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Identification of *Staphylococcus* Species with the API STAPH-IDENT System†

WESLEY E. KLOOS* AND JANA F. WOLFSHOHL

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27650

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The API STAPH-IDENT system was compared with conventional methods for the identification of 14 *Staphylococcus* species. Conventional methods included the Kloos and Schleifer simplified scheme and DNA-DNA hybridization. The API STAPH-IDENT strip utilizes a battery of 10 miniaturized biochemical tests, including alkaline phosphatase, urease, β-glucosidase, β-glucuronidase, and β-galactosidase activity, aerobic acid formation from D-(+)-mannose, D-mannitol, D-(+)-trehalose, and salicin, and utilization of arginine. Reactions of cultures were determined after 5 h of incubation at 35°C. Results indicated a high degree of congruence (>90%) between the expedient API system and conventional methods for most species. The addition of a test for novobiocin susceptibility to the API system increased the accuracy of identification of *S. saprophyticus*, *S. cohnii*, and *S. hominis*, significantly. Several strains of *S. hominis*, *S. haemolyticus*, and *S. warneri* which were difficult to separate with the Kloos and Schleifer simplified scheme were accurately resolved by the API system.

The identification of *Staphylococcus* species in human and veterinary clinical laboratories is important as some are frequently encountered in infections and often demonstrate a high level of resistance to various antibiotics (1, 2, 5, 7–9, 11–13, 18, 20–24, 26). Most surveys indicated a higher frequency of *S. aureus*, *S. epidermidis*, and *S. saprophyticus* strains associated with human infections compared with other species, and *S. aureus*, *S. intermedius* and *S. hyicus* were species more often associated with animal infections. Some of the other species are of questionable or undetermined clinical importance.

Extensive phenotypic character analyses and DNA-DNA hybridization studies (reviewed by Kloos, 13; Kloos and Schleifer, 16) have provided sound taxonomic criteria for the classification of at least 16 different *Staphylococcus* species. Shortly after the formal designation of most of the human-adapted species, Kloos and Schleifer (14) developed a simplified taxonomic scheme for application in the routine laboratory. The key characters used in this scheme included coagulase activity, hemolysis, nitrate reduction, and aerobic acid production from β-D-(−)-fructose, D-(+)-xylose, L-(+)-arabinose, D-(−)-ribose, maltose, α-lactose, sucrose, D-(+)-trehalose, D-mannitol, and xylitol. Susceptibility to novobiocin and lysostaphin, alkaline phosphatase activity, anaerobic growth pattern in thioglycolate, and colony morphology were considered as secondary characters to improve accuracy in identification. The above combination of characters was quite accurate in predicting DNA homology groups. However, even though the simplified scheme provided the clinical bacteriologist with a workable system for identifying species, it still required the preparation of a variety of media and reagents and from 2 to 3 days of incubation for interpretation of results.

To further facilitate the identification of *Staphylococcus* species, API system S.A., Montalieu-Vercieu, France, developed an API STAPH strip system combining 20 miniaturized biochemical tests, many of which were derived from the above simplified scheme. The API system was particularly advantageous in that it provided preformed strips containing the test substrates and made available necessary reagents. Furthermore, in most cases, reactions could be interpreted after incubation at 37°C for 24 h. Several investigators have reported on the use of this system (4, 8, 9).

Just recently, Analytab Products, Plainview, N.Y., has introduced a rapid and sensitive API STAPH-IDENT strip system that combines 10 miniaturized biochemical tests which can be interpreted after only 5 h of incubation at 35 to 37°C. The purpose of this investigation is to compare the new API STAPH-IDENT system

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with conventional methods for the identification of *Staphylococcus* species.

MATERIALS AND METHODS

Bacterial cultures. A total of 75 *S. epidermidis*, 59 *S. hominis*, 48 *S. haemolyticus*, 34 *S. warneri*, 46 *S. capitis*, 24 *S. auricularis* (sp. nov., W. E. Kloos and K. H. Schleifer, submitted for publication), 23 *S. simulans*, 24 *S. saprophyticus*, 25 *S. cohnii*, 20 *S. xylosus*, 22 *S. sciuri*, 22 *S. aureus*, 20 *S. intermedius*, and 26 *S. hyicus* strains were used in this study. Strains included those isolated from healthy skin and a variety of infections and the species type strains and other reference strains described previously (6, 10, 15, 17, 25). Approximately 25% of the strains tested were from fresh human or veterinary clinical sources.

