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Routine Procedures for Isolation and Identification of Enterococci and Fecal Streptococci†

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Over the past 6 years, a revised classification of the streptococci and enterococci, based primarily on molecular techniques such as 16S rRNA sequencing and DNA-DNA hybridization, emerged. However, little attention was placed on routine physiological tests that could be used in food and clinical laboratories to differentiate between species of a new genus, *Enterococcus*, and fecal *Streptococcus* spp. The purpose of this study was to devise a convenient and reliable system to identify enterococci and fecal streptococci by using conventional procedures. Fifty-nine strains of 13 *Enterococcus* spp., including the type strains and many strains used by previous investigators, were characterized by using conventional tube tests, the API Rapid Strep system, and MicroScan Pos ID panels. Results were compared with each other and with previously published results. A comparison of conventional tube tests versus published tube test results yielded 17 discrepancies. Although not all tests were done with each of the three systems, 28 discrepancies between results obtained with the API system and those obtained with conventional tube tests were found. There were 24 discrepancies between results obtained with the MicroScan Pos ID panel and those obtained with conventional tube tests. There were 12 discrepancies between the results with the API Rapid Strep system and those with the MicroScan Pos ID panels. We devised flow charts of key tests that might be used to identify cultures without resorting to nucleic acid analysis and other labor- and equipment-intensive analyses.

Enterococcus spp. inhabit the intestinal tracts of warm-blooded animals and of insects (16). Therefore, these bacteria have been useful as indicators of fecal contamination in water and in foods (5). Because of the prevalence of certain species of enterococci in the intestinal tracts of swine, enterococci are implicated in the spoilage of pork products (20). Enterococci and fecal streptococci are also receiving increased attention because of their role in serious human infections, such as endocarditis and bacteremia (17) and diarrheal diseases in neonates (7).

In 1984, the genus *Streptococcus* was divided into three genera: *Enterococcus*, *Lactococcus*, and *Streptococcus* (19). The genus *Enterococcus* now contains 18 species, differentiated primarily by the results of 16S rRNA and DNA-DNA hybridization studies. In addition to the 13 species described in Table 1, five new species have been proposed: *Enterococcus sulfureus* (15), which includes yellow-pigmented strains isolated from plants; *E. columbae* (6), a species that dominates the intestinal flora of domestic pigeons; *E. dispar* (4), composed of two strains of human origin; *E. seriolicida* (14), a fish pathogen; and *E. saccharolyticus* (18), previously named *Streptococcus saccharolyticus*.

Initially, these species were phenotypically characterized by using API 50CH and 20S systems in conjunction with conventional tube tests (1–4, 6, 12, 14, 15, 18). However, the API data base includes only 6 of the 13 tested enterococcal species, and the data base for another system, the MicroScan system, contains only 4 of the species. An identification system with conventional tube tests exclusively was introduced in 1989 by Facklam and Collins (9). However, conventional tube tests are cumbersome, costly, and labor intensive, and the results are often difficult to reproduce accurately in different laboratories.

In this study, we tested 59 strains of 13 species of enterococci and fecal streptococci. These included selected strains of each enterococcal species used in the original 16S rRNA studies as well as each type strain. Two different rapid systems and the conventional tube tests were compared. A scheme was developed to identify 13 species of enterococci and *Streptococcus bovis* and *S. equinus*. The test scheme uses either the API Rapid Strep or MicroScan system plus a minimum of supplemental tests.

MATERIALS AND METHODS

Strains. A total of 59 strains of 13 enterococci and *S. bovis* and *S. equinus* were collected (Table 1). The type strains used were obtained from the American Type Culture Collection (Rockville, Md.) or Richard Facklam (Centers for Disease Control, Atlanta, Ga.). Cultures were also obtained from the National Collection of Food Bacteria (Shinfield, Reading, England), MicroScan (Baxter Diagnostics, Deerfield, Ill.), Marcia Etheridge (Baltimore, Md.) (7), and the culture collection of Paul A. Hartman (Iowa State University, Ames).

