JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1987, p. 2017–2019
0095-1137/87/102017-03$02.00/0

Vol. 25, No. 10

Improved Methods for Isolation and Enumeration of *Malassezia furfur* from Human Skin

JOHN P. LEEMING†* AND FIONA H. NOTMAN‡

Department of Microbiology, University of Leeds, Leeds LS2 9JT, United Kingdom

Received 30 December 1986/Accepted 1 July 1987

A medium for the isolation and enumeration of *Malassezia furfur* is described. Incubation at 34°C yielded geometric mean counts (in CFU per square centimeter) of 2.6×10^3 on the forehead, 8.5×10^2 on the cheek, and 9.6×10^3 on the back. These counts compared favorably with microscopic counts and greatly exceeded those obtained with previously described media.

Yeasts of the genus *Malassezia* Baillon (synonym, *Pityrosporum* Sabouraud) constitute a major proportion of the saprophytic microflora of normal human skin, particularly in regions well supplied with sebaceous glands (11, 16). They have also been associated with a number of pathological conditions, including pityriasis versicolor (3), folliculitis (1, 21), seborrheic dermatitis and dandruff (5, 7, 15), and fungemia and systemic infections, most notably in low-birthweight infants provided with high-lipid-content infusions (12–14, 20). Most human-associated strains are members of the lipophilic species *Malassezia furfur*. *Malassezia pachydermatis* (nonlipophilic) strains have been isolated from the integuments and diseased ears of a variety of animals (4, 8) but are not frequently found on human skin (2, 11). Studies of the role of *M. furfur* in disease have been severely hampered by the lack of suitable culture media, particularly for its primary isolation and enumeration from either normal or diseased tissue. This investigation was undertaken in an attempt to formulate such a recovery medium.

Medium development. Different solid medium formulations were prepared, and their relative abilities to recover *M. furfur* as CFU from skin washings were assessed. Skin washings were obtained by swabbing the forehead with a moistened sterile microbiological cotton swab and dispersing yeast cells into 0.9 ml of phosphate-buffered 0.05% (vol/vol) Triton X-100 solution (pH 7.9) by vortex mixing for 30 s. Viable counts were determined by spreading 0.1-ml samples of 10-fold dilutions of each sample evenly over the surface of each medium with an L-shaped glass rod. In these initial experiments, the plates were incubated at 37°C for 10 days. Isolates were identified as *M. furfur* on the basis of colonial and microscopic form and the inability to grow on Sabouraud dextrose agar without lipid supplement (16, 23). The media yielding highest viable counts were retained, modified, and retested. In this manner, 164 formulations were considered in 30 stages.

The formula of the final medium (arbitrarily denoted medium A) is shown in Table 1. All components except Tween 60, milk, and antimicrobial agents were added to distilled water, left to stand for approximately 10 min, and then adjusted to pH 6.2 by adding 1 M HCl dropwise. The Tween 60 was warmed to 60 to 70°C before being added to the mixture, which was then heated briefly to boiling to dissolve all components. The milk and antimicrobial solutions were added after the mixture was autoclaved (20 min at 121°C) and cooled to approximately 50°C; the plates were poured immediately. Qualitatively, many components of previously described media were retained in this new formulation, but concentrations were altered to maximize colony counts. Alterations which produced larger colonies did not necessarily give improved counts; for example, ox bile was markedly stimulatory at low concentrations, but when concentrations were increased, counts were depressed despite an increased colony size. A variety of more purified bile-salt preparations such as sodium taurocholate proved less useful in isolating *M. furfur* from some individuals. The incorporation of cow's milk gave better results than a variety of other fat and oil sources (including olive oil, lard oil, lard, lanolin, and Tween 20, 40, and 80) and produced a homogeneous medium which required no lipid overlay. Commercially prepared ultra-high-temperature-treated milk was used for long-term stability. For reproducibility during the experiments, small samples of milk were stored at −20°C until use.

Incubation conditions. Medium A was used to determine the optimum incubation temperature for recovery of colonies from skin washings. Washings were prepared and processed as described above. Samples from clinically normal skin of the upper backs and foreheads of 10 human volunteers were studied. Multiple sets of count plates were prepared from each sample for incubation at 27, 30, 32, 34, 35, and 37°C. The plates were incubated aerobically for 14 days in covered (but not sealed) containers with moistened absorbent paper to prevent desiccation of media.