Conventional identification methods. All phenotypic character determinations were performed according to methods described in the Kloos and Schleifer simplified scheme (14). In addition to the 13 key characters making up this scheme, the supporting characters of colony morphology, aerobic acid production from D-(+)-cellobiose, anaerobic growth in thioglycolate, and susceptibility to novobiocin were also examined. Selected strains from each species were further verified on the basis of their DNA relatedness to species reference or type strains, using DNA-DNA hybridization techniques described previously (3, 19).

API STAPH-IDENT strip system. The API STAPH-IDENT strips consist of a series of 10 microcupules containing dehydrated substrates, nutrient media, or both. The alkaline phosphatase (Phs), β-glucosidase (Gls), and β-glucuronidase (Glc) microcupules contain chromogenic substrates and positive reactions are noted by the liberation of *p*-nitrophenol from them. The urease (Ure) and arginine utilization (Arg) microcupules contain phenol red as an indicator to detect alkaline end products. The mannose (Mne), mannitol (Man), trehalose (Tre), and salicin (Sal) microcupules contain cresol red as an indicator to detect acid production. The β-galactosidase (Ngp) microcupule contains 2-naphthol-β-D-galactopyranoside and requires the addition of STAPH-IDENT reagent (0.35% fast blue BB salt in 2-methoxyethanol) to detect free β-naphthol.

Recommended procedures of the manufacturer were followed for the preparation of strips and inoculum and the inoculation, incubation, and reading of strips. Test organisms were removed from an overnight culture inoculated on Trypticase soy agar plates containing 5% sheep blood, using a sterile cotton swab. The inoculated swab was agitated in 2 ml of sterile 0.85% saline so that the final turbidity was approximately that of a no. 3 McFarland ($BaSO_4$) turbidity standard. Each microcupule on the strip was filled with three drops (approximately 80 μl) of the above-described bacterial suspension. After inoculation, strips (placed into plastic trays with lids) were incubated for 5 h at 35°C in air and then read. Two drops of STAPH-IDENT reagent were added to the Ngp microcupule, and color development was recorded after 30 s.

Positive reactions were converted to a four-digit profile for species identification according to instructions of the manufacturer. The following 10 biochemical tests are divided into the four groups arranged in order on the strip: Phs, Ure, and Gls; Mne, Man, and Tre; Sal, Glc, and Arg; and Ngp. A value of one, two, and four is assigned to the first, second, and third positive biochemical test, respectively, in each group. A value of zero is assigned to a negative result. Each resulting profile number represents a unique combination of positive reactions.

RESULTS

Identification according to the Kloos and Schleifer simplified scheme. Of the 376 staphylococcal strains isolated from humans and belonging to the recognized species *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. warneri*, *S. capitis*, *S. simulans*, *S. saprophyticus*, *S. cohnii*, *S. xylosus*, and *S. aureus*, 370 (98%) could be classified by the simplified scheme of Kloos and Schleifer (14). The six (2%) strains not classified by the scheme were identified as two coagulase-negative, encapsulated *S. aureus* strains and one strain each of *S. epidermidis*, *S. warneri*, *S. capitis*, and *S. simulans* by DNA-DNA hybridization and the API STAPH-IDENT system according to the currently available STAPH-IDENT profile register. Four strains of *S. hominis*, two strains of *S. warneri*, and one strain of *S. haemolyticus* were misclassified by the simplified scheme. Misclassification was suspected first by results of the API STAPH-IDENT system and then verified by DNA-DNA hybridization.

Modification of the existing simplified scheme was necessary to identify the animal species *S. sciuri* (coagulase negative, acid from cellobiose, resistant to novobiocin at 1.6 μg/ml) (17), *S. intermedius* (coagulase positive, weak acid from maltose, usually weak acid from mannitol) (10, 23) and *S. hyicus* (coagulase variable, susceptible to novobiocin, no acid from maltose, usually no acid from mannitol) (6, 23, 24) and the newly proposed human species *S. auricularis* (coagulase negative, susceptible to novobiocin, acid from trehalose, usually weak acid from maltose, no acid from mannitol, xylose, arabinose, xylitol, cellobiose, or ribose, weak anaerobic growth in thioglycolate, very slow growing, unpigmented colonies; W. E. Kloos and K. H. Schleifer, manuscript submitted for publication). With the indicated modifications, all strains of *S. auricularis*, *S. sciuri*, and *S. intermedius* were classified correctly. Of the 26 *S. hyicus* strains tested, 20 (77%) were also correctly classified. The remaining six unclassified strains were among the coagulase-negative *S. hyicus*. Their identity was determined by DNA-DNA hybridization and the API STAPH-IDENT system.

