The Antibiotic Relationships of Some Yeasts from Soil and Leaves

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SUMMARY

The antibiotic powers of some common soil and leaf bacteria, streptomyces and moulds were tested against soil and leaf yeasts. Twelve of the seventeen bacteria used, ten of the eleven streptomyces strains and seven of the twenty-two moulds were inhibitory. *Pseudomonas chlororaphis*, *Aeromonas* sp., five streptomyces and *Trichoderma viride* inhibited 70 % or more of the twenty-five yeast species used. The most frequently isolated soil yeasts were amongst those most sensitive to antibiotics. Only one species of leaf yeast but several soil yeasts were inhibited by leaf bacteria and moulds. As streptomyces do not occur on leaves, it seems that the wide seasonal fluctuations in kinds and numbers of yeasts on leaves are due rather to nutritional and physical factors than to antibiotic ones.

INTRODUCTION

In New Zealand it has been found that the soil yeast flora varies qualitatively from place to place with soil type and vegetation but not with season (di Menna, 1955, 1957, 1958, 1960*a, b*), whilst the yeast flora on the leaves of pasture plants changes in type with season but not with locality (di Menna, 1959*a*). Yeasts from pasture leaves reach the soil under them in large numbers but are unable to grow there or even to survive for long (di Menna, 1960*b*). An examination of some physiological characters of soil and leaf yeasts showed little difference between the two groups (di Menna, 1959*b*), so it was suggested that antagonistic factors originating from other members of the soil population kept the two floras distinct. The present work reports a study of some interactions between yeasts common in soil or on leaves and the bacteria, streptomyces and moulds which have been found to occur most frequently in soil and on pasture plant leaves in New Zealand.

ORGANISMS USED

The yeasts used as test organisms were much the same as those used in the examination of physiological characters (di Menna, 1959*b*). Some modifications of the list, deletions, additions and changes in probable habitat, have been made with progress in the survey work. Twenty-five species were used, eleven from soil, seven from leaves, and seven whose habitat is still uncertain but which have appeared sufficiently frequently in cultures from soil and leaves to warrant inclusion under the heading Uncertain Habitat. Two isolates of each species were used.

From soil: *Hansenula saturnus* (Klocker) H. & P. Sydow, *H. californica* (Lodder) Wickerham, *H. mrakii* Wickerham, *Cryptococcus albidus* (Saito) Skinner, *C. diffluens* (Zach) Lodder & Kreger-van Rij, *C. terreus* di Menna, *Candida humicola* (Daszewska) Diddens & Lodder, *C. curvata* (Diddens & Lodder) Lodder & Kreger-van Rij, *C. muscorum* di Menna, *Trichosporon pullulans* (Lindner) Diddens & Lodder, *T. cutaneum* (de Beurm., Gougerot & Vaucher) Ota.

From leaves: *Sporobolomyces roseus* Kluyver & van Neil, *Cryptococcus laurentii* (Kufferath) Skinner, *Torulopsis ingeniosa* di Menna, *Rhodotorula glutinis* (Fres.) R. Harrison, *R. mucilaginis* (Jorg.) Harrison, *R. marina* Phaff, Mrak & Williams, *R. graminis* di Menna.

Uncertain habitat: *Saccharomyces delbrueckii* Lindner, *Debaryomyces hansenii* Guill. & Peju, *Cryptococcus luteolus* (Saito) Skinner, *Candida tropicalis* (Cast.) Berkhout, *C. guilliermondii* (Cast.) Langeron & Guerra, *C. parapsilosis* (Ashf.) Langeron & Talice, *Schizoblastosporion starkeyi-henricii* Ciferri.

One isolate of each of the antagonist organisms was used. Eleven strains of bacteria from soil and six from leaves were selected and provided by Dr J. D. Stout (see Stout, 1958, 1960*a, b*). The nomenclature is used according to *Bergey's Manual* (1957).

From soil: *Pseudomonas chlororaphis*, *P. fluorescens*, *Aeromonas* sp., *Achromobacter* sp., *Aerobacter cloacae*, *Serratia* sp., *Bacillus cereus* 'smooth' form, *B. cereus* 'rough' form, *B. sphaericus*, *Nocardia* sp., *Myxococcus* sp.

From leaves: *Xanthomonas* sp., *Flavobacterium* sp. 1, *Flavobacterium* sp. 2, *Aerobacter* sp., *Micrococcus* sp. 1, *Micrococcus* sp. 2.

