

## DIAGNOSTIC VALUE OF THE CHARACTERISTICS OF STAPHYLOCOCCUS HYICUS AND STAPHYLOCOCCUS CHROMOGENES

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### Abstract

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The value of the following 12 characteristics of *Staphylococcus hyicus* and *Staphylococcus chromogenes* for their routine identification was assessed: inhibition of staphylococcal delta haemolysin, hyaluronidase production, Tween 80 hydrolysis, proteolytic, phosphatase and urease activity, pigment production and aerobic acid production from sucrose, trehalose, mannose, maltose and mannitol. Of a total of 937 novobiocin-sensitive staphylococci used in the study 544 were *S. hyicus* strains, 322 were *S. chromogenes* strains and 71 showed characteristics classifying them as intermediaries between the two species. The characteristic shared by all of them was their ability to inhibit staphylococcal delta haemolysin. All *S. hyicus* strains produced hyaluronidase and hydrolysed Tween 80. On the basis of their differing proteolytic activity and carbohydrate utilization they were divided into three groups unequal in number. All *S. chromogenes* strains were intensely proteolytic, did not produce hyaluronidase and did not hydrolyse Tween 80. On the basis of their differing pigment production and acid production from carbohydrates they were divided into three groups unequal in number. The 71 strains originally not classified within either of the two species hydrolysed Tween 80. In view of this characteristic they were classified by us within the species *S. hyicus* although they did not produce hyaluronidase.

*Staphylococcus hyicus*, *Staphylococcus chromogenes*, inhibition of delta haemolysin, hyaluronidase, hydrolysis of Tween 80, proteolysis, phosphatase, urease, pigment, sucrose, trehalose, mannose, maltose, mannitol

Since 1986 (Kloss and Schleifer 1986; Hájek et al. 1986) *Staphylococcus hyicus* and *Staphylococcus chromogenes* have enlarged the number of coagulase-positive and coagulase-negative staphylococcal species which can be very often encountered upon routine examination of materials particularly of animal provenience (Skalka 1990b). The identification of the two species is complicated by the possibility of their being taken for other staphylococci as has been pointed out by a number of writers cited in our previous study (Skalka 1988a) and by further investigators (Gahrn-Hansen et al. 1987; Geary et al. 1989). Our previous studies on *S. hyicus* and *S. chromogenes* strains were concerned not only with the generally accepted characteristics of the two species (Skalka 1988a; 1990b) but also with the detection and description of some additional activities shared by these two species, namely their haemolytic activity (Skalka 1988b) and their ability to inhibit the haemolytic effect of staphylococcal delta haemolysin (Skalka 1990a; 1991). The present study on *S. hyicus* and *S. chromogenes* is focused on their major characteristics detectable in the laboratory with respect to their diagnostic value.

### Materials and Methods

#### Nutrient Media

The bases used for liquid nutrient media were Sugar Fermentation Series Base No. 2 (IMUNA, Czechoslovakia) and Nutrient Broth (OXOID) and those used for solid media were Blood Agar

Base No. 4 (IMUNA, Czechoslovakia) and Blood Agar Base (OXOID). For determination of the characteristics under study the afore-mentioned based were supplemented with respective ingredients.

#### Bacterial Strains

A total of 937 novobiocin-sensitive staphylococci were examined. Of these, 544 (58 %) were *S. hyicus* strains including culture collection strains CCM 2368, CNCTC M 51/88, CNCTC M 52/88 and CNCTC M 69/89, and 322 (34.4 %) were *S. chromogenes* strains including culture collection strains CCM 3387, CNCTC M 45/88, CNCTC M 46/88 and CNCTC M 47/88. The remaining 71 (7.6 %) strains with characteristics classifying them as intermediaries between the two species were tentatively referred to as Group "X". All the 937 strains were isolated during a 5-year period from pigs, cattle and domestic fowls kept on 35 different farms.

#### Characteristics Examined

Each strain was examined for the following 12 characteristics: ability to inhibit staphylococcal delta haemolysin, hyaluronidase activity, lipolytic activity (hydrolysis of Tween 80), proteolytic activity, phosphatase activity, urease activity, pigment production and ability to produce acid from sucrose, trehalose, mannose, maltose and mannitol under aerobic conditions. The techniques of the tests were described in detail previously (Skalka 1977; 1988a; 1990a; 1990b; 1991).

## Results

All *S. hyicus* strains inhibited staphylococcal delta haemolysin, produced hyaluronidase, hydrolysed Tween 80 and did not produce pigment. On the basis of their further characteristics they were divided into three groups designated as A, B and C.

