ACTA VET. BRNO, 55, 1986: 333-342

TYPING OF STAPHYLOCOCCUS AUREUS, STAPHYLOCOCCUS INTERMEDIUS AND COAGULASE-NEGATIVE STAPHYLOCOCCI BY MEANS OF STAPHYLOCOCCAL BACTERIOCINS

B. SKALKA

Department of Epizootiology and Microbiology, University of Veterinary Science, 612 42 Brno

Received October 31, 1985

Abstract

Skalka B.: Typing of Staphylococcus aureus, Staphylococcus intermedius and Coagulase-negative Staphylococci by means of Staphylococcal Bacteriocins. Acta vet. Brno, 55, 1986: 333-342.

Sensitivity to staphylococcal bacteriocins was examined in 1,314 staphylococcal strains, isolated from man and animals. The strains under study did not produce bacteriocins. S. aureus UT0002, S. intermedius OP-42, S. intermedius OP-12, and S. epidermidis K14 were used as producers of bacteriocins. The set of examined strains was represented by 730 S. aureus biovar C, 111 S. aureus biovar A, 34 S. aureus biovar B, 5 S. aureus biovar D, 224 S. intermedius and 210 coagulase-negative staphylococci. The deferred method was employed for the assays. The strains of biovar C were characterized by sensitivity to bacteriocins of all four producers, whilst S. aureus strains of other biovars were susceptible to bacteriocins UT0002 and K14, but resistant towards OP-42 and OP-12. S. intermedius strains were characterized by resistance to UT0002 bacteriocin but 53 % of them were sensitive to bacteriocins K14, OP-42 and OP-12. 31 % of S. intermedius strains were sensitive exclusively to K14 and 8 % strains were found quite resistant. A similar complete resistance was shown by 23 % strains in the framework of coagulase-negative staphylococci, and 75 % of them were susceptible exclusively to K14 bacteriocin.

Typing by means of staphylococcal bacteriocins, S. aureus, S. intermedius, coagulase-negative staphylococci.

Staphylococcal bacteriocins, also called staphylococcins, have already been described and characterized in detail (Tagg et al. 1976; Pulverer and Jeljaszewicz 1976; Brandis 1981; Ivanov 1983; Skalka 1985). Their lethal effect is not observed merely in the framework of the genus Staphylococcus, some of them also affect corynebacteria (Parker and Simmons 1959; Skalka et al. 1983 a,b) listeriae (Dajani et al. 1970; Jetten and Vogels 1972), streptococci and bacilli (Dajani and Wannamaker 1973; Jetten and Vogels 1973) and even some gramnegative species, as Neisseria

gonorrhoeae (Morris et al. 1978) or Escherichia coli (Kader et al. 1984). It is apparent from the reviews and from the recent studies about staphylococcins (Šmarda and Obdržálek 1981; Balusek and Hájek 1985; Skalka 1986) that the main effort is directed towards the discovery as many bacteriocin producing strains as possible what can be used in characterization of staphylococcal strain. Bacteriocin synthesis is a valuable character of some staphylococcal strains, though it is not always as important as in the case of the exfoliatin producing S. aureus strains (Parker and Simmons 1959; Anthony et al. 1972; Rogolsky et al. 1974; Warren et al. 1974). On the other hand, studies on the possibility of typing Staphylococcus spp. on the basis of their sensitivity to bacteriocins are published rarely (Ivanov 1970; Pulverer and Jeljaszewicz 1976). The purpose of the present study was to ascertain whether or not a possibility exists to type staphylococcal strains by means of staphylococcal bacteriocins.

Materials and Methods

Media

Oxoid brain heart infusion CM 225, Oxoid brain heart infusion agar CM 375, and agar blood base No. 4 Imuna were used.

Bacterial strains

Bacteriocin producers

Four staphylococcal strains producing bacteriocins were used, namely S. aureus UT0002 (kindly provided by Dr. Rogolsky), S. intermedius OP-42, S. intermedius OP-12, and S. epidermidis K14. The last mentioned three strains were selected from our collection of bacteriocin producers and they are deposited in the Czechoslovak National Collection of Type Cultures as M 23/25, M 24/85 and M 25/85.

Basic indicator strains

Corynebacterium renale CCM 5740, Corynebacterium pseudodiphtheriticum CNCTC Psdi 5/78, S. aureus (Oxford 209P) CNCTC Mau 28/58 and S. aureus CB-27, all previously described (Skalka et al. 1983b; Skalka 1986), were used as basic indicator strains.