Eleven strains of streptomyces from soil were used. Ten of these had been selected for their antibiotic producing ability and were provided by T. R. Vernon and one was provided by J. D. Stout. Three of these strains have subsequently proved to be of *Streptomyces hygroscopicus* and one of *S. pilosus*. Streptomyces are virtually absent from pasture plant leaves in New Zealand (T. R. Vernon, personal communication).

Fourteen moulds characteristic of soil and eight characteristic of leaves were selected and provided by R. H. Thornton (see Thornton, 1958, 1960).

From soil: *Absidia glauca* Hagem, *Mortierella alpina* Peyronel, *M. elongata* Linm, *Mucor hiemalis* Wehmer, *Zygorhynchus moelleri* Vuill, *Trichoderma viride* Pers. ex Fr., *Penicillium janthinellum* Biourge, *Gliocladium roseum* Bain, *Fusarium oxysporum* Schlecht emend. Snyder & Hansen, *F. culmorum* (W. G. Smith) Sacch., *Cylindrocarpon radiclecola* Wr., *Papulospora* sp., *Rhizoctonia* sp., unidentified isolate SB 235.

From leaves: *Hormodendron cladosporioides* (Fr.) Sacc., *Fusarium avenaceum* (Fr.) Sacc., *F. nivale* (Fr.) Ces., *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis, *Stemphylium* sp. and unidentified isolates LA 190, LA 270 and HU 47.

In addition, the inhibitory effects of the following purified antibiotics were tested: gliotoxin, frequentin, patulin, viridin, mycophenolic acid and mixed *Gliocladium roseum* antibiotics (all supplied by Dr P. W. Brian, I.C.I. Laboratories, Welwyn, Hertfordshire, England), griseofulvin (Glaxo Laboratories, Greenford, Middlesex, England), actidione (Upjohn Co.) and polymyxin B sulphate (Pfizer Co.).

One medium was used, both for preliminary growth of the antagonists and for the growth of test organism with antagonist. This was the soy bean agar (SBA) of

Smith, Gordon & Clark (1952). All organisms used grew well on it, and although it is high in total nutrients it is low in reducing sugars (about 2.8 g./l.) which may interfere with antibiotic phenomena. The final pH value of the cultures varied between pH 6 and 8.5, but was usually on the alkaline side of neutrality.

A slightly different technique was used with each group of antagonists. Bacteria were streaked across the dried surfaces of SBA plates, four streaks per 10 cm. diam. plate. They were incubated for 4 days. Suspensions of yeasts in sterile tapwater were then spread between, but not touching, the bacterial colonies. There was no evidence that the preliminary bacterial growth had exhausted the nutrients in the medium.

Streptomyces were grown on SBA plates for 14 days. Plugs 0.6 cm. in diameter were cut from the cultures and placed, growth side uppermost, on SBA plates seeded with yeasts.

Moulds were grown on SBA plates for 7 days. Half the culture was then cut away along a diameter of each plate and replaced with sterile molten SBA. On this solidified surface yeast suspensions were streaked up to the cut edge of the mould culture.

Purified antibiotics were diluted to 1/1000. Sterile water was used as a diluent, except for some of the sparingly water-soluble compounds where the primary dilution of 1/100 was made up in a 1 + 4 by vol. acetone + water mixture. Sterile filter paper disks soaked in antibiotic solution were placed on the surface of SBA plates seeded with yeasts.

All cultures were incubated at 20°. Yeast cultures used as inocula were 2–7 days old. Yeast-antagonist cultures were read after 2 days of incubation and read again after 3 days of incubation.

RESULTS AND DISCUSSION

Results are given in Tables 1, 2 and 3 and are summarized in Table 4.

Twelve of the seventeen bacterial strains used showed inhibitory powers. *Aeromonas* sp. was most active, strongly inhibiting all the yeasts tested except *Trichosporon cutaneum* which it inhibited only weakly. *Pseudomonas chlororaphis* also inhibited all yeasts, but inhibition of *Candida muscorum* and *T. cutaneum* was weak. Only seven of the twenty-two moulds showed antibiotic powers. *Trichoderma viride* was most active; *Papulospora* sp. and *Cylindrocarpon radiculicola* were the only others used in which more than a very low degree of activity could be demonstrated. Eight of the eleven streptomyces used inhibited more than half the yeasts tested.

Actidione, gliotoxin and viridin were the most strongly zymostatic of the purified antibiotics used. Polymyxin B, frequentin and mixed *Gliocladium roseum* antibiotics were moderately so. The antibiotic spectrum of viridin was similar to that of the isolate of *Trichoderma viride* used. At a concentration of 1/1000 the purified antibiotic inhibited both isolates of *Hansenula mrakii* which the growing culture used did not affect; the culture did not inhibit any yeasts resistant to the antibiotic. Mixed *Gliocladium roseum* antibiotics inhibited eleven yeast isolates belonging to eight species but the culture of *G. roseum* used inhibited none. Either the method employed was unfavourable to the production of *G. roseum* antibiotics, or else the strain used produced antibiotics at only a low concentration.

