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DETECTION OF ENTEROTOXINS GENES IN COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM FOODS

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ABSTRACT

Staphylococcal food poisoning is caused by ingestion of enterotoxins preformed in the food contaminated essentially through human manipulation or raw material obtained from animals. Although coagulase-positive *Staphylococcus aureus* is the main agent responsible for food intoxication, some researches emphasise that coagulase-negative staphylococci (CNS) are able to produce staphylococcal enterotoxins and may be a potential cause of food poisoning. In the present study CNS were isolated from foods and the toxigenic capacity of the strains determined. A total of 88 food samples were analysed and 22.7% were positive for CNS strains. Staphylococcal counts ranged from 3.0×10^2 to 1.4×10^6 CFU/g or mL of food examined. *S. epidermidis* predominated among the isolates (40%). Further isolates included *S. xylosus* (20%), *S. warneri* (20%), *S. saccharolyticus* (15%), and *S. hominis* (5%). Four isolates were positive for enterotoxin genes, as detected by polymerase chain reaction, with *se*a being the predominant gene. Although no enterotoxin production was detected by the reverse passive latex agglutination method, the data showed that the toxigenic capacity of CNS should not be ignored, requiring investigation of this group of microorganisms in food.

Key words: coagulase-negative staphylococci, PCR, enterotoxins

INTRODUCTION

Staphylococcus aureus enterotoxins are the most frequent causes of food poisoning, with outbreaks caused by mishandling of foods after heat treatment (21,23). The heat destroys the vegetative bacterial microbiota in food, and the non competitive staphylococci, introduced by inadequate handling process, may grow.

Although enterotoxins are produced mainly by coagulase-positive staphylococci, some coagulase-negative staphylococci (CNS), involved in a variety of human and animal infections (11), have also raised interest. Very little is known about the growth of CNS in foods. These strains have rarely been implicated in food poisoning because they do not grow rapidly in foods. Nevertheless CNS can contaminate foods because humans are common carriers of these microorganisms and some may be related to specific human infections (4).

Since immunoassays require a detectable amount of toxin, molecular techniques can complement the assay through detection of staphylococcal enterotoxin genes as important tool in the microbiology laboratory. Taking into account that the toxigenic potential of CNS is often neglected, in the present study enterotoxin genes in CNS strains isolated from foods were analysed by PCR and the results correlated with latex agglutination assay data.

MATERIALS AND METHODS

Food samples

A total of 88 food samples were analysed, including bakery goods (n=38), milk (n=18), snacks (n=11), white cheese (n=10), sandwich (n=8,) and pork meat (n=3), purchased from local markets or delicatessens in Botucatu, SP (Brazil) for a period of twelve months (2003). The interval between the sampling and the analysis was less than one hour.

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Isolation of staphylococci

Twenty-five grams of each sample was suspended in 225 mL peptone water, and 0.1 mL of 10^{-1} to 10^{-6} dilutions was spread on the surface of Baird-Parker agar. The plates were incubated for 48 h at 37ºC and typical colonies (black to dark grey, smooth, convex, well-defined contours, surrounded or no by a dull halo) were counted and the number expressed in CFU/g or mL.

Identification of coagulase-negative staphylococci

Representatives of each colony type were checked by Gram method for purity and morphology, and submitted to catalase and coagulase tests. The genus *Staphylococcus* was differentiated from *Micrococcus* by glucose oxidation and fermentation tests, resistance to bacitracin (0.04 U, absence of an inhibition halo or formation of a halo of up to 9 mm), and sensitivity to furazolidone (100 mg, inhibition halo of 15 to 35 mm) (2).

CNS were identified by simplified biochemical test scheme proposed by Kloos and Schleifer (10) and Kloos and Bannerman (11), which is based on the utilization of xylose, arabinose, sucrose, trehalose, mannitol, maltose, lactose, xylitol, ribose, and fructose, characterization of hemolysis, reduction of nitrate, presence of urease and ornithine decarboxylase, and resistance to novobiocin.