The recovery of *M. furfur* at different temperatures is shown in Fig. 1. Although optimum temperatures varied according to site (counts obtained from the forehead samples were markedly more sensitive to deviations in temperature than were counts from the back samples), 34°C yielded high recovery rates at both sites and so was used as the incubation temperature for the remainder of the study. This finding is in contrast with standard descriptions of growth temperature optima in the range of 35 to 37°C (16, 23). However, a low optimum temperature is consistent with conditions which *M. furfur* is likely to encounter on human skin (9, 11) and may be an important factor in limiting more deep-seated infections.

Plates examined by eye after 5, 7, 10, 14, and 20 days of incubation demonstrated that colony counts increased up to 14 days but not beyond this period (even on plates incubated at 27 or 30°C).

* Corresponding author.
† Present address: Department of Microbiology, Bristol Royal Infirmary, Bristol BS2 8HW, United Kingdom.
‡ Present address: MRC Dental Research Unit, The London Hospital Medical College, London E1 2AA, United Kingdom.

TABLE 1. Formulas of media compared in this study[a]

Component	Amt added per liter of medium:		
	A	F	V
Bacteriological peptone[b]	10 g	10 g	
Mycological peptone[b]			6 g
Glucose[c]	5 g	40 g	
Malt extract[b]			36 g
Yeast extract[b]	0.1 g	0.1 g	
Ox bile, desiccated[b]	4 g		20 g
Glycerol[c]	1 ml		2 ml
Glycerol monostearate[d]	0.5 g	2.5 g	
Tween 40[c]			10 ml
Tween 60[c]	0.5 ml		
Tween 80[c]		2 ml	
Oleic acid (92%)[d]			2 ml
Olive oil[c]		20 ml	
Whole-fat cow's milk[e]	10 ml		
Agar no. 1[b]	12 g	12 g	10 g

[a] Chloramphenicol (50 μg/ml) and cycloheximide (200 μg/ml) were added to all media after autoclaving. The pHs of media A, F, and V before autoclaving were 6.2, 6.0, and 6.2, respectively.
[b] Oxoid Ltd., Basingstoke, United Kingdom.
[c] Sigma Chemical Co. Ltd., Poole, United Kingdom.
[d] BDH, Poole, United Kingdom.
[e] Average fat content, 3.7% (wt/vol); homogenized, ultra-high-temperature treated (140°C for 3 s); Associated Dairies, Leeds, United Kingdom.

Comparative study. The ability of the developed medium A to recover *M. furfur* from clinically normal skin was compared with that of previously described formulations. Samples were obtained from the foreheads, cheeks, and upper backs (midline interscapular) of 20 healthy volunteer laboratory workers (10 male, 10 female; age range, 21 to 32 years) by the scrub-wash method of Williamson and Kligman (22). A stainless steel ring (5-cm^2 internal area) was held firmly against an area of skin. Two successive 1-min washings of the area enclosed were made with 1-ml volumes of phosphate-buffered 0.1% (vol/vol) Triton X-100 solution (pH 7.9). During each wash, the skin was agitated with the flat end of a Teflon rod. Viable counts were made as described above with medium A, the olive-oil-containing medium F of Faergemann and Fredriksson (6), and the ox-bile-containing medium (V) of van Abbe (19) as modified by G. Midgley (personal communication). The formulas of these media are compared in Table 1. The media of Ushijima et al. (18) were excluded after preliminary studies demonstrated only sparse growth on these plates. Plates were incubated at 34°C for 14 days.

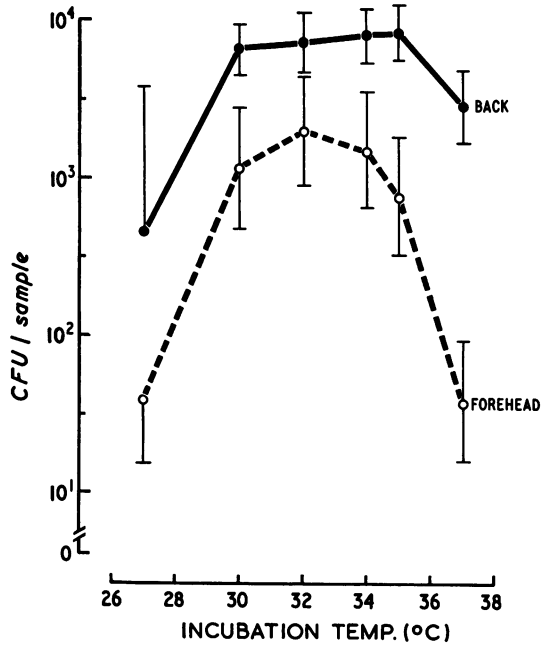

FIG. 1. Effect of incubation temperature on recovery of *M. furfur*. Geometric mean counts (CFU per sample + 1) were obtained from swabs of back (interscapular) and forehead skin. Vertical bars represent 95% confidence limits; n = 10 at each site.