Cryptococcus laurentii, *C. albidus*, *C. luteolus*, *Candida curvata* and *Schizoblastosporion starkeyi-henricii* were the most sensitive of the yeasts examined, being inhibited by twenty or more of the fifty antagonist organisms used. The least

Table 1. Sensitivity of soil and leaf yeasts to soil and leaf bacteria

	Yeasts from soil										Yeasts from leaves										Uncertain habitat									
From soil																														
<i>P. chlororaphis</i>																														
<i>P. fluorescens</i>																														
<i>Aeromonas</i> sp.																														
<i>Aerobacter</i> sp.																														
<i>Aerobacter cloacae</i>																														
<i>Serratia</i> sp.																														
<i>B. cereus</i> 'S' form																														
<i>B. sphæricus</i>																														
<i>Nocardia</i> sp.																														
From leaves																														
<i>Flavobacterium</i> sp. 1																														
<i>Aerobacter</i> sp.																														
<i>Micrococcus</i> sp. 1																														

+, Inhibition of both yeast isolates by antagonist; ±, inhibition of one yeast isolate by antagonist, or weak inhibition of both.
 No sensitivity was observed to *Bacillus cereus* 'rough' form or to *Myxococcus* sp. from soil, or to *Xanthomonas* sp., *Flavobacterium* sp. 2 or *Micrococcus* sp. 2 from leaves.

Table 2. Sensitivity of soil and leaf yeasts to soil streptomycetes

Uncertain habitat	<i>S. starkeyi-henricii</i>	+	+	+	+	+	+	+	+	+	+	+
	<i>C. parapsilosis</i>	+	+	+	.	.	+	+	.	.	+	.
	<i>C. guilliermondii</i>	+	+	.	.	.	.	.	.	.	.	+
	<i>C. tropicalis</i>	+	+	+	.	+	+	+	.	.	+	+
	<i>C. luteolus</i>	+	+	+	+	+	+	+	.	+	+	+
	<i>D. klockeri</i>	+	+	+	.	.	+	+	.	.	+	.
Yeasts from leaves	<i>S. delbrueckii</i>	.	.	.	.	.	.	+	.	+	+	.
	<i>R. graminis</i>	+	+	.	+	+	+	.	+	+	+	+
	<i>R. marina</i>	+	+	.	+	+	+	+	+	+	+	.
	<i>R. mucilaginosa</i>	+	+	+	+	+	+	+	.	+	+	.
	<i>R. glutinis</i>	+	+	.	+	+	+	+	.	+	+	.
	<i>S. roseus</i>	+	+	+	+	+	+	+	.	+	+	+
	<i>T. ingensiosa</i>	+	+	+	+	+	+	+	.	+	+	+
Yeasts from soil	<i>C. laurentii</i>	+	+	+	+	+	+	+	.	+	+	.
	<i>T. cutaneum</i>	+	.	+	+	.	.	.	.	+	+	.
	<i>T. pullulans</i>	+	+	+	+	+	+	+	+	+	+	+
	<i>C. muscorum</i>	+	+	+	+	+	+	.	.	+	+	.
	<i>C. curvata</i>	+	+	+	+	+	+	.	+	+	+	.
	<i>C. humicola</i>	.	.	+	+	+	+	.	+	+	+	.
	<i>C. terreus</i>	.	+	+	+	+	+	.	.	+	+	.
	<i>C. diffluens</i>	+	+	+	+	+	+	+	.	+	+	.
	<i>C. albidus</i>	+	+	+	+	+	+	.	+	+	+	+
	<i>H. mrakii</i>	.	.	.	.	.	.	.	.	+	+	.
	<i>H. californica</i>	.	+	+	+	+	.	.	.	+	+	+
	<i>H. saturnus</i>	.	+	.	+	+	+	.	.	+	+	+

×4.3

×4.4

×22.2

×37.2

×59.2

×60.1

×62.1

×62.2

×62.3

70/71

+, Inhibition of both yeast isolates by antagonist; $\pm$, inhibition of only one isolate. One strain of streptomycetes, $\times 11.1$, showed no antibiotic powers. Strain $\times 4.3$ has been identified as *Streptomyces pilosus*; strains $\times 37.2$, $\times 62.2$ and $\times 62.3$ as *S. hygroscopicus*.