Stock cultures were maintained on brain heart infusion (BHI) slants at 5 to 10°C and transferred monthly. Stock cultures were also frozen in 10% glycerol and stored at −70 to −100°C.

Tests. All cultures were Gram stained as they were obtained to verify that they were gram-positive cocci. Each culture was also tested for the presence of catalase before being inoculated to the test panels.

The *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) test (21) was used to determine the presence of β-galactosidase. All other conventional tube tests were performed as

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TABLE 1. List of strains used[a]

Species	Strain(s)
E. avium	ATCC 14025, 49462, 49463, 49465; NCDO 2366; SS-1[b]; A[b]; B[b]
E. casseliflavus	ATCC 25788, NCDO 2376, 7765F,[b] 443,[b] A8[b]
E. cecorum	ATCC 43198, NCDO 2674
E. durans	ATCC 19432, NCDO 498, PAH 940,[b] 15-20[b]
E. faecalis	ATCC 4082, 19433, 35038, 49477, 49478; NCDO 581; R187a[b]; 334-2[b]
E. faecium	ATCC 349, 19434; NCDO 502; R169a[b]; R281a[b]; 2100[b]; 2124[b]
E. gallinarum	ATCC 49573; NCDO 2311, 2315, 2704
E. hirae	ATCC 9790; NCDO 1631, 1648, 2683; ME[c]
E. malodoratus	ATCC 43197, NCDO 847
E. mundtii	ATCC 43186; NCDO 582, 2374, 2377
E. pseudoavium	SS-1277,[d] NCDO 2138
E. raffinosus	SS-1278[d]
E. solitarius	SS-1279[d]
S. bovis	ATCC 9809, 33317; H-12[b]; H-24[b]
S. equinus	ATCC 9812

[a] See the text for sources not listed in footnotes *b* through *d*.
[b] From the culture collection of Paul A. Hartman.
[c] From M. E. Etheridge.
[d] From R. R. Facklam.

described by Facklam and coworkers (8, 9, 10) and Gross et al. (13). Motility was determined by using modified Difco motility medium (Difco Laboratories, Detroit, Mich.) or wet mounts of cultures grown in BHI broth at 30 and 37°C (21). Cultures were monitored for yellow pigmentation on cotton swabs used to pick up growth from BHI agar plates incubated overnight.

Serogrouping for groups A, B, C, D, F, and G was accomplished by using the Streptex grouping kit (Wellcome Diagnostics, Research Triangle Park, N.C.). β-Hemolysis was determined by observation of 24-h growth on a plate of tryptone soy agar–5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.).

Inoculum preparation for the API Rapid Strep system (Analytab Products, Inc., Plainview, N.Y.) was carried out on blood agar plates as indicated in the manufacturer's directions but without anaerobic incubation. The test strips were inoculated, overlaid with mineral oil where specified, incubated, and read as indicated in the manufacturer's directions. After 4 h of incubation, Zyme A and B reagents (Analytab) were added to the enzyme tests, ninhydrin solution was added to the hippurate test, and reagents A and B were added to the Vogues-Proskauer (VP) test; the results of these tests were recorded. The results of the remaining tests were determined after incubation of the panel for 18 to 24 h.

Inoculum preparation for the MicroScan Pos ID panels (Baxter Diagnostics, Deerfield, Ill.) was carried out by the log-phase technique specified by the manufacturer. The panels were inoculated, covered, and incubated at 37°C. After 18 to 24 h of incubation, appropriate reagents were added and the tests were interpreted as indicated in the manufacturer's instructions.

RESULTS AND DISCUSSION

Table 2 shows the results of conventional tube tests. A comparison of our results with published results (9) revealed 17 discrepancies among 87 instances in which comparisons could be made. Every attempt was made to perform the tests as specified in the literature, but the discrepancies appeared repeatedly. These discrepancies are probably due to the difficulty in accurately reproducing tube test results in different laboratories.