Staphylococcal strains under study

A total od 1 314 staphylococcal strains was tested. The strains were classified according to our previous description (Skalka 1985). The set of S. aureus strains was composed of 111 biovar A strains isolated from man, 34 biovar B strains isolated from poultry, 730 biovar C strains isolated from secrets of bovine mastitic udders, and 5 biovar D strains isolated from hares. Furthemore 224 S. intermedius strains and 210 coagulase-negative staphylococci were tested. The last mentioned strains were classified as 44 S. epidermidis, 38 S. haemolyticus, 35 S. hominis, 23 S. simulans, 15 S. saprophyticus, 13 S. capitis, 9 S. xylosus, 6 S. cohnii, 6 S. warneri, 4 S. gallinarum, 2 S. caprae, and 15 strains could not be alloted to any staphylococcal species.

Nature of bacteriocins of the producer strains

The producer strains were tested as on their activity on basic indicator strains, as on the mutual antagonistic action. Sen-

sibility of bactericidal exosubstances to chloroform and trypsin (Brandis 1981) were also determined.

Bacteriocin sensibility assay

A modification of deferred method was employed in the following way. Spots of producer (active) strains were given in a diametric line on the surface of the agar medium and the plates were incubated at 37 °C for 48 h. Then the strains under investigation were inoculated on the plates. In order to obtain a close lawn, a drop of a 24 h broth culture was spread by means of an L-shaped wire in such a way that the edge of the lawn ran alongside of the spots of bacteriocin producers. After that a 24 h reincubation at 37 °C followed, then the results were evaluated. The result was considered positive, if there was a growth-inhibition zone of 2 mm and broader in the lawn of the investigated strain.

Results

Properties of antagonistic exosubstances of four testing strains are presented in Table 1. Growth of the corynebacterial and staphylococcal strains used as indicators was inhibited by all active strains. Exosubstances of all active strains were found to be trypsin-sensitive. The bacteriocin UT0002 was inactivated by chloroform, while the bacteriocins OP-42, OP-12 and K14 were chloroform-resistant.

Table 1

Properties of bacteriocins under study

Producer strain		UT0002	OP-42	OP-12	K14
Staphylococcus		aureus	intermedius	intermedius	epidermidis
Relation to	trypsin	S	S	S	S
	chloroform	S	R	R	R
Mutual antagonistic effect on strains	UT0002	-	+	+	+
	OP-42	-	-	-	+
	OP-12	-	-	-	+
	K14	-	-	-	-
Effect on basic indicator set					
C. renale		+	+	+	+
C. pseudodiphtheriticum		+	+	+	+
S. aureus OX 209P		+	+	+	+
S. aureus CB-27		+	+	+	+

Legends: S = sensitive R = resistant
+ = inhibition of growth - = without effect

Most of the tested staphylococcal strains were susceptible to the bacteriocin of the strain K14 which inhibited 99.31 % strains S. aureus biovar C, 97.33 % S. aureus biovars A, B, D, 91.96 % S. intermedius, and 77.14 % coagulase-negative staphylococci. The bacteriocin UT0002 acted on 99.45 % S. aureus biovar C and 98.66 % S. aureus biovars A, B, D, but on only 1.33 % S. intermedius. The coagulase-negative staphylococci were resistant to its action. Susceptibility towards the bacteriocin OP-12 was observed in 95.61 % S. aureus biovar C, and 61.16 % S. intermedius, but in no more than 7.33 % S. aureus biovars A, B, D, and 1.9 % coagulase--negative strains. In the case of the bacteriocin OP-42, susceptibility was observed in 94.79 % S. aureus biovar C and 54.46 % S. intermedius, but in only 6.66 % S. aureus biovars A, B, D, and in 1.9 % coagulase-negative staphylococcal strains.

