Acetoin Production in the Identification of Isolates as Members of *Staphylococcus intermedius* Hájek

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A total of 50 staphylococcal strains of animal origin were identified as members of *Staphylococcus intermedius* Hájek on the basis that they possessed all of the characteristics originally reported by Hájek for *S. intermedius* except one, acetoin production. The Barritt method failed to demonstrate acetoin production by these isolates, whereas in the case of a micromethod in which pyruvate was used as substrate, acetoin production was detected. We discuss both the discrepancy observed between the results of the two methods and the taxonomic implications of our findings.

After studies on *Staphylococcus aureus* strains from animals, including humans, Hájek and Maršálek (10) suggested a six-biovar (bio-type) classification for staphylococci (biovars A through F). Biovars E and F include strains isolated from dogs, horses, and mink (biovar E), as well as from pigeons and foxes (biovar F), and differ from the other biovars in their biochemical and structural characteristics (11), thus positioning these biovars between *S. aureus* and the coagulase-negative staphylococci. Consequently, Hájek (9) advocated the recognition of a new species, *Staphylococcus intermedius*, for the strains of biovars E and F.

Acetoin production was 1 of the 17 tests selected by Hájek (9) for species differentiation. To detect acetoin production, Hájek used two methods, the conventional technique of Baird-Parker (2) and the more rapid Davis-Hoyling test (6). Hájek found that *S. intermedius* did not produce acetoin.

In a study on coagulase-positive biovar E staphylococci of animal origin, we used two methods for the detection of acetoin, the conventional method of Baird-Parker (2), who utilized the Barritt method, and a micromethod (5). Our results did not agree with those of Hájek.

The purpose of this study was to draw attention to the problems that occur when attempts are made to use for taxonomic purposes characters whose determinations are based on the sensitivities of the methods employed.

MATERIALS AND METHODS

Bacterial strains. We studied 50 coagulase-positive *Staphylococcus* biotype E strains, which were identified by the criteria and methods described by Hájek and Maršálek (9, 11). Of these, 49 were from

dogs and one was from poultry (Table 1). *S. intermedius* CCM 5739, *S. aureus* Cowan I (=NCTC 8530), and *Staphylococcus epidermidis* CCM 2124 were included as reference strains.

Methods. A total of 16 of the biochemical and morphological characters described by Hájek for *S. intermedius* were examined by the methods used by Hájek (9). These characters included the production of coagulase and thermonuclease, the anaerobic utilization of mannitol, the production of pigment, the susceptibility to phages of human origin, and the presence of α - and β -ribitol teichoic acids. Also, the presence of protein A was investigated by two techniques, conditioned hemagglutination (8) and gel precipitation (14). Susceptibility to novobiocin was tested by using 30- μ g disks. Mannitol, maltose, mannose, and trehalose acidifications, nitrate reduction, and phosphatase production were examined by using a micromethod (5), the API Staph System (La Balme les Grottes, Montalieu Vercieu, France), and an 18-h culture prepared by the method of Brun et al. (5) as the inoculum. The production of acetoin was investigated by three methods. In the Barritt method (4), we used Clark and Lubs (Difco Laboratories, Detroit, Mich.) medium (glucose peptone medium buffered with dipotassium phosphate). In the Barritt method as modified by Abd-el-Malek and Gibson (1), we used glucose peptone medium without phosphate, and acetoin production was detected after incubation at 37°C for 2, 7, and 14 days. To 1 ml of culture, 0.6 ml of 6% α -naphthol in ethyl alcohol and 0.2 ml of 40% potassium hydroxide (water solution) were added (2). And in the micromethod (5), sodium pyruvate was used as the substrate, and after 18 h of incubation acetoin production was detected by adding 1 drop each of α -naphthol and 40% potassium hydroxide.

RESULTS AND DISCUSSION

In 14 of the 16 tests performed, the *S. intermedius* reference strain and our 50 strains produced the same results. However, these strains did not show two of the characteristics described

