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# Genome Analysis of the Meat Starter Culture Bacterium *Staphylococcus carnosus* TM300▽†

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**The *Staphylococcus carnosus* genome has the highest GC content of all sequenced staphylococcal genomes, with 34.6%, and therefore represents a species that is set apart from *S. aureus*, *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus*. With only 2.56 Mbp, the genome belongs to a family of smaller staphylococcal genomes, and the *ori* and *ter* regions are asymmetrically arranged with the replichores I (1.05 Mbp) and II (1.5 Mbp). The events leading up to this asymmetry probably occurred not that long ago in evolution, as there was not enough time to approach the natural tendency of a physical balance. Unlike the genomes of pathogenic species, the TM300 genome does not contain mobile elements such as plasmids, insertion sequences, transposons, or STAR elements; also, the number of repeat sequences is markedly decreased, suggesting a comparatively high stability of the genome. While most *S. aureus* genomes contain several prophages and genomic islands, the TM300 genome contains only one prophage, ΦTM300, and one genomic island, νSCA1, which is characterized by a mosaic structure mainly composed of species-specific genes. Most of the metabolic core pathways are present in the genome. Some open reading frames are truncated, which reflects the nutrient-rich environment of the meat starter culture, making some functions dispensable. The genome is well equipped with all functions necessary for the starter culture, such as nitrate/nitrite reduction, various sugar degradation pathways, two catalases, and nine osmoprotection systems. The genome lacks most of the toxins typical of *S. aureus* as well as genes involved in biofilm formation, underscoring the nonpathogenic status.**

It has been known for a long time that staphylococci play a role in the fermentation of dry sausage (52). At first, they were regarded as micrococci, but it turned out that these micrococci were wrongly classified and were in fact staphylococci. Based on DNA/DNA hybridization, biochemical properties, and cell wall composition, these staphylococci formed a new species, which was named *Staphylococcus carnosus* because the bacteria can be isolated from meat fermentation products and have been used since the 1950s as a starter culture (64).

One of the main advantages of starter cultures in fermented-food processing is that the fermentation and ripening process can be carried out under controlled conditions. In this way, food poisoning and food spoilage microorganisms can be suppressed, and the course of the fermentation process and its termination can be more reliably monitored. During the ripening process of dry sausage, *S. carnosus* exerts several desired functions (5, 14, 40). First, *S. carnosus* gradually reduces nitrate to nitrite (50). The advantages of this reaction are that the nitrate concentration is lowered and that nitrite can combine with myoglobin to form nitrosomyoglobin, which results in the typical red color. In the second step, nitrite is then further reduced to ammonia, thus lowering the unbound nitrite concentration (51). Other advantages are development of characteristic flavor, moderate decrease of the pH, and the capacity to reduce hydrogen peroxide produced by the catalase-negative lactobacilli that were frequently used in combination with *S. carnosus*, thus preventing odors. It is thought that the high catalase and superoxide dismutase (SOD) activities contribute to the antioxidant capacities of *S. carnosus* (5). Commercial starter cultures of *S. carnosus* are manufactured in many European countries, and therefore *S. carnosus* is regarded as a "food grade" staphylococcal species.

Currently, more than 36 species and several subspecies are recognized in the genus *Staphylococcus*, and the genus *Macrococcus* comprises 4 species (23). A comparative 16S and 23S rRNA analysis shows that *S. carnosus* forms together with *S. piscifermentans* and *S. condimentii* in a cluster (23, 57, 62). *S. carnosus* TM300 is one of the strains used in the food industry and is phenotypically indistinguishable from the type strain DSM20501.

We have little information regarding the natural habitat of *S. carnosus*. Unlike for *S. epidermidis*, *S. aureus*, and *S. hominis*, there are no reports that *S. carnosus* has ever been isolated from human skin or mucosa. However, it might be present on the skin of certain animals, which would explain its natural occurrence in meat products. On the other hand, *S. piscifermentans* is found predominantly in fermented marine fish (62, 70), and its close phylogenetic relationship based on both 16S rRNA (23) and the CydA and CydB proteins (72) leads to the speculation that *S. carnosus* also could originate from marine fish.

As S. *carnosus* is apathogenic and an important organism in

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food manufacturing, in the 1980s we began to establish a cloning system for one of the *S. carnosus* strains to study gene expression and virulence factors. One prerequisite for this is an efficient transformation system. However, we failed to make *S. carnosus* strains competent to take up DNA as was described for calcium-induced competence in *S. aureus* (41). In that respect, *S. carnosus* differed from most of the *S. aureus* strains. The only way to transform *S. carnosus* is to use either protoplast transformation (24) or electroporation (4). We also constructed a number of plasmid vectors that are used by many groups studying staphylococci (32, 34, 60). Over time, the established host-vector system for *S. carnosus* (22) appeared to be more attractive to the scientific community, and therefore physical and genetic maps of the genome of *S. carnosus* TM300 were constructed (74).