Table 2

Susceptibility patterns of investigated staphylococci

Antagonistic effect of bacteriocinogenic strain				Tested strains			
				Staphylococcus			
UT0002	OP-42	OP-12	K14	aureus biovar A,B,D	aureus biovar C	inter-medius	coagulase-negative spp.
+		+	+	8	685	1	0
+	-	-	+	135	24	2	0
+	-	+	+	2	9	0	0
+	+	-	+	1	4	0	0
+	+	+	-	1	2	0	0
+	-	-	-	1	2	0	0
+	-	-	-	1	2	0	0
-	+	+	+	0	1	119	4
-	-	+	+	0	1	15	0
-	-	-	+	0	1	69	158
-	-	-	-	2	1	18	48
Total				150	730	224	210

Legends: + = inhibition of growth - = without effect

From the point of view of their sensitivity to the bacteriocin used, the strains of S. aureus biovar C fell into ten groups. The most numerous group was characterized by susceptibility to all four bacteriocins and comprised 93.83 % strains of the biovar C. Susceptiblity to the bacteriocins UT0002 and K14, but resistance to those of the strains OP-42 and OP-12 were characteristic for 90 % strains S. aureus biovars A, B, D. The largest group of S. intermedius comprised 53.12 % strains resistant to the bacteriocin UT0002 but susceptible to the action of other three, while 30.8 % strains of this species were susceptible to the bacte-

riocin K14 and 8.03 % S. intermedius strains were resistant to all four bacteriocins. Susceptibility patterns of coagulase-negative staphylococci were not related to species competence. The most numerous group of them comprised 75.23 % strains. Resistance to all four bacteriocins was found in 22.85 % coagulase-negative staphylococci. The remaining combinations of susceptibility applied only to a small number of the strains under study (Table 2). Typical results are presented on Figures 1 - 3.

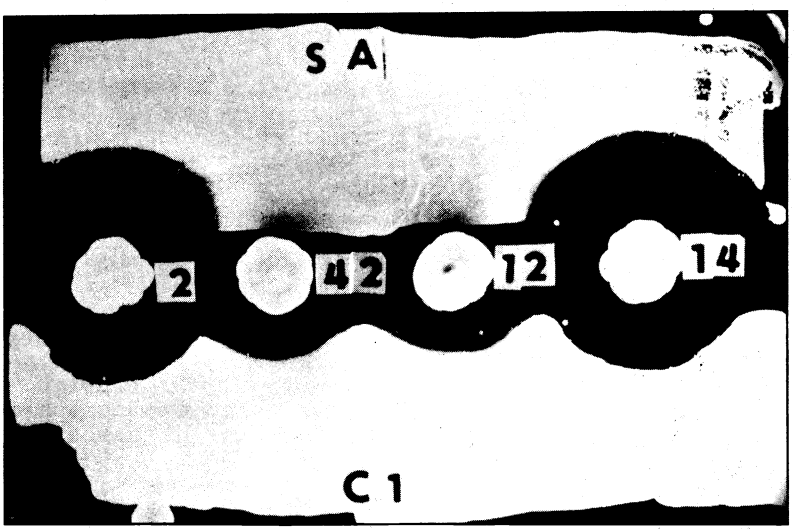

Fig. 1. Strains of S. aureus biovar A (SA) and biovar C (C_1) are tested against bacteriocin producers S. aureus UT0002 (2), S. intermedius OP-42 (42), S. intermedius OP-12 (12), and S. epidermidis K14 (14)

Discussion

Following up our previous studies (Skalka et al. 1983a, b; Skalka 1985, 1986), we obtained a collection of bacteriocin producing staphylococci, from which we selected three strains for the present work and we added the well known bacteriocin producer strain S. aureus UT0002 (Rogolsky et al. 1974; Warren et al. 1974) to obtain a set. The choice of producer strains was determined by our working hypothesis that strains optimal for bacteriocinotyping are not those with a broad effect, but rather with a differential one.

Bacteriocins are always described to be chloroform resistant. Their sensitivity to chloroform is only occasional (Tagg et al. 1975), and chloroform-method (Dajani and Wannamaker 1973) is employed for search of new active strains (Balusek