Toxin production

Staphylococcal enterotoxin was produced using the dialysis bag method described by Donnelly *et al.* (8). A 30 to 40 cm dialysis bag (Cut-Off 12,000-16,000 MW, Inlab) was previously washed with distilled water, closed at one end, filled with 50 mL of double-concentrated brain heart infusion broth (BHI), and the other end closed. The bag, bended to a "U" shape, was placed in a 250 mL Erlenmeyer flask and autoclaved for 15 min at 121ºC. The flask was added of 18 mL of 0.02 M phosphate buffer pH 7.4 in 0.9% NaCl, inoculated with staphylococci previously cultured in 5 mL BHI at 37ºC/18 h. After incubation at 37ºC, 200 rpm for 24 h, the culture was centrifuged at 8000 *g*/ 10 min at 4ºC and the supernatants stored at -20ºC and in liquid nitrogen until use.

Detection of enterotoxins

For the detection of toxins by reverse passive latex agglutination (RPLA) method, the supernatants concentrated in dialysis bags (8) were filtered through an 8 µm Millipore membrane to avoid nonspecific reactions. Extracellular enterotoxins were detected by RPLA method as described by Shingaki *et al*. (20) using the SET-RPLA-T900 kit for the detection of SEA, SEB, SEC and SED (Oxoid Diagnostic Reagents). Briefly, microplate wells with a V-shaped bottom were inoculated with 25 µL of the supernatant and 25 µL latex sensitised with anti-enterotoxins. Standard toxins (provided by manufacturer, Oxoid Diagnostic Reagents) were used as positive controls and the occurrence of nonspecific reactions was tested by addition of 25 µL of the supernatant to 25 µL of control latex. The plates were covered with cellophane and homogenised in a micromixer for 3 min. After incubation for 20 to 24 h at environmental temperature, the results were recorded according to the agglutination pattern described by the manufacturer. Positive reactions were classified as (+), (++), and (+++), while formation of a pink bud was interpreted as a negative result.

Detection of enterotoxin genes

DNA extraction

Total DNA was extracted from CNS strains cultured on blood agar, inoculated individually into BHI broth and incubated at 37ºC/ 24h. The GFX kit (Amersham Biosciences) was used for DNA extraction by manufacturer's protocol, which consists of initial digestion of the staphylococcal cells with lysozyme (10 mg/mL) and proteinase K (20 mg/mL). Then 500 µL of the extraction solution was added, and the mixture was centrifuged (5000 *g*/ 1 min). The supernatant was then transferred to a GFX column and centrifuged (5000 *g*/ 1 min). The eluent was discarded, and 500 µL of extraction solution was again added to the column. After centrifugation and disposal of the collected eluent, 500 µL of the wash solution was added to the column and the column was centrifuged at 20800 *g*/ 3 min. The column was then transferred to a 1.5-mL tube and 200 µL Milli-Q water heated at 70ºC was used for elution under centrifugation (5000 *g*/ 1 min).

PCR

PCR was carried out in 0.5-mL microcentrifuge tubes added of 20 pmol of each primer (Table 1), 2.5 U Taq DNA polymerase, 200 µM dNTPs, 20 mM Tris-HCl, pH 8.4, 0.75 mM $MgCl_2$, and 5 µL DNA (total volume of 50 µL). A negative control in which DNA was replaced with water was run in parallel in all reactions. Amplification was performed with an MJ Research PTC-100 thermocycler as described by Johnson *et al.* (9) with some modification, which consisted of a first cycle at 94ºC for 4 min, denaturation at 94ºC for 2 min, annealing at 55ºC for 1 min and 30 sec, and extension at 72ºC for 1 min and 30 sec, followed by a second cycle of denaturation at 94ºC for 2 min, annealing at 53ºC for 1 min and 30 sec, and extension at 72ºC for 1 min and 30 sec. In the third cycle, the annealing temperature was reduced to 51ºC, followed by additional 37 cycles at 94ºC for 2 min, 51ºC for 1 min and 30 sec and 72ºC for 1 min and 30 sec. At the end of the 40 cycles, the tubes were incubated at 72ºC for 7 min and stored at 4ºC.

