JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1995, p. 1005–1007 Vol. 33, No. 4
0095-1137/95/$04.00+0

Improved Detection of *Malassezia* Species in Lipid-Supplemented Peds Plus Blood Culture Bottles

SUSAN C. NELSON, YVONNE C. W. YAU, SUSAN E. RICHARDSON, AND ANNE G. MATLOW*

Department of Microbiology, Hospital For Sick Children, and Department of Microbiology, University of Toronto, Toronto, Canada

Received 29 August 1994/Returned for modification 15 October 1994/Accepted 20 December 1994

The growth of *Malassezia* species in BACTEC Peds Plus blood culture bottles was optimized by using various lipid supplements. Palmitic acid (3%, wt/vol) was superior and overcame the inhibitory effect of blood in mock clinical specimens. Palmitic acid (3%) supplementation of Peds Plus bottles may improve recovery of *Malassezia* species in the BACTEC NR 660.

Malassezia furfur is an important agent of catheter-associated sepsis, primarily in low-birth-weight infants receiving lipids through a central venous line (1, 10–12). The syndrome is characterized by fever, respiratory distress, and thrombocytopenia (6). Recently, *Malassezia pachydermatis* has been implicated in a febrile syndrome similar to that described for *M. furfur* (5, 6, 8).

M. furfur requires lipid-supplemented media for in vitro growth. The Dupont Isolator system has been advocated as the optimal system for its isolation from blood (7). Failure of the organism to generate sufficient CO_2 for detection in unsupplemented BACTEC 6B aerobic broth media (Becton Dickinson and Company, Sparks, Md.) has been reported (7), but there is no published information on the isolation of *Malassezia* spp. from the pediatric Peds Plus (PP) bottle in the BACTEC nonradiometric (NR) system.

Recently in our laboratory, *M. furfur* was recovered from a blood culture in a PP bottle which had not registered a significant (≥20) growth index (GI) value. This prompted us to examine the viability of *Malassezia* species in PP blood culture bottles and to maximize detection in the BACTEC NR 660 system by supplementing the bottles with lipid under mock clinical conditions.

Three clinical strains of *Malassezia* (two *M. furfur* strains [MF1 and MF2] and one *M. pachydermatis* strain) were studied. Isolates were grown at 37°C on Sabouraud's dextrose agar (Difco, Detroit, Mich.) to which several drops of olive oil were added prior to inoculation. Colonies were suspended in sterile phosphate-buffered saline (PBS, Dulbecco A; Oxoid, Basingstoke, Hampshire, England) with 0.1% Triton X-100 (Sigma, St. Louis, Mo.) added to aid organism emulsification (7). The suspensions were adjusted to an optical density at 540 nm of 0.1 (determined by viable counts to be approximately 10^6 CFU/ml), and appropriate dilutions were made in PBS–0.1% Triton X-100 to desired concentrations for inoculation. Inoculated PP bottles were incubated for 7 days in the NR 660 (35°C), with agitation for the initial 48 h. GI values were recorded after 24 h and daily for 7 days. Bottles with positive (≥20) GI values were examined microscopically either by direct examination or by Gram stain, and aliquots were subcultured onto blood agar and Sabouraud's dextrose agar-olive oil overlay and incubated at 35°C for 3 days. All experiments were performed in duplicate, and all positive cultures were confirmed as *Malassezia* species.

We first questioned the ability of the PP bottle to support the viability of *Malassezia* species. Blood (0.5 ml; fresh, unclotted, unheparinized, adult human), which may be toxic to *M. furfur* (7), was added to half the bottles to assess any inhibitory effect. PP bottles were inoculated aseptically with a 1-ml (10^2 yeast cells per ml) suspension of MF1. The viability of *M. furfur* (determined by viability counts) decreased rapidly, with a 50% decrease in cell counts by 48 h and no viable yeasts detected after 5 days of incubation. This decrease was hastened in the presence of blood, with over 80 and 100% cell death after 2 and 3 days of incubation, respectively. GI values remained at <20 throughout the incubation period.

