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1981

Nordisk Veterinaermedicin 33(9-11):434-40

<http://hdl.handle.net/1975/966>

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The Effect of Various Stress Treatments on the Coagulase Test in *Staphylococcus aureus*

by H. Korkeala and Varpu Hirvelä

False-negative coagulase tests were observed in Staphylococcus aureus strains stressed by low pH, heat treatment or repeated freezing and lyophilization carried out directly from Baird-Parker agar on which the strains were plated after stress treatments.

The processing of foods in modern food technology causes a number of problems to the food microbiologist, since many of the surviving bacteria may be damaged and their recovery and growth impaired (e.g. Busta 1978, Ray 1979). The debilitating effects of physical and chemical stress on staphylococcal cells have been well documented (Baird-Parker & Davenport 1965, Brewer et al. 1977, Flowers et al. 1977, Andrews & Martin 1979, Korkeala 1980).

In carrying out the coagulase test, the recommended procedure is by subculturing the suspected *Staphylococcus aureus* colonies from the primary isolation agar in brain heart infusion broth before the cultures are inoculated to plasma to examine for coagulase production (Food and Drug Administration 1976, Bear et al. 1976, The International Commission on Microbiological Specifications for Foods 1978). The purpose of the present study was to find out whether the coagulase test can be performed directly from the colonies from the primary isolation medium, particularly when the bacteria have previously been subjected to various stress treatments.

Material and methods

The test organisms

A total of 77 coagulase-positive strains of *S. aureus* were used in these experiments, of which 57 were enterotoxin-producing and 20 were not. The strains were obtained from A. Niskanen, D.V.M. and S. Lindroth, Ph.D., Technical Research Centre of Finland, Food Research Laboratory, Espoo, Finland.

Treatments

Every staphylococcal strain was inoculated into brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich., USA). From the overnight culture, 0.1 ml of each staphylococcal suspension was inoculated into 5 ml of skim-milk for the purpose of stress treatments. Each stress treatment was studied with the same strains. After treatment one loopful of cells was spread on Baird-Parker agar (Difco) and incubated for two days at 35° C.

Low pH: The pH of the skim-milk was adjusted to 5.0 with 4 N-HCl. The inoculated tubes containing skim-milk were incubated for 24 h at 35° C.

Table I. Coagulase production of *Staphylococcus aureus* strains after incubation in skim-milk at pH 5, for 24 h at 35° C and spreading on Baird-Parker agar.

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from BHI broth ^{a)}											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
¼	1	2	3	4	24	¼	1	2	3	4	24			
Enterotoxigenic strains	A single colony	33	0	82	100	100	100	100	0	45	94	97	100	100
	Heavy inoculum	27	0	59	78	78	93	100	0	37	74	74	93	100
Non-toxigenic strains	A single colony	10	0	70	100	100	100	100	0	40	100	100	100	100
	Heavy inoculum	20	0	40	50	50	70	100	0	40	45	45	70	100

Table I (continued).

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from Baird-Parker agar											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
¼	1	2	3	4	24	¼	1	2	3	4	24			
Enterotoxigenic strains	A single colony	33	0	0	9	36	42	94	0	0	0	15	36	88
	Heavy inoculum	27	0	7	48	56	63	96	0	4	37	44	63	93
Non-toxigenic strains	A single colony	10	0	0	30	80	100	100	0	0	30	40	70	100
	Heavy inoculum	20	0	10	50	75	90	100	0	0	15	50	70	90

a) strains were first subcultured into BHI broth from Baird-Parker agar.

b) small organized clot.

c) entire content of tube coagulates and is not displaced when tube is inverted.

Heat treatment: The inoculated tubes containing skim-milk were incubated for 24 h at 35° C. After the incubation the tubes were heated for 15 minutes at 52° C.

Freezing: The inoculated tubes containing skim-milk were incubated for 24 h at 35° C. Thereafter the tubes were frozen and kept two weeks at -25° C.

Repeated freezing and lyophilization: The inoculated tubes containing skim-milk were incubated for 24 h at 35° C, frozen and kept one week at -25° C.

Then the cells were thawed, refrozen and kept one week at -25° C, rethawed and lyophilized. The dried cells were rehydrated with 5 ml of physiological saline and spread on Baird-Parker agar.

