Brazilian Journal of Microbiology (2003) 34 (Suppl.1):125-127
ISSN 1517-8382

IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS*, *S. INTERMEDIUS* AND *S. HYICUS* BY PCR AMPLIFICATION OF *COA* AND *NUC* GENES

Wladimir Padilha da Silva[1]*; Jorge Adolfo Silva[2]; Márcia Raquel Pegoraro de Macedo[1]; Márcia Ribeiro de Araújo[1]; Márcia Magalhães Mata[1]; Eliezer Avila Gandra[1]

[1]Laboratório de Microbiologia de Alimentos, Departamento de Ciências e Tecnologia Agro-industrial, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, RS, Brasil.
[2]Laboratório de Biotecnologia de Alimentos, Departamento de Ciências e Tecnologia Agro-industrial, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, RS, Brasil.

This paper corresponds to an "extended abstract" selected for oral presentation in the 22nd Brazilian Congress of Microbiology, held in Florianópolis, SC, Brazil, in November 17-20, 2003

ABSTRACT

Sixty-five strains of coagulase positive staphylococci (*Staphylococcus aureus*, *S. intermedius* and *S. hyicus*) were identified at species level by PCR amplification of the *coa* gene, specific for *S. aureus,* and of the *nuc* gene, specific for *S. intermedius* and for *S. hyicus*.

Key words: coagulase positive staphylococci, PCR, *coa* gene, *nuc* gene.

INTRODUCTION

S. aureus, S. hyicus and *S. intermedius* are species of *staphylococcus* associated with food intoxication outbreaks (5) that present very similar phenotypic characteristics, which makes the identification and differentiation through traditional culture techniques defficult (5,7). Despite the recommendation for the use of methods based on the polymerase chain reaction (PCR) to select pathogens among other bacterial species (2), very limited research using molecular methods to differentiate *S. aureus*, *S. hyicus* and *S. intermedius* has been reported. This work ained to evaluate the amplification of the gene sequences of *coa* and *nuc* for the identification of *S. aureus*, *S. intermedius* and *S. hyicus*.

MATERIALS AND METHODS

Sixty-five strains previously characterized as coagulase positive *staphylococci* (CPS) and identified as *S. aureus* (55 strains), *S. intermedius* (4 strains) and *S. hyicus* (6 strains) were used. The genomic DNA extraction from all strains was done according to the protocol proposed by Matthews *et al*. (6). The primers used in the PCR were: COAG2 and COAG3, specific for the *coa* gene of *S. aureus*; NUC1 and NUC2, specific for the *nuc* gene of *S. intermedius* and NUC3 and NUC4, specific for the *nuc* gene of *S. intermedius* (Table 1).

The PCR reactions were prepared with 20 nM of extracted DNA, 1 μM of each primer, 200 μM of each triphosphate desoxinucleotide (dNTP, Gibco BRL), 1 U of Taq DNA Polymerase (Pharmacia, 5 $U.\mu L^{-1}$), 2 mM of $MgCl_2$ (Pharmacia) and 4 μL of 10 X buffer (Pharmacia), making a total volume of 40 μL. The program consisted of 50 seconds at 95ºC, 2 minutes at 55ºC (for primers COAG) or at 42ºC (for primers NUC) and 4 minutes at 72ºC, for 40 cycles (4). The electrophoresis was performed in agarose gel (p/v) stained with 0.5 $mg.mL^{-1}$ of ethidium bromide. The size of the amplified fragments was determined under UV light, through comparison with the molecular weight standard (1 Kb Plus DNA Ladder or 100 bp DNA Ladder, Gibco BRL).

*Corresponding author. Mailing address: Laboratório de Microbiologia de Alimentos, Departamento de Ciências e Tecnologia Agro-industrial, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Campus Universitário s/n, Caixa Postal 374. 96010-900, Pelotas, RS, Brasil. Fax: (+5553) 2759031. E-mail: silvawp@ufpel.tche.br

Table 1. Oligonucleotide used for molecular identification and differentiation among *S. aureus*, *S. intermedius* and *S. hyicus*, PCR products and target species.

