Myxococcus</i> sp.	1	20	0	0	0	22	0	0	0	0	0	0	22	0	0	0	0	64
Total inhibition		20	0	0	84	22	194	0	0	0	0	0	42	24	0	0	0	386
No. taxa inhibited		1	0	0	2	1	8	0	0	0	0	0	2	1	0	0	0	9

Fungi tested: *Aspergillus fumigatus* (1), *Penicillium nigricans* (2), *Penicillium janthinellum* (3), *Penicillium frequentans* (4), *Gliocladium roseum* (5), *Trichoderma viride* (6), *Absidia glauca* (7), *Mucor hiemalis* (8), *Zygorhynchus moelleri* (9), *Mortierella elongata* (10), *Rhizoctonia* sp. (11), *Cylindrocarpum radiculicola* (12), *Fusarium culmorum* (13), *Fusarium avenaceum* (14), *Phthomycescharitum* (15), *Stemphylium* sp. (16).

The first is associated with decaying herbage and the last three with growing herbage. The remainder are soil fungi.

Table 7. *Growth of bacterial inocula on 16 plates previously streaked with bacteria*

Medium: soya bean agar. Measure of growth: 0, no growth; 1, poor growth; 2, moderate growth; 3, good growth.

Bacteria tested. Taxa	No. of strains	Degree of growth by inocula																Control	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Pseudomonadaceae																			
<i>Pseudomonas aeruginosa</i>	1	1	2	1	3	1	2	1	2	2	2	2	2	2	2	0	2	2	2
<i>P. fluorescens</i> I	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1
<i>Pseudomonas</i> sp. III	1	0	0	0	2	0	1	0	0	1	0	0	0	0	2	0	2	0	1
<i>Xanthomonas</i> sp.	1	2	3	0	0	1	1	2	2	2	1	2	2	0	2	0	2	2	2
<i>Aeromonas</i> sp.	1	3	3	3	3	1	2	3	3	3	3	3	2	3	3	2	3	3	3
Achromobacteriaceae																			
<i>Flavobacterium</i> sp. I	1	2	2	0	2	0	2	2	0	2	2	2	1	1	2	2	2	2	2
<i>Flavobacterium</i> sp. II	1	2	3	0	3	1	0	2	3	1	2	3	2	0	2	0	3	2	2
<i>Achromobacter</i> sp.	1	0	1	0	0	0	1	1	1	0	0	1	0	0	0	0	2	1	1
Rhizobiaceae																			
<i>Chromobacterium lividum</i>	1	2	3	1	3	1	1	1	2	2	1	1	1	2	3	1	2	2	2
Enterobacteriaceae																			
<i>Escherichia coli</i>	1	3	3	3	3	1	3	3	3	3	3	2	2	3	3	0	3	3	3
<i>Aerobacter cloacae</i>	1	3	3	3	3	1	3	3	3	2	2	2	2	3	3	1	3	3	3
<i>Serratia</i> sp.	1	3	3	3	3	1	2	2	3	3	2	2	1	3	3	3	3	3	3
Micrococaceae																			
<i>Micrococcus</i> sp.	1	0	3	1	0	0	0	1	1	1	0	0	1	0	1	0	2	2	2
<i>Micrococcus</i> sp.	1	1	2	1	3	1	1	2	2	2	2	2	1	2	2	0	2	2	2
<i>Sarcina</i> sp.	1	0	2	0	0	0	0	0	0	1	0	0	1	0	0	0	1	2	2
Bacillaceae																			
<i>Bacillus megaterium</i>	1	1	2	0	0	0	2	2	2	2	1	1	1	0	2	0	2	2	2
<i>Bacillus cereus</i>	1	0	2	0	0	0	1	2	0	2	0	1	1	0	2	0	2	2	2
<i>Bacillus mycoides</i>	1	1	2	0	0	1	2	2	2	1	0	1	1	1	1	0	2	2	2
<i>Bacillus polymyxa</i>	1	2	2	2	2	1	3	3	2	2	1	1	1	2	3	1	2	3	3
<i>Bacillus circulans</i>	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1
<i>Bacillus sphaericus</i>	1	0	2	1	0	0	1	1	1	1	0	2	1	0	1	0	1	2	2
Actinomycetaceae																			
<i>Nocardia</i> sp. I	1	1	2	1	1	2	2	2	2	2	2	2	2	1	2	0	2	2	2
<i>Nocardia</i> sp. III	1	1	2	1	0	1	2	2	2	2	2	2	1	2	3	0	2	2	2
Myxobacteriaceae																			
<i>Myxococcus</i> sp.	1	0	2	0	0	0	0	1	1	0	0	0	2	0	0	0	2	2	2
Total	24	28	49	21	31	14	31	39	37	38	27	31	30	27	41	13	45	49	49

Streaked bacteria: *Pseudomonas fluorescens* I (1), *Pseudomonas* sp. III (2), *Pseudomonas chlororaphis* (3), *Xanthomonas* sp. (4), *Aeromonas* sp. (5), *Micrococcus* sp. (6), *Sarcina* sp. (7), *Flavobacterium* sp. I (8), *Flavobacterium* sp. III (9), *Flavobacterium* sp. IV (10), *Aerobacter cloacae* (11), *Serratia* sp. (12), *Bacillus cereus* (13, 14), *Bacillus polymyxa* (15), *Nocardia* sp. I (16).