There was also a poor correlation between results obtained with the API and MicroScan systems and those obtained with conventional tube tests (Table 2). There were 28 discrepancies between API results (see Table 4) and conventional tube test results (Table 2). There were 24 discrepancies between MicroScan results (Table 3) and conventional tube test results (Table 2).

Twelve discrepancies between the API Rapid Strep results and MicroScan Pos ID system results were observed (Tables 2 and 3). VP test results were more variable when the API Rapid Strep strips were used than when MicroScan panels were used. *Enterococcus cecorum* and *S. bovis* test results varied unpredictably on the API panels, but they were

TABLE 2. Conventional tube test results

Species (no. of strains)	Test result[a]										
	β-Galactosidase	Raffinose	Sorbitol	Pyruvate	Sucrose	Mannitol	Hippurate	Lactose	Sorbose	L-Arabinose	Arginine
E. avium (8)	(+)	−	+	(+)[b]	+	+	−	+	+	+	−
E. casseliflavus (5)	+	(+)	−[c]	−	+	+	−	+	−	+	−[b,c,d]
E. cecorum (2)	+	+	−	−	+	−	−[d]	+	−	−	−
E. durans (4)	+	(−)	−	−	−	−	v	+	−	−	−[b,c,d]
E. faecalis (8)	+[d]	−	+	v[b]	+[c]	+	−[c,d]	+	−	−	+
E. faecium (7)	+[d]	(−)	−[c]	−	v	+	(−)[d]	+	−	+	+
E. gallinarum (4)	+	+	−	−	+	+	+[c]	+	−	+	−[b,c,d]
E. hirae (6)	+	+[b,d]	−	−	+	−	−[d]	+	−	−	+
E. malodoratus (2)	+	+	v[c,d]	−	+	+	−	+	+	−	+[b,c,d]
E. mundtii (4)	+	+[b,d]	+[b,c]	v[b]	+	+	−	+	−	+	+
E. pseudoavium (2)	(+)[d]	−	+	v[b]	+	+	−	+	+	−	−
E. raffinosus (1)	−	+	+	−	+	+	−[c,d]	+	+	+	+[b,c,d]
E. solitarius (1)	−	−	v[b,c,d]	+[b]	+	+	−[c,d]	−	−	+[b,c,d]	+
S. bovis (4)	+[b,d]	+	−	−	+	v	v[d]	+	−	+[b,d]	+[b,d]
S. equinus (1)	+[b,d]	−	−	−	+	+	−	+[b,d]	−	+[b,d]	+[b,d]

[a] +, Positive reaction (100%); −, negative reaction (100%); (+), 75% or greater show positive reaction; (−), 75% or greater show negative reaction; v, variable (some strains positive, some strains negative).
[b] Discrepancy between Table 3 MicroScan results and Table 2 results.
[c] Discrepancy between published tube test results and Table 2 results.
[d] Discrepancy between Table 4 API results and Table 2 results.