Table 3. Sensitivities of soil and leaf yeasts to soil and leaf moulds and to some purified antibiotics

	Yeasts from soil												Yeasts from leaves										Uncertain habitat											
Moulds from soil																																		
<i>C. radicicola</i>																							<i>S. starkeyi-henricii</i>	+	+	+	+	+	+	+	+	+	+	+
<i>F. culmorum</i>																							<i>C. parapsilosis</i>	.	.	.	.	.	.	.	.	.	.	.
<i>F. oxysporum</i>																							<i>C. guilliermondii</i>	.	.	.	.	.	.	.	.	.	.	.
<i>Papulospora</i> sp.																							<i>C. tropicalis</i>	.	.	.	.	.	.	.	.	.	.	.
<i>Rhizoctonia</i> sp.																							<i>C. luteolus</i>	+	+	+	+	+	+	+	+	+	+	+
<i>T. viride</i>																							<i>D. klockeri</i>	.	.	.	.	.	.	.	.	.	.	.
Moulds from leaves																																		
LA 190																							<i>S. delbrueckii</i>	.	.	.	.	.	.	.	.	.	.	.
Purified antibiotics																																		
Actidione																							<i>R. graminis</i>	.	.	.	.	.	.	.	.	.	.	.
Frequentin																							<i>R. marina</i>	.	.	.	+	.	.	.	.	.	.	.
Mixed Gliocladium antibiotics																							<i>R. mucilaginosus</i>	.	.	.	.	.	+	.	.	.	.	.
Gliotoxin																							<i>R. glutinis</i>	.	.	.	+	.	.	.	.	.	.	.
Viridin																							<i>S. roseus</i>	.	.	.	+	.	.	.	.	.	.	.
Polymyxin B																							<i>T. ingensiosa</i>	.	.	.	+	.	.	.	.	.	.	.
																							<i>C. laurentii</i>	+	+	+	+	+	+	+	+	+	+	+
																							<i>T. cutaneum</i>	.	.	.	.	.	.	.	.	.	.	.
																							<i>T. pullulans</i>	.	.	.	.	.	.	.	.	.	.	.
																							<i>C. muscorum</i>	.	.	.	.	.	.	.	.	.	.	.
																							<i>C. curvata</i>	+	+	.	.	.	.	.	.	.	.	.
																							<i>C. humicola</i>	+	.	.	.	.	.	.	.	.	.	.
																							<i>C. terreus</i>	.	.	.	+	.	.	.	.	.	.	.
																							<i>C. diffluens</i>	.	.	.	.	.	.	.	.	.	.	.
																							<i>C. albidus</i>	+	+	.	.	+	+	+	+	+	+	+
																							<i>H. mrakii</i>	.	.	.	.	.	.	.	.	.	.	.
																							<i>H. californica</i>	.	.	.	.	.	.	.	.	.	.	.
																							<i>H. saturnus</i>	.	.	.	.	.	.	.	.	.	.	.

+, Inhibition of both yeast isolates by antagonist; ±, inhibition of only one isolate or weak inhibition of both.

No sensitivity was observed to *A. glauca*, *M. alpina*, *M. elongata*, *M. hiemalis*, *Z. moelleri*, *P. janthinellum* or unidentified SB235 from soil, to *F. acenaceum*, *F. nivale*, *H. cladosporoides*, *P. chartarum*, *Stemphylium* sp. or the unidentified LA 270 and HU 47 from leaves, or to the purified antibiotics patulin, mycophenolic acid or griseofulvin.

Table 4. Total sensitivities of soil and leaf yeasts to bacteria, streptomycetes and moulds from soil and leaves

	Yeasts from soil												Yeasts from leaves					Uncertain habitat							
	<i>H. saturnus</i>	<i>H. californica</i>	<i>H. mrakii</i>	<i>C. albidus</i>	<i>C. diffluens</i>	<i>C. terreus</i>	<i>C. humicola</i>	<i>C. curvata</i>	<i>C. muscorum</i>	<i>T. pullulans</i>	<i>T. cutaneum</i>	<i>C. laurentii</i>	<i>T. ingeniosa</i>	<i>S. roseus</i>	<i>R. glutinis</i>	<i>R. mucilaginosus</i>	<i>R. marina</i>	<i>R. graminis</i>	<i>S. delbrueckii</i>	<i>D. kloeckeri</i>	<i>C. luteolus</i>	<i>C. tropicalis</i>	<i>C. guilliermondii</i>	<i>C. parapsilosis</i>	<i>S. starkeyi-henricii</i>
From soil																									
Bacteria (11)	3	3	5	8	4	7	2	7	2	3	2	5	3	3	2	2	3	3	9	4	6	3	2	2	6
Streptomycetes (11)	6	6	2	9	8	6	6	8	7	10	5	8	9	9	7	8	8	8	3	4	9	6	2	4	9
Moulds (14)	1	1	0	4	1	2	2	3	1	1	1	5	2	2	2	1	2	1	1	1	3	0	0	0	3
Total soil strains	10	10	7	21	13	15	10	18	10	14	8	18	14	14	11	11	13	12	13	9	18	9	4	6	18
From leaves																									
Bacteria (6)	0	0	1	3	0	2	0	2	0	0	0	2	0	0	0	0	0	0	2	0	2	1	1	1	2
Moulds (8)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total all strains	10	10	8	24	13	17	11	20	10	14	8	20	14	14	11	11	13	12	15	9	20	10	5	7	20