Analysis of the amplified products

Amplification efficiency was determined on 2% agarose gels in 1X TBE buffer stained with ethidium bromide. The size of the amplified products was compared with 50- and 100-kb standards and the gels were photographed under UV transillumination.

RESULTS

Table 2 shows the distribution of CNS according to species and food analysed, slowing that twenty food samples were positive for CNS species. Of the 20 CNS isolates, 12 were isolated from bakery goods, 2 from milk, 1 from cheese, 2 from sandwiches, and 3 from pork meat.

CNS counts in the foods ranged from 3.0 x 10^2 to 1.4 x 10^6 CFU/g or mL, except for snacks, where no staphylococci were isolated (Table 3).

S. epidermidis was the most predominant specie, accounting for 40% of all CNS, followed by *S. xylosus* (20%), *S. warneri* (20%), *S. saccharolyticus* (15%), and *S. hominis* (5%).

The gene specific analysis by PCR for enterotoxin production revealed *se*a gene in three CNS isolates, while *se*c-1 gene was detected in only one isolate (Table 4). Despite potentially toxigenic strains of CNS were detected by molecular tool, the analysis of enterotoxin by RPLA method showed negative results.

The *se*a gene was detected in one *S. epidermidis* isolated from chocolate cream-filled puffs, in one *S. xylosus* isolate from cream-filled puffs and in one *S. hominis* from apple pie. The *sec*-1 gene was detected in only one *S. xylosus* isolated from fruit pie.

DISCUSSION

As shown in Table 2, *S. epidermidis* was the most frequently isolated species (40%). This species is a common inhabitant of human skin and mucous membranes of individuals manipulating food and animals, and is able to contaminate raw products and processed foods (4).

Both *S. warneri* and *S. xylosus* were the second most frequent species in our study (20%). In a study carried out by Udo *et al.* (24) with restaurant workers, the prevalent CNS species on the hands of food handlers were *S. hominis* (23.6%), *S. warneri* (20.6%) and *S. epidermidis* (3.4%). Since human nares and fingers are the main sources of *S. aureus* (16,22), and because CNS inhabit the human skin and mucous membranes, these microorganisms can contaminate food if these are not handled properly. Therefore, enterotoxigenic CNS strains may contribute to staphylococcal food poisoning (4).

In the present study, staphylococcal enterotoxin genes were detected in one *S. epidermidis* isolate, two *S. xylosus* isolates and one *S. hominis* isolate (Table 4).

Valle *et al.* (25) determined the enterotoxigenic character of staphylococci isolated from the skin, nasal mucosa and milk of

Table 1. Primers used for the detection of staphylococcal enterotoxin genes.

Primers	5' to 3' nucleotide sequence	Target	Amplicon size (bp)
sea1	TTG GAA ACG GTT AAA ACG AA	Enterotoxin A	120
sea2	GAA CCT TCC CAT CAA AAA CA	Enterotoxin A	
seb1	TCG CAT CAA ACT GAC AAA CG	Enterotoxin B	478
seb2	GCA GGT ACT CTA TAA GTG CC	Enterotoxin B	
sec1	GAC ATA AAA GCT AGG AAT TT	Enterotoxin C	257
sec2	AAA TCG GAT TAA CAT TAT CC	Enterotoxin C	
sed1	CTA GTT TGG TAA TAT CTC CT	Enterotoxin D	317
sed2	TAA TGC TAT ATC TTA TAG GG	Enterotoxin D	

Source: Johnson *et al*., 1991 (9).

Table 2. Frequency of coagulase-negative staphylococci (CNS) isolated from foods.

Species	Isolates n=20	Bakery goods n=38	Snack n=11	Milk n=18	White cheese n=10	Sandwich n=8	Pork meat n=3
S. epidermidis	8 (40.0)	4 (20.0)	-	2 (10.0)	-	2 (10.0)	-
S. xylosus	4 (20.0)	4 (20.0)	-	-	-	-	-
S. hominis	1 (5.0)	1 (5.0)	-	-	-	-	-
S. warneri	4 (20.0)	3 (15.0)	-	-	1 (5.0)	-	-
S. saccharolyticus	3 (15.0)	-	-	-	-	-	3 (15.0)
Total	20 (100.0)	12 (60.0)	-	2 (10.0)	1 (5.0)	2 (10.0)	3 (15.0)

N= Number of isolates CNS of the 88 samples analysed; n = Total number of samples analysed.