Addition of lipid supplements to BACTEC bottles enhances growth and detection of *M. furfur* (7). To assess the threshold of *Malassezia* detection in olive oil-supplemented bottles, 1-ml suspensions of MF1, MF2, and *M. pachydermatis* (10^1, 10^2, and 10^3 yeasts per ml) in PBS–Triton X-100 were inoculated into PP bottles with or without sterile olive oil (1% [vol/vol] final concentration) and incubated as described above. An initial inoculum of as few as 5 CFU per bottle (determined by viable counts) registered a GI of >20 by day 4 in olive oil-supplemented bottles. Positive GI values were obtained within 3 days of incubation for inocula of ≥50 CFU per bottle. No positive GIs were observed for unsupplemented bottles or for the olive oil control. Results were similar for all *Malassezia* strains studied.

Optimal lipid supplementation of PP bottles was assessed by supplementing the bottles with olive oil (5, 0.5, or 0.05% [vol/vol]), palmitic acid (3, 0.3, or 0.03% [wt/vol]), oleic acid (0.3 or 0.03% [wt/vol]), or Tween 40 (3 or 0.3% [vol/vol]). One milliliter containing 10^2 yeasts (MF1, MF2, or *M. pachydermatis*) was inoculated separately into these bottles as well as the unsupplemented control bottles. For Tween 40, only MF1 was tested. Palmitic acid was superior to all supplements tested in supporting the growth of all three isolates. Figure 1 shows results for MF1; all three concentrations of palmitic acid yielded positive GIs, with 3% palmitic acid supplementation allowing detection of *Malassezia* organisms within 24 to 48 h. Viability counts correspondingly increased to 10^6 to 10^7 CFU/ml in positive samples (data not shown). Oleic acid and Tween 40 did not enable *Malassezia* detection. Olive oil enhanced the growth of all three isolates, although 0.5% olive oil supplementation produced consistently higher and earlier positive GI values than 5% olive oil. No positive GIs were ob-

* Corresponding author. Mailing address: Department of Microbiology, Hospital for Sick Children, 555 University Ave., Toronto, Canada M5G 1X8.

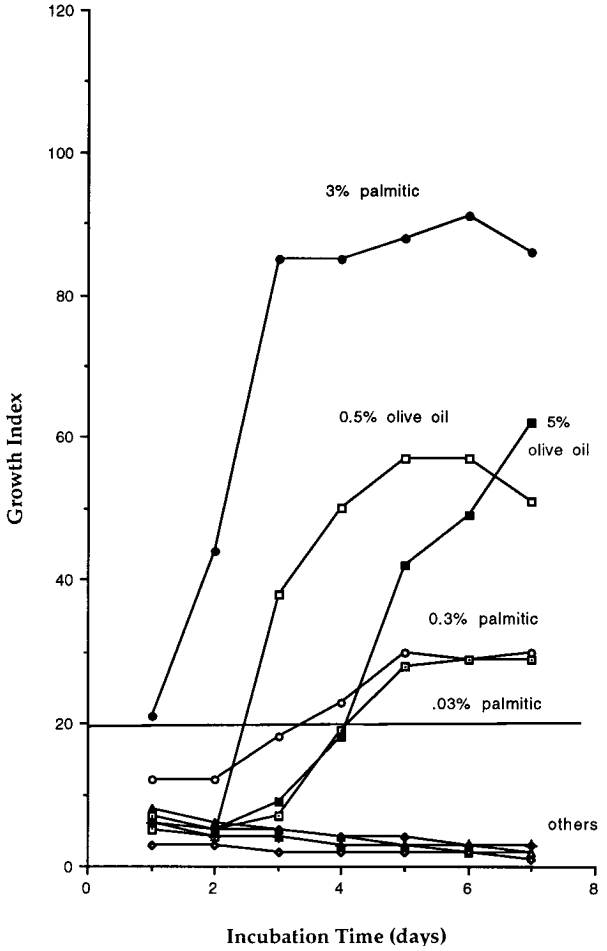

FIG. 1. Growth of *M. furfur* in supplemented PP bottles. A GI of 20 (horizontal rule) is considered significant. "Others" include 0.05% olive oil (+), 0.03% oleic acid (▲), 0.3% oleic acid (▲), 3% Tween 40 (◆), and 0.3% Tween 40 (◆).

