

The distribution and ecology of *Malassezia furfur* and cutaneous bacteria on human skin

J.P. LEEMING*, F.H. NOTMAN† & K.T. HOLLAND *Department of Microbiology, University of Leeds, Leeds LS2 9JT, UK*

Received 28 November 1988 and accepted 24 January 1989

LEEMING, J.P., NOTMAN, F.H. & HOLLAND, K.T. 1989. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria on human skin. *Journal of Applied Bacteriology* 67, 47-52.

The prevalence and abundance of *Malassezia furfur* on clinically normal skin at 20 sites has been determined. All of the 16 subjects studied (young adults) had high counts on the upper trunk and on at least one site on the head. Counts obtained from the lower trunk and upper thighs of male subjects were considerably higher than those from female volunteers. Organisms isolated, grouped according to their colony morphologies, had distributions consistent with those noted for '*Pityrosporum ovale*' and '*P. orbiculare*' in previous studies. Mean *M. furfur* counts were compared with aerobic and anaerobic bacterial counts performed at the same sites. Propionibacteria and *M. furfur* distributions were similar but not identical. Some factors which might limit the range of *M. furfur* are discussed.

Malassezia furfur (synonyms *Pityrosporum ovale* and *P. orbiculare*) has been recognized for many years as both a saprophyte of human skin (predominantly in its yeast phase) and as a cause of the mild but chronic skin disorder pityriasis versicolor (in which it exhibits a mixed hyphal and yeast morphology). Interest in this organism has increased in recent years, however, as it has been implicated in the pathogenesis of various other dermatological conditions, including dandruff, seborrhoeic dermatitis and folliculitis, by the favourable response of these conditions to anti-fungal therapy (Shuster 1984; Faergemann 1986). *Malassezia furfur* can also cause intravenous catheter-associated systemic infections, particularly in low birth weight infants receiving lipid-rich parenteral nutrition (Powell *et al.* 1984; Danker *et al.* 1987).

Strains of *M. furfur* are nutritionally fastidious and require, to a varying extent, lipid supplementation of growth media. As a

consequence there have been few attempts to define experimentally the range of *M. furfur* on clinically normal human skin and the most extensive have relied on microscopical examination rather than viable counting methods (Roberts 1969). Faergemann, Aly & Maibach (1983) performed a limited cultural survey which did not include any sites on the head, but the medium used fails to isolate many strains of *Malassezia* (Leeming & Notman 1987). The purpose of this investigation was to exploit improved isolation and enumeration methods to perform a comprehensive survey of the distribution of *M. furfur* on human skin and to compare this with the distribution of other cutaneous micro-organisms.

Materials and Methods

SUBJECTS

Sixteen volunteer laboratory workers were studied. All had clinically normal skin and, with the exception of cosmetic applications, none had recently received topical or systemic antimicrobial therapy. The types of soap, deodorants and

* Present address and address for correspondence: Department of Microbiology, Bristol Royal Infirmary, Bristol BS2 8HW, UK.

† Present address: MRC Dental Research Unit, The London Hospital Medical College, London E1 2AA, UK.

shampoos used by subjects were noted; none of these preparations had been used for at least 6 h before skin sampling except on the hands.

SKIN SAMPLING

For each individual 20 samples were taken at skin sites indicated in Table 1. When possible (i.e. on flat sites free of terminal hair) the scrub-wash technique of Williamson & Kligman (1965) was used. An area defined by a stainless steel ring (5 cm² internal area) was washed with two successive 1 ml volumes of phosphate-buffered 0.1% (v:v) Triton X-100 solution (pH 7.9; 'wash fluid'). The skin surface was agitated for 1 min with the flat end of a Teflon rod during each washing and the resultant microbial suspensions were pooled before processing. All other sites were sampled by swabbing the area of interest with a fibre-tipped swab moistened in

wash fluid. The tip of each swab was then dropped into 0.9 ml half-strength wash fluid (wash fluid diluted 1:1 with distilled water) and agitated for 30 s using a vortex mixer to disperse micro-organisms. Attempts were made to sample similar areas of skin at the same site on each individual, but no attempt was made to quantify this accurately and results from swabbed sites are therefore expressed as counts per swab rather than per unit area (see Table 1).