TABLE 1. *Strains of staphylococci used in this study*

Serial no.	Laboratory no.	Received as:		Source	Habitat
1	791104	<i>S. intermedius</i>	CCM 5739	CCM ^a	Nasal swabs from a healthy pigeon
2	800426	<i>S. aureus</i> Cowan I	NCTC 8530	CCM	Human
3	791103	<i>S. epidermidis</i>	CCM 2124	CCM	Human nose
4	SC1	<i>Staphylococcus</i> sp.		Pilet ^b	Canine
5	SC2	<i>Staphylococcus</i> sp.		Pilet	Canine
6	SC3	<i>Staphylococcus</i> sp.		Pilet	Canine
7	SC4	<i>Staphylococcus</i> sp.		Pilet	Canine
8	SC5	<i>Staphylococcus</i> sp.		Pilet	Canine
9	SC6	<i>Staphylococcus</i> sp.		Pilet	Canine
10	SC7	<i>Staphylococcus</i> sp.		Pilet	Canine
11	SC8	<i>Staphylococcus</i> sp.		Pilet	Canine
12	323 C	<i>Staphylococcus</i> sp.		Devoyot ^c	Canine
13	21855	<i>Staphylococcus</i> sp.		Desmettre ^d	Canine
14	21723	<i>Staphylococcus</i> sp.		Desmettre	Canine
15	21890	<i>Staphylococcus</i> sp.		Desmettre	Canine
16	21684	<i>Staphylococcus</i> sp.		Desmettre	Canine
17	21714	<i>Staphylococcus</i> sp.		Desmettre	Canine
18	21644	<i>Staphylococcus</i> sp.		Desmettre	Canine
19	21869	<i>Staphylococcus</i> sp.		Desmettre	Canine
20	21861	<i>Staphylococcus</i> sp.		Desmettre	Canine
21	21762	<i>Staphylococcus</i> sp.		Desmettre	Canine
22	21740	<i>Staphylococcus</i> sp.		Desmettre	Canine
23	21807	<i>Staphylococcus</i> sp.		Desmettre	Canine
24	21734	<i>Staphylococcus</i> sp.		Desmettre	Canine
25	21875	<i>Staphylococcus</i> sp.		Desmettre	Canine
26	21834	<i>Staphylococcus</i> sp.		Desmettre	Canine
27	21800	<i>Staphylococcus</i> sp.		Desmettre	Canine
28	21679	<i>Staphylococcus</i> sp.		Desmettre	Canine
29	21853	<i>Staphylococcus</i> sp.		Desmettre	Canine
30	21687	<i>Staphylococcus</i> sp.		Desmettre	Canine
31	21851	<i>Staphylococcus</i> sp.		Desmettre	Canine
32	21667	<i>Staphylococcus</i> sp.		Desmettre	Canine
33	21704	<i>Staphylococcus</i> sp.		Desmettre	Canine
34	21735	<i>Staphylococcus</i> sp.		Desmettre	Canine
35	21761	<i>Staphylococcus</i> sp.		Desmettre	Canine
36	21768	<i>Staphylococcus</i> sp.		Desmettre	Canine
37	21774	<i>Staphylococcus</i> sp.		Desmettre	Canine
38	21808	<i>Staphylococcus</i> sp.		Desmettre	Canine
39	21833	<i>Staphylococcus</i> sp.		Desmettre	Canine
40	21839	<i>Staphylococcus</i> sp.		Desmettre	Canine
41	21876	<i>Staphylococcus</i> sp.		Desmettre	Canine
42	21889	<i>Staphylococcus</i> sp.		Desmettre	Canine
43	21891	<i>Staphylococcus</i> sp.		Desmettre	Canine
44	21893	<i>Staphylococcus</i> sp.		Desmettre	Canine
45	21681	<i>Staphylococcus</i> sp.		Desmettre	Canine
46	21707	<i>Staphylococcus</i> sp.		Desmettre	Canine
47	21710	<i>Staphylococcus</i> sp.		Desmettre	Canine
48	21718	<i>Staphylococcus</i> sp.		Desmettre	Canine
49	21760	<i>Staphylococcus</i> sp.		Desmettre	Canine
50	21826	<i>Staphylococcus</i> sp.		Desmettre	Canine
51	21843	<i>Staphylococcus</i> sp.		Desmettre	Canine
52	21884	<i>Staphylococcus</i> sp.		Desmettre	Canine
53	1547	<i>Staphylococcus</i> sp.		Desmettre	Poultry

^a CCM, Czechoslovak Collection of Microorganisms, JE Purkyne University Trída Obránců Miru, 1066243 Brno, Czechoslovakia.

^b X. Pilet, Laboratory of Bacteriology, Veterinary School, Maisons Alfort, France.

^c X. Devoyot, INRA, Aurillac, France.

^d X. Desmettre, Laboratory of Bacteriology, IFFA Merieux, Lyon, France.

by Hájek (Table 2); they acidified maltose and produced acetoin. With the Barritt method, acetoin production was detected in only two of our strains and was not detected in the reference strain. The presence of phosphate in the medium did not modify the result. In the two test strains that produced acetoin, the reaction was not detected until the day 7, and the intensity increased between the days 7 and 14 (Table 3). With the micromethod, acetoin production was detected in all strains (Table 2).

The characteristics of our strains were in good agreement with those described by Hájek (9) for *S. intermedius*, and therefore our strains could be assigned to this species. As mentioned previously (17), the production of acid from maltose as determined by the micromethod did not agree with the results of Hájek, perhaps because phenol red was used as an indicator.