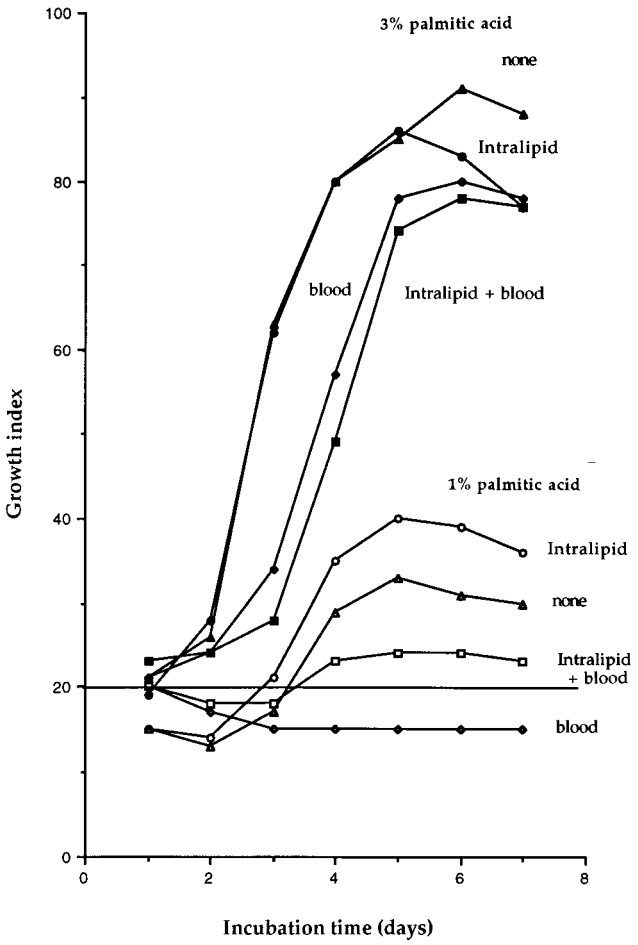

FIG. 2. Growth of *M. furfur* in the presence of two concentrations of palmitic acid with and without blood and Intralipid 20 supplementation. A GI of 20 (horizontal rule) is considered significant. Results obtained with all *Malassezia* strains tested were similar.

served for the control bottles lacking lipid supplementation (data not shown).

The growth-enhancing effect of palmitic acid was then assessed in experiments designed to test the impact of clinical variables, i.e., blood and Intralipid 20 (a parenteral lipid supplement; Clintec Nutrition Company, Mississauga, Ontario, Canada). Palmitic acid at 1 or 3% (wt/vol) was tested with Intralipid 20 (100 μl of a 1/80 dilution in PBS, equivalent to a 0.5-ml blood specimen containing 0.5 g of fat per liter of blood), human blood (0.5 ml; fresh, unclotted, unheparinized), both, or neither. All bottles were inoculated aseptically with 10^2 yeasts. Controls with Intralipid 20, blood, or palmitic acid (3%) alone were included. Bottles with 3% palmitic acid were tested further with 3 ml of blood to assess whether the inhibitory effect of blood was volume dependent.

As seen in Fig. 2 for MF1, in the presence of 1% palmitic acid, the addition of Intralipid 20 increased GI values slightly, whereas the addition of blood (in the presence or absence of Intralipid 20) yielded values that were barely above the threshold GI of 20. However, with 3% palmitic acid, the inhibitory effect of blood was overcome and positive GI values were obtained in 24 to 48 h. Results obtained with 3 ml of blood were the same as those obtained with 0.5 ml. Results for all *Malassezia* strains tested were similar.

Detection of *M. furfur* in PP bottles in the BACTEC NR 660 required lipid supplementation, a phenomenon previously described for the BACTEC 6B bottle (7). Palmitic acid, a fatty acid with a carbon chain length of 16, was superior to olive oil, oleic acid (C_{18}), and Tween 40 as a supplement, in terms of both speed of detection and growth enhancement. The GI appeared to reflect conidiogenesis, as cell numbers increased to 10^6 to 10^7/ml. We report here that olive oil, which enhances the growth and detection of *M. furfur* in vitro, may be inhibitory at higher concentrations. This phenomenon has also been reported for supplementation of *Malassezia* growth medium with Intralipid (3, 4). This study corroborates the toxic effect of human blood on yeast growth; preliminary work in our laboratory suggests that this inhibitory effect is serum dependent and not cell dependent. Antibodies to *Malassezia* spp., which have been detected in adults (6), may have been responsible. Three percent palmitic acid was able to overcome the inhibitory effect of both small (0.5-ml) and larger (3-ml) volumes of blood.

