


do Nitrophenyl-β-D-Galactopyranoside–Urease–Indole Broth, a New Composite Tube Medium for Salmonella Screening

FRANCESCA BERLUTTI,1 BENEDETTO DAINELLI,2 ANNARITA MARTINI,3 AND MARIA C. THALLER1*

Istituto di Microbiologia, Università La Sapienza,1 Dipartimento di Medicina Sperimentale e Scienze Biochimiche, II Università,2 and Istituto Superiore di Sanità,3 00185 Rome, Italy

Received 29 March 1986/Accepted 25 June 1986

A new composite broth medium combining o-nitrophenyl-β-D-galactopyranoside (ONPG) and urease and indole tests in a single tube is described. High-level agreement with individual conventional tests was recorded in comparative studies with 2,412 cultures of members of the family Enterobacteriaceae, i.e., 100% agreement with the exception of Hafnia spp. (96.3% agreement) for the ONPG test and Citrobacter, Enterobacter, and Hafnia spp. (75, 86.4, and 98.2% agreement, respectively) for the urease test. The new medium seems especially promising as a screen for Salmonella subgroup I which encompasses most pathogenic Salmonella species other than the Arizona subgroup.

Numerous composite media aimed at combining different biochemical reactions into a one-tube system have been developed and are used in microbiological laboratories (1, 3, 8, 10).

In this study, a new single-tube broth medium combining the hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG), urease production, and indole production (β-GUI medium) is described. The results obtained with β-GUI medium for a large number of members of the family Enterobacteriaceae were compared with those obtained by conventional individual tests. A total of 2,412 cultures of Enterobacteriaceae were examined. These strains included 1,639 clinical isolates obtained from several diagnostic laboratories in Rome, 748 strains from the collections of our institutes, and 25 standard cultures from official collections (Collection de L’Institut Pasteur and American Type Culture Collection). Strain identification was done by methods described in Bergey’s Manual of Systematic Bacteriology (2), with reference to newer reports for the new species (4, 6, 7).

The strains included 2 Cedececa isolates, 56 Citrobacter isolates, 59 Enterobacter isolates, 124 Escherichia isolates, 54 Hafnia isolates, 45 Klebsiella isolates, 1 Kluyvera isolate, 273 Morganella isolates, 1,273 Proteus isolates, 224 Providencia isolates, 224 Salmonella isolates (220 from subgroup I and 4 from subgroup III), 69 Serratia isolates, 3 Shigella isolates, and 5 Yersinia isolates. β-GUI medium was prepared by the following formula: Trypticase peptone (BBL Microbiology Systems, Cockeysville, Md.), 1 g; glucose, 0.5 g; tryptophan, 0.1 g; NaCl, 5 g; K3HPO4, 0.7 g; KH2PO4, 0.125 g; 1-naphtholphthalein (catalog no. 6246; E. Merck AG, Darmstadt, Federal Republic of Germany), 0.03 g. To dissolve the last compound, 30 ml of ethanol was added before 920 ml of deionized water. The medium was sterilized at 121°C for 15 min and allowed to cool. A 50-ml volume of a filtered solution of urea (40%) and ONPG (1%) was then added. The final medium, which had a pH of 7.3 ± 0.1 and appeared clear and light brownish, was distributed into sterile tubes (5 ml per tube). Each tube was inoculated with a single colony from a fresh isolate of a test strain. After overnight incubation at 37°C, readings were performed. The results were as follows, on the basis of the color obtained: (i) turquoise-blue, urease positive; (ii) yellow, ONPG positive; (iii) shining green, both urease and ONPG positive; (iv) colorless, both urease and ONPG negative. Kovacs reagent was then added to the tubes to reveal indole production.

In comparative trials, all the strains were examined for their responses both to β-GUI medium and to individual ONPG, urease, and indole tests performed by standardized procedures (5, 9). A 100% agreement was obtained with the following exceptions. Two Hafnia strains were false-negative in the ONPG test (agreement, 96.3%), and Citrobacter, Enterobacter, and Hafnia spp. recorded an agreement of 75, 86.4, and 98.2%, respectively, in the urease test. Besides the high levels of agreement between the results with β-GUI medium and individual reference tests, the complete absence of false-positive reactions in β-GUI medium must be emphasized. One interesting aspect of this study was that a triple-negative reaction in β-GUI medium most often denoted Salmonella subgroup I, which includes most human enteric pathogens other than the Arizona subgroup; when only lactose-negative cultures (totaling 2,236) were considered, of 251 strains yielding a triple-negative reaction, 220 were all of the isolates examined that belonged to Salmonella subgroup I, 16 were Hafnia spp., 2 were Klebsiella spp. (K. ozrenae, K. rhinoscleromatis), 9 were Proteus spp., 2 were Serratia spp., and 2 were Shigella spp.

These findings suggest that β-GUI medium might be used in diagnostic laboratories as a practical and inexpensive screen for Salmonella spp.

LITERATURE CITED


* Corresponding author.


