Food Control 66 (2016) 93–99

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Safety assessment of selected *Staphylococcus carnosus* strains with regard to their application as meat starter culture

Anne Müller [a], Regina Reichhardt [a], Grit Fogarassy [a], Ramona Bosse (née Danz) [b], Monika Gibis [b], Jochen Weiss [b], Herbert Schmidt [a], Agnes Weiss [a, *]

[a] Department of Food Microbiology and Hygiene, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany

[b] Department of Food Physics and Meat Science, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 25, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form
26 January 2016
Accepted 28 January 2016
Available online 29 January 2016

Keywords:
Staphylococcus carnosus
Safety assessment
Antibiotic resistance
Biogenic amines
Haemolysis
Starter culture

ABSTRACT

In this study, 39 different *Staphylococcus carnosus* strains were analysed for virulence and pathogenicity determinants with regard to their application as starter cultures for the fermentation of meat products. Therefore, the Qualified Presumption of Safety concept of the European Food Safety Authority was used as a guideline to estimate their potential health risk for consumers, with emphasis on antibiotic resistance, presence of toxin genes and production of biogenic amines. The resistance against 17 antibiotics was analysed with an agar disc diffusion test according to the Clinical and Laboratory Standards Institute. Ten strains were resistant or intermediate resistant against cefotaxime, chloramphenicol, oxacillin or trimethoprim/sulfamethoxazole. Of these, only two strains were resistant to more than one antibiotic. None of the tested strains was PCR-positive with primers targeting the staphylococcal enterotoxin genes (*sea-see*, *seh*), the exfoliative toxin gene (*eta*) or the toxic shock syndrome toxin gene (*tst-1*). Two strains showed β-haemolysis on human blood agar plates and were excluded from the study. The remaining 22 antibiotic-sensitive and non-toxigenic *S. carnosus* strains were tested for the production of biogenic amines by HPLC-analysis. None of the strains produced cadaverine, putrescine and histamine under the experimental conditions used, but 12 strains produced phenethylamine in concentrations ranging between 2.6 and 15.0 μg/mL. The results of this study indicate that safety risks, such as antibiotic resistance and biogenic amine production, are quite common among strains of the species *S. carnosus*. Consequently, each strain should be analysed individually before it is applied as starter culture in meat products.

1. Introduction

The genus *Staphylococcus* currently includes 52 species and 28 subspecies (Euzéby, 1997), which can be divided into coagulase positive (CPS) and coagulase negative (CNS) staphylococci. While the CPS species *Staphylococcus aureus* often causes food intoxications and severe human infections, most CNS species, such as *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, are only known as opportunistic pathogens. Some CNS like *Staphylococcus xylosus* and *Staphylococcus carnosus* are used as starter cultures in the fermentation of meat products (Corbiere Morot-Bizot, Leroy, & Talon, 2007).

To estimate the risk associated with microorganisms used in food two standards were established: the “Generally Recognized as Safe” (GRAS) status from the US Food Drug Administration (FDA; Burdock & Carabin, 2004) and the “Qualified Presumption of Safety” (QPS) status from the European Food Safety Authority (EFSA). The QPS system relies on four pillars: taxonomy, familiarity, pathogenicity and end use. These pillars basically include the precise identification of the microorganism, to subspecies level if necessary, the history of use and industrial application as well as the investigation for known toxins and antibiotic resistances (EFSA, 2007). Several studies focused on the safety assessment of CNS used

* Corresponding author.
E-mail addresses: mueller.a@uni-hohenheim.de (A. Müller), g.fogarassy@uni-hohenheim.de (G. Fogarassy), ramona.danz@uni-hohenheim.de (R. Bosse (née Danz)), gibis@uni-hohenheim.de (M. Gibis), j.weiss@uni-hohenheim.de (J. Weiss), herbert.schmidt@uni-hohenheim.de (H. Schmidt), agnes.weiss@uni-hohenheim.de (A. Weiss).

http://dx.doi.org/10.1016/j.foodcont.2016.01.042
0956-7135/

in foodstuffs in the last years (e.g., Jeong, Han, & Lee, 2014; Martín et al., 2006; Marty et al., 2012). *S. xylosus* and *S. carnosus* were even proposed for QPS status by Talon and Leroy (2011), but did not achieve it until now.

Although *S. carnosus* is used as a starter culture in sausage production for several decades, and besides the fact that it was proposed for QPS status, no study focused on the safety assessment of this species taking the two subspecies *S. carnosus* subsp. *carnosus* and *S. carnosus* subsp. *utilis* into account. Even if it is not often the case, it is worth of notice that clinical isolates of *S. carnosus* have been described previously (Couto, Pereira, Miragaia, Santos Sanches, & De Lencastre, 2001) and that just recently the first clinical case of bloodstream infection caused by *Staphylococcus condimenti*, the closest relative of *S. carnosus*, was described (Misawa, Yoshida, Okugawa, & Moriya, 2015). Moreover, *S. carnosus* can be a possible reservoir for antibiotic resistance genes and different studies (e.g., Chajecka-Wierzchowska, Zadernowska, Nalepa, Sierpinska, & Laniewska-Trokenheim, 2015; Kastner et al., 2006; Marty et al., 2012) showed that antibiotic resistant CNS strains are widely distributed, even among commercial starter cultures, and the demand for new starters without antibiotic resistances is high.