Group A included 281 (51.6 %) strains that were proteolytic, produced acid from sucrose and trehalose but not from mannitol. Phosphatase was produced by 98.9 %, urease by 77.9 % and acid from mannose and maltose by 66.2 % and 25.6 % of Group A strains, respectively. Group B included 187 (34.4 %) strains. None of them was proteolytic, which was a marked difference from the strains of the preceding group. Similarly to Group A strains, Group B strains all produced acid from sucrose and trehalose. Phosphatase and urease were produced by 93.6 % and 93 % of the strains, respectively. Acid from mannose, maltose and mannitol was produced by 69.5 %, 34.8 % and 5.9 % of Group B strains, respectively. The least numerous Group C included 76 (14 %) strains that differed from the preceding two groups by their inactivity in carbohydrates, particularly in sucrose and trehalose. Phosphatase was produced by 96 %, proteolytic activity was shown by 55.3 %, whereas urease was produced by as few as 6.6 % of Group C strains.

The properties shared by all *S. chromogenes* strains were inhibition of staphylococcal delta haemolysin, markedly intense proteolysis, lack of hyaluronidase activity and inability to hydrolyse Tween 80. On the basis of their further characteristics *S. chromogenes* strains were divided into three groups designated as I, II and III.

Group I included 66 (20.5 %) strains that were markedly chromogenic and produced acid from sucrose. Phosphatase activity was shown by 94 %, hydrolysis of urea by 88 % and production of acid from trehalose also by 88 % of the strains. Acid from mannose, maltose and mannitol was produced by 68 %, 38 % and 18 % of Group I strains, respectively. Group II including 219 (68 %) strains differed from Group I by lack of pigmentation and by production of acid from both sucrose and trehalose. Phosphatase activity was shown by 91 % and urease activity by 89.5 % of the strains. Acid from mannose, maltose and mannitol was produced by 86.8 %, 24.7 % and 9 % of Group II strains, respectively. Group III

Table 1  
 Characteristics of staphylococcal strains examined in the present study

Species	Group	No. of positively reacting strains												
		No. of strains	IDH	HYA	LIP	PRO	PIG	PHO	URE	SUC	TRE	MAN	MAL	MIT
<i>S. hyicus</i>	A	281	281	281	281	281	0	278	219	281	281	186	72	0
	B	187	187	187	187	0	0	175	174	187	187	130	65	11
	C	76	76	76	76	42	0	73	5	0	0	0	0	0
<i>S. chromogenes</i>	I	66	66	0	0	66	66	62	58	66	58	45	25	12
	II	219	219	0	0	219	0	200	196	219	219	190	54	20
	III	37	37	0	0	37	16	14	10	0	0	0	0	0
Unclassified "X" strains		71	71	0	71	69	0	71	71	71	60	45	14	0

Explanatory notes: IDH, inhibition of staphylococcal delta haemolysin; HYA, hyaluronidase; LIP, hydrolysis of Tween 80; PRO, proteolysis; PIG, pigment; PHO, phosphatase; URE, urease; SUC, sucrose; TRE, trehalose; MAN, mannose; MAL, maltose; MIT, mannitol.

including 37 (11.5 %) strains differed from the foregoing *S. chromogenes* groups by inactivity in carbohydrates. Pigment was produced by 43 %, phosphatase by 38 % and urease by 27 % of Group III strains, respectively.

Group "X" strains all inhibited staphylococcal delta haemolysin similarly to *S. hyicus* and *S. chromogenes* strains. All of them also hydrolysed Tween 80 similarly to *S. hyicus* but, like *S. chromogenes*, did not produce hyaluronidase. Most of them (97 %) were proteolytic but the intensity of their proteolytic activity was comparable to that shown by *S. hyicus* proteolytic strains, never reaching the intensity shown by *S. chromogenes* strains. All Group "X" strains were achromogenic, but they showed phosphatase and urease activity and produced acid from sucrose. Acid from trehalose, mannose and maltose was produced by 84.5 %, 63.4 % and 19.7 % of Group "X" strains, respectively. No strain of this group produced acid from mannitol.

The results are summarized in Table 1.

