THE DISTRIBUTION OF KERATINOPHILIC FUNGI IN SOILS FROM NEW ZEALAND, AND FROM TWO POLYNESIAN ISLANDS

by

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(with 1 fig.)

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Surveys carried out in many parts of the world have demonstrated that the soil is a reservoir for certain fungal species, potentially pathogenic to mammals. Different techniques have however been used to determine the ecological importance of soil for species capable of causing systemic and superficial infections. Dermatophytes and other keratinophilic fungi have usually been isolated from soil by the well-tried hair-baiting technique introduced by VANBREUSEGHEM in 1952.

In the following paragraphs the results obtained by this method from soil samples collected in New Zealand are reported. A small number of soils collected inRarotonga, Cook Islands and in Tokelau Islands has also been examined and the results obtained from this material are reported here. The figure is a map to show the location of these islands in relation to those of Australasia.

The two Polynesian island groups are very different in character. Rarotonga is the main island of the Southern Cook group. It is a volcanic island with an area of 16,602 acres, partially surrounded by a fringing coral reef. It rises to a height of 2,140 ft. in the centre and has approximately 8,600 human inhabitants, living mainly in coastal villages. The Tokelau islands are three small atolls situated almost on the equator. Their total area is 1,500 acres. The population of the different islands varies from 500 to 700. Although the available material from these areas is limited, the results obtained are of considerable interest, since little is known of the distribution of dermatophytes in Polynesia.

The results obtained from the New Zealand and Island soil collections have, in the discussion, been compared only with those

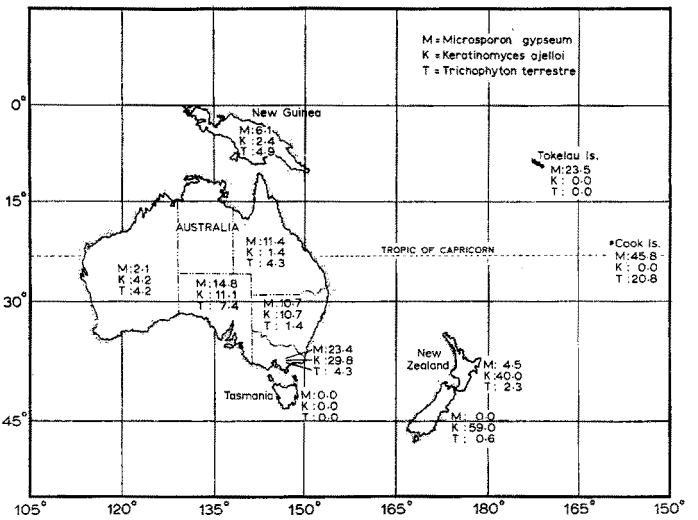

Fig. 1. Map showing the percentage incidence of *Microsporon gypseum, Keratinomyces ajelloi* and *Trichophyton terrestre* in Australasia and two Pacific islanf groups. (Australian figures from DURIE & FREY 1962)

reported from other countries in the Southern Hemisphere. Very many soil surveys have been carried out in nothern latitudes, some in heavily populated countries. It is difficult to equate conditions in these areas with those of sparsely populated countries such as New Zealand. It has therefore seemed more profitable and less pedestrian to confine comparison of the present results to those obtained in surveys carried out elsewhere in the Southern Continents.

MATERIALS AND METHODS

A total of 312 soil samples have been investigated for the presence of keratinophilic fungi. Of these 271 were obtained from the North and South Islands of New Zealand, 24 from Rarotonga and 17 from the Tokelau Islands.

The New Zealand material consisted of surface soils collected in plastic bags from different areas of the two islands. Each sample was retained in its bag and stored at 4° C if it could not be processed immediately. At the time of collection, the origin and type of soil was noted, and the pH of 125 samples was measured, using a Cambridge pH meter (No. L 297, 813).

Approximately 50 g of each sample was placed in a sterile petri dish, moistened with distilled water and baited with autoclaved fragments of human hair, guinea pig hair and hedgehog quills. The plates were incubated at 27° C, and examined at intervals for 4 weeks, after which they were discarded. Material from colonised

hairs or quills was examined microscopically. Hairs which appeared to be carrying a dermatophyte were transferred to plates of Sabouraud glucose agar, containing cycloheximide and chloramphenicol (GEORG & CAMP, 1957) but supplemented with traces of thiamine and inositol. Dermatophyte species were identified by the macroscopic and microscopic features observed in these subcultures.