DNA-DNA hybridization. In addition to identifying the few strains that were unclassified by the Kloos and Schleifer simplified scheme (14),

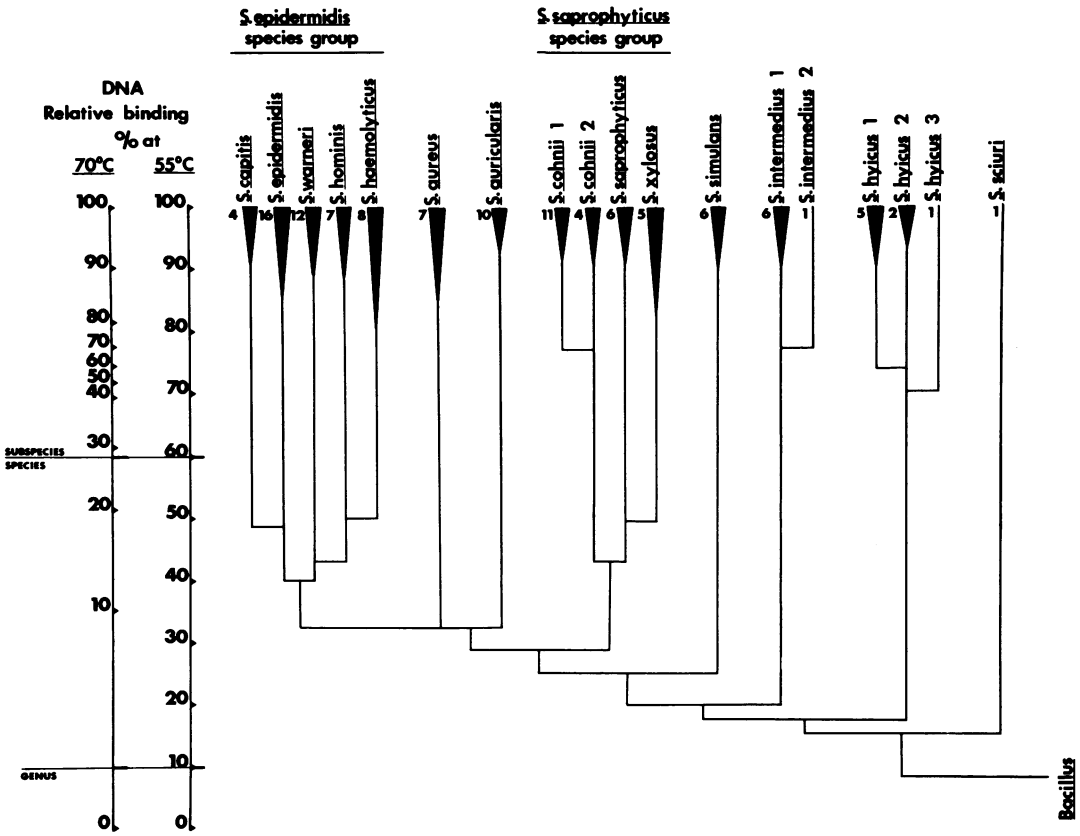

FIG. 1. DNA relationships of *Staphylococcus* species. The number of strains tested in each species is indicated just below the species name and to the left of the dendrogram line. DNA relatedness can be determined by the relative binding indicated on the ordinates (left). Subspecies are given a numerical (e.g., 1, 2, etc.) designation due to the absence of a formally proposed epithet.

DNA-DNA hybridization techniques were used to verify the identity of a representative sample of different classified strains in each species. Various selected strains were used as sources of unlabeled DNA, whereas known species reference or type strains were used as sources of tritiated thymidine-labeled DNA. Reassociation reactions were performed at optimal (55°C) and stringent (70°C) conditions and the relative binding of labeled to unlabeled DNA was determined (Fig. 1). Strains belonging to the same species exhibited ≥75% relative DNA binding at optimal and stringent reassociation conditions, with the exception of strains belonging to different subspecies in *S. cohnii*, *S. intermedius*, and *S. hyicus*, where under stringent conditions relative DNA binding was between 50 and 70%.

