GERMAN

DAS AUFTRETEN UND DIE BEDEUTUNG VON KOAGULASE-POSITIVEN STAPHYLOKOKKEN BEI WILTSHIRE-PÖKELBRÜHEN

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Zusammenfassung

Siebenunddreißig 'reife' Immersionsbrühen wurden auf Staphylokokkus aureus untersucht. 22 (59%) waren positiv. Zählungen nach dem Baird-Parker EGPTA-Medium (1962) schwankten zwischen 4 bis 200/ml mit einem Durchschnitt von 27/ml. Von den diesem Medium entnommenen 104 Isolaten erwiesen sich 63 (60,5 %) als koagulase-positiv. Zweiundsechzig der letzteren waren bakteriophage Typen. Zwanzig (32 %) davon gehörten zum Phagentypus der lytischen Gruppe II; Typus 3c/55 war der am häufigsten gefundene Einzeltypus. Fünfunddreißig (56 %) der restlichen 42 waren nicht zu typisieren.

FRENCH

FREQUENCE ET IMPORTANCE DES STAPHYLOCOQUES POSITIFS A LA COAGULASE (CPS) DANS LES SAUMURES DE SALAISON DE TYPE WILTSHIRE

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Résumé

On a examiné trente-sept saumures d'immersion "mûres" afin de trouver le Staphylococcus aureus; 22 (59%) ont été positives. Les comptes effectués sur le bouillon de culture EGPTA de Baird-Parker (1962) s'échelonnaient entre 4 et 200/ml avec une moyenne de 27/ml. Sur 104 individus prélevés sur ce bouillon, 63 (60,5%) se sont avérés positifs à la coagulase. Soixante-deux sur les 104 ont été déterminés par phages. Vingt (32%) de ceux-ci ont été déterminés par les phages du Groupe lytique II; Le groupe 3C/55 a été le groupe le plus fréquemment trouvé. Trente-cinq (56%) des 42 qui restaient n'ont pas pu être déterminés.

THE OCCURRENCE AND SIGNIFICANCE OF COAGULASE-POSITIVE STAPHYLOCOCCI (CPS) IN WILTSHIRE-TYPE CURING BRINES

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Summary

Thirty seven 'mature' immersion brines were examined for Staphylococcus aureus; 22 (59%) were positive. Counts on EGPTA medium of Baird-Parker (1962) ranged from 4 to 200/ml with a mean of 27/ml. Of 104 isolates picked off this medium, 63 (60.5%) proved to be coagulase-positive. Sixty-two of the latter were phage-typed. Twenty (32%) of these were typed by phages of lytic Group II; type 3C/55 was the single most common type found. Thirty five (56%) of the remaining 42 were non-typable.

Нахождение и значимость стафилококков содержащих коагулазу в вильтширском соляном растворе.

Резюме.

Исследовали Staphylococcus aureus в 37 «зрелых» соляных растворах: 22 (59%) оказались позитивными. Подсчёты на среде EGPTA Бэрд-Паркера (1962) колебались от 4 до 200/мл при среднем числе 27/мл. Из 104 проб изолированных из этой среды 63 (60.5%) содержали коагулазу. 62 из этих были типа бактериофага. 20 из них являлись бактериофагами литической группы II; тип 3C/55 был самым частым из [illegible] найденных. У 35 (56%) из 42 остальных не было возможно определить их тип.