TABLE 3. MicroScan Pos ID panel results

Species (no. of strains)	Test result[a]																									
	Crystal violet	*Micrococcus* screen	Nitrate	VP	Optochin	Phosphatase	40% bile esculin	Pyrrolidonylase	Arginine	β-Galactosidase	Urea	Raffinose	Lactose	Trehalose	Mannose	6.5% NaCl	Sorbitol	Arabinose	Ribose	Inulin	Mannitol	Bacitracin	Pyruvate	Hemolysis	Pigment	Motility
E. avium (8)	v	+	−	+	+	−	+	+	−	v	−	−	+	+	+	v	+	+	+	−	+	+	−	−	−	−
E. casseliflavus (5)	v	+	−	+	+	−	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+
E. cecorum (2)	v	+	−	+	+	+	+	−	−	+	−	+	+	+	+	−	−	−	+	+	−	+	−	−	−	−
E. durans (4)	v	+	−	+	+	−	+	+	+	+	−	−	+	(+)	+	+	−	−	+	−	−	+	−	−	−	−
E. faecalis (8)	+	+	−	+	+	+	+	+	+	+	−	−	(+)	+	+	+	(+)	(−)	+	−	+	+	−	−	−	−
E. faecium (7)	v	+	−	+	+	−	+	+	+	+	−	−	+	+	+	+	−	+	+	−	+	+	−	−	−	−
E. gallinarum (4)	v	+	−	+	+	−	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	−	−	+
E. hirae (6)	v	+	−	+	+	−	+	+	+	+	−	−	+	+	+	+	−	−	+	−	−	+	−	−	−	−
E. malodoratus (2)	v	+	−	+	+	−	+	+	(−)	+	−	+	+	+	+	+	v	−	+	v	+	+	−	−	−	−
E. mundtii (4)	v	+	−	+	+	−	+	+	+	+	−	−	+	+	+	+	−	+	+	−	+	+	−	−	+	−
E. pseudoavium (2)	+	+	−	+	+	−	+	+	−	+	−	−	+	+	+	+	+	−	+	−	+	+	−	−	−	−
E. raffinosus (1)	v	+	−	+	+	−	+	+	−	−	−	+	+	+	+	+	+	+	+	−	+	+	−	−	−	−
E. solitarius (1)	v	+	−	+	+	−	+	+	+	−	v	−	−	+	+	+	+	−	−	−	+	+	−	−	−	−
S. bovis (4)	+	+	−	+	+	−	+	−	−	−	−	+	+	v	+	−	−	−	−	v	v	+	−	−	−	−
S. equinus (1)	+	−	−	−	+	−	+	−	−	−	−	−	−	+	+	−	−	−	−	+	−	+	−	−	−	−

[a] +, Positive reaction (100%); −, negative reaction (100%); (+), 75% or greater show positive reaction; (−), 75% or greater show negative reaction; v, variable (some strains positive, some strains negative).

always positive on the MicroScan Pos ID panels. On the other hand, *S. equinus* was VP positive when tested with API strips but VP negative on MicroScan panels. Because the same α-naphthol and KOH solutions were used for the VP tests on both panels, these discrepancies are caused by something other than the detection reagents.

β-Galactosidase results for *Enterococcus faecalis* and *E. pseudoavium* were negative on API Rapid Strep panels, whereas positive results were obtained for both species with MicroScan Pos ID panels. Alkaline phosphatase test results also differed. *E. faecalis* was alkaline phosphatase negative when run on the API panel but positive when tested with the MicroScan Pos ID panel. These discrepancies probably are the results of differences between the methodologies of the API Rapid Strep and MicroScan system β-galactosidase and alkaline phosphatase tests. The API Rapid Strep panel utilizes a naphthol-linked substrate, and color development is detected after the addition of Zyme A and B reagents furnished by API. The MicroScan Pos ID β-galactosidase and alkaline phosphatase tests use *para*-nitrophenyl-β-D-galactopyranoside (PNPG) and *para*-nitrophenyl phosphate, respectively, as substrates. Both enzyme reactions release *para*-nitrophenol, which generates a yellow color. β-Galactosidase tests were also conducted in test tubes (21) with ONPG. ONPG is very susceptible to cleavage by β-galactosidase and, as in the MicroScan test, releases a yellow product. The product in this case, however, is *ortho*-nitrophenol. The only difference between the two tests is the orientation of the nitrogen on the phenyl group, *para* or *ortho*. The ONPG tube test results, except for those with *S. bovis* and *S. equinus*, correlated well with the MicroScan results (Table 2). It seems that the API Rapid Strep methodology, at least for the β-galactosidase test, is not as sensitive as either the PNPG methodology of the MicroScan system or the ONPG tube methodology.