Coagulase test

The production of coagulase was measured by the tube method with pig plasma. Each strain studied after the various treatments was inoculated into BHI broth from Baird-Parker agar, using either a single colony or a heavy inocu-

Table II. Coagulase production of *Staphylococcus aureus* strains after heating in skim-milk 15 minutes at 52° C and spreading on Baird-Parker agar.

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from BHI broth ^{a)}											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
			¼	1	2	3	4	24	¼	1	2	3	4	24
Enterotoxigenic strains	A single colony	33	0	82	100	100	100	100	0	52	94	97	100	100
	Heavy inoculum	27	0	85	89	89	100	100	0	78	89	89	100	100
Non-toxigenic strains	A single colony	10	0	80	100	100	100	100	0	50	100	100	100	100
	Heavy inoculum	19	0	26	63	74	84	100	0	16	42	68	84	100

Table II (continued).

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from Baird-Parker agar											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
			¼	1	2	3	4	24	¼	1	2	3	4	24
Enterotoxigenic strains	A single colony	33	0	0	27	45	45	70	0	0	9	36	39	67
	Heavy inoculum	27	0	30	48	67	67	100	0	19	44	59	67	96
Non-toxigenic strains	A single colony	10	0	0	60	100	100	100	0	0	40	50	70	100
	Heavy inoculum	19	0	11	63	79	89	100	0	5	53	74	79	100

a) strains were first subcultured into BHI broth from Baird-Parker agar.

b) small organized clot.

c) entire content of tube coagulates and is not displaced when tube is inverted.

lum. From each culture, grown overnight in BHI broth at 35° C, 0.1 ml was added to 0.5 ml of plasma. For purposes of comparison, the test was also carried out directly from Baird-Parker agar, using either a single colony or a heavy inoculum. The tubes containing plasma were incubated at 35° C and examined after ¼, 1, 2, 3, 4 and 24 h. Two parallel tests were done in each case. The results were interpreted according to the guidelines given by *The International Commission on Microbiological Specifications for*

Foods (1978). A '2+' reaction or greater was considered positive evidence of coagulase production. If the parallel tubes did not show the same coagulase production the result was recorded according to the slower reaction. When no coagulase production was observed, both parallel tubes always gave a negative coagulase result.

Results

The effects of different stress treatments on the coagulase production of

Table III. Coagulase production of *Staphylococcus aureus* strains after freezing in skim-milk two weeks at -25° C and spreading on Baird-Parker agar.

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from BHI broth ^{a)}											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
			¼	1	2	3	4	24	¼	1	2	3	4	24
Enterotoxigenic strains	A single colony	33	0	88	100	100	100	100	0	12	76	85	85	97
	Heavy inoculum	27	4	81	89	100	100	100	0	59	89	89	96	100
Non-toxigenic strains	A single colony	10	0	50	100	100	100	100	0	10	70	100	100	100
	Heavy inoculum	20	0	80	100	100	100	100	0	45	90	100	100	100

Table III (continued).

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from Baird-Parker agar											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
			¼	1	2	3	4	24	¼	1	2	3	4	24
Enterotoxigenic strains	A single colony	33	0	0	48	82	91	100	0	0	9	39	64	100
	Heavy inoculum	27	0	4	81	89	93	100	0	0	52	70	85	96
Non-toxigenic strains	A single colony	10	0	20	80	90	100	100	0	0	20	90	90	100
	Heavy inoculum	20	0	5	65	90	100	100	0	0	10	60	90	100

a) strains were first subcultured into BHI broth from Baird-Parker agar.

b) small organized clot.

c) entire content of tube coagulates and is not displaced when tube is inverted.

S. aureus strains carried out after plating on Baird-Parker agar, either 1) subculturing first in BHI broth or 2) directly from the agar are shown in Tables I, II, III and IV. One non-toxigenic strain did not grow on Baird-Parker agar after heat treatment.

Discussion

The different stress treatments had no effect on coagulase production in the tests carried out with BHI broth (Tables I-IV). The repair after stress occurred

during the growth of cells on Baird-Parker agar and in the BHI broth. Positive coagulase reactions were seen after four h incubation at 35° C. In a few cases 24 h incubation was needed for the positive reaction.