THE OCCURRENCE AND SIGNIFICANCE OF COAGULASE-POSITIVE STAPHYLOCOCCI (CPS) IN WILTSHIRE-TYPE CURING BRINES

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Introduction

Staphylococcus aureus is so ubiquitous that it's occurrence on handled food can hardly be avoided. There have been already reports that it occurs in curing brines (Buttiaux & Moriamez, 1958; Dempster, Reid & Cody, 1973) where it might be expected to survive (Eddy & Ingram, 1962). However, no case of food-poisoning attributable to the presence of staphylococcal enterotoxin in 'bacon' has been recorded in the United Kingdom (Gilbert, 1975, pers. comm.). 'Bacon' is the product of the 'Wiltshire' cure or short-time, sweet cures. It is sliced before sale and may be vacuum-packaged. Eddy & Ingram (1962) have suggested that the absence of toxin in bacon may be due to any one or combination of the following reasons. Firstly, toxin-producing staphylococci may always be absent, secondly, they may be present but unable to develop sufficiently to constitute a hazard and thirdly the organism may have grown but is unable to produce toxin due to the presence of salt (NaCl) and nitrite.

The objectives of the present investigation were to estimate the incidence of S. aureus in commercial bacon curing brines and characterize the strains recovered by phage-typing.

Materials

Thirty seven 'mature' immersion brines were collected during a 5 week period from 18 factories. Each sample was taken from a full tank of brine and placed in a 120 ml sterile, plastic jar and dispatched by post to the laboratory within 2 hrs of collection. The samples were held at 4°C until tested. A mature brine is one which is used repeatedly. Before re-use, it is filtered or otherwise clarified and brought back to strength.

Methods

a) Staph. aureus
The method described by Dempster et al. 1973 was used, except that 0.25 ml of brine, instead of 0.1 ml, was spread on a plate of EGPTA medium (Baird-Parker, 1962).

b) Escherichia Coli I and Clostridium spp. were enumerated by the method described by Dempster et al. 1973.

c) Viable counts
Serial dilutions of the brines in ¼ Ringers diluent + 0.1% of added peptone, were plated on PCA (Oxoid). Dilutions for the PCA + 4% of added NaCl (4PCA) counts and PCA + 10% NaCl (10 PCA) counts were made in 20% (W/v) NaCl. The plates were counted after 5 days at 25°C.

d) Coagulase test
A tube coagulase test was carried out on the growth from nutrient agar slopes (48h./37°C) by the method of Fisk (1940).

e) Phage-typing
This was carried out by the method described by Blair & Williams (1961) using the international basic set of phages. Phages 187, 42D, 88, 89, 90 and 92 were also included. Phage preparations and propagating strains were kindly supplied by the Cross-infection reference laboratory, Colindale, London.

Results and Discussion

In Table 1 are shown the bacteriological analyses of the bacon brines. Fifteen (40%) of the 37 contained no S. aureus per 0.25 ml brine tested. The highest recovery was 50/0.25 ml (200/ml) and the mean was 27/ml. These results confirmed earlier observations that staphylococci occur frequently in ordinary bacon brines (Eddy & Ingram, 1962) and hence might be expected to occur on bacon. These workers examined 8 factory brines; 7 had less than 40 phosphatase-positive staphylococci per ml. Coagulase-negative staphylococci rarely produce the enzyme phosphatase (Barber & Kuper, 1951).

The brines in this experiment were mature, that is, they were used over long periods. The high indicator (E. Coli) counts and plate counts are normal for an immersion brine which has been repeatedly used. Dempster et al (1973) reporting on beef curing brines which were frequently replaced showed that 25 (81%) out of 31 samples contained less than 10 S. aureus per ml.

Patterson (1965) reported that the addition of sulphamezathine (50 µg/ml) by Smith & Baird-Parker (1964) to the original medium of Baird-Parker (1962) did not completely inhibit Proteus spp. He obtained his isolates from steak and kidney, liver, beef and pork sausage and suggested that Proteus spp. are probably not so prevalent in cured meats as in fresh meats; this may have accounted for his much better (81%) results obtained with the recovery of S. aureus from pork sausage. We enumerated all colonies after 24h./37°C exhibiting typical characteristics (black, shiny, convex, 1.0 - 1.5 m.m. in diameter, with narrow white margins and surrounded by clear zones extending from 2.0 to 5.0 m.m. into the opaque medium) as S. aureus. On examination however, 40% proved to be coagulase negative staphylococci. It is therefore necessary when enumerating presumptive coagulase-positive staphylococci on selective media to confirm that a culture is S. aureus. Various tests have been suggested (fermentation of mannitol, production of nucleases, phosphatases, haemolysins, lysozymes and coagulases) for S. aureus. Of these, the coagulase test and nuclease test are the most specific (Baird-Parker, 1970).