SAMPLE PROCESSING

Serial 10-fold dilutions of each sample were made in half-strength wash fluid. *Malassezia* counts were made by drying four 25 µl drops of appropriate dilutions on the surface of media containing (g or ml/l): bacteriological peptone (Oxoid L37), 10; glucose, 5; ox bile (Oxoid L50), 4; yeast extract (Oxoid L21), 0.1; glycerol

Table 1. The prevalence and mean count of *Malassezia furfur* at 20 body sites. Sites are ranked according to mean count. Where a sex difference was noted, results from males and females are listed separately. Mean counts are calculated for colonized sites only, i.e. zero values are excluded

Site (numbers are those used in Figs 1 & 2)	Prevalence (%)	Geometric mean count†	Range‡		Sampling method
			Lower limit	Upper limit	
6 Chest (lower sternum)	100	1.1×10^4	5.2×10^3	8.4×10^4	Scrub-wash
17 Upper inner thigh, males	100	9.3×10^3	2.6×10^3	3.6×10^5	Scrub-wash
15 Upper back (mid-line)	100	8.9×10^3	1.9×10^3	3.7×10^4	Scrub-wash
4 Forehead (central)	87.5	4.8×10^3	<4	4.7×10^4	Scrub-wash
11 Abdomen (left iliac fossa), males	87.5	1.1×10^3	<4	1.4×10^4	Scrub-wash
16 Lower back (posterior iliac crest), males	100	1.0×10^3	4.0	2.0×10^4	Scrub-wash
5 Cheek	94	1.0×10^3	<4	2.4×10^4	Scrub-wash
16 Lower back, females	62.5	8.9×10^2	<4	3.2×10^3	Scrub-wash
7 Forearm (dorsal)	44	3.8×10^2	<4	2.1×10^3	Scrub-wash
14 Ante cubital fossa	81	7.1×10^1	<4	1.4×10^3	Scrub-wash
17 Upper thigh, females	100	5.8×10^1	4.0	5.2×10^2	Scrub-wash
8 Forearm (volar)	50	2.2×10^1	<4	3.7×10^2	Scrub-wash
9 Hand (dorsal)	31	1.9×10^1	<4	4.4×10^1	Scrub-wash
19 Sole of foot (instep)	44	1.6×10^1	<4	2.2×10^3	Scrub-wash
10 Hand (palm)	56	1.4×10^1	<4	2.0×10^2	Scrub-wash
18 Calf	31	1.4×10^1	<4	8.3×10^1	Scrub-wash
11 Abdomen, females	62.5	1.1×10^1	<4	4.4×10^1	Scrub-wash
2 Ear (external auditory meatus)	100	3.0×10^4	5.4×10^1	5.7×10^5	Swab
12 Umbilicus, males	100	2.3×10^3	9.0	2.6×10^5	Swab
1 Scalp	100	1.4×10^3	9.0	1.8×10^5	Swab
3 Anterior nares	94	1.1×10^3	<9	7.0×10^4	Swab
12 Umbilicus, females	87.5	2.3×10^2	<9	3.1×10^3	Swab
13 Axilla	69	1.4×10^2	<9	9.4×10^3	Swab
20 4th toe cleft*	80	4.0×10^1	<9	6.8×10^2	Swab

† Counts are expressed as cfu per cm² (scrub wash samples) or cfu per swab (swab samples).

* One subject was excluded because cultures from this site yielded confluent, multi-focal cultures of *Trichophyton rubrum*.

monostearate (BDH), 0.5; Tween 60 (Sigma, 0.5; glycerol, 1; whole-fat cows' milk, 10; agar (Oxoid L11) 12 (Leeming & Notman 1987). Bacterial counts were made by depositing four 15 μ l drops on the surfaces of heated blood agar (Oxoid Blood Agar Base No. 2 supplemented with 50 ml/l defibrinated horse blood) and Reinforced Clostridial Agar (Oxoid) supplemented with 0.1 ml/l Tween 80 (Sigma) and 6 mg/l furazolidone (Sigma). *Malassezia* plates were incubated at 34°C for 14 d, heated blood agar plates at 37°C for 2 d and reinforced clostridial agar plates anaerobically at 37°C for 6 d. Organisms were identified by colony type, Gram reaction and cell morphology.

Results

The *Malassezia* counts obtained at 20 body sites are shown in Table 1. Each subject yielded positive cultures at at least 10 sites; prevalences and mean counts were highest at sites on the upper trunk and head, and lowest on areas at the distal reaches of the limbs.