## Discussion

Only few writers have been concerned with systematic investigation of the characteristics of *S. hyicus* and *S. chromogenes* and their respective diagnostic value. But even before Kloos and Schleifer (1986) published their nowadays generally accepted identification scheme, attention had been drawn to the possibility that *S. hyicus* and its then known subspecies might be confused with *S. aureus*, *S. intermedius*, *S. epidermidis*, *S. simulans*, *S. capitis* and *S. hominis* as was discussed by us previously. More recently, this possibility has been considered repeatedly (Gahrn-Hansen et al. 1987; Geary et al. 1989). When identification of staphylococci is based merely on their fermentation activity, it is also not possible to exclude the confusion between *S. hyicus* and *S. chromogenes*. Table 2 shows comparison of our results with those reported by writers dealing with similar problems.

The results reported in the present study support our previous observations on the capability of *S. hyicus* and *S. chromogenes* to inhibit staphylococcal delta haemolysin. This capacity was also shown by all strains classified by us within

Table 2  
Results and data reported by other writers

Species	Author Year of publications	No. of strains	Percentage of positively reacting strains											
			IDH	HYA	LIP	PRO	PIG	PHO	URE	SUC	TRE	MAN	MAL	MIT
<i>S. hyicus</i>	D. (1985)	49	n	98	100	100	0	100	96	100	86	100	0	0
	K. S. (1986)	n	n	100	100	100	0	100	80	100	100	100	0	10
	Z. P. (1986)	n	n	n	n	n	0	100	94	100	92	100	10	10
	G. (1987)	10	n	n	100	n	0	100	100	100	100	100	0	0
	Our study	544	100	100	100	59	0	97	73	86	86	58	26	2
<i>S. chromogenes</i>	D. (1985)	54	n	0	0	98	60	100	91	98	96	96	50	43
	K. S. (1986)	n	n	0	0	100	90	100	90	100	100	100	50	58
	Z. P. (1986)	n	n	n	n	n	95	100	90	100	90	100	50	50
	G. (1987)	9	n	n	0	89	100	100	100	100	100	100	22	44
	Our study	322	100	0	0	100	25	87	82	89	86	73	24	10
Group "X"	Our study	71	100	0	100	97	0	100	100	100	85	63	20	0

Explanatory notes: D., Devriese et al. (1985); K. S., Kloos and Schleifer (1986); Z. P., Zdražilék and Petráš (1986); G., Goodfellow et al. (1987); n, not specified; IDH, inhibition of staphylococcal delta haemolysin; HYA, hyaluronidase; LIP, hydrolysis of Tween 80; PRO, proteolysis; PIG, pigment; PHO, phosphatase; URE, urease; SUC, sucrose; TRE, trehalose; MAN, mannose; MAL, maltose; MIT, mannitol.

Group "X". A similar, though less intense, effect can be observed in most strains of *S. sciuri* and *S. lentus* (Skalka 1990a, 1991) as well as in some micrococci (Liu 1954; Skalka 1991). However, *S. sciuri* and *S. lentus* are resistant to novobiocin (Kloos and Schleifer 1986) and this characteristic itself differentiates them from the species considered in our study. Micrococci, on the other hand, have no phosphatase activity (Kocur 1986), which differentiates them, among other characteristics, from Group "X" strains. No data for comparison with our results of the staphylococcal delta haemolysin inhibition test are available; this characteristic was observed and described by us quite recently (Skalka 1990a, 1991).

Hyaluronidase is produced as a rule only by two species of the genus *Staphylococcus*, namely *S. aureus* and *S. hyicus*. Only quite exceptionally and then only in small quantities it can be also produced by some *S. epidermidis* strains (Kloos and Schleifer 1986) but these strains do not inhibit staphylococcal delta haemolysin; on the contrary, they generally produce it similarly to an overwhelming majority of *S. aureus* strains (Skalka 1990a, 1990b, 1991).

Our finding that Tween 80 was hydrolysed by all *S. hyicus* strains but by no *S. chromogenes* strains is in keeping with the data reported by most writers. This activity was also shown by Group "X" strains. No explanation can be offered of the solitary finding of Tween 80 hydrolysis by *S. chromogenes* as reported by Goodfellow et al. (1987); as a matter of fact, this finding remained unexplained by the afore-mentioned writers themselves. Although Tween 80 hydrolysis may also be shown by some strains of *S. aureus* and *S. intermedius*, their activity in this respect is invariably less intense than that of *S. hyicus* and Group "X" strains. The delta haemolysin inhibition test, however, provides a reliable differentiation of the afore-mentioned groups of staphylococci.