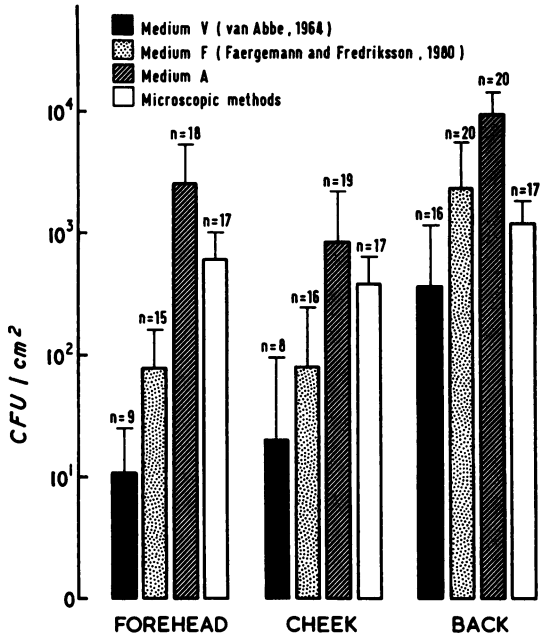

FIG. 2. Geometric mean *M. furfur* colony counts and numbers of positive cultures (n) obtained from scrub-wash samples (see text) from three body sites of 20 individuals by using various recovery media. Zero values were excluded from mean calculations. Vertical bars represent 95% confidence limits.

Microscopic counts of each sample were also performed. Three 0.1-ml samples were dried onto a 12-mm-diameter well of a multispot microscope slide (C. A. Hendley, Essex, United Kingdom). Films were heat fixed and Gram stained, and 40 3 × 10^{-4} cm^2 fields were counted. Yeast cells were counted individually; clumps were only rarely observed and, when observed, consisted of only 2 to 3 cells. Hyphae were not observed.

All statistical comparisons were made by the Wilcoxon matched-pairs signed-rank test (17).

Results of the comparative study are shown in Fig. 2. At each site, medium A yielded counts significantly higher than either media F and V (all $P \leq 0.0002$) or microscopic counts ($P \leq 0.03$). Medium F gave higher counts than medium V ($P < 0.0001$), but comparisons between counts on these media and microscopic counts varied greatly according to site. Medium F yielded significantly lower counts than did microscopic methods on samples from the forehead ($P = 0.002$) but higher counts on samples from the back ($P = 0.02$), with intermediate results on samples from the cheek. A similar trend was noted with medium V but not with medium A. The observation of viable counts which were

higher than microscopic counts may be due to the occlusion of some yeast cells by skin squames and other debris in samples and the failure to identify visible yeast cells as such if they did not conform to the expected form (particularly if they were not budding).

Most strains isolated on medium A had the microscopic form associated with isolates traditionally identified as "*Pityrosporum orbiculare*." However, strains of "*P. ovale*" and "*P. pachydermatis*" authenticated by the National Collection of Pathogenic Fungi, London, United Kingdom (NCPF 3380 and NCPF 3381, respectively), also grew well when subcultured onto this medium.

A variety of colony types were noted on medium A. The relative abundances of different colony forms appeared to vary according to the body site sampled. Comprehensive taxonomic investigations would be useful in determining whether the strain variations noted in this and previous studies (10) result from stable, taxonomically significant genotypic heterogeneity or from less stable phenotypic adaptations to different environments. This information would be of great interest in the elucidation of the pathogenicity and epidemiology of infections with *M. furfur*, as well as its normal ecology. It is hoped that the availability of improved culturing methods will facilitate such studies and promote investigations into the distribution and activities of *M. furfur* in healthy and diseased tissue.

We thank members of the research laboratory of K. T. Holland for help during this investigation.

We were supported by a grant from the United Kingdom Medical Research Council.