All subjects reported regular or occasional application of antiperspirant/deodorant preparations to the axilla. No difference in *Malassezia* carriage was attributable to the frequency of use of these preparations, but it is nevertheless possible that their application might depress counts. One male subject used a zinc pyrithione-containing shampoo up to three times a week and yielded very low *M. furfur* counts from the scalp and ear, and a negative culture from the forehead. There was no evidence of suppression of bacterial counts by zinc pyrithione.

Several groups of *Malassezia* strains could be differentiated according to colony type. These different groups were stable on repeated subcultivation but could not be reliably differentiated according to microscopic morphology. Large colony, apparently less nutritionally fastidious strains (Leeming & Notman 1987) were particularly prevalent on the upper trunk whereas medium and small colony strains usually predominated at other sites.

A marked sex difference was noted in the carriage of *Malassezia* at sites on the lower trunk and upper thigh. Counts were significantly higher (Mann-Witney U test) on male subjects at the abdomen ($P < 0.05$), lower back ($P < 0.025$) and upper thigh ($P < 0.001$). Mean counts were over 10-fold higher from the umbil-

ical swabs of males than those of females, but this difference was not significant at the 0.05 probability level. There was no indication of sex differences in *Malassezia* carriage at other sites. No statistically significant sex differences in bacterial carriage were noted except at the upper thigh where males yielded higher aerobic bacterial counts ($P < 0.01$). Comparisons of mean *Malassezia* counts with mean *Propionibacterium* and aerobic bacterial counts are shown in Figs 1 and 2. In each case there was a statistically significant association of mean densities: $P < 0.01$ for the *Propionibacterium* vs. *Malassezia* comparison and $P < 0.05$ for aerobic bacterial count vs. *Malassezia* analysis (Spearman's rank correlation). Figures 1 and 2, however, show that although the relationship between *Propionibacterium* and *Malassezia* counts was fairly consistent, the association between mean aerobic bacterial and *Malassezia* count was not consistent. In general, sites associated with elevated hydration (foot, umbilicus and axilla) had high aerobic bacterial counts but low *Malassezia* counts; the reverse was observed at sites on the trunk; sites on the head yielded high counts of both groups and areas with low levels of hydration and sebum production (forearm, hand and calf) had low counts of both groups. Exceptions from these general rules were noted at sites exhibiting the sex differences described previously.

Discussion

This study confirms that *M. furfur* is a major component of the microbial flora of clinically normal skin on young adults. The range of *M. furfur* is clearly more similar to that of the propionibacteria than staphylococci and aerobic coryneform bacteria. An obvious factor which could determine their range is the availability of lipid, which appears to be an essential nutrient for *M. furfur*. McGinley *et al.* (1980) reported that regional variations in *Propionibacterium acnes* population sizes correlate with sebum excretion rates (SERs) but Cove *et al.* (1980) reported that individual variation in propionibacterial counts at a single site (the face) did not correlate with SER. In a study similar to, but larger than that of Cove *et al.*, Kearney *et al.* (1984) observed that individuals with high SERs invariably yielded high propionibacterial counts, but that individuals with low SERs

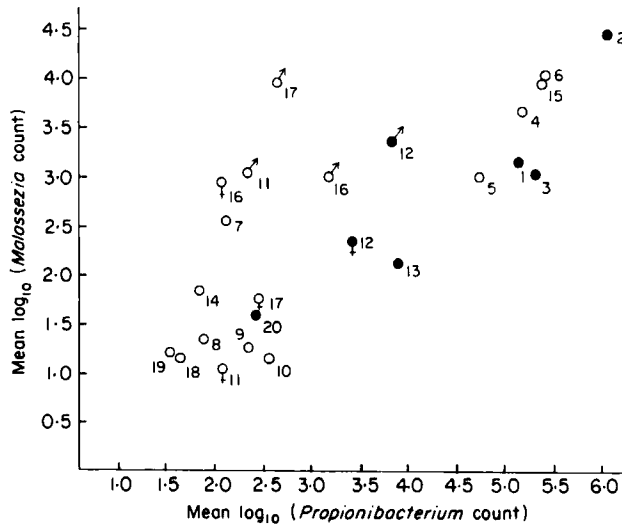


Fig. 1. A comparison of mean *Malassezia* and *Propionibacterium* counts at 20 skin sites. Site numbers are identified in Table 1. ○, scrub-wash samples (cfu/cm²); ●, swab samples (cfu/swab); Where results from male and female subjects are plotted separately, ♂ or ♀ are appended as appropriate.

might have either high or low counts. They concluded that any effect of SER upon bacterial population sizes was likely to be an indirect one. Whether this is also the case with *M. furfur* remains to be proven. *Malassezia furfur*, propionibacteria and staphylococci are known to colonize the lumen of pilosebaceous units (Wolff & Plewig 1976; Noble 1981; Leeming et al. 1984). The profound anatomical differences

between pilosebaceous units at skin sites with differing SERs should therefore also be considered as these will affect the availability of physical niches suitable for microbial habitation.

