Use of the Rapid STREP System for Identification of Viridans Streptococcal Species

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A commercially available streptococcal identification system, Rapid STREP (API System S.A., Montalieu-Vercieu, France), was used to identify 119 viridans strains at the species level. A total of 92% of the strains tested were identified correctly with the Rapid STREP system, and 81% of the correct identifications were made at percentages of identification of 90 or greater. From these data it appears that the Rapid STREP system is a fairly accurate and rapid (24-h incubation) method for identifying species of viridans streptococci.

Along with an increasing interest in identification at the species level of clinically important viridans streptococci, there have been numerous reports describing simplified identification schemes. Most of these schemes have concentrated on reducing the number of tests required for viridans species identification (4, 11, 13), and some have also employed commercially available substrate disks as an alternative to the many types of conventional media required (6, 10). Although they are logistical improvements over classical identification schemes, the aforementioned schemes still require the use of unusual media and incubation periods of from 2 to 5 days.

Commercially available systems used for viridans streptococcal identification provide a simple and rapid approach to identifying viridans streptococci, but reports published to date indicate that they are fairly inaccurate tools for the identification of these streptococci (7, 9, 14). Our interest in viridans streptococci led us to test a commercially available product, Rapid STREP (API System S.A., Montalieu-Vercieu, France), with 119 viridans strains. A previous report (12) noted excellent agreement between results obtained with a prototype of this system and conventional methods.

MATERIALS AND METHODS

Bacterial strains. The viridans strains tested consisted of 105 clinical isolates and 14 stock strains. The clinical strains were either fresh isolates or had been stored at −70°C on glass beads (8). The stock strains were stored at −70°C as suspensions in horse blood. Stock strains SS909 and SS980 (Streptococcus mutans) and SS1076 and SS912 (S. sanguis I) were originally obtained from R. Facklam, Centers for Disease Control, Atlanta, Ga. Stock strains BHT, 6715, AHT and GS-5 (S. mutans), 5, 6, and 7 (S. sanguis I), and RG34 and H7PR (S. sanguis II) were originally obtained from R. Gibbons, Forsythe Dental Infirmary, Boston, Mass. The streptococci were maintained on brucella agar plates containing 5% horse blood (GIBCO Diagnostics, Madison, Wis.) and were routinely incubated at 35°C in the presence of 3 to 5% CO2.

Conventional identification methods. Strains of alpha- or nonhemolytic streptococci that either were nongroupable or possessed F, H, or K Lancefield antigens were identified by previously described methods (10; K. L. Ruoff, J. A. Fishman, S. B. Calderwood, and L. J. Kunz, Am. J. Clin. Pathol., in press), and when discrepancies between results obtained with these methods and with Rapid STREP occurred, the methods of Facklam (2, 3) and Facklam and coworkers (5) were used to obtain definitive species identifications.

Identification by Rapid STREP. Rapid STREP strips and other required reagents were purchased from DMS Laboratories, Inc., Flemington, N.J. The strips are each composed of 20 cups containing dehydrated substrates used for the determination of the following biochemical activities: acidification of ribose, L-arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, starch, and glycogen; hydrolysis of arginine and hippurate; acetoin production; and production of the enzymes β-glucosidase, pyrrolidonyl arylamidase, α-galactosidase, β-glucuronidase, β-galactosidase, alkaline phosphatase, and leucine arylamidase.

The strips were inoculated, read, and interpreted according to the instructions of the manufacturer, with one exception. The recommended procedure for specimen preparation involves choosing a single colony, which is subsequently propagated on Columbia sheep blood agar for 24 to 48 h in an anaerobic atmosphere. We used multiple colonies from pure cultures which were streaked on one or more brucella horse blood agar plates and incubated aerobically with 3 to 5% CO2.
to obtain sufficient inocula for the strips. After incubation of the inoculated strips at 35°C for 4 h, the specified reagents were added to eight of the cupsules, and the reactions were recorded. The strips were reincubated overnight before the remaining 12 reactions were read. A seven-digit profile was formulated by adding the numerical values given to each test result. This seven-digit profile was then compared to those provided in an identification codebook.

