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Fluorogenic Assay for Differentiating *Staphylococcus warneri* and *Staphylococcus hominis* Strains of Bovine Origin†

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A fluorogenic assay for the detection of β-glucosidase was developed as part of a simplified conventional method to distinguish *Staphylococcus warneri* and *Staphylococcus hominis* isolated from bovine body sites. The assay is based on the fact that strains of *S. warneri* produce β-glucosidase, while strains of *S. hominis* do not.

We have observed problems distinguishing *Staphylococcus hominis* and *Staphylococcus warneri* among bovine isolates being studied in our laboratory (3, 4). Many of the rapid identification systems give profiles which result in a low discrimination of these two species. To improve our ability to distinguish these two species, we developed a fluorogenic assay for the detection of β-glucosidase. The assay is based on the fact that strains of *S. warneri* produce β-glucosidase, while strains of *S. hominis* do not (2). P agar (5) supplemented with 4-methylumbelliferyl-β-D-glucopyranoside (150 mg/liter; Sigma Chemical Co., St. Louis, Mo.) was radially streaked with up to six cultures per plate, and the plates were incubated at 35°C for 24 h. After incubation, the plates were examined under long-wave UV (366-nm) light for fluorescence surrounding the streaks. Fluorescence is due to 4-methylumbelliferone released from 4-methylumbelliferyl-β-D-glucopyranoside by β-glucosidase (1, 6, 7). The assay was tested with 74 isolates identified as either *S. hominis* or *S. warneri* with the API Staph-Trac system (Analytab Products, Plainview, N.Y.) (4). The β-glucosidase reactions from the fluorogenic assay were compared with the chromogenic β-glucosidase reaction, which was one of the 10 biochemical characteristics in the API Staph-Ident system (Analytab Products).

All 34 isolates identified as *S. hominis* were observed to be β-glucosidase negative by the fluorogenic β-glucosidase assay. Positive reactions in the β-glucosidase test of the Staph-Ident system were observed for 21 of these 34 isolates. Thus, only 38% of the *S. hominis* strains were found to be β-glucosidase negative by the Staph-Ident system. The 40 isolates identified as *S. warneri* were found to be β-glucosidase positive by both the fluorogenic assay and the Staph-Ident system. A difficulty with the API Staph-Ident system is the subjective scoring of the wells by the individual who is reading the strip. The β-glucosidase well can be misinterpreted because of subtle color changes. The fluorogenic assay is very easy to interpret, since only the presence or absence of fluorescence when viewed under UV light must be determined. The fluorogenic assay is a rapid and sensitive method for detecting β-glucosidase and is suitable for distinguishing β-glucosidase-positive *S. warneri* from β-glucosidase-negative *S. hominis*. The fluorogenic assay can be easily incorporated into an identification regime.

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