The reduced pH, the heat treatment and the repeated freezing and lyophilization caused false-negative coagulase reactions when carried out directly from Baird-Parker agar (Tables I, II and IV). Increasing the size of the inoculum into the plasma reduced the false-negative

Table IV. Coagulase production of *Staphylococcus aureus* strains after repeated freezing at -25°C and lyophilization in skim-milk and spreading on Baird-Parker agar.

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from BHI broth ^{a)}											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
			¼	1	2	3	4	24	¼	1	2	3	4	24
Enterotoxigenic strains	A single colony	33	0	39	61	85	85	100	0	12	39	55	64	100
	Heavy inoculum	27	0	85	89	100	100	100	0	74	85	89	93	100
Non-toxigenic strains	A single colony	10	0	0	20	100	100	100	0	0	0	0	50	100
	Heavy inoculum	20	0	85	100	100	100	100	0	50	100	100	100	100

Table IV (continued).

Strain	Size of inoculum	Number of strains tested	Positive reactions as function of time (h) expressed as percentage											
			Inoculation from Baird-Parker agar											
			'2+' reaction ^{b)}						'4+' reaction ^{c)}					
			¼	1	2	3	4	24	¼	1	2	3	4	24
Enterotoxigenic strains	A single colony	33	0	0	6	36	52	94	0	0	3	12	27	88
	Heavy inoculum	27	0	44	81	93	96	100	0	11	59	85	89	93
Non-toxigenic strains	A single colony	10	0	0	0	0	10	100	0	0	0	0	0	100
	Heavy inoculum	20	0	10	75	100	100	100	0	0	40	75	90	100

a) strains were first subcultured into BHI broth from Baird-Parker agar.

b) small organized clot.

c) entire content of tube coagulates and is not displaced when tube is inverted.

reactions, although they did not totally disappear. Most of the false-negative reactions occurred after heat treatment when a single colony was used for inoculation. False-negative reactions occurred in spite of the classification of a '2+' reaction as coagulase-positive. Baird-Parker agar has been shown previously to give better recovery values for both injured and uninjured staphylococci than other selective media (Gray *et al.* 1974, Stiles & Clark 1974, Niskanen &

Aalto 1978). It is thus apparent that Baird-Parker agar gives better results, due to the lack of inhibition of injured staphylococci, than other selective media for coagulase production, if the coagulase test is performed directly from the primary isolation medium.

Two weeks' freezing gave no false-negative reactions, even when a single colony was used for inoculation in testing (Table III). This implies that foods not heavily processed can be examined

for coagulase production directly from Baird-Parker agar. In general it is necessary to incubate the coagulase tubes for 24 h to avoid false-negative reactions when carried out directly from Baird-Parker agar.

Hurst *et al.* (1973) have observed that cells of *S. aureus* may recover their salt tolerance while various membrane functions are still damaged. It is also possible in the present case that the repair of different functions did not occur at the same time. Some disturbances were left, even though the *S. aureus* cells were able to grow on Baird-Parker agar. If the cells are first subcultured into BHI broth before coagulase testing, the false-negative reactions are eliminated and the coagulase reaction is more rapid.

It is possible to reduce the time for identification of *S. aureus* by performing the coagulase test directly from Baird-Parker agar, when the foods in question are not heavily processed. In the case of heavily processed foods, false-negative reactions are possible. These may sometimes be reduced by using a heavy inoculum; this, however, is not often possible.

There seem to be no major differences between toxigenic and non-toxigenic strains of *S. aureus* in terms of the coagulase reaction.

Acknowledgements

The authors wish to thank Aimo Niskanen, D.V.M. and Seppo Lindroth, Ph.D., Technical Research Centre of Finland, Food Research Laboratory, Espoo, Finland, for supplying the *S. aureus* strains.

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tion of unheated and heated staphylococci. Can. J. Microbiol. 1974, 20, 1735—1744.

Summary

Coagulase production of 77 coagulase-positive *Staphylococcus aureus* strains stressed before plating on Baird-Parker agar were studied directly from Baird-Parker agar and from BHI broth on strains subcultured first from Baird-Parker agar.

Reduced pH, heat treatment and repeated freezing and lyophilization caused false-negative coagulase reactions carried out directly from Baird-Parker agar. No false-negative reactions were observed after simple freezing. The different stress treatments had no effect on coagulase production carried out from BHI broth. Coagulase tubes inoculated directly from Baird-Parker agar have to be incubated for longer time for a positive reaction than tubes inoculated from BHI broth.