RESULTS

Correctly identified strains. The strains of viridans species tested that were identified correctly by the Rapid STREP system are shown in Table 1, along with indications of the likelihood of correct identification. In the Rapid STREP system the qualification “excellent” refers to a percentage of identification equal to or greater than 99.9, “very good” corresponds to percentages between 99.0 and 99.8, “good” corresponds to percentages between 90.0 and 98.9, and “acceptable” indicates percentages of identification between 80.0 and 89.9. The qualification “species identification” is applied to strains which can be identified at the species level, but not at the level of a distinct biotype within the species. The “low discrimination” category applies when the highest percentage of identification is less than 80.0, but the sum of the two or three highest percentages of identification is greater than 80.0. In this case additional biochemical tests are required to identify the isolate with certainty.

The data in Table 1 indicate that 109 (92%) of the 119 viridans strains examined were correctly identified by the Rapid STREP system. A total of 88 of these 109 strains were correctly identified at percentages of identification of 90.0 or better, and in only four cases was additional testing required due to the low-discrimination qualification. The unclassified strains in Table 1 could not be identified with conventional methods, and since their profile numbers were absent from the codebook, they were considered to be correctly identified by the Rapid STREP system. The identification profiles of two strains of S. sanguis I were not found in the codebook, but these profiles were located by the DMS Computer Service.

Discrepancies between conventional and Rapid STREP results. Of the 10 strains which were not correctly identified by the Rapid STREP system, 7 strains had identification profiles which were not included in the codebook. When the DMS Computer Service was contacted, three of the profiles yielded “doubtful” identifications, and the remaining four could not be found in the extended list of profile numbers. These seven strains were accounted for by two isolates identified by conventional methods as S. mitis and one strain each of S. mutans, S. sanguis I, S. sanguis II, S. MG-intermedius and S. anginosus-constellatus.

Two strains of S. MG-intermedius were misidentified by Rapid STREP; one was given a species identification as S. sanguis I, and the other was given a low-discrimination identification for S. sanguis I and S. sanguis II. One S. mitis strain was misidentified as S. sanguis I in the “good” category of likelihood of identification.

DISCUSSION

From the examination of the 119 strains included in our study, it appears that the Rapid STREP system is a fairly accurate method for attaining species identifications for viridans streptococcal strains. A total of 92% of the strains tested were correctly identified by Rapid STREP. The system is simple to use, and reactions were usually easy to interpret. While Rapid STREP may be used to identify groupable streptococci in 4 h, the manufacturer recommends a 24-h incubation of strips before certain reactions are read when Rapid STREP is used with viridans strains. The 24-h incubation period still is
shorter than those required by simplified conventional schemes (4, 6, 10, 11, 13).

The Rapid STREP system allows the identification of biotypes within the species S. mitis, S. sanguis I, S. sanguis II, and S. milleri. Within the S. milleri species, it appears that biotype 1 corresponds to beta-hemolytic S. milleri, biotype 2 includes strains designated as either S. MG-intermedius or S. anginosus-constellatus according to Facklam’s nomenclature (4), and biotype 3 corresponds to S. milleri strains described by Ball and Parker (1) and referred to as “unidentified urine isolates” in the present and previous studies (10). In the study reported here, three of the nine correctly identified S. anginosus-constellatus strains produced profiles consistent with S. milleri biotype 1, while the remaining six strains, along with 20 of 24 correctly identified S. MG-intermedius isolates, were identified as S. milleri biotype 2. All 13 of the unidentified urine isolates tested were identified as S. milleri biotype 3. It was difficult to correlate the Rapid STREP biotypes of S. mitis, S. sanguis I, and S. sanguis II with biotypes revealed by conventional testing because the Rapid STREP biotypes were often determined by characteristics not assayed in conventional identification schemes. Nevertheless, the biotyping capacity of the Rapid STREP system could conceivably be useful in studying the epidemiological aspect of infections caused by viridans streptococci.

LITERATURE CITED