Proteolytic activity differentiating Group B strains from Group A and Group C strains of *S. hyicus* was markedly positive in all *S. chromogenes* strains. This characteristic is also shown by some *S. aureus*, *S. intermedius* and *S. epidermidis* strains (Kloos and Schleifer 1986; Skalka 1977, 1990b) that can be differentiated from *S. hyicus* and *S. chromogenes* by the delta haemolysin inhibition test. Also most *S. sciuri* strains are proteolytic but this species is novobiocin-resistant.

Our observations on the incapability of *S. hyicus* strains to produce pigment agree with those reported by other writers. In contrast to them, however, the proportion of pigmented *S. chromogenes* strains was lower than generally reported and the differing pigment production differentiated these strains into Groups I and II.

The results of the remaining tests used in our study did not differ substantially from those reported by other writers, particularly from the data recorded in the generally accepted classification of staphylococci (Kloos and Schleifer 1986).

Of interest, on the other hand, are our results obtained for *S. hyicus* Group C and *S. chromogenes* Group III strains which proved inactive in carbohydrates, particularly sucrose and trehalose. The proportions of these strains within *S. hyicus* and *S. chromogenes* were 14 % and 11.5 %, respectively. This inactivity may give rise to diagnostic mistakes where *S. hyicus* and *S. chromogenes* strains are identified merely on the basis of their fermentation activity.

From our results it can be concluded that the most important characteristics for routine identification of *S. hyicus* are sensitivity to novobiocin, inhibition of staphylococcal delta haemolysin, production of hyaluronidase and hydrolysis of Tween 80, together with the absence of pigment production. Routine identification of *S. chromogenes* is possible on the basis of sensitivity to novobiocin, inhibition of staphylococcal delta haemolysin, intense proteolytic activity, absence of hyaluronidase production and inability to hydrolyse Tween 80.

Group "X" strains were classified by us within the species *S. hyicus* in view of their hydrolysis of Tween 80 in spite of their negative hyaluronidase production.

### **Diagnostický význam vlastností *Staphylococcus hyicus* a *Staphylococcus chromogenes***

Sledoval se význam dvanácti vlastností pro určení *Staphylococcus hyicus* a *Staphylococcus chromogenes*, a to schopnost inhibovat stafylokokový hemolyzin delta, tvorba hyaluronidázy, hydrolyza Tween 80, aktivita proteolytická, fosfatázová a ureázová, tvorba pigmentu, aerobní tvorba kyseliny v sacharóze, trehalóze, mannóze, maltóze a manitu. Použilo se 937 novobiocinsenzitivních stafylokokových kmenů, ze kterých náleželo 544 do druhu *S. hyicus*, 322 do druhu *S. chromogenes* a 71 se řadilo mezi oba druhy. Společnou vlastností všech použitých kmenů byla schopnost inhibovat stafylokokový delta hemolyzin. Všechny kmeny *S. hyicus* produkovaly hyaluronidázu a hydrolyzovaly Tween 80. Na podkladě rozdílné proteolytické aktivity a užitizace karbohydrátů se rozdělily do tří nestejně početných skupin. Všechny kmeny *S. chromogenes* byly intenzivně proteolytické, netvořily hyaluronidázu a nehydrolyzovaly Tween 80. Na podkladě různé produkce pigmentu a tvorby kyseliny v uhlohydrátech se rozdělily do tří různé početných skupin. 71 kmenů původně nepřirazených k žádnému z obou druhů se vyznačovalo schopností hydrolyzovat Tween 80. Pro tuto vlastnost byly tyto kmeny přirazené k *S. hyicus*, vzdor jejich negativní produkci hyaluronidázy.

## Диагностическое значение свойства *Staphylococcus hyicus* и *Staphylococcus chromogenes*

Проводили исследования двенадцати свойств с целью определения *Staphylococcus hyicus* и *Staphylococcus chromogenes*, а именно способности торможения стафилококкового гемолиза дельта, образования гиалуронидазы, гидролиза Tween 80, протеолитической активности, фосфатазной и уреазной активностей, образования пигмента, аэробного образования кислоты в сахарозе, маннозе, мальтозе и маните. Применены 937 новобиоцинсенситивных стафилококковых штаммов, из числа которых 544 принадлежали *S. hyicus*, 322 — *S. chromogenes* и 71 — к обоим видам. Совместное свойство всех применяемых штаммов сводилось к способности к ингибированию стафилококкового дельта-гемолизина. Все штаммы *S. hyicus* выделяли гиалуронидазу и гидролизвали Tween 80. На основе разной протеолитической активности и утилизации карбогидратов их разделили на три, по численности неодинаковые группы. Все штаммы *S. chromogenes* отличались интенсивной протеолитичностью, не образовали гиалуронидазу и не гидролизвали Tween 80. На основе разной продукции пигмента и образования кислоты в углеводах они делились на три разные по численности группы. 71 штамм, не принадлежавших первоначально ни к одному из двух видов, отличались способностью к гидролизу Tween 80. Упомянутые штаммы, исходя из приведенного свойства, были отнесены к *S. hyicus*, несмотря на их негативную продукцию гиалуронидазы.