Discrepancies in carbohydrate fermentation tests of the API and MicroScan systems were also observed. Three discrepancies in the sorbitol test were observed: *Enterococcus malodoratus* showed a positive API result but was variable on MicroScan panels; *E. mundtii* was also positive for sorbitol when run on the API panel, but all four strains were negative on the MicroScan panel; and *E. solitarius* was sorbitol negative in the API tests and sorbitol positive in the MicroScan tests. When a conventional tube method for sorbitol fermentation was used (9), *E. malodoratus*, *E. mundtii*, and *E. solitarius* produced variable, positive, and variable results, respectively (Table 2). Thus, manual test results failed to support the validity of the results of either panel as superior to those of the other. Two discrepancies were observed in the inulin test (Tables 3 and 4). *E. malodoratus* was uniformly inulin negative on API panels; variable results were obtained when MicroScan panels were used. Only one discrepancy existed in the raffinose test: variable results were obtained with *Enterococcus hirae* on API Rapid Strep test strips, whereas negative results were obtained on MicroScan Pos ID panels.

The six discrepancies seen in the carbohydrate fermentation tests for sorbitol, inulin, and raffinose are highly variable. There was no consistent pattern wherein one panel was positive and the other was negative. Both systems use phenol red as the pH indicator, but there is a slight difference in methodology. The API Rapid Strep system requires a sterile mineral oil overlay on all carbohydrate tests to reduce oxidative metabolism of the carbohydrates. The MicroScan system does not utilize a mineral oil overlay. Therefore, the discrepancies could be caused by the ability of some bacteria to oxidatively metabolize the carbohydrates in the MicroScan system but not in the API system. If this was the only cause of the discrepancies, one would expect more negative results from the API system and more positive results from the MicroScan system. As was pointed out above, however, this did not occur.

Also included in Tables 3 and 4 are two tests that were not performed on either panel. Pigmentation is important for

TABLE 4. API Rapid Strep test results

Species (no. of strains)	Test result[a]																						
	VP	Hippurate hydrolysis	Esculin	Pyrrolidonylase	α-Galactosidase	β-Glucuronidase	β-Galactosidase	Alkaline phosphatase	Leucine arylamidase	Arginine dehydrolase	Ribose	L-Arabinose	Mannitol	Sorbitol	Lactose	Trehalose	Inulin	Raffinose	Starch	Glycogen	Hemolysis	Pigment	Motility
E. avium (8)	+	−	+	+	−	−	v	−	+	−	+	+	+	+	+	+	−	−	v	−	−	−	−
E. casseliflavus (5)	+	−	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+
E. cecorum (2)	v[b]	v	+	−	+	+	+	+	+	−	+	−	−	−	+	+	+	+	+	−	−	−	−
E. durans (4)	+	v	+	+	(+)	−	+	−	+	+	+	−	−	−	+	(+)	−	(−)	v	−	−	−	−
E. faecalis (8)	+	v	+	+	−	−	−[b]	−[b]	+	+	+	(−)	+	+	(+)	+	−	−	+	−	−	−	−
E. faecium (7)	+	v	+	+	v	−	+	−	+	+	+	+	+	−	+	+	(−)	−	+	−	−	−	−
E. gallinarum (4)	+	+	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	v	−	−	+
E. hirae (6)	+	v	+	+	(+)	−	+	−	+	+	+	−	−	−	+	+	−	v[b]	+	−	−	−	−
E. malodoratus (2)	+	−	+	+	+	−	+	−	+	−	+	−	+	+[b]	+	+	−[b]	+	v	−	−	−	−
E. mundtii (4)	+	−	+	+	(+)	−	+	−	+	+	+	+	+	+[b]	+	+	−	−	v	−	−	+	−
E. pseudoavium (2)	+	−	+	+	v	−	−[b]	−	+	−	+	−	+	+	+	+	−	−	−	−	−	−	−
E. raffinosus (1)	+	v	+	+	+	−	−	−	+	−	+	+	+	+	+	+	−	+	v	−	−	−	−
E. solitarius (1)	v[b]	v	+	+	+	−	−	−	+	+	−	−	+	−[b]	−	+	−	−	−	−	−	−	−
S. bovis (4)	+	−	+	−	v	−	−	−	+	−	−	−	v	−	+	v	(+)	+	+	+	−	−	−
S. equinus (1)	+[b]	−	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−[b]	−	−	−	−	−	−

