CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM A SLAUGHTERHOUSE FOR PIGEONS BY RAPD-PCR AND REA-PFGE

Losito P.[1], Blaiotta G.[2], Vergara A.[1], Villani F.[2], Ianieri A.[1]

[1] Department of Food Science, Piazza Aldo Moro 45, 64100, University of Teramo, Italy;
[2] Department of Food Science, Via Università, 100, 80055 Portici, University of Naples "Federico II", Italy.

Background

S. aureus is a common etiologic factor of foodborne infections and intoxication and is a significant marker of food quality and surface cleanliness (Losito *et al*., 2004). Recently, a variety of molecular methods have been used to clarify staphylococcal identification, including RAPD-PCR and REA-PFGE (Restriction Endonucleases Analysis-Pulsed Field Gel Electrophoresis) (Matthews *et al*., 1997). However, no information is available concerning the intraspecies differentiation of *S. aureus* isolated from a slaughterhouse for pigeons by these genetic procedures.

Objectives

In the present study, RAPD-PCR and REA-PFGE were used to characterize *S. aureus* strains originating from a slaughterhouse for pigeons and to assess the practical value of these typing methods for the discrimination among strains.

Materials and methods

Bacterial strains. The source of isolation of the 38 wild strains used in this study is shown in Table 1. Strains were grown in Brain Heart Infusion broth (BHI) (Oxoid Ltd., Basingstoke, Hampshire, UK) at 37°C. Before DNA extraction, cultures were streaked on BHI agar plates and grown overnight at 37°C.

DNA isolation for PCR assays. DNA extraction was carried out from a single colony by using an InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA) following the conditions described by the supplier. About 25 ng of DNA were used for PCR amplification.

Identification of *S. aureus* strains by PCR. The thermostable nuclease (*nuc*) was amplified for the identification of *S. aureus* strains. Sequences of the primer set for the *nuc* gene were first described by Brakstad *et al*. (1992) (Table 2).
PCR mixtures were prepared as described in Blaiotta *et al*. (2004). After the denaturing step for 3 min at 95°C, 35 amplification cycles (5 s at 95 °C for the denaturing step, 30 s at 58 °C for the annealing-extension step) were performed for detection of *nuc* gene. The PCR products were resolved by agarose (2 % w/v) gel electrophoresis at 7 V cm^{-1} for 2 h.

RAPD-PCR amplifications. RAPD-PCR analysis was performed in a total volume of 25 µl using primers PRIM 239 (CTGAAGCGGA) and XD9 (GAAGTCGTCC) in the same reaction. RAPD-PCR mixtures were prepared as reported by Moschetti *et al*. (2000). Amplification was carried out in a programmable heating incubator for 40 cycles (1 min at 94 °C, 1 min at 31 °C, 2 min at 72 °C per cycle). Finally, a 7 min extension period at 72 °C was performed. Amplified products (25 µl) were resolved by electrophoresis on a 2 % (wt/vol) agarose gel at 7 V cm^{-1} for 2 h.

REA-PFGE (Restriction Endonucleases Analysis-Pulsed Field Gel Electrophoresis). Genomic DNA of high molecular weight was prepared as previously described Blaiotta et al. (2001). Inserts of intact DNA were digested in 200 µl of appropriate buffer supplemented with 40 U of *Sma* I (Promega). Electrophoresis of the restriction digests was performed by using the CHEF system (Bio-Rad Laboratories, Hercules, CA, USA) with 1% (wt/vol) agarose gels and 0.5 x TBE as running buffer, at 10 °C. Restriction fragments < 560 kb were resolved in a single run, at constant voltage of 6 V cm^2 and an orientation angle of 120° between electric fields, by a single phase procedure for 24 h with a pulse ramping between 1 and 50s.