The 41 samples from the Pacific Islands were collected in the same way. They were sent to the laboratory by surface mail, and were therefore stored for considerably longer periods, and not always at refrigerator temperatures, before being investigated. It is possible that this prolonged storage may have affected the results obtained by hair-baiting. GRIFFIN (1960) has shown that there is a succession of colonising fungi in the keratin-soil micro-habitat, and it is possible that species, such as *Microsporon gypseum*, which are late colonisers were at an advantage in these stored samples.

No detailed information of the source of the island samples was available, so that this material could not be included in the classification of the samples into soil types. Since the Tokelaus are atolls, they have very little surface soil, and the appearance of the samples indicated that they had been collected from areas heavily contaminated by animals.

RESULTS

The overall results of hair-baiting are shown in table I. Five keratinophilic species were isolated from New Zealand soils and 3 from the Islands samples. Table II shows the distribution of the species, found in New Zealand, in different kinds of soil. The samples were grouped as:– (1) garden soil, taken from areas in surrounding houses and supporting the growth of flowers or vegetables; (2) farmyard soil, collected from areas in and about stables, cattle houses, hen runs etc. and therefore subject to high animal contamination; (3) woodland and ploughed areas with a low animal population.

The keratinophilic species were not evenly distributed in the different areas, nor in the different soil types within New Zealand. *M. gypseum* was recovered much more frequently from the Pacific Island than the New Zealand collections. This species was isolated from 45.8% of Rarotongan and 23.5% of Tokelau soils, so that 36.6% of the 41 Pacific samples yielded strains of this species. *M. gypseum* was only isolated from 1.5% of the 271 New Zealand samples, all strains being recovered from soil collected in the North Island. The difference in incidence of *M. gypseum* in Polynesian and New Zealand soils is significant ($p < .001$). Neither the difference in incidence in the two Polynesian areas nor in the two islands of New Zealand is significant. One of the strains of *M. gypseum* recovered from a Rarotongan soil, spontaneously produced perfect stages on the soil plates but it could not be assigned to any of the

TABLE I

The distribution of keratinophilic species in soils of New Zealand, Rarotonga and Tokelau Islands

Area	Number of Samples	*Microsporon gypseum*		*Microsporon cookei*		*Trichophyton terrestre*		*Keratinomyces ajelloi*		*Chrysosporium* sp.	
		No.	%	No.	%	No.	%	No.	%	No.	%
North Island	88	4	4.5	1	1.1	2	2.3	35	40.0	1	1.1
South Island	183	0	—	20	10.9	1	0.6	108	59.0	1	0.6
Total New Zealand	271	4	1.5	21	7.7	3	1.1	143	52.4	2*	0.7
Rarotonga	24	11	45.8	0	—	5	20.8	0	—	1	4.2
Tokelau	17	4	23.5	0	—	0	—	0	—	0	—
Total Pacific Is.	41	15	36.6	0	—	5	12.2	0	—	1	2.4

*) *Chrysosporium keratinophilum.*

TABLE II

The distribution of keratinophilic fungi in different soil types in New Zealand

Soil type	Region	Number of samples	*Microsporon gypseum*		*Microsporon cookei*		*Trichophyton terrestre*		*Keratinomyces ajelloi*		*Chrysosporium keratinophilum*	
			No.	%	No.	%	No.	%	No.	%	No.	%
Garden	N. Island	49	3	6.1	0	—	2	4.1	29	59.2	1	2.0
	S. Island	68	0	—	0	—	1	1.5	49	72.1	0	—
	New Zealand	117	3	2.6	0	—	3	2.6	78	66.6	1	0.8
Farmyard	N. Island	5	0	—	1	20.0	0	—	4	80.0	0	—
	S. Island	78	0	—	20	25.6	0	—	53	68.0	0	—
	New Zealand	83	0	—	21	25.3	0	—	57	68.7	0	—
Ploughed & woodland (low animal population)	N. Island	34	1	2.9	0	—	0	—	2	5.9	0	—
	S. Island	37	0	—	0	—	0	—	6	16.2	1	2.7
	New Zealand	71	1	1.4	0	—	0	—	8	11.3	1	1.4
Total	New Zealand	271	4	1.5	21	7.7	3	1.1	143	52.4	2	0.7

Nannizzia species described by STOCKDALE (1962).