API STAPH-IDENT strip system. The distribution of API four-digit profiles among strains of various *Staphylococcus* species is shown in Table 1. A total of 115 different profiles were encountered in this study of which 85 to 90% were unique for a single species. From 8 to 14% of the profiles overlapped two species, and 1 to 3% overlapped three species. If novobiocin susceptibility and coagulase testing (as recommended by the manufacturer) were added to the battery, 95 to 96% of the profiles generated were unique. Profile overlap was particularly noted between the species *S. hominis*, *S. cohnii*, and *S. saprophyticus*. By the addition of novobiocin susceptibility as a test, the accuracy in identification of *S. cohnii* and *S. saprophyticus* increased to nearly 100%.

In all but four species, 50% or more of the strains were distributed among one or two major species profiles. In *S. warneri*, *S. simulans*, *S. xylosus*, and *S. hyicus*, 50% or more of the strains were distributed among three to four major species profiles. More than 50% of the total, and all major, profiles were tested and verified according to species identity by DNA-DNA hybridization of representative strains bearing them. It was interesting to find that the API STAPH-IDENT strip system could accurately resolve two different subspecies popula-

TABLE 1. Frequency of API STAPH-IDENT four-digit profiles among *Staphylococcus* species

Staphylococcus species	No. of strains	API profile[a]	% with profile	Species with same profile (differentiating character[s])[b]
Coagulase-negative				
S. epidermidis	75	**3040** (3)	33–47	
		3000 (6)	21–25	
		3140 (2)	9–13	
		7140 (1)	7–9	
		3100	4–5	
		7040	1–9	
		2040 (2)	3–4	*S. hominis* (Ana$^-$)
		2140	1	
		3400 (1)	1	
		5040	1	
		1040	1	
		6000 (1)	0–1	
		2000 (1)	0–1	*S. hominis* (Ana$^-$) (1) *S. cohnii* (Nov$^+$, Suc$^-$)
		6040	0–1	*S. warneri* (Nit$^-$)
S. hominis	59	***2400*** (3)	42–52	*S. saprophyticus* (Nov$^+$, Xyt$^+$)
		2000 (1)	17–25	(1) *S. cohnii* (Nov$^+$, Suc$^-$)
		2040 (3)	12	*S. epidermidis* (Ana$^+$)
		0400 (1)	5	*S. auricularis* (Cm^s) (1) *S. cohnii* (Nov$^+$, Suc$^-$)
		2440	<2	
		2620	<2	
		2700	<2	
		2600	<2	
		0600	<2	*S. cohnii* (Nov$^+$, Suc$^-$)
		0700 (1)	<2	*S. cohnii* (Nov$^+$, Suc$^-$)
		0040	<2	*S. auricularis* (Cm^s)
S. haemolyticus	48	**0460** (3)	25–31	
		4440 (2)	25	
		0640 (1)	15–17	
		0660	8–12	
		4460	10	
		4640	4	
		0440 (1)	2–4	*S. auricularis* (Cm^s, Hem$^-$)
		4660	2	
		4400	2	
S. warneri	34	**6600** (7)	32	
		6420 (2)	15	
		6660 (3)	9–12	
		6460	9–12	
		6400	9–12	
		6620 (1)	6	
		6040	3	
		4600	3	
		6440	0–9	
		6260 (1)	0–3	
		6120	0–3	
		6520	0–3	
S. capitis	46	**0340** (4)	64	
		0300 (2)	24	
		0240	4	
		0140	2–4	
		0200	2	*S. cohnii* (Nov$^+$)
		0040	0–2	*S. auricularis* (Cm^s, Mal$^\pm$) (1) *S. hominis* (Mai$^+$)
S. auricularis	24	***0440*** (6)	21–42	*S. haemolyticus* (Cm^l, Hem$^+$)