Routinely, acetoin production is determined for taxonomic and diagnostic purposes, especially in the *Enterobacteriaceae*. Within the *Micrococcaceae*, this character is useful in differentiating among *Staphylococcus* species (13). Baird-Parker (3) also used it to distinguish among the four biovars of *S. epidermidis* and

among *Staphylococcus saprophyticus* strains. According to Hájek, it appears that *S. intermedius* is the only coagulase-positive *Staphylococcus* that does not produce acetoin; this fact induced Hájek (9) to select this character as the main criterion for differentiating *S. intermedius* from the other coagulase-positive staphylococci. Acetoin is produced from pyruvate in one of the metabolic pathways of glucose catabolism (20). When the enzymes of this pathway are present, the induction of these enzymes does not occur spontaneously but depends on cultural conditions (7, 20). Acetoin is not the only end product of glucose catabolism; when the oxidoreduction potential is low (for instance, in a glucose medium), acetoin is reduced to 2,3-butanediol, and when it is high (as in the presence of pyruvate), acetoin is oxidized to diacetyl (12). These reactions are reversible (18). The role of acetoin as a source of carbon has not been established (16). According to Barritt (4), acetoin can be detected by adding α -naphthol and potassium hydroxide to a culture. A positive reaction is also obtained with diacetyl but not with 2,3-butanediol.

Therefore, the failure to detect acetoin production in an organism may be explained in the following ways: the organism does not possess a metabolic pathway for acetoin, the production of acetoin is not triggered because of cultural conditions, or acetoin and diacetyl are metabolized, in which case the acetoin reaction is positive at first and later becomes negative (19).

S. intermedius has a metabolic pathway for acetoin production, as demonstrated by the results which we obtained by using the micromethod and pyruvate as the substrate. Induction of this pathway by pyruvate seems to occur with staphylococci in general. Páková and Kocur (15) have shown that production of acetoin by different bacterial species is more rapid when pyruvate rather than glucose is used as the substrate; moreover, acetoin production occurs more fre-

TABLE 2. Characters of 50 biovar *E. staphylococcal* test strains and three reference strains

Character	No. of test strains giving positive reaction	Reaction of:		
		<i>S. intermedius</i> CCM 5739	<i>S. aureus</i> NCTC 8530	<i>S. epidermidis</i> CCM 2124
Acid from maltose	50	+	+	+
Acetoin production				
Barritt method	2	—	+	+
Barritt method without phosphate	2	—	+	+
Micromethod	50	+	+	+

TABLE 3. Acetoin production by three staphylococcal strains as determined by three different methods

Strain	Acetoin production as determined by the Barritt method after: ^a			Acetoin production as determined by the Barritt method without phosphate after: ^b			Acetoin production as determined by the micromethod after 18 h
	2 Days ^c	7 Days	14 Days	2 Days	7 Days	14 Days	
<i>S. intermedius</i> 16	—	+	+++	—	++	+++	++
<i>S. intermedius</i> 45	—	+	+++	+	++	+++	++
<i>S. aureus</i> NCTC 8530	—	+	+	—	+	++	++

^a Clark and Lubs medium.

^b Glucose peptone medium without phosphate.

^c Incubation time.

quently in staphylococci than in other organisms. Our observations confirm these findings (Tables 2 and 3).

It seems that the negative reactions obtained when the conventional method for detecting acetoin production was used were not due to the metabolism of acetoin and diacetyl because the positive reactions of two of the strains were enhanced with time (Table 3). Hájek used the following two methods for determining acetoin metabolism (9): the conventional technique of Baird-Parker (2), with glucose as the substrate (the same negative results were obtained as we obtained for our strains); and the Davis-Hoyling method (6), in which the substrate was pyruvate on a paper disk placed on the surface of a glucose agar plate for 3 h (the results of this test were also negative). In the micromethod described previously (5), the technique was quite different; the strain was grown for 18 h in an appropriate medium containing pyruvate as the specific substrate.

Hájek and Maršálek (9, 11) failed to demonstrate acetoin production in 90 to 100% of the biovar E staphylococcal strains tested by two methods (the conventional technique of Baird-Parker [2] and the more rapid Davis-Hoyling test [6]). This led them to advocate that this negative reaction should be used as a reliable taxonomic criterion for *S. intermedius*. Generally, negative test results are not conclusive, since it cannot be assumed that optimum experimental conditions were used to determine a given characteristic.

In the case of staphylococcal acetoin production, the micromethod in which pyruvate is used as the substrate seems to be the more sensitive method and thus is the preferred method.

REPRINT REQUESTS

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