The percentage of colonies picked off the plating medium which were coagulase-positive are shown in Table 2. Of 104 isolates, 63 (60.5%) proved to be positive. This compares with the 66% of positives picked off the SETGPA medium of Smith & Baird-Parker (1964) by Patterson (1965).

Sixty-two isolates of S. aureus were phage-typed (Table 3). Nine strains were typable at Routine test dilution (R.T.D.) and 18 were typable at R.T.D. X 100. Two strains were of phage-type 42D and three gave a wide pattern with phages of lytic Groups I and III. This wide pattern is characteristic of bovine staphylococci and the present strains are probably derived from animal sources. Twenty (32%) were typed by phages of Group II of which the most common type found was 30/55. Cultures of S. aureus are classified according to their susceptibility to a set of phages. Phage-typing is therefore a method of bacterial classification based on a single set of characters (Parker, 1972) and as such was used here. The majority of the strains belonged to lytic Group II. Oeding et al (1972) found that 84% of 71 strains of S. aureus isolated from the nasal cavities of healthy pigs belonged to this group and they refer to the work of Mori (1971) who isolated similar strains from pigs in Japan. This suggests that some S. aureus isolated from the brine here are derived from the commensal flora of the pig. However, further study is necessary to establish whether strains found in bacon curing brines are also derived from human sources.

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TABLE I

BACTERIOLOGICAL ANALYSES OF 37 WILTSHIRE-TYPE IMMERSION CURING BRINES

	Colony counts per ml on					
	CPS*	*Clostridium* spp.	*E. Coli*	PCA X 10^3	4PCA X 10^3	10PCA X 10^3
Range	nil - 200	nil - 13	nil - 200	8 - 970	15 - 1,480	10 - 3,430
Mean	27	2	17	149	342	831

*Coagulase-positive *Staphylococcus aureus* on Baird-Parker medium (1962)

TABLE 2

PERCENTAGE OF COAGULASE-POSITIVE S. AUREUS IN IMMERSION CURING BRINES

Brine No.	Count/0.25 ml on EGFTA medium[1]		% positive	Brine No.	Count/0.25 ml on EGFTA medium		% positive
1							
2	45	(10/10)[2]	100.0	12	33	(10/10)	100.0
3	1	(1/1)	100.0	13	12	(7/11)	63.6
4	50	(1/5)	20.0	14	1	(0/1)	0.0
5	6	(0/6)	0.0	15	4	(4/4)	100.0
6	5	(0/5)	0.0	16	1	(0/1)	0.0
7	2	(0/7)	0.0	17	4	(4/4)	100.0
8	37	(10/10)	100.0	18	2	(0/2)	0.0
9	2	(0/2)	0.0	19	11	(8/11)	72.7
10	1	(0/1)	0.0	20	2	(1/2)	50.0
11	6	(0/6)	0.0	21	1	(1/1)	100.0
	1	(1/1)	100.0	22	14	(5/10)	50.0

Total (63/104) = 60.5%

1. Medium of Baird-Parker (1962)
2. Figures in parenthesis show the number of colonies confirmed as coagulase-positive out of the number tested

TABLE 3

PHAGE-TYPES OF 62 STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM BRINE

No.	Phage types at R.T.D.	No.	Phage types at R.T.D. X 100
3	80/6/42E/47/54/75/77/84/85/81	7	3C/55
1	3A/3C/55/71	5	55
1	3A/3C/71	1	3A/55
1	3A/71	1	3C
1	3C/55	2	42D
1	55	1	52
1	3C	1	95
		35	non-typable