Microsporon cookei was isolated from 21 (7.7%) of New Zealand samples, 20 of which came from the South Island. Although this might appear to be a significant difference in geographical distribution ($.001 < p < .01$), this finding is misleading. All strains of *M. cookei* were recovered from farmyard soils, and unfortunately only 5 such samples were included in the North Island collection. *M. cookei* was isolated from 1 out of these 5 soils and from 20 out of 78 South Island farmyard samples. It appeared to be closely associated with an animal contaminated substrate. The species was not isolated from the polynesian material.

Trichophyton terrestre was isolated from 3 New Zealand samples, all of garden origin and from 5 samples derived from Rarotonga, but was not recovered from Tokelau material. The incidence in Polynesia was significantly greater than in New Zealand ($p < .001$). These polynesian strains of *T. terrestre* also spontaneously produced perfect stages in primary culture.

Keratinomyces ajelloi was far the most common species which could be recovered by baiting New Zealand soils. It was isolated from 52.4% of the 271 samples, and did not differ significantly in its distribution in the two islands. It was found to be equally prevalent in garden and farmyard soils, being recovered from 66.6% and 68.7% respectively. It was much less frequently encountered in woodland and ploughed soils being recovered from only 11.3% of 71 such samples. It appeared, however, to be associated with acid substrates. Table III shows the distribution of *K. ajelloi* in relation to pH in 125 samples. Only 15 of these had a pH of less than 6, and 12 of these yielded isolates of *K. ajelloi*. The species was only isolated from 30 of the 110 samples with a pH greater than 6. This association with strongly acid conditions is significant ($p < .01$).

TABLE III

Distribution of Keratinomyces ajelloi in relation to pH of soil

Soil pH	No. of samples	*Keratinomyces ajelloi*	
		present	absent
< 6.0	15	12	3
> 6.0	110	30	80
Total	125	42	83

Keratinomyces ajelloi was not isolated from any of the polynesian island samples. This was an unexpected result. Unfortunately the pH values of these soils was not measured, so that the bearing of the hydrogen-ion concentration on this result cannot be discussed.

The prolonged storage of the samples might have been unfavourable for the survival of *K. ajelloi*, but this seems unlikely, since GRIFFIN (1960) regards *K. ajelloi*, like *M. gypseum*, as a late coloniser of hair baits.

Two strains of *Chrysosporium* were isolated from New Zealand soils, one from each island. These strains were sent to Miss P. M. STOCKDALE and Dr. J. W. CARMICHAEL, who kindly consented to examine them. They were identified as *Chrysosporium keratinophilum*. A third strain, isolated from a Rarotongan soil was assigned to this genus by Dr. L. AJELLO, who was good enough to examine it, but its species has not yet been determined.

DISCUSSION

The results reported above provide some interesting problems of distribution, and much further and more detailed investigations are necessary for their elucidation. In table IV the distribution of *M. gypseum*, *K. ajelloi* and *T. terrestre* reported from different areas of the Southern Hemisphere is tabulated. It will be seen that the incidence of these species in the different countries varies considerably, and it is possible to postulate a number of factors which might contribute to this variation. In the following paragraphs individual species are discussed in turn.

TABLE IV.

The distribution of some keratinophilic species in soils of certain areas in the Southern Hemisphere.

Country	No. of samples	*M. gypseum*	*K. ajelloi*	*T. terrestre*	Publication
Australia mainland	623	11.1	10.4	2.4	DURIE & FREY (1962)
Tasmania	7	0	0	0	
New Guinea	82	6.1	2.4	4.9	
Brazil	27	29.6	11.1	—	LONDERO & RAMOS (1961)
Uruguay	140	40.7	25.7	—	YARZABEL (1961)
Pacific Is.	41	36.6	0	12.2	This survey
New Zealand	271	1.5	52.4	1.1	

Microsporon gypseum

The results obtained in the present survey suggest that *M. gypseum* is prevalent in the soils of tropical polynesian islands, sparse in the subtropical North Island of New Zealand and rare or absent from the soils of its temperate South Island. If no other figures were available, it could be inferred that the distribution of this dermatophyte was related to climatic conditions, and that

its incidence was increased by high environmental temperatures. This conclusion is shown to be incorrect when the results of surveys conducted in other countries are considered. The figure shows the percentage incidence of *M. gypseum* in different areas of Australia and in New Guinea, as reported by DURIE & FREY (1962), together with the findings of the present investigation. It can readily be seen that latitude alone does not account for the distribution of this species, and this conclusion is confirmed by the findings of LONDERO & RAMOS (1961) and YARZABEL (1961) in South America, which are included in table IV.