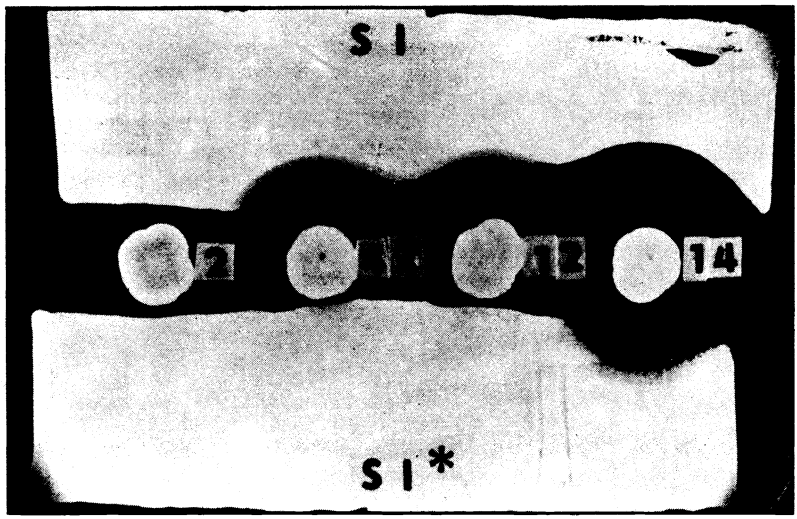

Fig. 2. Two strains of S. intermedius (SI, SI*) differently susceptible are tested against the same producers as on Figure 1

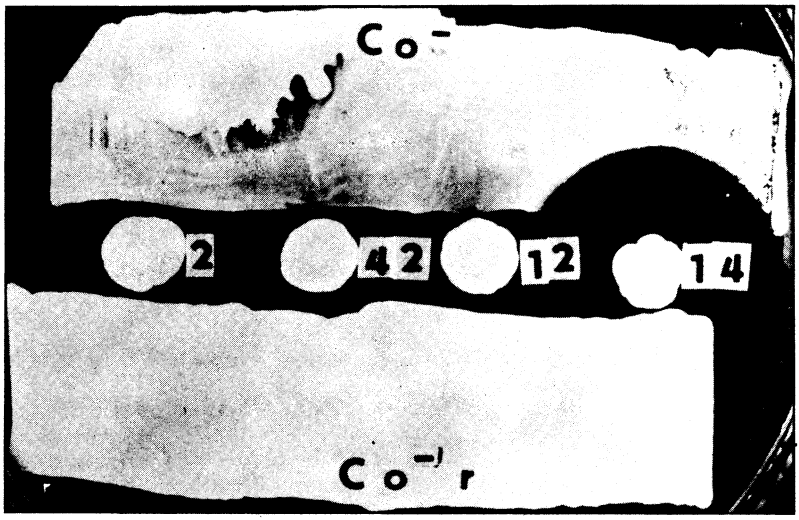

Fig. 3. Two strains of coagulase-negative staphylococci (Co⁻, Co⁻r) are tested against the same strains as on Figures 1 and 2

and Hájek 1985). Our observation about chloroform sensitivity of the exosubstance of UT0002 strain is surprising and it has not been described till now.

Our effort to use the bacteriocinotyping of staphylococci is not unique, but unlike previous studies (Ivanov 1970; Pulverer and Jeljaszewicz 1976), it considers as the existence of two coagulase-positive species as the biovars in the framework of S. aureus.

Larger sets of active strains, six and seven respectively, were used in comparable studies (Ivanov 1970; Pulverer and Jeljaszewicz 1976), and six groups of sensitivity patterns were ascertained in one of them (Ivanov 1970), whilst nine groups in the other (Pulverer and Jeljaszewicz 1976). Nevertheless, only S. aureus strains were typed and the group susceptible to all active staphylococci comprised 96 %.

The use of the set of four active strains described in this paper made it possible to differentiate the majority of S. aureus, S. intermedius and coagulase negative strains, further within these three groups of staphylococci several susceptibility types could be established. With the exception of the strains resistant to all four bacteriocins, 99.2 % of the staphylococcal strains under study were sensitive to the K14 bacteriocin. The susceptibility to the bacteriocin UT0002 differentiated almost all S. aureus strains from those of S. intermedius and coagulase-negative ones. Bacteriocins of the S. intermedius strains OP-42 and OP-12 facilitated the formation of groups within the framework of S. aureus and S. intermedius, and they inhibited growth of biovar C strains above all. The use of the bacteriocin K14 is turned to account of broad activity spectrum of this exosubstance.

Though further developments are likely, it is felt that the results obtained represent a contribution to the field of staphylococcal bacteriocins in general, and in particular to their practical use.