Table 3. Number of coagulase-negative staphylococci (CNS) in foods.

Food	Isolates N (%)	Species	Average Count CFU/g or mL
Bakery goods (n=38)	4 (20.0)	*S. epidermidis*	1.3×10^3
	4 (20.0)	*S. xylosus*	6.0×10^4
	1 (5.0)	*S. hominis*	5.5×10^4
	3 (15.0)	*S. warneri*	3.2×10^4
Milk (n=18)	2 (10.0)	*S. epidermidis*	3.2×10^4
White cheese (n=10)	1 (5.0)	*S. warneri*	1.4×10^6
Sandwich (n=8)	2 (10.0)	*S. epidermidis*	7.2×10^4
Pork meat (n=3)	3 (15.0)	*S. saccharolyticus*	3.0×10^2

n = Total number of samples analysed.

Table 4. Detection of toxin genes by PCR in CNS species isolated from foods.

Species	*se*a	*se*b	*se*c-1	*se*d
S. epidermidis	1	-	-	-
S. xylosus	1	-	-	-
S. xylosus	-	-	1	-
S. hominis	1	-	-	-
S. warneri	-	-	-	-
S. saccharolyticus	-	-	-	-
Total	3	-	1	-

*se*a, *se*b, *se*c-1, and *se*d: enterotoxins genes A, B, C and D, respectively.

133 healthy goats. Twenty-two percent of CNS strains produced enterotoxins, including *S. caprae*, *S. xylosus*, *S. warneri* and *S. epidermidis*.

Analysis of the toxigenic profile of CNS isolated in our study by PCR revealed the presence of the *sea* gene in one *S. xylosus* isolate, one *S. hominis* isolate and one *S. epidermidis* isolate (Table 4). It has been widely accepted that most staphylococcal food intoxications are caused by the ingestion of enterotoxin A (17,18,26). The presence of the *se*c-1 gene was observed in one *S. xylosus* isolate. All theses toxigenic CNS were isolated from bakery goods. Bakery goods covered and filled with cream are leading causes of food poisoning worldwide (1,5,7).

The enterotoxigenicity of CNS has been described by several investigators (6,14,25), and questioned by others (3), but few studies have been conducted to determine the enterotoxigenic capacity of CNS in foods. In this study, a small number of CNS harboured enterotoxin genes but, their detection was important because confirms that CNS isolated from foods can produce enterotoxins. These results confirm the findings reported by Crass and Bergdoll (6) who isolated enterotoxin- producing CNS from food implicated in a food poisoning outbreak.

Comparing the PCR and RPLA data, the isolates were shown to be PCR-positive for enterotoxin gene did not evidence detectable production of enterotoxin by RPLA method. Similar findings have been reported by others (9,19) and might be due to toxin production below the detection limit of the RPLA method or to the non-expression of genes. According to Schmitz *et al.* (19), in clinical practice, staphylococcal strains carrying a toxin gene should be considered potential producers of this toxin since toxin production *in vivo* cannot be excluded. Many factors affect *S. aureus* growth and enterotoxin production in foodstuffs, such as the presence of specific amino acids that vary with the strains (15), glucose, pH, acetic acid, lactic acid, sodium chloride and competing microorganisms (12). Thus considerable research effort is still required for better understanding of the interactions between CNS and the food matrix, and the mechanisms of enterotoxin production in foodstuffs.

PCR was found to be a rapid and reliable method for the identification of genes responsible for the production of staphylococcal toxins. The determination of the enterotoxigenicity of strains is mainly based on immunological methods, which depend on the concentration of the toxin produced and, therefore, might not detect low-producing strains.

The importance of our results lies not only in scientific aspects considering the scarcity of available data in this respect, but in providing practical information about food safety, which is of great importance since some CNS species, such as *S. xylosus*, are used as a starter culture in fermented meat products (13). In addition, the present results confirm the need for further studies in order to better characterize the enterotoxigenic potential of CNS in foods.