The superior efficacy of palmitic acid in our studies is consistent with reports which have suggested that free fatty acids whose carbon chain length is greater than 10 (2, 13), particularly C_{16} (palmitic acid) and C_{18} (oleic acid), are required for *M. furfur* growth (9). Even in the presence of blood, palmitic

acid allowed the detection of *Malassezia* spp. in 24 to 48 h, a time significantly shorter than that reported for the pediatric Isolator system (48 to 72 h) or for BACTEC 6B bottles supplemented with other lipids (72 to 96 h) (7). Although there are disadvantages to adding 3% palmitic acid to PP bottles (risk of contamination and difficulty in manipulating the thick stock solution), the potential benefits must be seriously entertained. Certainly, before routine lipid supplementation of blood culture bottles can be considered, determination of the effects of such lipids on the growth of other bloodstream pathogens is necessary.

In conclusion, increasing awareness of fungemia due to *Malassezia* species has prompted the assessment of current blood culture systems for *Malassezia* detection. Inclusion of 3% palmitic acid in PP bottles may be useful in improving *Malassezia* recovery in the BACTEC NR 660.

We thank Barb Morrison for her help in the preparation of the manuscript and Betty Taylor and Mohamed Karmali for useful discussions.

REFERENCES

1. **Azimi, P. H., K. Levernier, L. M. Lefrak, A. M. Petru, T. Barrett, H. Schenck, A. S. Sandhu, G. Duritz, and M. Valesco.** 1988. *Malassezia furfur*: a cause of occlusion of percutaneous central venous catheters in infants in the intensive care nursery. Pediatr. Infect. Dis. J. **7:**100–103.
2. **Benham, R. W.** 1941. Cultural characteristics of *Pityrosporum ovale*—a lipophilic fungus. Nutrient and growth requirements. Proc. Soc. Exp. Biol. **46:**176–178.
3. **Dankner, W. M., S. A. Spector, J. Fierer, and C. E. Davis.** 1987. *Malassezia* fungemia in neonates and adults: complication and hyperalimentation. Rev. Infect. Dis. **9:**743–753.
4. **Klotz, S. A., M. Huppert, and D. J. Drutz.** 1982. Pulmonary vasculitis on intralipid therapy. N. Engl. J. Med. **306:**994. (Letter.)
5. **Larocco, M., A. Dorenbaum, A. Robinson, and L. K. Pickering.** 1988. Recovery of *Malassezia pachydermatis* from eight infants in a neonatal intensive care nursery: clinical and laboratory features. Pediatr. Infect. Dis. J. **7:**398–401.
6. **Marcon, M. J., and D. A. Powell.** 1992. Human infections due to *Malassezia* spp. Clin. Microbiol. Rev. **5:**101–119.
7. **Marcon, M. J., D. A. Powell, and D. E. Durrell.** 1986. Methods for optimal recovery of *Malassezia furfur* from blood culture. J. Clin. Microbiol. **24:**696–700.
8. **Mickelsen, P. A., M. C. Viano-Paulson, D. A. Stevens, and P. S. Diaz.** 1988. Clinical and microbiological features of infection with *Malassezia pachydermatis* in high-risk infants. J. Infect. Dis. **157:**1163–1168.
9. **Porro, M. N., S. Passi, F. Caprilli, P. Nazzaro, and G. Morpurgo.** 1976. Growth requirements and lipid metabolism of *Pityrosporum orbiculare*. J. Invest. Dermatol. **66:**178–182.
10. **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.
11. **Redline, R. W., and B. B. Dahms.** 1981. *Malassezia* pulmonary vasculitis in an infant on long-term intralipid therapy. N. Engl. J. Med. **305:**1395–1398.
12. **Shek, Y. H., M. C. Tucker, A. L. Viciana, H. J. Manz, and D. H. Conner.** 1989. *Malassezia furfur*—disseminated infection in premature infants. Am. J. Clin. Pathol. **92:**595–603.
13. **Wilde, P. F., and P. S. Stewart.** 1968. A study of the fatty acid metabolism of the yeast *Pityrosporum ovale*. Biochem. J. **108:**225–231.