[a] +, Positive reaction (100%); −, negative reaction (100%); (+), 75% or greater show positive reaction; (−), 75% or greater show negative reaction; v, variable (some strains positive, some strains negative).
[b] Discrepancy between the API Rapid Strep and the MicroScan system.

differentiating pigmented *Enterococcus casseliflavus* and *E. mundtii* from nonpigmented enterococci and fecal streptococci. Motility tests are used to distinguish motile *Enterococcus gallinarum* and *E. casseliflavus* from nonmotile species.

Figures 1 through 3 depict selected tests that can be used to identify all 13 species of enterococci as well as *S. bovis* and *S. equinus*. The tests shown in Tables 3 and 4 were used to construct the identification schemas, with the exception of the sucrose test (Table 2), which is needed to distinguish *Enterococcus hirae* (positive) from *E. durans* (negative). Also included in Fig. 1 through 3 are the Lancefield group D reactions. They were included to aid in the differentiation of *E. cecorum* and *E. pseudoavium* (group D negative) from enterococci and fecal streptococci that produce the group D antigen.

Not all of the tests that could be used to differentiate between species were included in these schemas. Each strain was tested several times, and some tests yielded inconsistent results (i.e., a positive result one time and a negative result the next). To obtain the most consistent and reliable identification, only tests that produced the most

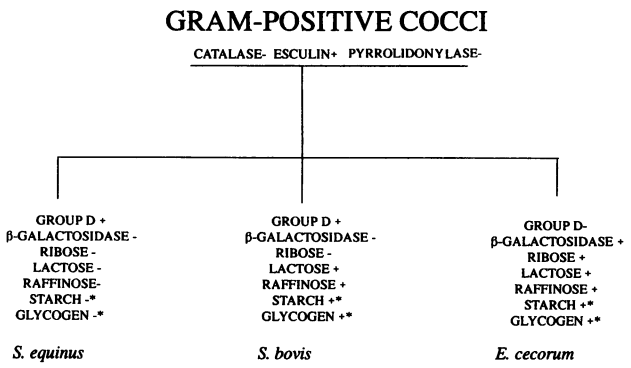

FIG. 1. Flow chart for differentiating the pyrrolidonylase-negative enterococci and fecal streptococci. The data shown are condensed from Tables 3 and 4. The asterisk (*) indicates that a test is available with the API Rapid Strep system but not with the MicroScan system.

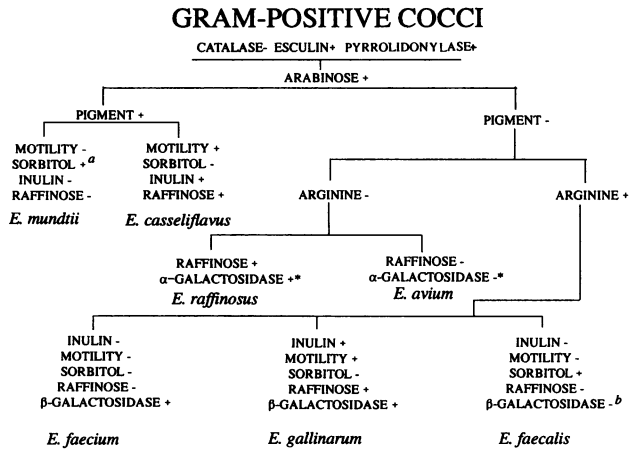

FIG. 2. Flow chart for differentiating the pyrrolidonylase- and arabinose-positive enterococci. The data shown are condensed from Tables 3 and 4. The asterisk (*) indicates that a test is available with the API Rapid Strep system but not with the MicroScan system. A superscript *a* indicates a test that has a positive result with the API Rapid Strep system and a negative result with the MicroScan system. A superscript *b* indicates a test that has a negative result with the API Rapid Strep system and a positive result with the MicroScan system.