Results and discussion

Results obtained by RAPD-PCR and REA-PFGE are depicted in Table 3. Examples of RAPD PCR and REA-PFGE patterns are showed in Fig.1 and Fig.2 respectively. RAPD PCR technique produced 9 different pattern-types among the 38 strains of *S. aureus* analysed. The most common RAPD PCR profile was the A one. The strains mostly isolated from the defeathering machine and worker's overall during all samplings produced this pattern. The number of the patterns went up to 25 using REA-PFGE. The strains isolated from the defeathering machine during the three samplings showed six different REA-PFGE patterns; one of them was isolated before slaughtering. The four strains from worker's hand produced three REA-PFGE patterns. The profile G was the only one common to different samplings. It was found on worker's overall during the first and the third sampling.

Conclusions

Results obtained during this study confirm that REA-PFGE technique is more discriminatory than RAPD (Raimundo *et al*., 2002; Al-Thawadi *et al*., 2003). A good discrimination among the strains is a very important step for the contamination monitoring above all in the slaughterhouse environment where the microbial contamination levels are always high. Our results underline the elevated differentiation percentage of the strains of *S. aureus* present in a slaughterhouse for pigeons. The findings also point out that the REA-PFGE technique could be a useful tool to reveal the contamination sources.

References

Al-Thawadi, S.I., Kessie, G., Dela Cruz, D., Al-Ahdal, M.N. 2003. A comparative study on the application of 3 molecular methods in epidemiological typing of bacterial isolates using MRSA as a prototype. Saudi Med. J. 24, 12: 1317-1324.

Brakstad, O. G., Aasbakk, K. and Maelamd, J. A. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of nuc gene. Journal of Clinical Microbiology 30, 1654-1660.

Blaiotta, G., Moschetti, G., Simeoli, E., Andolfi, R., Villani, F., Coppola, S. 2001. Monitoring lactic acid bacteria during "cacioricotta" cheese production by Restriction Endonucleases Analysis and Pulsed-Field Gel Electrophoresis. J Dairy Res 68, 139-144.

Blaiotta, G., Ercolini, D., Pennacchia, C., Fusco, V., Casaburi, A., Pepe, O., Villani, F. 2004. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products. Evidence for new variants of seG and seI in *S. aureus* AB-8802. Journal of Applied Microbiology (In Press).

Losito, P., Muscariello, T., Vergara, A., Ianieri, A. (2004): Staphylococcus aureus in a slaughterhouse for pigeons. Proceedings of International Conference – Farm to Fork Food Safety, Athens, Greece.

Matthews, K.R., Roberson, J., Gillespie, B.E., Luther, D.A., Oliver, S.P. 1997. Identification and differentiation of coagulase-negative *Staphylococcus aureus* by polymerase chain reaction. Journal of Food Protection 60, 6: 686-688.

Moschetti, G., G. Blaiotta, F. Villani, and S. Coppola. 2000. Specific detection of *Leuconostoc mesenteroides* subsp. *mesenteroides* with DNA primers identified by randomly amplified polymorphic DNA analysis. Appl. Environ. Microbiol. 59: 695-700.

Raimundo, O., Heussler, H., Bruhn, J.H., Suntraranchun, S., Kelly, N., Deighton, M.A., Garland, S.M. 2002. Molecular epidemiology of coagulase-negative staphylococcal bacteraemia in a newborn intensive care unit. J. Hosp. Infect. 51, 1: 33-42.

Tab.1. tested surfaces contaminated by *St. aureus*

Tested surfaces	I sampling		II sampling		III sampling	
	B	**A**	**B**	**A**	**B**	**A**
Crop rinsing tube	+	+				
Defeathering machine		+	+	+		+
Leg defeathering machine (I finger)		+			+	+
Leg defeathering machine (V finger)	+	+				
Gutting machine		+				+
Tunnel entrance		+				+
Tunnel exit		+				+
Worker's hand	+	+				
Worker's overall		+		+		+

B: before slaughtering; **A**: after slaughtering

Table 2. PCR primers for the *nuc* gene used in this study

Primer	Sequence (5'-3')	References	Target gene	Product (bp)
nucF	GCGATTGATGGTGATACGGTT	Bakstad et al. 1992	*nuc*	279
nucR	AGCCAAGCCTTGACGAACTAAAGC			

Table 3. RAPD PCR and REA-PFGE types of *S. aureus* strains used in the present study.