DURIE & FREY (1962) found the highest incidence of keratinophilic fungi in soils frequented by man and his domestic animals, but they do not analyse the distribution of individual species in relation to their soil types. YARZABEL and his colleagues (1960) found *M. gypseum* to be common in soils of Uruguay, for they isolated it from 54% of 53 samples, but its distribution did not appear to be affected by the type of substrate examined (YARZABEL, 1961). LONDERO & RAMOS (1961) found that shade was important in the distribution of keratinophilic fungi. The majority of their isolates came from shady cultivated soils and they suggested that direct sunlight had an inhibitory effect on these species.

It is difficult to account for the rarity of *M. gypseum* in New Zealand soil. Many of the samples examined were contaminated by animal products, for 83 came from farmyards and 117 from gardens, most of whose soils would have been enriched from time to time by the application of manure. Yet *M. gypseum* was not isolated from any of the farmyard samples and one of the 4 strains isolated was recovered from a woodland soil. One possibility to be considered was that failure to isolate *M. gypseum* was due to the effects of competition for the keratin bait by *K. ajelloi*, which appears to be prevalent in New Zealand soils. This does not provide a satisfactory explanation, when the results obtained in the other surveys are examined. Although DURIE & FREY (1962) isolated *K. ajelloi* from 29.8% of 47 soils collected in Victoria, they were able to isolate *M. gypseum* from 23.4% of the same samples. YARZABEL (1961) recovered strains of *M. gypseum* from 40.1% of 140 soils, in spite of the presence of *K. ajelloi* in 25% of his samples. Competition between the two species does not therefore appear to account for the low incidence of *M. gypseum* in New Zealand.

It seems possible that exposure to direct sunlight may be associated with the relative absence of the species in this country. Although much of New Zealand was originally covered with dense bush, this has largely been removed, and most of the soil in the South Island is exposed to strong sunshine. Shade trees are rather more common in the North Island, as they are required for the protection of domestic animals. Shade may play a part, also, in promoting the presence of *M. gypseum* in the Pacific Island soils.

Both Rarotonga and the Tokelaus are tropical and have a high rainfall, fairly evenly distributed throughout the year. It is probable that the soils sampled were protected from the effects of direct sunlight.

The pH of the soil may also play a part in determining the distribution of *M. gypseum*. Further investigations of the relation of pH of the substrate to the distribution of keratinophilic fungi are at present in progress.

Although *M. gypseum* is a relatively common member of the soil flora in the polynesian islands, it does not appear to be more important as a cause of human ringworm than has been found elsewhere. Very few scrapings from ringworms in Rarotonga have been available for examination, but none have yielded *M. gypseum* in culture. On the other hand more than 300 specimens collected in the Tokelau Islands from human lesions, clinically suspected to be of mycotic origin, have been examined in this laboratory. *M. gypseum* was identified in only 0.6% of positive cultures (MARPLES unpublished). The presence of this species in soil of other Pacific Islands can be assumed from the fact that it was isolated in 1.6% of 306 positive cultures of material from human ringworms occurring in the Solomon Islands (SMITH & MARPLES, 1964).

In New Zealand, in spite of the rarity of its isolation from the soil, *M. gypseum* appears to make some contribution to the aetiology of human ringworm. In the Auckland area it has formed 0.8% of 1,380 positive cultures. Its annual incidence however, varies considerably, and most of the cases have come from one suburb (RUSH-MUNRO, personal communication). In the South Island although infections with *M. gypseum* do occur from time to time they are far from common. In one diagnostic laboratory in Dunedin, the species was not isolated at all during the years 1963 and 1964 (FITZGERALD, personal communication).

Keratinomyces ajelloi

The incidence of *K. ajelloi* in New Zealand soils appears to be unusually high, since strains of this species were isolated from more than 50% of the samples. Much of New Zealand consists of grassland, which supports populations of sheep, varying in density with the type of cover. It seems possible that the constant increments of wool, which the soil receives, serve as an enrichment of the substrate for *K. ajelloi*. The association of the species with strongly acid soils is suggestive and its widespread dominance may be related to pH of the substrate.

The absence of *K. ajelloi* from the soil samples of the Pacific Island is curious. Although their small number makes it impossible for any conclusions to be drawn, the following factors might play a part in its distribution and are worthy of further investigation. High environmental temperatures may be inhibitory to the presence of *K. ajelloi* in soils. In DURIE & FREY'S (1962) survey the species

was isolated from only 2 out of 81 New Guinea soils and 1 out of 70 samples from Queensland, while 45 strains were isolated from 451 samples in the less tropical New South Wales. But *K. ajelloi* was isolated in 11.1% of samples from Brazil (LONDERO & RAMOS, 1961) so that ambient temperature alone does not appear to account for the distribution of the species.