TABLE 1—*Continued*

Staphylococcus species	No. of strains	API profile[a]	% with profile	Species with same profile (differentiating character[s])[b]
		0400 (3)	29–38	*S. hominis* (Cm^m) (1) *S. cohnii* (Nov^+, Cm^l)
		0040 (2)	8–17	(1) *S. hominis* (Cm^m)
		0000	4	
		0541 (1)	4	
		0501	4	
		0441 (1)	0–12	
		0041	0–8	
S. simulans	23	**2461** (1)	22	
		3601 (2)	13	
		2541	9–17	
		2761 (1)	9	
		2741	9	
		2061	9	
		2441	4–17	
		3441 (1)	4–9	
		3461	4	
		2421 (1)	4	
		3041 (1)	0–4	
S. saprophyticus	24	**2401** (1)	33	
		2400 (3)	17–21	*S. hominis* (Nov^-, Xyt^-)
		6401 (1)	12–21	
		2001 (2)	17	
		6001	4–12	
		2601	4	
		2600	0–4	(1) *S. hominis* (Nov^-, Xyt^-)
S. cohnii subsp. 1	19	***0600*** (7)	42–63	(1) *S. hominis* (Nov^-, Suc^+)
		0200 (1)	11–16	(1) *S. capitis* (Nov^-)
				S. auricularis (Nov^-, Cm^s)
		1600 (1)	0–11	
		1000 (1)	0–5	
		0000 (1)	0–5	(1) *S. auricularis* (Nov^-, CM^s)
S. cohnii subsp. 2	6	**3721** (2)	33	
		3021 (1)	17–33	
		2421 (1)	0–33	(1) *S. simulans* (Nov^-, Suc^+)
		3421 (1)	0–33	
		3061 (1)	0–17	*S. simulans* (Nov^-, Suc^+)
S. xylosus	20	**7021** (2)	25–45	
		7421 (1)	10–20	
		7501 (1)	10	(1) *S. intermedius* ($Coag^+$, Nov^-)
		6421	5–10	
		6021	5–10	
		7721 (1)	5–10	
		7521	5	
		7020	5	
		7121	0–5	
		7001	0–5	
		6721 (1)	0–5	
		6731	0–5	
		6031	0–5	
S. sciuri	22	**5710** (1)	68–77	
		5310	9	
		4710 (1)	5–9	
		5700	5–9	(1) *S. aureus* ($Coag^+$, Nov^-)
		5300	5	
Coagulase-positive *S. aureus*	20	**7740** (1)	55–60	

TABLE 1—*Continued*

Staphylococcus species	No. of strains	API profile[a]	% with profile	Species with same profile (differentiating character[s])[b]
		0700 (1)	5–11	(1) *S. hominis* (Nov$^-$, Suc$^+$)
		2000 (1)	5	*S. hominis* (Nov$^-$ Suc$^+$)
		0400	5	*S. hominis* (Nov$^-$ Suc$^+$)
		5700 (1)	5	*S. sciuri* (Coag$^-$, Nov$^+$)
		3740 (1)	0–5	
S. intermedius	20	**7541** (5)	45–55	
		3541 (1)	20–35	
		7141	5	
		3141 (1)	5	
		3401 (1)	5	
		7501 (1)	5	*S. xylosus* (Coag$^-$, Nov$^+$)
		3501	0–5	
		5740	5–10	
		3700 (1)	5–10	
		1740 (2)	5–10	
		1700 (1)	5–10	
		7540	5	(1) *S. hyicus* (Mal$^-$)
S. hyicus	26	**3560** (2)	19–23	
		1560 (2)	8–23	
		7560	8–19	
		3540 (3)	15	
		1540 (2)	8–12	
		5560 (1)	4–12	
		5540	4	
		5420	4	
		5000	4	
		7540	4	(1) *S. aureus* (Mal$^+$)
		2560	0–4	

[a] Major species profiles (including ≥20% of strains) are indicated by boldface type, and profiles representing more than one species are indicated by italics. Numbers in parentheses indicate the number of strains with a given profile verified by DNA-DNA hybridization. More than one profile (usually two) were assigned to strains exhibiting borderline positive reactions.

[b] The small number 1 in parentheses in front of the species name indicates that only one strain showed the same profile. Abbreviations: Nov, novobiocin resistance (+, MIC ≥ 1.6 μg/ml); Coag, coagulase; Ana, dense anaerobic growth in thioglycolate; Suc, acid formation from sucrose; Xyt, acid formation from xylitol; Mal, acid formation from maltose; Nit, nitrate reduction; Cm, colony size (^{s}, small, very slow growing; ^{m}, medium size; ^{l}, large); and Hem, hemolysis (14–16, 18, 19).