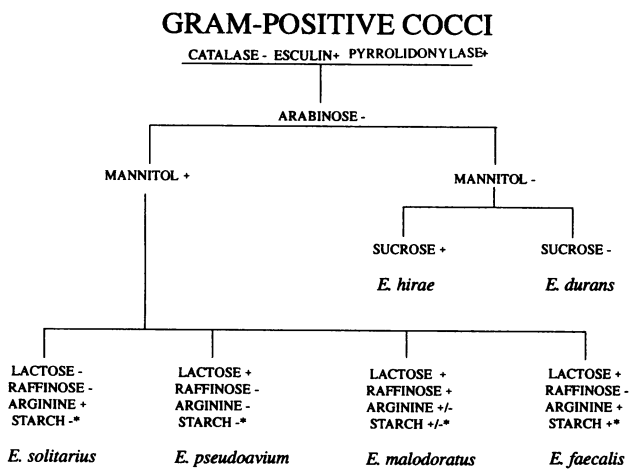

FIG. 3. Flow chart for differentiating the pyrrolidonylase-positive and arabinose-negative enterococci. The data shown are condensed from Tables 2, 3, and 4. The asterisk (*) indicates that a test is available with the API Rapid Strep system but not with the MicroScan system.

consistent and reproducible results were included in the identification schemas. These flow charts can be used with either the API Rapid Strep system or the MicroScan system. Some tests depicted are only available on the API Rapid Strep strips and are noted with an asterisk (*). Figure 2 shows that *E. mundtii* and *E. casseliflavus* are differentiated by the sorbitol test. As stated above, *E. mundtii* is only sorbitol positive when tested with the API Rapid Strep kit; when *E. mundtii* is tested with the MicroScan system, the result is negative. Thus, when the MicroScan system is used, differentiation between these two species must be determined by using the motility, inulin, and raffinose results only. Figure 2 also shows that *E. faecalis* is β-galactosidase negative. As discussed above, this is true only when *E. faecalis* is tested on the API Rapid Strep panel; when it is tested with the MicroScan panel or the ONPG tube test, the result is positive.

The enterococci can be distinguished from other gram-positive catalase-negative cocci. The pyrrolidonylase (pyrrolidonyl arylamidase or pyrrolidonyl peptidase) test differentiates *Enterococcus* spp. from *Leuconostoc*, *Lactococcus*, and *Pediococcus* spp. Another test, which is included in the API Rapid Strep panels or can be conducted as a tube test, is for leucine aminopeptidase activity. This test differentiates *Enterococcus* spp. from all other nonstreptococcal isolates (11).

In conclusion, we have described key tests that can be used to differentiate between species of enterococci and fecal streptococci. These tests can be performed with either the API Rapid Strep panel or the MicroScan system panel. Additional tests include motility, pigmentation, and sucrose tests. The Lancefield group D determination is optional. This test scheme is a rapid and reproducible way to differentiate the enterococci and fecal streptococci.