In this study, a safety analysis of 39 *S. carnosus* strains was performed, including phenotypic and genotypic analysis of antibiotic resistances, testing for relevant staphylococcal toxin genes, potential to form biogenic amines and potential of haemolysis. We aimed at using a combination of methods which can be used as basis for screening *S. carnosus* strains for their safe application as starter culture in fermented foods. The technologically relevant physiological activities for the use as starter culture were already investigated for this strain set in a former study (Müller et al. 2016). Additionally, the distribution of virulence and pathogenicity determinants in the two subspecies was evaluated and compared.

2. Material and methods

2.1. Bacterial strains and identification

Thirty-nine different *S. carnosus* strains were identified on subspecies level in a former study by amplification of a 384 bp fragment of the superoxide dismutase gene *sodA* (Müller et al., 2016). A list of these strains stating their subspecies affiliation and origin is given in Table 3 together with the corresponding results of each test performed in this study.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the pour plating method modified from the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2007). Therefore, 100 μL of a bacterial 0.9% NaCl solution with a bacterial count of 1.0×10^8 cfu/mL was used to inoculate 5 mL liquid Mueller-Hinton soft-agar (21 g/L Mueller Hinton broth (Merck KGaA, Darmstadt, Germany), 7 g/L Bacto™ agar (Becton Dickinson GmbH, Heidelberg, Germany), 45–50 °C). The suspension was mixed and poured on previously prepared Mueller-Hinton agar plates (Merck KGaA). After 15 min drying of the agar, the antibiotic filter discs (all Oxoid Deutschland GmbH, Wesel, Germany) were applied to the agar with a dispenser (Oxoid Deutschland GmbH). Seventeen antibiotics commonly used in human and animal medicine were tested at the amounts given in brackets: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), cefotaxime (30 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), imipenem (10 μg), kanamycin (30 μg), linezolid (30 μg), oxacillin (1 μg), quinupristin/dalfopristin (15 μg), rifampin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), tetracycline (30 μg) and vancomycin (30 μg). The plates were incubated for 24 h at 37 °C before the diameters of the inhibition zones were measured. The mean value of the diameters of the inhibition zones of two independent measurements per strain was compared to the values given by the CLSI (2007) (Table S1) and strains were classified as susceptible, intermediate resistant or resistant.

2.3. PCR analysis for the detection of selected toxin-, haemolysin- and antibiotic resistance genes

All *S. carnosus* strains and a control strain for each toxin (Table 1) were tested for the presence of the staphylococcal enterotoxin genes *sea*, *seb*, *sec*, *sed*, *see*, *seh,* the toxic shock syndrome toxin gene *tst-1* and the exfoliative toxin gene *eta*. The DNA of the test and control strains was isolated as described by Müller et al. (2016). Amplification of the toxin genes was performed in a T1 thermocycler (Biometra GmbH, Göttingen, Germany) as described in Table 2. Additionally to that, all *S. carnosus* strains were examined by PCR for the presence of the antibiotic resistance genes *blaZ* (encoding β-lactamase), *mecA* (penicillin binding protein 2a) and *tetK* (tetracycline efflux pump) and for the haemolysin genes *hla* (encoding α-haemolysin), *hlb* (β-haemolysin) and *hld* (δ-haemolysin) as described in Table 2.

The PCR products and the GeneRuler™ 100 bp DNA ladder (Fisher Scientific GmbH, Schwerte, Germany) were separated electrophoretically on 2% (w/v) or 3% (w/v) agarose gels (LE agarose, Biozym Scientific GmbH, Hessisch Oldendorf, Germany) at 120 V for 1 h. The gels were stained in a solution with 1 μg/mL ethidium bromide (Carl Roth GmbH + Co KG, Karlsruhe, Germany) for 20 min and photographed (E.A.S.Y Win32 Analyse Software, Herolab GmbH, Wiesloch, Germany).

2.4. Investigation of β-haemolysis on blood agar plates

The test for haemolysis was performed in duplicate as described by Zell et al. (2008). Briefly, all test strains and the control strain LTH 5915 (Table 1) were streaked on human blood agar plates. These were prepared with Difco™ Tryptose Blood Agar Base (Becton Dickinson GmbH), which was supplemented with 5% sterile defibrinated human blood (B, rh^+, human erythrocyte concentrate, leucocytes depleted; Centre for Clinical Transfusion Medicine Tuebingen (ZKT), Tuebingen, Germany). The plates were incubated aerobically at 37 °C for 48 h and another 24 h at 4 °C. Beta-haemolysis was visible as clear zone around single colonies.

2.5. Determination of biogenic amines by HPLC

The ability to produce biogenic amines was examined for 22 selected strains which did not show a antibiotic resistance or β-haemolysis. The method of Seitter, Geng, and Hertel (2011) for growing cells was used with minor modifications. Briefly, overnight cultures of the strains (Standard 1 broth, 37 °C, 180 rpm) were adjusted to an $OD_{600\ nm}$ of 0.1. One hundred μL of these were used to inoculate 10 mL Standard 1 broth with either 0.1 g/L L-phenylalanine, 0.127 g/L L-ornithine monohydrochloride, 0.124 g/L L-lysine monohydrochloride or 0.1 g/L L-histidine (all Sigma–Aldrich Chemie GmbH, Taufkirchen, Germany), respectively. Incubation, centrifugation and precipitation of proteins were performed as described by Seitter, Geng et al. (2011), except that 0.4 M perchloric acid was used instead of 70% perchloric acid. For each strain, 200 μL of the four Standard 1 preparations were combined to gain one sample per strain. Each strain was inoculated two times at different days, to gain two biological replicates. Standard 1 medium supplemented with 2-phenethylamine, putrescine, cadaverine or