Typizace Staphylococcus aureus, Staphylococcus intermedius a koaguláza-negativních stafylokoků použitím stafylokokových bakteriocinů

Senzitivita ke stafylokokovým bakteriocinům byla zjišťována u 1 314 kmenů stafylokoků animální a humánní provenience, u kterých se neprokázala produkce bakteriocinů. Jako producenti bakteriocinů se použily kmeny S. aureus UT0002, S. intermedius OP-42, S. intermedius OP-12 a S. epidermidis K14. Soubor testovaných kmenů tvořilo 730 kmenů S. aureus biovar C, 111 kmenů S. aureus biovar A, 34 kmenů biovar B, 5 kmenů biovar D, dále 224 kmenů S. intermedius a 210 koaguláza-negativních stafylokoků. Pro vyšetření byla použita vlastní modifikace metody předkultivace producentů. Pro kmeny S. aureus biovar C byla charakteristická citlivost k bakteriocinům všech čtyř testačních kmenů, zahrnující 94 % kmenů této biovar. Pro kmeny S. aureus ostatních biovar byla charakteristická senzitivita k bakteriocinům kmenů UT0002 a K14 a současná necitlivost k bakteriocinům kmenů OP-42 a OP-12, pozorovaná u 90 %. V rámci druhu S. inter-

medius bylo 53 % kmenů citlivých k substancím kmenů OP-42, OP-12 a K14, při současné necitlivosti k bakteriocinu kmene UTOOO2, 31 % bylo citlivých jen k bakteriocinu K14 a 8 % kmenů bylo necitlivých ke všem bakteriocinům použitého testačního setu. Podobnou úplnou rezistenci mělo 23 % kmenů koaguláza-negativních stafylokoků, zatím co 75 % bylo citlivých výlučně k bakteriocinu kmene K14. Odlišné kombinace senzitivity, než jaké byly uvedeny, pozorovaly jen u malých počtů vyšetřovaných stafylokoků.

Типизация Staphylococcus aureus, Staphylococcus intermedius и коагулаза-отрицательных стафилококков применением стафилококковых бактериоцинов

Проводились исследования чувствительности к стафилококковым бактериоцинам у 1314 штаммов стафилококков животного и человеческого происхождения, у которых не была установлена продукция бактериоцинов. В качестве продуцентов бактериоцинов были использованы штаммы *S. aureus* UTOOO2, *S. intermedius* OP-42, *S. intermedius* OP-12 и *S. epidermidis* K14. В комплекс проверяемых штаммов входили 730 штаммов *S. aureus* biovar C, 111 штаммов *S. aureus* biovar A, 34 штамма biovar B, 5 штаммов biovar D, далее, 224 штамма *S. intermedius* и 210 коагулаза-отрицательных стафилококков. Для исследований был использован метод предварительной культивации продуцентов собственной модификации. Дла штаммов *S. aureus* biovar C была характерной чувствительность к бактериоцинам всех четырех проверяемых штаммов, включающих 94% штаммов данной биоварианты. Дла штаммов *S. aureus* и остальных биовариант была характерна чувствительность бактериоцидным штаммам UTOOO2 и K14 и одновременно нечувствительность к бактериоцинам штамм OP-42 и OP-12, наблюдаемая у 90%. В рамках штамма *S. intermedius* 53% штаммов отличалось чувствительностью к веществам штаммов OP-42, OP-12 и K14 при одновременной нечувствительности к бактериоцину штамма UTOOO2, 31% отличался чувствительностью лишь к бактериоцину K14 и 8% штаммов - нечувствительностью ко всем бактериоцинам используемого набора. Аналогичной полной стойкостью отличались 23% штаммов коагулаза-отрицательных стафилококков, между тем как 75% - чувствительностью исключительно к бактериоцину штамма K14. Различные от приведенных комбинации чувствительности наблюдались лишь у небольшого числа исследуемых стафилококков.

References

ANTHONY, B. F. - GIULIANO, D. M. - OH, W.: Nursery outbreak of staphylococcal scalded skin syndrome. Rapid indentification of the epidemic bacterial strains. Am. J. Dis. Child., 124, 1972, 41 - 44.

BALUSEK, J. - HÁJEK, V.: Antagonistic activities of coagulase positive staphylococci. J. Hyg. Epidemiol. Microbiol. Immunol. (Prague), 28, 1985: 147 - 154.

BRANDIS, H.: Bacteriocins with special consideration of staphylococcins. Zbl. Bakt. Hyg. I. Abt., Suppl. 10, 1981, 719 - 729.