Figures in table are numbers of organisms to which a yeast species has shown some sensitivity. Figures in parentheses are numbers of antagonist organisms used.

sensitive were *Hansenula saturnus*, *H. californica*, *H. mrakii*, *Debaryomyces kloederi*, *Candida tropicalis*, *C. guilliermondii*, *C. parapsilosis*, *C. muscorum* and *Trichosporon cutaneum* which were inhibited by ten or less. Some of these sensitivities are perhaps predictable in the light of what is known of yeast ecology, others seem paradoxical. Almost all the fermenting species tested (*Hansenula* spp., *S. delbrueckii*, *D. kloederi*, *C. tropicalis*, *C. guilliermondii* and *C. parapsilosis*) are included in the low sensitivity group. Fermenting yeasts generally appear to be commonest in habitats such as spoiling food, rotting fruit and fungus fruiting bodies, tree exudates and the animal gut, where nutrients are high, competition intense, and lack of sensitivity to antibiotics probably has high survival value.

Soil yeasts were relatively sensitive to antibiotics produced *in vitro* by other soil micro-organisms; indeed the most frequently isolated were the most sensitive. Of the soil yeasts with low sensitivities *Hansenula* spp. seem to be common in New Zealand only in those rarely occurring soils that are both water-logged and with a pH approaching neutrality (di Menna, 1960*b*; and unpublished). *Candida muscorum* has been found as the dominant yeast of only a few rather acid soils (Wright, 1959) where, according to the evidence of Jackson (1958), inhibitory effects will be decreased and resistance to antibiotics not a critical factor. *Trichosporon cutaneum* can be found in most New Zealand soils, but never as more than a small percentage of the total yeast population. It seems that resistance to antibiotics is not in itself of paramount importance in determining the ability of a yeast to live in soil.

It is perhaps significant that of the two rather similar soil species, *Candida humicola* and *C. curvata*, *C. humicola* was sensitive to eleven antagonist organisms and *C. curvata* to twenty. In New Zealand *C. curvata* has been the dominant yeast in a number of mineral soils and some acid peats. *C. humicola* on the other hand has been found mainly in the root zones of tussock plants (di Menna, 1958) and in the fermenting layer of forest litter (di Menna, 1960*b*), both relatively rich and presumably competitive environments.

Leaf yeasts as a group were almost completely insensitive to either leaf bacteria or moulds. The only demonstrable effects were that one isolate of *Cryptococcus laurentii* was inhibited by *Micrococcus* sp. 1 and that both *C. laurentii* isolates were weakly inhibited by *Aerobacter* sp. *Aerobacter* sp. and *Flavobacterium* sp. 1 showed moderate antibiotic activity against soil yeasts and yeasts of uncertain habitat, but of the group of eight leaf moulds the only one with any inhibitory powers was the unidentified LA 190 which inhibited *Candida humicola*. Although leaf bacteria may prevent some soil yeasts from establishing themselves in the phyllosphere, it seems that the great quantitative and qualitative changes which occur seasonally in the leaf yeast population are due to alterations in physical conditions and availability of nutrients rather than to antibiotic activities of the rest of the leaf microflora.

Leaf yeasts as a whole appeared only slightly more sensitive to soil bacteria, streptomycetes and moulds than soil yeasts, so that it seems as though antibiotics play little part in preventing leaf species from establishing themselves in soil. However, the data obtained here show only the antibiotic producing potentialities of the antagonists tested, not the pattern of antibiotic activity in soil. The effective antibiotic producers in soil may be, for instance, *Papulospora* sp. and the streptomycetes x4.3 and x60.1 which inhibit leaf rather than soil species. It will be necessary to work with soil itself to clarify this point.

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