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RESUMO

Detecção de genes de enterotoxinas em estafilococos coagulase-negativa isolados de alimentos

A intoxicação alimentar estafilocócica ocorre devido à ingestão de alimentos contaminados com enterotoxinas. Essa contaminação tem sido oriunda, principalmente, da manipulação humana, ou de matérias-primas procedentes de animais portadores. Embora *Staphylococcus aureus* coagulase positiva, seja o principal agente de intoxicação alimentar, alguns

pesquisadores enfatizam que os estafilococos coagulase-negativa (ECN) podem produzir as enterotoxinas estafilocócicas, podendo contribuir para a intoxicação alimentar. Este estudo teve como objetivos isolar os ECN de alimentos e verificar a capacidade enterotoxigênica dessas linhagens. Foram estudadas 88 amostras de alimentos, sendo que 22,7% foram positivas para ECN com crescimento entre 10^2 e 10^6 UFC/g or mL. A espécie predominante dentre as linhagens isoladas foi *S. epidermidis* (40%), seguido por *S. warneri* (20%), *S. xylosus* (20%), *S. saccharolyticus* (15%) e *S. hominis* (5%). Entre as linhagens isoladas, quatro apresentaram genes para produção de enterotoxinas pelo método de Reação da Polimerase em Cadeia (PCR), com predominância do gene *se*a. Não se detectou a produção de enterotoxina pelo método de aglutinação em látex (RPLA). Através dos resultados obtidos, observou-se que os ECN isolados de alimentos não devem ser ignorados quanto à sua capacidade toxigênica, necessitando de maior estudo e atenção para melhor caracterização desse grupo de microrganismos em alimentos.