The extent to which *M. furfur* can multiply in clinically normal stratum corneum outside pilosebaceous units is unknown. It does infiltrate this niche extensively in skin affected with

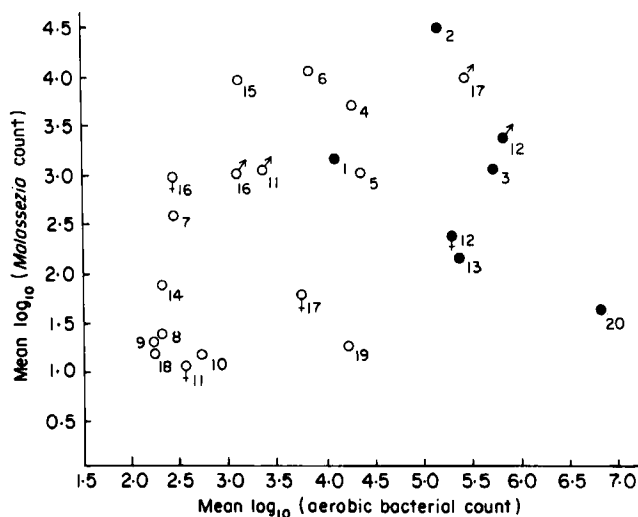


Fig. 2. A comparison of mean *Malassezia* and aerobic bacterial counts at 20 skin sites. Site numbers are identified in Table 1. ○, scrub-wash samples (cfu/cm²); ●, swab samples (cfu/swab); Where results from male and female subjects are plotted separately, ♂ or ♀ are appended as appropriate.

pityriasis versicolour but this is observed only at sites known to harbour large populations of *M. furfur* under normal conditions (particularly the upper trunk). This may well reflect a nutritional dependence upon sebaceous secretions from adjacent pilosebaceous units. Such an explanation would also be consistent with the observation that occluded sites did not have elevated *M. furfur* counts in this study whilst Faergemann *et al.* (1983) reported that population increases were observed after artificial occlusion of the back. This is in contrast with Micrococcaceae and coryneform populations which are clearly sustained at high levels in the occluded stratum corneum of the sole of the foot and fourth toe cleft, which are devoid of pilosebaceous units (Malcolm & Hughes 1980; Marshall *et al.* 1987).

Although *Malassezia* counts correlated much more closely with propionibacterial counts than with aerobic bacterial counts, there were sufficient differences in their distributions to suggest that the niches these groups occupy on the skin differ, as noted at the follicular level in previous studies (Wolff & Plewig 1976; Leeming *et al.* 1984). The most notable divergence was the marked sex difference in *Malassezia* carriage on the lower trunk and upper thigh which was not observed for *Propionibacterium* counts. Insufficient information about the anatomy and physiology of skin in this region is available to predict whether lipid availability, hydration or other factors might be responsible for the observed sex difference.

In much of the published work about the ecology of *M. furfur*, these organisms were divided into *Pityrosporum ovale* and *P. orbiculare*. Although the basis of this differentiation, cell morphology, is now recognized to be unstable and therefore taxonomically dubious (Salkin & Gordon 1977), it is interesting that differential distributions of these groups have been noted by microscopic examination of skin samples (Roberts 1969). Roberts observed that *P. ovale* predominated on the head whereas *P. orbiculare* was more abundant on the trunk. Similar distributional variation was observed in this investigation on the basis of stable colony morphology heterogeneity. Different colony morphotypes were not reliably reflected by different cell morphologies, perhaps due to the culture medium used. The evidence that organisms classified as *M. furfur* (Yarrow &

Ahearn 1984) form a single coherent species is limited and inconclusive, particularly as many strains have only recently been isolated *in vitro* (Leeming & Notman 1987). Midgley (1988) has described subgroups within *M. furfur* based on stable morphological and antigenic differences even amongst the low proportion of strains isolated on Dixon's recovery medium (Van Abbe 1964). Resolution of the taxonomic status of morphological groups within this genus would be of great value not only in the evaluation of future ecological studies but also in consideration of the pathogenesis of the various diseases in which *M. furfur* is increasingly implicated.

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