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REFERENCES

1. **Collins, M. D., R. R. Facklam, J. A. E. Farrow, and R. Williamson.** 1989. *Enterococcus raffinosus* sp. nov., *Enterococcus solitarius* sp. nov. and *Enterococcus pseudoavium* sp. nov. FEMS Microbiol. Lett. **57**:283–288.
2. **Collins, M. D., J. A. E. Farrow, and D. Jones.** 1986. *Enterococcus mundtii* sp. nov. Int. J. Syst. Microbiol. **36**:8–12.
3. **Collins, M. D., D. Jones, J. A. E. Farrow, R. Kilpper-Bälz, and K. H. Schleifer.** 1984. *Enterococcus avium* nom. rev., comb. nov.; *E. casseliflavus* nom. rev., comb. nov.; *E. durans* nom. rev., comb. nov.; *E. gallinarum* comb. nov.; and *E. malodoratus* sp. nov. Int. J. Syst. Microbiol. **34**:220–223.
4. **Collins, M. D., U. M. Rodrigues, N. E. Pigott, and R. R. Facklam.** 1991. *Enterococcus dispar* sp. nov. a new *Enterococcus* species from human sources. Lett. Appl. Microbiol. **12**:95–98.
5. **Deibel, R. H.** 1964. The group D streptococci. Bacteriol. Rev. **28**:330–366.
6. **Devriese, L. A., K. Ceyssens, U. M. Rodrigues, and M. D. Collins.** 1990. *Enterococcus columbae*, a species from pigeon intestines. FEMS Microbiol. Lett. **71**:247–251.
7. **Etheridge, M. E., R. H. Yolken, and S. L. Vonderfecht.** 1988. *Enterococcus hirae* implicated as a cause of diarrhea in suckling rats. J. Clin. Microbiol. **26**:1741–1744.
8. **Facklam, R. R.** 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. J. Clin. Microbiol. **23**:1131–1139.
9. **Facklam, R. R., and M. D. Collins.** 1989. Identification of enterococcus species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27**:731–734.
10. **Facklam, R. R., L. G. Thacker, B. Fox, and L. Eriquez.** 1982. Presumptive identification of streptococci with a new test system. J. Clin. Microbiol. **15**:987–990.
11. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci, p. 238–257. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
12. **Farrow, J. A. E., and M. D. Collins.** 1985. *Enterococcus hirae*, a new species that includes amino acid assay strain NCDO 1258 and strains causing growth depression in young chickens. Int. J. Syst. Microbiol. **35**:73–75.
13. **Gross, K. C., M. P. Houghton, and L. B. Senterfit.** 1975. Presumptive speciation of *Streptococcus bovis* and other group D streptococci from human sources by using arginine and pyruvate tests. J. Clin. Microbiol. **1**:54–60.
14. **Kusuda, R., K. Kawai, F. Salati, C. R. Banner, and J. L. Fryer.** 1991. *Enterococcus seriolicida* sp. nov., a fish pathogen. Int. J. Syst. Bacteriol. **41**:406–409.
15. **Martinez-Murcia, A. J., and M. D. Collins.** 1991. *Enterococcus sulfureus*, a new yellow-pigmented *Enterococcus* species. FEMS Microbiol. Lett. **80**:69–74.
16. **Mundt, J. O.** 1986. Enterococci and lactic acid streptococci, p. 1063–1066. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology. The Williams & Wilkins Co., Baltimore.
17. **Murray, B. E.** 1990. The life and times of the *Enterococcus*. Clin. Microbiol. Rev. **3**:46–65.
18. **Rodrigues, U., and M. D. Collins.** 1990. Phylogenetic analysis of *Streptococcus saccharolyticus* based on 16S rRNA sequencing. FEMS Microbiol. Lett. **71**:231–234.
19. **Schleifer, K. H., and R. Kilpper-Bälz.** 1984. Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. Int. J. Syst. Bacteriol. **34**:31–34.
20. **Sharpe, M. E., and B. G. Fewins.** 1960. Serological typing of strains of *Streptococcus faecium* and unclassified group D streptococci isolated from canned hams and pig intestines. J. Gen. Microbiol. **23**:621–630.
21. **Smibert, R. M., and N. R. Krieg.** 1981. General characterization, p. 409–443. *In* P. Gerhardt, R. G. E. Murray, R. S. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.