Strains	**Source**	**D/P**	**Sampling**	**PCR *nuc***	**RAPD PCR**	***Sma*I PFGE**
2D	Defeathering machine	D	III	+	A	?
3D1	Leg defeathering machine (I finger)	D	III	+	A	A
5D1	Gutting machine	D	III	+	B	O
6D1	Tunnel entrance	D	III	+	A	B
7D1	Tunnel exit	D	III	+	A	C
9D3	Worker's overall	D	III	+	A	G
10D2	Defeathering machine	D	III	+	A	B
8	Tunnel entrance	D	I	+	B	J1
9	Leg defeathering machine (I finger)	D	I	+	B1	J3
10	Gutting machine	D	I	+	A2	T
11	Leg defeathering machine (IV finger)	P	I	+	A	G1
12	Defeathering machine	D	I	+	A	D
13	Tunnel exit	D	I	+	A	D
14	Leg defeathering machine (I finger)	D	I	+	B2	N
15	Leg defeathering machine (IV finger)	D	I	+	B4	S
16	Tunnel entrance	D	I	+	A	D
17	Defeathering machine	D	I	+	B2	P
18	Crop rinsing tube	D	I	+	B3	R
19	Leg defeathering machine (IV finger)	D	I	+	B1	J3
20	Leg defeathering machine (IV finger)	P	I	+	A	E
21	Crop rinsing tube	D	I	+	B	M
22	Tunnel exit	D	I	+	B1	J3
23	Crop rinsing tube	P	I	+	A	F
24	Worker's hand	P	I	+	B	J2
25	Worker's hand	P	I	+	B	L
26	Worker's hand	D	I	+	A	E1
27	Worker's overall	D	I	+	B2	Q
28	Worker's hand	D	I	+	A	E1
29	Worker's overall	D	I	+	A	G
101	Worker's overall	D	II	+	A	H
102	Worker's overall	D	II	+	A	H
103	Worker's overall	D	II	+	A	H
104	Defeathering machine	D	II	+	A	H
105	Defeathering machine	D	II	+	A1	I
106	Defeathering machine	D	II	+	A1	I
107	Defeathering machine	D	II	+	A	?
108	Defeathering machine	D	II	+	A	H
109	Defeathering machine	P	II	+	A3	K

Fig.1. RAPD-PCR patterns

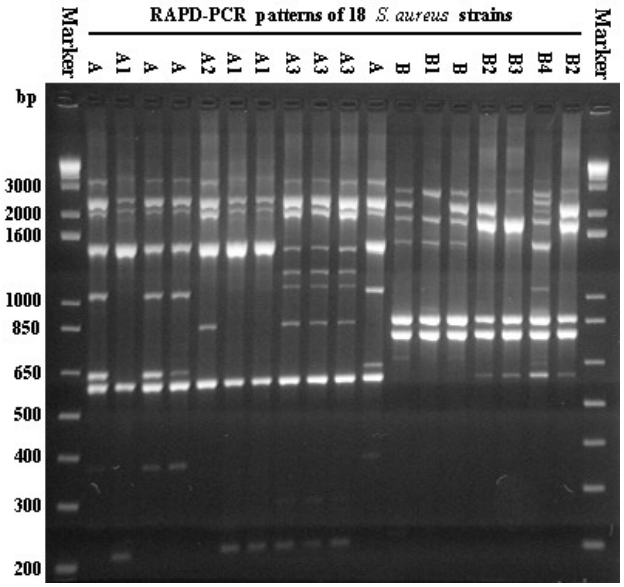

Fig. 2. REA-PFGE patterns