The rarity of domestic mammals in the Pacific Islands could contribute to the scarcity of *K. ajelloi* in these soils. There are however dense populations of rats, at least in the Tokelau islands, and these could be expected to provide adequate mammalian supplements to the substrate. On the other hand, poultry is an important part of the economy of these islands, and the effect on the soil could be to inhibit the growth of keratinophilic fungi. DE VRIES (1962) states that litter from chicken houses is extremely poor in these species. The high incidence of *M. gypseum* in the Polynesian samples indicates that these soils were suitable for the survival of keratinophilic fungi. Moreover, *K. ajelloi* was recovered from 4 out of 15 samples taken from New Zealand hen runs in the present survey, so that the species appears to be able to withstand conditions provided by bird litter.

The pH of the soil may play a part in the difference in incidence of *K. ajelloi* in Pacific Island and New Zealand soils. In view of the preponderance of coral and its widespread use for flooring domestic buildings, it is probable that the village soils in the Pacific Islands have an alkaline reaction. The association of *K. ajelloi* with strongly acid soils in New Zealand was observed during the present investigation, and it is possible that while acid conditions favour the species, alkaline substrates are less readily colonised. Studies of the affects of altering the soil pH on the keratinophilic flora are in progress in Dunedin, as are more extensive investigations of soils from other areas in Oceania.

Other keratinophilic fungi

In New Zealand, *M. cookei* has occasionally been isolated from the hair of non-domestic animals (MARPLES, 1960), but it is not common in this habitat. Nevertheless it seems probable that the skin and hair of mammals rather than the soil itself, is the primary habitat of the species, for the 21 strains isolated during the present survey all came from broken up soils, heavily contaminated by animal products. DURIE & FREY (1962) in Australia did not isolate *M. cookei* from their soil samples, but RIDLEY (1962) has recorded its presence in a survey carried out in Queensland. ROGERS & BENEKE (1964) have recently reported the isolation of 2 strains of *M. cookei* from 202 soil samples collected in Brazil, but it was not isolated in the earlier South American surveys, so that the species must be somewhat rare in the Southern hemisphere.

Trichophyton terrestre has also been isolated from animals in

New Zealand. Strains resembling soil isolates were recovered from the hair or quills of 4 out of 187 hedgehogs (*Erinaceus europaeus*), while a pigment-producing variant was found in 10 of these animals and was regarded as a member of the normal cutaneous flora (MARPLES & SMITH, 1962). The strains reported in the present survey were all of the non-pigmented soil type. No explanation of the relatively high incidence of the species in Pacific Island soils can be offered.

The isolation of two strains of *Chrysosporium keratinophilum* from New Zealand soil and of the genus from the Pacific Islands requires no comment. The genus is obviously not of importance in the keratinophilic flora, of these areas at least as demonstrated by hair-baiting techniques. The results obtained in the preliminary survey, here reported underline the necessity for far more detailed investigation of the source and character of the soil samples, before the factors controlling the distribution of keratinophilic fungi can be elucidated.

Summary

(1) A total of 312 soils, 271 from New Zealand, 24 from Rarotonga, Cook Islands and 17 from Tokelau Islands was examined by the hair-baiting technique.

(2) *Microsporon gypseum* was isolated from 1.5% of New Zealand soils and from 36.6% of Pacific Island soils. The difference in incidence in the two areas is significant.

(3) *Keratinomyces ajelloi* and *Microsporon cookei* were recovered from 52.8% and 7.7% of New Zealand soils respectively but neither species was isolated from the Island samples. *K. ajelloi* was found to be significantly associated with strongly acid soils, and *M. cookei* with a farmyard substrate.

(4) *Trichophyton terrestre* was isolated from 1.1% of New Zealand and from 12.2% of Pacific Island soils. Two strains of *Chrysosporium keratinophilum* were isolated from New Zealand soils and an unidentified *Chrysosporium* from one Rarotongan sample.

(5) The distribution of the different species is discussed and compared with those reported in surveys carried out in other countries of the Southern Hemisphere. The effects of ambient temperature, and source and pH of the soil, on the distribution of keratinophilic fungi are considered, but there is insufficient detailed evidence to determine the importance of these factors in the ecology of these organisms.

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