LITERATURE CITED

1. **Back, O., J. Faergemann, and R. Hornqvist.** 1985. *Pityrosporum* folliculitis: a common disease of the young and middle-aged. J. Am. Acad. Dermatol. **12**:56–61.
2. **Barnett, J. A., R. W. Payne, and D. Yarrow.** 1983. Yeasts: characteristics and identification, p. 363. Cambridge University Press, Cambridge.
3. **Burke, R. C.** 1961. Tinea versicolor: susceptibility factors and experimental infections in human beings. J. Invest. Dermatol. **36**:389–401.
4. **Dufait, R.** 1985. Présence de *Malassezia pachydermatis* (syn. *Pityrosporum canis*) sur les poils et les plumes d'animaux domestiques. Bull. Soc. Fr. Mycol. Med. **1**:19–22.
5. **Faergemann, J.** 1986. Seborrhoeic dermatitis and *Pityrosporum orbiculare*: treatment of seborrhoeic dermatitis of the scalp with miconazole-hydrocortisone (Daktacort), miconazole and hydrocortisone. Br. J. Dermatol. **114**:695–700.
6. **Faergemann, J., and T. Fredriksson.** 1980. Age incidence of *Pityrosporum orbiculare* on human skin. Acta Dermato-Venereol. **60**:531–533.
7. **Ford, G. P., P. M. Farr, F. A. Ive, and S. Shuster.** 1984. The response of seborrhoeic dermatitis to ketoconazole. Br. J. Dermatol. **111**:603–607.
8. **Kuttin, E. S., and I. Glas.** 1985. Mycotic otitis externa in animals. Mykosen **28**:61–65.
9. **Marples, M. J.** 1965. The ecology of human skin, p. 135–148. Charles C Thomas, Publisher, Springfield, Ill.
10. **Midgley, G.** 1985. Comparison of *Pityrosporum* (*Malassezia*) isolates by morphology and immunoelectrophoresis. Br. J. Dermatol. **113**:783–784.
11. **Noble, W. C.** 1981. Microbiology of human skin, 2nd ed, p. 17–20, 264–268. Lloyd-Luke, London.
12. **Oberle, A. D., M. Fowler, and W. D. Grafton.** 1981. *Pityrosporum* isolate from the upper respiratory tract. Am. J. Clin. Pathol. **76**:112–116.
13. **Powell, D. A., J. Aungst, S. Snedden, N. Hansen, and M. Brady.** 1984. Broviac catheter-related *Malassezia furfur* sepsis in five infants receiving intravenous fat emulsions. J. Pediatr. **105**: 987–990.
14. **Redline, R. W., S. S. Redline, B. Boxerbaum, and B. B. Dahms.** 1985. Systemic *Malassezia furfur* infections in patients receiving Intralipid therapy. Hum. Pathol. **16**:815-822.
15. **Shuster, S.** 1984. The aetiology of dandruff and the mode of action of therapeutical agents. Br. J. Dermatol. **111**:235–242.
16. **Slooff, W. C.** 1970. Genus 6. *Pityrosporum* Sabouraud, p. 1167–1186. *In* J. Lodder (ed.), The yeasts: a taxonomic study, 2nd ed. North-Holland Publishing Co., Amsterdam.
17. **Sokal, R. R., and F. J. Rohlf.** 1981. Biometry, 2nd ed., p. 448–449. W. H. Freeman, San Francisco.
18. **Ushijima, T., M. Takahashi, and Y. Ozaki.** 1981. Selective and differential media for isolation and tentative identification of each species of *Pityrosporum* residing on normal or diseased human skin. Microbiol. Immunol. **25**:1109–1118.
19. **van Abbe, N. J.** 1964. The investigation of dandruff. J. Soc. Cosmet. Chem. **15**:609–630.
20. **Wallace, M., H. Bagnall, D. Glen, and S. Averill.** 1979. Isolation of lipophilic yeast in "sterile" peritonitis. Lancet **ii**:956.
21. **Weary, P. E., C. M. Russell, H. K. Butler, and Y. T. Hsu.** 1969. Acneform eruption resulting from antibiotic administration. Arch. Dermatol. **100**:179–183.
22. **Williamson, P., and A. M. Kligman.** 1965. A new method for the quantitative investigation of cutaneous bacteria. J. Invest. Dermatol. **45**:498–503.
23. **Yarrow, D., and D. G. Ahearn.** 1984. Genus 7. *Malassezia* Baillon, p. 882–885. *In* N. J. W. Kreger-van Rij (ed.), The yeasts: a taxonomic study, 3rd ed. Elsevier Science Publishers B.V., Amsterdam.