TABLE 2. Differentiation of *Staphylococcus* species by API STAPH-IDENT system biochemical tests.

Staphylococcus species	No. of strains tested	API STAPH-IDENT system test[a]										Additional tests	
		Phs	Ure	Gls	Mne	Man	Tre	Sal	Glc	Arg	Ngp	Nov	Coag
Coagulase negative													
S. epidermidis	75	94	97–99	8–19	21–24	0	1	0	0	65–79	0	0	0
S. hominis	59	0	90–92	0	3	8	59–66	0	0	19	0	2	0
S. haemolyticus	48	0	0	44	0	31–40	100	0	48	98	0	0	0
S. warneri	34	0	97	97–100	3	53	91–97	0	44–47	26–32	0	0	0
S. capitis	46	0	0	0	91–96	96–98	0	0	0	74	0	0	0
S. auricularis	24	0	0	0	8	0	75–83	0	0	54–62	8–29	0	0
S. simulans	23	17–26	100	0	26–35	17	74	0	61	96	100	0	0
S. saprophyticus	24	0	100	25	0	0–4	71–83	0	0	96	79	100	0
S. cohnii a subsp. 1	19	0–16	5	0	5–16	84	79–84	0	0	0	0	100	0
S. cohnii b subsp. 2	6	67–100	100	0	33	33	67	0	100	0–17	100	100	0
S. xylosus	20	75–80	100	100	25–35	10–15	45–55	5	85–90	0	95	100	0
S. sciuri	22	91–95	0	100	100	100	86	86–91	0	0	0	100	0
Coagulase positive													
S. aureus	20	100	60–70	80	100	90	100	0	0	80–85	0	0	100
S. intermedius	20	100	100	55–65	95	0	90	0	0	85–90	100	0	100
S. hyicus	26	96–100	54–58	27–46	92	0	96	0	62–65	92	0	0	46–58

[a] Abbreviations: Phs, alkaline phosphatase; Ure, urease; Gls, β-glucosidase; Mne, acid formation from mannose; Man, acid formation from mannitol; Tre, acid formation from trehalose; Sal, acid formation from salicin; Glc, β-glucuronidase; Arg, utilization of arginine; Ngp, β-galactosidase; Nov, resistance to novobiocin (MIC ≥ 1.6 μg/ml); and Coag, coagulase. Values are the percentages of positive reactions. Two values indicate a difference in results that may be obtained due to borderline positive reactions.

tions of *S. cohnii* (13). The API strip was unable to resolve certain subspecies within *S. intermedius* or *S. hyicus*.

A summary of the percentage of strains in each species giving positive results for each of the biochemical tests in the API STAPH-IDENT strip and the additional tests of novobiocin susceptibility and coagulase activity is given in Table 2. Most biochemical tests included on the strip were very useful in resolving members of the *S. epidermidis* species group (composed of *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. warneri*, and *S. capitis*) and members of the *S. saprophyticus* species group (composed of *S. saprophyticus*, *S. cohnii*, and *S. xylosus*). The three coagulase-positive species *S. aureus*, *S. intermedius*, and *S. hyicus* could be resolved on the basis of acid formation from mannitol and β-glucuronidase and β-galactosidase activities.

DISCUSSION

The API STAPH-IDENT system provided a rapid and accurate method for identifying the various *Staphylococcus* species examined in this study. The 12-test system proposed (including the 10 miniaturized biochemical tests, novobiocin susceptibility, and coagulase production) had an overall congruence of 95% with conventional methods. Preliminary results (unpublished data) suggest that this system can also identify at least two other new animal *Staphylococcus* species, currently under investigation, and *Micrococcus kristinae*, the species most often confused with staphylococci by virtue of its ability to produce acid from a variety of carbohydrates and anaerobic growth.

Some of the results of certain biochemical tests used in the API STAPH-IDENT strip are different from those obtained by conventional methods. This discrepancy is in most part, due to differences in incubation time, substrate concentration and indicator sensitivity. For example, *S. saprophyticus* is usually mannitol negative by the API test, but mannitol positive with conventional methods (14, 25). Similarly, only those few strains of *S. epidermidis* producing rapid acid from mannose will be recorded as positive by the API test, whereas by longer conventional methods, most strains of *S. epidermidis* will be mannose positive. These discrepancies did not alter the outcome of the API system in identifying species.

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