Similarly, *Micrococcus* but not *Sarcina* was inhibited by the fungus though both were sensitive to viridin; and the flavobacteria and nocardias were inhibited by the fungus though they were not particularly sensitive to viridin.

The same bacterial strains used in the fungi tests were also tested against eight yeasts (*Cryptococcus laurentii*, *C. albidus*, *Rhodotorula marina*, *Candida curvata*, *C. humicola*, *Hansenula saturnis*, *Trichosporon cutaneum*, *Sporobolomyces roseus*). The results were inconclusive. There was no evidence of inhibition on the streak plates but on the plug plates some bacteria (*Xanthomonas*, *Aeromonas*, Flavobacterium) showed a very slight zone of inhibition round the plug. Since the yeasts grew on the sides of the plug and on to the surface of the plate after inoculation the significance of this very limited inhibition is not clear. It may have been due to competition for nutrients, but since *Aeromonas*, a facultative anaerobe, was affected it could not have been due in this case at least to induced anaerobiosis under the plug.

Inhibitory effect of bacterial streak plates

The results are shown in Table 7. Some bacteria (e.g. the aerogenic fermenters) grew well while others (e.g. some pseudomonads and bacilli) grew very poorly on the soya bean agar pre-streaked with bacteria. This was due partly to the limited nutrient status of the agar and partly to its desiccation during the period of incubation when the bacterial streaks were growing. Consequently, these plates did not give a clear picture of the relative sensitivity of the different taxa tested. However, the results did indicate that certain of the streaked bacteria, namely *Bacillus polymyxa*, *Aeromonas*, and *Pseudomonas chlororaphis*, did possess appreciably greater inhibitory powers than the other strains. *Aeromonas* and *P. chlororaphis* also proved to be two of the most effective bacteria in inhibiting the growth of yeasts (di Menna, 1961).

DISCUSSION

The present evidence does not support the thesis that sensitivity to antibiotics is a major factor in the discontinuous distribution of free-living bacteria. The principal taxon found on pasture leaves, often in enormous numbers and commonly absent from soil, namely *Flavobacterium* Type I (see Stout, 1960*b*), is no more sensitive to these antibiotics than the common soil pseudomonads. Other minor leaf taxa, however, are more sensitive and consequently may be excluded from the soil by this factor. The most sensitive group are the aerobic spore-forming bacilli which are amongst the most widespread and numerous of soil organisms. Clearly other attributes are more important in determining the ecology of these bacteria. One such attribute may be the capacity to produce antibiotics (see Brian, 1957). In this respect the bacilli and nocardias, typical soil bacteria, are the most commonly recorded bacterial sources of antibiotics (Spector, 1957). In the present experiments both *Bacillus polymyxa* and *Pseudomonas chlororaphis* appeared to have some inhibitory effect on bacterial growth. This conforms to their known production of polymyxin and chlororaphin, respectively (Florey *et al.* 1949). It is interesting to note that polymyxin also inhibited the growth of *B. polymyxa*. A similar situation obtains with megacin and *B. megaterium* (Ivánovics & Nagy, 1958). A possible explanation of this curious anomaly, at variance with the fungal evidence (Brian,

1957), is that the antibiotic is produced during spore formation (Bernlohr & Novelli, 1959) and the spores, as distinct from the vegetative cells, may be unaffected.

Although *Pseudomonas fluorescens* is often dominant in the indigenous grassland soils of New Zealand (Stout, 1958) and in the exotic pastures developed from them (Stout, 1960*a*), *P. aeruginosa* has not been isolated from these soils. There does not appear to be any distinction in their sensitivity to antibiotics to account for this difference in distribution between the saprophyte and the parasite. Similarly, although *Escherichia coli* may be recovered from herbage and from soil in a grazed pasture it does not appear to persist in either habitat, despite its relative tolerance of antibiotics.

The suggestion that sensitivity to antibiotics may afford a useful taxonomic criterion (Shewan, Hodgkiss & Linton, 1954) receives some support from this study, though a wider range of strains of the individual taxa would need to be tested before it could be applied with confidence. Amongst suggestive results the complete insensitivity of both strains of *Bacillus sphaericus* tested to streptomycin at the concentrations used, was noteworthy, while some of the sharpest contrasts between different taxa were obtained with bacitracin and tyrothricin.

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