Primer	Sequence 5′- 3′	Estimated amplification product (pb)	Species
NUC1[a]	GCCCCTGCAATGAGAGG	334	*S. hyicus*
NUC2[a]	CGGACCACTTTCCGTC		
NUC3[a]	CGCCGTTCTCTCTTTGG	431	*S. intermedius*
NUC4[a]	CGCCTCTCACATCCG		
COAG2[b]	ACCACAAGGTACTGAATCAACG	Variable	*S. aureus*

[a] Design from the complete sequence of *nuc,* obtained from GenBank/NCBI (Accession number L23973 for *S. hyicus* and X67678 for *S. intermedius*); [b] Previously described by Aarestrup *et al*., (1); [c] According to Aarestrup *et al*., (1).

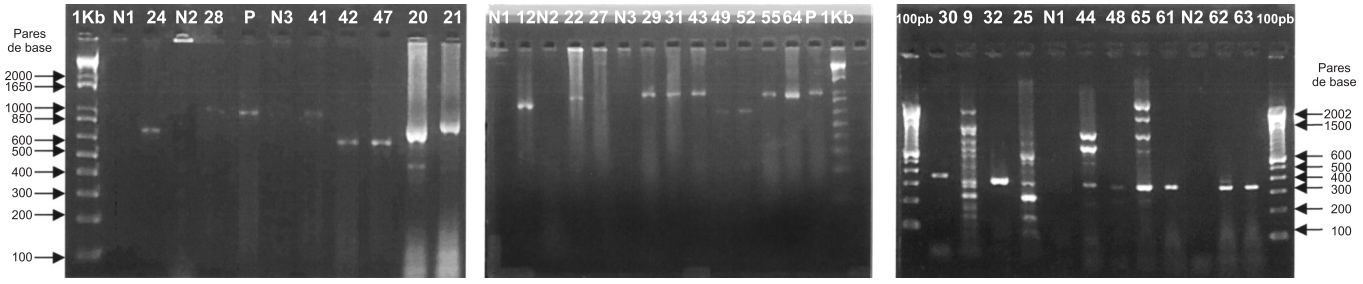

Figure 1. Agarose gel 1%, stained with ethidium bromide, under ultraviolet light. A – 1 kb – Plus DNA Ladder, N1 – sterile distilled water, N2 – *S. intermedius*, N3 – *S. hyicus*, P – *S. aureus* ATCC 10832. B – 1 kb-Plus DNA Ladder, N1- sterile distilled water, N2 – *S. intermedius*, N3 - *S. hyicus*, P - *S. aureus* ATCC 10832. C - 100 bp - DNA Ladder, N1- sterile distilled water, N2- *S. aureus* ATCC 10832. 12, 22, 20, 21, 24, 27, 28, 29, 31, 41, 42, 43, 47, 49, 52, 55 and 64 are PCR products obtained with cultures of *S. aureus*. 30, 32, 48, 61, 62 and 63 are PCR products obtained with cultures of *S. hyicus* and 9, 25, 44 and 65 are PCR products from cultures of *S. intermedius*. A and B amplifications with COAG2-COAG3 primers, C amplifications with NUC1-NUC2 and NUC 3-NUC4 primers.

RESULTS AND DISCUSSION

It was verified that COAG2 and COAG3 primers showed specificity for *S. aureus*, as amplifications were obtained in all the reactions in which DNA from this species (3) was used (Fig. 1). These results are in accordance with Aarestrup *et al*. (1), who studied the amplification of sequences of the *coa* gene in 187 strains of *S. aureus*, 10 strains of *S. intermedius*, 3 strains of *S. hyicus*, 1 strain of *S. delphini* and 1 strain of *S. schleiferi* subspecies *coagulans* and verified the presence of bands only in *S. aureus*. The variability in the size of the amplified fragments with primers COAG2 e COAG3 may be due to the existence of structurally different gene forms of coagulase in *S. aureus*, allowing one strain to produce one or more of these variants (4). However, the reason for this polymorphism is not clear yet (4).

