



Genetic diversity and population structure of food-borne *Staphylococcus carnosus* strains

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ABSTRACT

The species *Staphylococcus carnosus* is a non-pathogenic representative of the coagulase negative staphylococci. Specific strains are applied as meat starter cultures. The species consists of two subspecies, *S. carnosus* ssp. *carnosus* and *S. carnosus* ssp. *utilis*. In order to place *S. carnosus* strains, characterized in former studies, in a genetic background that allows a typing of candidates for starter cultures, a Multilocus Sequence Typing (MLST) scheme was developed. Internal fragments of the genes *tpiA*, *xprT*, *dat*, *gmk*, *glpK*, *narG*, *cstA*, encoding triosephosphate isomerase, xanthine phosphoribosyltransferase, D-amino acid aminotransferase, guanylate kinase, glycerol kinase, the α -chain of the respiratory nitrate reductase, and a carbon starvation protein were chosen. Genes were selected based on their equal distribution in the genome, taxonomic value in subspecies differentiation and metabolic function. This MLST was applied to 44 *S. carnosus* strains, most of them previously analyzed for their suitability as starter cultures.

The number of alleles varied between zero (*tpiA*) and five (*cstA*) and allowed the definition of nine sequence types (ST). ST1 was most abundant (18 strains), followed by ST2 (8) and ST4 (6). The nine STs confirmed a close relationship of all strains despite their isolation source and year, but lacked correlation with physiological activities relevant for starter cultures. The low amount of STs in the strain set lets us suggest that recombination between strains is rare. Thus, it is hypothesized that evolutionary events seem to be due to single point mutations rather than intrachromosomal recombination, and that the species possesses a clonal population structure.

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Introduction

The coagulase negative *Staphylococcus* (CNS) species *Staphylococcus carnosus* can be isolated from various meat products and fish, and has a long tradition as meat starter culture, especially in the processing of raw sausages [4]. Different enzymes, such as nitrate-reductases, catalases, superoxide dismutases, lipases and proteases, can be produced by specific *S. carnosus* strains. These influence the formation of the typical fermented meat color and flavor, as well as the reduction of hydrogen peroxide [8,1,38]. *S. carnosus* has been subdivided into the two subspecies *S. carnosus* ssp. *carnosus* [37], which was first isolated from dry-fermented sausage, and *S. carnosus* ssp. *utilis* [33], which was first isolated from fermenting fish and shrimp sauce. Together with *Staphylococcus*

similans, *Staphylococcus condimenti* and *Staphylococcus piscifermentans*, *S. carnosus* forms the so-called “Similans – Carnosus cluster group” [15]. Despite its importance as a starter culture and compared to other *Staphylococcus* species, only few genetic- and population data are available about members of this group and especially of the species *S. carnosus*. Only two complete- and two draft genomes of *S. carnosus*, the complete genome of *S. carnosus* ssp. *carnosus* strain TM300 [35], the complete genome of *S. carnosus* strain LTH 3730 [28], the draft genome of *S. carnosus* ssp. *utilis* strain LTH 7013 [27] and the draft genome of *S. carnosus* strain 336 (National Center for Biotechnology Information, BioSample: SAMN03998523) are available in the databases.

The standard molecular typing techniques applied for *S. carnosus* are randomly amplified polymorphic DNA PCR (RAPD) [26,20] and Pulsed Field Gel electrophoresis (PFGE) [21]. However, these techniques are based on the comparison of band patterns, which are not well portable and by this, results obtained in different laboratories are difficult to compare [34]. To overcome the difficulty of data transferability, Multilocus Sequence Typing (MLST) was first established in 1998 [19] and proved to be the standard method

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for microbial typing ever since. MLST usually targets an internal fragment of five to seven housekeeping genes, depending on the bacterial species examined. Sequence variations in these housekeeping genes are detected and are designated as different alleles of the particular gene. The allele numbers are combined in a specific order and this defines the so-called sequence type (ST) [18]. MLST is already established as a method for strain characterization of different *Staphylococcus* species, namely *Staphylococcus aureus* [5], *Staphylococcus epidermidis* [41], *Staphylococcus equorum* [12], *Staphylococcus haemolyticus* [2], *Staphylococcus hominis* [46], *Staphylococcus lugdunensis* [3] and *Staphylococcus saprophyticus* [16]. The population structures in different *Staphylococcus* spp. are diverse. While *S. epidermidis* shows an epidemic population structure, where recombination has a major impact on evolution [25], *S. aureus* is rather evolving by point mutations and shows a clonal population structure [6].

In this study, we developed a MLST scheme to examine the genetic diversity and population structure of *S. carnosus* strains. A differentiation between the two subspecies has also been an integral feature of the developed scheme.

Material and methods

Bacterial strains and growth conditions

A total of 44 *S. carnosus* strains was analyzed in this study. The strains originated from various sources, such as fermented fish sauce, fish brine, raw ham, starter cultures and salami, and were isolated at different points in time (Table 1). The majority of strains has been identified, characterized and typed on strain level by RAPD-PCR in a former study [26]. All *S. carnosus* strains were cultivated

Table 1

List of the 44 investigated *S. carnosus* strains. The origin of each strain, year of addition to the strain collection of the University of Hohenheim, allelic profile, sequence type, groups defined by eBURST and clonal complex are displayed. ST = sequence type; s = singleton; CC = clonal complex; n.d. = none declared.