### References

- DEVRIESE, L. A.—SCHLEIFER, K. H.—ADEGOKE, G. O.: Identification of coagulase-negative staphylococci from farm animals. *J. Appl. Bacteriol.*, **58**, 1985: 45—55
- GAHRN-HANSEN, B.—HELTBERG, O.—ROSDAL, T. H.—SOGAARD, P.: Evaluation of a conventional routine method for identification of clinical isolates of coagulase-negative *Staphylococcus* and *Micrococcus* species. Comparison with API-Staph and API-Staph-Ident. *Acta Pathol. Microbiol. Immunol. Scand. Sect. B*, **95**, 1987: 283—292
- GEARY, C.—STEVENS, M.—SNEATH, P. H. A.—MITCHELL, C. J.: Construction of a database to identify *Staphylococcus* species. *J. Clin. Pathol.*, **42**, 1989: 298—294
- GOODFELLOW, M.—HARWOOD, C. R.—NAHAIE, M. R.: Impact of plasmids and genetic change on the numerical classification of staphylococci. *Zbl. Bakt. Hyg., A*, **266**, 1987: 60—85
- HÁJEK, V.—DEVRIESE, L. A.—MORDARSKI, M.—GOODFELLOW, M.—PULVERER, G.—VARALDO, P. E.: Elevation of *Staphylococcus hyicus* subsp. *chromogenes* (Devriese et al. 1978) to species status: *Staphylococcus chromogenes* (Devriese et al. 1978) comb. nov. *Syst. Appl. Microbiol.*, **8**, 1986: 169—173
- KLOOS, W. E.—SCHLEIFER, K. H.: Genus IV. *Staphylococcus* Rosenbach 1884: p. 1 013 to 1 035. In: SNEATH, P. H. A.—MAIR, N. S.—SHARPE, M. E.—HOLT, J. G. (ed.): *Bergey's manual of systematic bacteriology*. 1st ed. Vol. 2. Baltimore, London, Los Angeles, Sydney, Williams and Wilkins 1986: p. 965—1 595
- KOCUR, M.: Section 12. Gram-positive cocci. Genus I. *Micrococcus* Cohn 1872: p. 1 004—1 008. In: SNEATH, P. H. A.—MAIR, N. S.—SHARPE, M. E.—HOLT, J. G. (ed.): *Bergey's manual of systematic bacteriology*. 1st ed. Vol. 2. Baltimore, London, Los Angeles, Sydney. Williams and Wilkins 1986: p. 965—1 595
- LIU, P.: Inhibition of a staphylococcal hemolysin by a soluble substance produced by a non-hemolytic *Micrococcus* species. *J. Bacteriol.*, **68**, 1954: 718—723
- SKALKA, B.: Diagnostics of coagulase-positive staphylococci with respect to the epizootologic importance (orig. in Czech). Research report. Brno, 1977: 51 p.
- SKALKA, B.: Isolation of *Staphylococcus hyicus* strains from healthy hosts and a diagnostic study of the strains (orig. in Czech). *Veter. Med. (Praha)*, **33**, 1988a: 607—616

- SKALKA, B.: Hemolytic activity of *Staphylococcus hyicus* and *Staphylococcus chromogenes*. Acta vet. Brno, 57, 1988b: 3–11
- SKALKA, B.: Hemolytic interactions of some gram-positive bacteria and their importance (orig. in Czech). Dissertation. Brno, 1990a, 204 p.
- SKALKA, B.: Staphylococcal species isolated from healthy farm and free-living animals (orig. in Czech). Research report. Brno, 1990b: 48 p.
- SKALKA, B.: Antagonistic effect of *Staphylococcus hyicus* and *Staphylococcus chromogenes* exhibited on staphylococcal delta hemolysin. Acta vet. Brno, 60, 1991: 61–69
- ZDRAŽÍLEK, J. – PETRÁŠ, P.: Genus *Staphylococcus* and Genus *Micrococcus*. p. 92–103. In: Anonym.: Classification of bacteria 1986. Praha, 1986: 134 p.