Palavras-chave: estafilococos coagulase-negativa, PCR, enterotoxinas

REFERENCES

1. Anunciação, L.L.C.; Linardi, V.R.; Carmo, L.S.; Bergdoll, M.S. Production of staphylococcal enterotoxin A in cream-filled cake. *Rev. Microbiol.*, 26, 259-263, 1995.
2. Baker, J.S. Comparison of various methods for differentiation of staphylococci and micrococci. *J. Clin. Microbiol.*, 19, 875-879, 1984.
3. Becker, K.; Haverkamper, G.; von Eiff, C.; Roth, R.; Peters, G. Survey of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene in non-*Staphylococcus aureus* species. *Eur. J. Clin. Microbiol. Infect. Dis*., 20, 407-409, 2001.
4. Bergdoll, M.S. Importance of staphylococci that produce nanogram quantities of enterotoxin. *Zbl Bakt.*, 282: 1-6, 1995.
5. Cerqueira-Campos, M.L.; Furlanetto, S.M.P.; Iaria, S.T.; Bergdoll, M.S. Staphylococcal food poisoning outbreaks in São Paulo (Brazil). *Rev. Microbiol*., 24, 261-264, 1993.
6. Crass, B.A.; Bergdoll, M.S. Involvement of coagulase-negative staphylococci in toxic shock syndrome. *J. Clin. Microbiol*., 23, 43-45, 1986.
7. Dias, R.S.; Carmo, L.S.; Silva, M.C.C. Surto de toxinfecção alimentar causado pela ação simultânea de enterotoxina estafilocócica e *Salmonella enteritidis* – Estudo de caso. *Rev. Inst. Adolfo Lutz*, 58, 7-11, 1999.
8. Donnelly, C.B.; Leslie, J.E.; Black, I.A.; Lewis, K.H. Serological identification of enterotoxigenic staphylococci from cheese. *Appl. Microbiol*., 15, 1382-1387, 1967.
9. Johnson, W.M.; Tyler, S.D.; Ewan, E.P.; Ashton, F.E.; Pollard, D.R.; Rozee, K.R. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *J. Clin. Microbiol*., 29, 426-430, 1991.
10. Kloos, W.E.; Schleifer, K.H. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Microbiol*., 1, 82-88, 1975.
11. 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. (eds.). *Manual of Clinical Microbiology*. American Society Microbiology, Washington, 1995, p.282-*298*.
12. Loir, Y.L.; Baron, F.; Gautier, M. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.*, 2, 63-76, 2003.
13. Montel, M.C. Fermented meat products. *In*: R.K. Robinson, C.A. Batt, P.D. Patel. *Encyclopedia of Food Microbiology*, Academic Press, London, 2000, p.744-753.
14. Olsvik, O.; Fossum, K.; Berdal, B.P. Staphylococcal enterotoxin A, B, and C produced by coagulase-negative strains within the family *Micrococcaceae*. *Acta. Pathol. Microbiol. Immunol.*, 2, 92-96, 1982.
15. Onoue, Y.; Mori, M. Amino acid requirement for growth and enterotoxin production by *Staphylococcus aureus* in chemically defined media. *Int. J. Food Microbiol.*, 36, 77-82, 1997.
16. Polledo, J.J.F.; Garcia, M.L.; Moreno, B.; Menes, I. Importance of food handlers as a source of enterotoxigenic staphylococci. *Zentrabl. Bacteriol. Mikrobiol. Hyg.*, 181, 364-367, 1985.
17. Rasooly, A.; Rasooly, R.S. Detection and analysis of staphylococcal enterotoxin A in food by Western immunoblotting. *Int. J. Food Microbiol.*, 41, 205-212, 1998.
18. Rasooly, A.; Ito, Y. Toroidal coil countercurrent chromatography separation and analysis of staphylococcal enterotoxin A (SEA) in milk. *J. Liq. Chrom. Rel. Technol*., 22, 1285-1293, 1999.
19. Schmitz, F.J.; Steiert, M.; Hofmann, B.; Verhoef, J.; Hadding, U.; Heinz, H.P.; Kohrer, K. Development of a multiplex-PCR for direct detection of the genes for enterotoxin B and C, and toxic shock syndrome toxin-1 in *Staphylococus aureus* isolates. *J. Med. Microbiol*., 47, 335-340, 1998.
20. Shingaki, M.H.; Igarashi, H.; Fujikawa, H.; Ushioda, H.; Terayama, T.; Sakai, S. Study on reverved passive latex agglutination for the detection of staphylococcal enterotoxins A-C. *Annu. Rep. Tokyo Metr. Res. Lab. Plubl. Heth.*, 32, 128-31, 1981.
21. Smith, J.L.; Buchanan, R.L.; Palumbo, S.A. Effect of food environment of staphylococcal enterotoxin synthesis: A review. *J. Food Protect.*, 46, 545-555, 1983.
22. Soares, M.J.S.; Miyazaki, N.H.T.; Noleto, A.L.S.; Figueiredo, A.M.S. Enterotoxin production of *Staphylococcus aureus* and detection of Brazilian epidemic MRSA clone (III:B:A) among isolates from food handlers. *J. Med. Microbiol*., 46, 214-221, 1997.
23. Soriano, J.M.; Font, G.; Moltó, J.C.; Mañes, J. Enterotoxigenic staphylococci and their toxins in restaurant foods. *Trends Food Sci. Tech.*, 13, 60-67, 2002.
24. Udo, E.E.; Al-Bustan M.A.; Jacob, L.E.; Chugh, T.D. Enterotoxin production by coagulase-negative staphylococci in restaurant workes from Kuwait City may be a potential cause of food poisoning. *J. Med. Microbiol*., 48, 819-823, 1999.
25. Valle, J.; Gomez-Lucia, E.; Piriz, S.; Goyache, J.; Orden, J.A.; Vadillo, S. Enterotoxin production by staphylococci isolated from goats. *Appl. Environ. Mirobiol*., 56, 1323-1326, 1990.
26. Zschöck, M.; Botzler, D.; Blöcher, S.; Sommerhäuser, J.; Hamann, H.P. Detection of genes for enterotoxins (*ent*) and toxic shock syndrome toxin-1 (*tst*) in mammary isolates of *Staphylococcus aureus* by polymerase chain reaction. *Int. Dairy J.*, 10, 569-574, 2000.