Use of Litmus Milk Agar for Presumptive Identification of Cutaneous Propionibacteria

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Presumptive identification of cutaneous propionibacteria can be achieved with litmus milk agar as medium.

The anaerobic propionibacteria are among the most numerous bacteria in humans. *Propionibacterium acnes*, *P. avidum*, and *P. granulosum* are the dominant members of cutaneous microflora (5); they also reside in the gut (7) and are frequently isolated from clinical samples. The works of Marples and McGinley (5) and Johnson and Cummins (3) have clearly defined these species and provide a set of tests for identifying propionibacterial isolates. Gram-positive, anaerobic nonsporeformers can be placed in the *Propionibacterium* genus and identified to species level by performing tests to detect beta-hemolysis (5), indole production, nitrate reduction, and gelatin liquefaction (2, 5), or by serological typing (1). It has also been shown that casein hydrolysis can be a valuable character for propionibacteria identification (5). As described originally, casein hydrolysis was detected on standard methods casein agar; zones of clearing around colonies indicated a positive reaction (5, 6). However, the potential usefulness of this medium is lessened by its relative unavailability and bothersome preparation. We have found that a modified litmus milk medium can be substituted for standard methods casein agar with favorable results.

Litmus milk agar (LMA) was prepared by adding 1.5% agar to 5% litmus milk medium (Baltimore Biological Laboratory, Cockeysville, Md.), sterilized for 15 min at 121°C with 15 lb (ca. 66.72 N) of pressure, and then poured into petri dishes to an approximate depth of 4 mm. Sterilization under these conditions does not adversely affect LMA, although this problem has been noted with conventional litmus milk medium.

In this study, *P. acnes* (ATCC 6919), *P. avidum* (VPI 0575; from the Anaerobe Laboratory culture collection at Virginia Polytechnic Institute and State University, courtesy of C. S. Cummins), and *P. granulosum* (ATCC 11829) strains were tested on LMA along with an addi-

tional 525 propionibacterial isolates from normal human skin. All isolates were identified by characteristics of indole production, nitrate reduction, gelatin liquefaction, hemolysis on sheep blood agar, and lysis by bacteriophage specific for *P. acnes* (ATCC 29399B; submitted to the American Type Culture Collection in August 1976) (4, 5). A colony of each strain was suspended in 1 ml of 1% peptone water to a visibly turbid suspension and spotted with a swab onto LMA, five strains to a plate, producing a 4- to 5-mm zone of uniform growth. All media were incubated anaerobically at 37°C, with plated media in GasPak jars (BBL). All tests were read after 7 days of incubation. Clearing zones sizes in the LMA were measured, correlated with the identification obtained via standard tests, and then averaged within each species.

As shown in Table 1, all *P. acnes* and *P. avidum* strains produced clearing in LMA; *P. avidum* zones ranged from 2.2 to 3.0 cm, and *P. acnes* zones ranged from 0.8 to 1.3 cm; *P. granulosum* strains failed to produce any detectable

TABLE 1. Correlation of conventional identification methods^a

Strains	No. tested	Minimum zone size (cm)	Maximum zone size (cm)	Avg zone size (cm)	Predictive zone size (cm)
<i>P. acnes</i> ATCC 6919	270	0.8	1.3	1.15	1.5
<i>P. avidum</i> VPI 0575	95	2.2	3.0	2.5	2.0
<i>P. granulosum</i> ATCC 11829	160	0	0	0	0

^a Methods of Marples and McGinley (5).

clearing. A predictive zone size is proposed for each species. This parameter was arrived at by adding to the maximum zone size for *P. acnes* and the minimum zone size for *P. avidum* a 2-mm variation factor to allow for strains with activity outside of limits found in this study.

In a second series of tests, 20 strains of each serotype of *P. acnes* and *P. avidum* (as identified by the method of Cummins [1]) were tested on LMA. The same correlation between zone size and species was demonstrated. There was no correlation between intraspecific serotype and zone size.

Clearing production in LMA, presumably due to casein hydrolysis, is thus a reliable biochemical character for cutaneous propionibacteria. The good reproducibility of this method combined with its low cost, when compared with standard methods, make it an attractive medium for presumptive identification of these organisms.

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