In the case of foods not heavily processed, it is possible to reduce the time for identification of *S. aureus* by performing the coagulase test directly from Baird-Parker agar.

Descriptors: low pH, heat treatment, freezing, repeated freezing and lyophilization.

Modtaget af redaktionen 1981.06.04.

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Sammanfattning

Effekten av olika stressbehandlingar på koagulas-test med *Staphylococcus aureus*

Koagulasproduktionen hos 77 koagulas-positiva *Staphylococcus aureus* stammar, som hade stressats före odlingen på Baird-Parker agar, undersöktes direkt från Baird-Parker agar och från BHI näringslösning, till vilken de undersökta stammarna hade ympats från Baird-Parker agar.

Sänkt pH, värmebehandling och upprepad djupfrysning och lyofilisering förorsakade falska-negativa koagulasreaktioner, då koagulas-testet företogs direkt från Baird-Parker agar. Inga falska-negativa reaktioner observerades efter djupfrysning. De olika stressbehandlingarna hade ingen effekt på koagulasproduktionen, om testet företogs från BHI näringslösningen. Koagulasrör, som hade inympats direkt från Baird-Parker agar, måste inkuberas längre än sådana rör, som inympades från BHI näringslösningen.

Då det gäller livsmedel som inte varit föremål för en extrem behandling, är det möjligt att förkorta tiden för identifiering av *S. aureus* genom att utföra koagulas-testen direkt från Baird-Parker agar.

Occurrence of *Campylobacter fetus* subsp. *jejuni* and *Yersinia enterocolitica* in Domestic Animals and in Some Foods of Animal Origin in Finland

by Marja-Liisa Hänninen and Markku Raevuori

This paper deals with the occurrence of Campylobacter fetus subsp. jejuni and Yersinia enterocolitica in fecal samples of some domestic animals and in samples of raw milk and ground meat.

Campylobacter fetus subsp. *jejuni* has recently become recognized as a common bacterial cause of diarrheal disease in man (Butzler *et al.* 1973, Skirrow 1977, Lindqvist *et al.* 1978). Reservoirs of infection are not well known. Organisms, indistinguishable by current criteria from strains of *C. fetus* subsp. *jejuni* which affect humans, have been isolated from many mammalian and avian species (Smibert 1978). Chickens have often been suspected to be sources of *Campylobacter* infection in humans (King 1957, Butzler *et al.* 1973, Brouwer *et al.* 1979). Milk has also been an incriminated vehicle in food-borne campylobacteriosis (Levy 1945, Robinson *et al.* 1979). A thermophilic campylobacter, earlier known as *V. coli*, has frequently been isolated from the intestinal contents of the pig. *Campylobacter enteritis* is also a recognized disease in Finland (Kosunen 1978).

Pigs are generally considered to be the most important reservoir of human yersinosis (Esseveld & Goudzwaard 1973, Toma & Diedrick 1975, Hurvell *et al.* 1977). The organism has also been isolated from meats originating from various animals (Leistner *et al.* 1975) as well as from milk products (Schiemann & Toma 1978, Schiemann 1978, Hughes 1979).

A survey was made in order to investigate the occurrence of *C. fetus* subsp. *jejuni* in rectal swabs of cattle and in the caecal contents of chickens. The occurrence of *C. fetus* subsp. *jejuni* and *Yersinia enterocolitica* in samples of porcine caecal contents, raw and pasteurised milk and ground meat was also investigated.

Materials

The 200 samples of rectal swabs from cattle were taken at the municipal slaughterhouse of Helsinki in May and June 1979. The 100 samples of chicken caecal contents from seven breeding farms were collected at a slaughterhouse, 400 km north of Helsinki, between September and November. The samples were transported in Stuart transport medium (Oxoid) to Helsinki. In total 81 unpasteurised samples of tank milk were taken in August and September. The 17 samples of pasteurised milk and the 16 samples of ground meat (12 samples of beef, 2 samples of mixed pork and beef) were taken from retail stores in Helsinki in October. The 20 samples of porcine caecal contents were collected at the Helsinki municipal slaughterhouse in October.