NUC1 and NUC2 primers demonstrated to be specific for *S. hyicus*, as there was amplification only when the DNA from this microorganism was used (Fig. 1). Although these results refer only six strains of *S. hyicus*, the observed specificity indicates that these primers have potential to be used in the differentiation between this species and *S. aureus* and *S. intermedius*.

A great variability in the size of the amplified fragments obtained with NUC3 and NUC4 primers was verified. However, the expected fragment (431 bp) was always obtained when the DNA from *S. Intermedius* was submitted to amplification (Fig. 1). This polymorphism may be related to the conditions used for the PCR, which could be enabling unspecific amplifications or, also allowing the primers to attach to other genes with similar sequences or partially homologous. Despite the small number of strains tested, these results showed that these primers have potential to be used in CPS identification and differentiation studies, but conditions of the reaction still need to be optimized.

ACKNOWLEDGEMENT

This work was supported by Capes and CNPq/Pibic schoolarship.

RESUMO

Identificação de *Staphylococcus aureus*, *S. intermedius* e *S. hyicus* através de seqüências dos genes *coa* e *nuc*.

Sessenta e cinco cepas de estafilococos coagulase positiva foram identificadas em nível de espécie, através da amplificação, por PCR, de seqüências do gene *coa*, específicas para *S. aureus*, e do gene *nuc,* específicas para *S. intermedius* e para *S. hyicus*.

Palavras-chave: estafilococos coagulase positiva, PCR, gene *coa*, gene *nuc.*

REFERENCES

1. Aarestrup, F.M.; Wegener, H.C.; Rosdahl, V.T. Evaluation of phenotypic and genotypic methods for epidemiological typing of *Staphylococcus aureus* isolates from bovine mastitis in Denmark. *Vet. Microbiol.*, 45:139-150, 1995.
2. Farber, J.M.; Gendel, S.M.; Tyler, K.D.; Boerlin, P.; Landry, W.L.; Fritschel, S.J.; Barrett, T.J. Molecular typing and differentiation. *In: Compendium of methods for the microbiological examination of foods*. APHA, Washington, 2001. p.127-158.
3. Gandra, E.A. *Identificação de* Staphylococcus aureus*,* S. hyicus *e* S. intermedius *através de testes bioquímicos e da amplificação por PCR de sequências dos genes* coa *e* nuc. (Dissertação de mestrado, Universidade Federal de Pelotas). Pelotas, 2003, p.101.
4. Goh, S.H.; Byrne, S.K.; Zhang, J.L.; Chow, A.W. Molecular typing of *Staphylococcus* on the basis of coagulase gene polymorphism. *J. Clin. Microbiol.*, 30:1642-1645, 1992.
5. Kloos, W.E.; Bannerman, T.L. *Staphylococcus* and *Micrococcus*. In: Murray, P.R.; Baron, E.J.; Pfaller, M.A.; Tenover, F.C.; Yolken, R.H. *Manual of Clinical Microbiology*. ASM, Washington, 1999. p.264-282.
6. Matthews, K.R.; Roberson, J.; Gillespie, B.E.; Luther, D.A.; Oliver, S.P. Identificação and differentiation of coagulase-negative *Staphylococcus aureus* by polymerase chain reaction. *J. Food Prot.*, 60:686-688, 1997.
7. Roberson, J.R.; Fox, L.K.; Hancock, D.D.; Besser, T.E. Evaluation of methods for differentiation of coagulase-positive Staphylococci. *J. Clin. Microbiol.*, 30:3217-3219, 1992.