DAJANI, A. S. - GRAY, E. D. - WANNAMAKER, L. W.: Bactericidal substance from Staphylococcus aureus. Biological properties. J. Exp. Med., 131, 1970, 1004 - 1015.

DAJANI, A. S. - WANNAMAKER, L. W.: In vitro and in vivo studies on a phage type 71 staphylococcal bacteriocin. p. 413 - 421. In: JELJASZEWICZ, J.: Staphylococci and staphylococcal infections. S. Karger, Basel, 1973, 658 p.

IVANOV, N. A.: Staphylococcins, their properties, classification and use for typing of staphylococci. Bull. Exp. Biol. Med., 69, 1970, 559 - 560.

IVANOV, N. A.: Baktěriocinogenija stafilokokov. Žurnal Mikrobiol., Epidemiol. Immunol., No. 9, 1983: 3 - 7.

JETTEN, A. M. - VOGELS, G. D.: Nature and properties of a Staphylococcus epidermidis bacteriocin. J. Bacteriol., 112, 1972: 243 - 250.

JETTEN, A. M. - VOGELS, G. D.: Characterization and extrachromosomal control of bacteriocin production in Staphylococcus aureus. Antimicrob. Ag. Chemother., 4, 1973: 49 - 57.

KADER, A. O. - SAHL, H. G. - BRANDIS, H.: Isolation and mode of action of a staphylococcin like substance active against gram-positive and gram-negative bacteria. J. Gen. Microbiol., 130, 1984: 2291 - 2300.

MORRISS, M. D. - LAWSON, J. W. - ROGOLSKY, M.: Effect of a staphylococcin on Neisseria gonorrhoeae. Antimicrob. Ag. Chemother., 14, 1978: 218 - 223.

PARKER, M. T. - SIMMONS, L. E.: The inhibition of Corynebacterium diphtheriae and other gram-positive organisms by Staphylococcus aureus. J. Gen. Microbiol., 21, 1959: 457 - 476.

PULVERER, G. - JELJASZEWICZ, J.: Staphylococcal micrococcins. Zbl. Bakt. Hyg. I. Abt., Suppl. 5, 1976: 599 - 621.

ROGOLSKY, M. - WARREN, R. - WILEY, B. B. - NAKAMURA, H. T. - - GLASGOW, L. A.: Nature of the genetik determinant controlling exfoliative toxin production in Staphylococcus aureus. J. Bacteriol., 117, 1974: 157 - 165.

SKALKA, B.: Ecology and cross-pathogenity of Staphylococcus aureus strains of diverse host origin (orig. in Czech). Research report. Brno, 1984.

SKALKA, B.: Staphylococcal bacteriocins - their present and future (orig. in Czech). Veterinářství, 35, 1985a: 316 - 317.

SKALKA, B.: An up to date scheme for the diagnostics of staphylococci (orig. in Czech). Veter. Med. (Praha), 30, 1985b: 477 - 484.

SKALKA, B.: Bacteriocin activity of Staphylococcus aureus, Staphylococcus intermedius and coagulase negative staphylococci. Acta vet. Brno, 55, 1986: 65-72.

SKALKA, B. - PILLICH, J. - POSPÍŠIL, L.: New possibilities of staphylococcin detection in the exfoliatin producing strains of Staphylococcus aureus. Zbl. Bakt. Hyg. I. Abt. Orig. A 254, 1983a: 34 - 41.

SKALKA, B. - PILLICH, J. - POSPÍŠIL, L.: Further observations of Corynebacterium renale as an indicator organism in the detection of the exfoliatin positive strains of Staphylococcus aureus. Zbl. Bakt. Hyg. A 256, 1983b: 168 - 174.

ŠMARDA, J. - OBDRŽÁLEK, V.: Staphylococcins: incidence and some characteristics of antibiotic action. Zbl. Bakt. Hyg. I. Abt., Suppl. 10, 1981: 407 - 411.

TAGG, J. R. - DAJANI, A. S. - WANNAMAKER, L. W.: Bacteriocins of gram positive bacteria. Bact. Rev., 40, 1976: 722 - 756.
WARREN, R. - ROGOLSKY, M. - WILEY, B. B. - GLASGOW, L. A.: Effect of ethidium bromide on elimination of exfoliative toxin and bacteriocin production in Staphylococcus aureus. J. Bacteriol., 118, 1974: 980 - 985.