Strain ^a	Origin	Year	Allelic profile							ST	eBURST	CC
			<i>glpK</i>	<i>tpiA</i>	<i>dat</i>	<i>xprT</i>	<i>gmk</i>	<i>narG</i>	<i>cstA</i>			
LTH 1574	Starter culture	1990 ^b	1	1	1	1	1	1	1	1	1	1
LTH 3697	–	1993	1	1	1	1	1	1	1	1	1	1
LTH 3724	Fish sauce (fermented)	1993 ^b	3	1	3	1	3	1	3	7	s	n.d.
DSM 11676 ^T	Fish sauce (fermented)	1993 ^b	2	1	2	2	2	2	2	2	2	2
LTH 3729	Fish sauce (fermented)	1993 ^b	2	1	2	2	2	2	2	2	2	2
LTH 3730	Fish sauce (fermented)	1993 ^b	3	1	3	1	3	1	3	7	s	n.d.
LTH 3739	–	1993	4	1	1	1	1	1	1	4	1	1
LTH 3740	–	1993	4	1	1	1	1	1	1	4	1	1
LTH 3741	–	1993	4	1	1	1	1	1	1	4	1	1
LTH 3742	–	1993	4	1	1	1	1	1	1	4	1	1
LTH 3743	–	1993	4	1	1	1	1	1	1	4	1	1
DSM 11677	Fish sauce (fermented)	1994	2	1	2	2	2	2	2	2	2	2
LTH 4407	Fish brine	1996	3	1	1	1	1	1	1	4	6	1
LTH 4408	Fish brine	1996	3	1	1	1	1	1	1	4	6	1
LTH 4409	Fish brine	1996	3	1	1	1	1	1	1	3	1	1
LTH 4410	Fish brine	1996	3	1	1	1	1	1	1	4	6	1
LTH 4411	Fish brine	1996	3	1	1	1	1	1	1	4	6	1
LTH 4412	Fish brine	1996	3	1	1	1	1	1	1	3	1	1
LTH 6175	Sausage (fermented)	2005	1	1	1	1	1	1	1	1	1	1
LTH 6176	Sausage (fermented)	2005	1	1	1	1	1	1	1	1	1	1
LTH 6180	Sausage (fermented)	2005	1	1	1	1	1	1	1	1	1	1
LTH 6181	Sausage (fermented)	2005	1	1	1	1	1	1	1	1	1	1
LTH 6182	Sausage (fermented)	2005	1	1	1	1	1	1	1	1	1	1
LTH 6183	Sausage (fermented)	2005	1	1	1	1	1	1	1	1	1	1
LTH 6184	Sausage (fermented)	2005	1	1	1	1	1	1	1	1	1	1
LTH 7012	Smoked raw ham	2013	2	1	2	2	2	2	2	2	2	2
LTH 7013	Smoked raw ham	2013	2	1	2	2	2	2	2	2	2	2
TM300	Starter culture	2013 ^c	4	1	1	1	1	1	1	4	1	1
LTH 7027	Starter culture	2013	1	1	1	1	1	1	1	1	1	1
LTH 7034	Salami	2013	1	1	1	1	1	1	1	1	1	1
LTH 7035	Salami	2013	1	1	1	1	1	1	1	1	1	1
LTH 7036	Salami	2013	1	1	1	1	1	1	1	1	1	1
LTH 7037	Salami	2013	2	1	2	2	1	2	2	8	2	2
LTH 7038	Starter culture	2013	2	1	2	2	2	2	2	2	2	2
LTH 7039	Starter culture	2013	4	1	1	1	2	1	1	5	1	1
LTH 7040	Starter culture	2013	2	1	2	2	1	2	2	8	2	2
LTH 7041	Starter culture	2013	2	1	2	2	2	2	2	2	2	2
LTH 7042	Starter culture	2013	1	1	1	1	1	1	1	1	1	1
LTH 7044	Starter culture	2013	1	1	1	1	1	1	1	1	1	1
LTH 7045	Starter culture	2013	2	1	2	2	2	2	2	2	2	2
LTH 7053	Starter culture	2013	1	1	1	1	1	1	1	1	1	1
LTH 7057	Starter culture	2013	1	1	1	1	1	1	1	1	1	1
DSM 20501 ^T	Sausage	2014 ^d	1	1	1	1	1	1	1	1	1	1
336 ^f	Beef	2014 ^e	3	1	2	3	4	1	5	9	s	n.d.

^a Alternative strain names: TM300 = LTH 7024 [35]; DSM 20501^T (type strain for *S. carnosus* ssp. *carnosus*) = LTH 7089; DSM 11677 = LTH 3838; DSM 11676^T (type strain for *S. carnosus* ssp. *utilis*) = LTH 3728.

^b First description 1998 [33].

^c First description 2009 [35].

^d First description 1982 [37].

^e Collection date as in National Center for Biotechnology Information, BioSample: SAMN03998523.

^f Sequence data obtained here are from GenBank accession no.: GCA.001276205.

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