Currently, *S. carnosus* has been proposed to be the first choice to study pathogenicity factors from pathogenic staphylococcal species. For example, numerous invasion factors and matrix-binding proteins have been expressed in *S. carnosus*, and their function has been studied (1, 25, 26, 28, 30, 33, 66). Also, *S. epidermidis*-derived virulence factors have been studied in *S. carnosus*, such as phenol-soluble modulin peptides (55), the methicillin resistance gene (71), and biofilm formation by the *S. epidermidis*-derived *ica* genes (29). Elements of the sugar phosphotransferase system (PTS) have been structurally analyzed (27, 45, 53), and specific pathways have been investigated (6). Many reports deal with the function and application of *S. carnosus* in the food industry (39, 61). Finally, the pathogenic and nonpathogenic staphylococcal species also differ with respect to the number of pathogenicity factors, which are clearly decreased in the nonpathogenic species. We demonstrated that *S. carnosus* together with other nonpathogenic species representatives lacks the *oatA* gene (OatA, peptidoglycan-specific *O*-acetyltransferase) and is therefore lysozyme susceptible (7). This apathogenic species group also has a pyocyanin- and cyanide-resistant cytochrome *bd* oxidase, which makes these species resistant to *Pseudomonas* supernatants, in contrast to the pathogenic species such as *S. aureus* or *S. epidermidis* (72).

*S. carnosus* has applications in the food industry, as an alternative cloning host in biotechnology, in the study of pathogenicity factors, and as a live vaccine delivery system. Because of its many applications, it has become more and more important to determine and analyze the genome sequence of this strain to learn more about what is in common with and what is different from other sequenced staphylococcal species.

## MATERIALS AND METHODS

High-molecular-weight genomic DNA from bacterial cells was prepared using guanidinium isothiocyanate-isobutanol precipitation on silica to purify DNA from a precleared proteinase K-sodium dodecyl sulfate incubation. Genomic DNA was sheared, concentrated, and desalted using standard protocols. The product was end repaired, desalted, and tailed with an extra A residue. The tailed DNA was size fractionated by electrophoresis. A 2- to 3-kb fraction was isolated and cloned into pGEM-T (Promega, Madison, WI). A 3- to 4-kb fraction was tagged in vitro with the artificial transposon KAN-2 using the EZ-TN KAN-2 Tnp kit from Epicentre (Madison, WI). The transposon-tagged DNA fragments were cloned into pGEM-T. We used *Escherichia coli* DH10b (Invitrogen, Karlsruhe) for transformations.

Plasmid DNAs were prepared on a RoboPrep2500 instrument (MWG-Biotech) using the Nucleospin MWG PlasmidPrep96. Plasmid clones were sequenced from both ends with standard primers using the Big Dye terminator chemistry on ABI 3700 capillary sequencers (Applied Biosystems, Foster City, CA).

The sequence was assembled from 42,925 reads (giving 11.8× coverage) using the Paracel Genome Assembler software (Paracel Inc., Pasadena, CA) as well as the Staden package (http://www.mrc-lmb.cam.ac.uk/pubseq/staden_home.html). Vectorette PCRs (3a), combinatorial PCRs, and walking reads on selected clones were used to assemble the sequence and fill in gaps. For the final contig assembly, we used the genome sequence of *S. aureus* N315 as a scaffold. Final gap closing and confirmation of the correct contig order and orientation were done by long-range PCR and sequencing on a LI-COR 4200 DNA sequencer (Lincoln Corporation, Lincoln, NE).

Prediction of open reading frames (ORFs) in the finished sequence and annotation were performed by using the GenDB annotation tool (48). The automated annotation provided by this system was thoroughly edited by manual inspection. rRNA gene regions were localized by comparison to rRNA genes of *S. aureus* by using BLASTN (2). tRNAs were identified by analyzing the genome with tRNASCAN-SE (43).

GC skew analysis and localization of start and terminator regions of chromosomal replication were performed with GenSkew (http://mips.gsf.de/services/analysis/genskew). Truncated genes were identified by comparison to the nr and SwissProt protein databases. The corresponding genome regions were checked by control sequencing. Full-length genome alignments were performed with the MUMmer software (37). In-house BLAST comparisons were done by using the stand-alone BLAST software available at the NCBI website (ftp://ftp.ncbi.nih.gov/blast). For reasons of comparability, the same threshold for homologous gene products as described by Gill et al. (20) was applied. Genome maps were drawn using the GenVision Software, and basic sequence analyses were performed with Lasergene (both from Dnastar, Inc, Madison, WI). Sequence repeats were localized with the Repeatfinder tool (73).

**Nucleotide sequence accession number.** The annotated genome sequence has been submitted to the EMBL nucleotide database under accession no. AM295250.

## RESULTS AND DISCUSSION

**General features of the genome.** The *S. carnosus* genome differs in various aspects from most other staphylococcal genomes (Table 1). With only 2,566,428 bp and 2,474 ORFs, it belongs to the smaller staphylococcal genomes. The coding density of the *S. carnosus* chromosome is 86.0%, which is rather high, and the GC content is 34.6%, which is the highest value described for any staphylococcal genome thus far. In all *S. aureus* genomes and many other genomes, the termination region (*ter*) is 180° from the *oriC*. However, in the *S. carnosus* genome, the *ter* region is not directly opposite the *oriC*; rather, it is 1.07 Mbp away (160°) in clockwise orientation (Fig. 1, innermost circle, GC skew). This appears to be typical for coagulase-negative staphylococcal species, being also seen in the genomes of S. *epidermidis*, *S. saprophyticus*, and *S. haemolyticus*, where the *ter* region also deviates from the 180° position.

The *oriC* was set at position 0. The immediate flanking genes are highly conserved, similar to the case for other staphylococcal genomes. In *S. carnosus* (like in *S. epidermidis* RP62A, *S. haemolyticus*, and *S. saprophyticus*), the *oriC* is inverted compared to that in the genomes of all *S. aureus* strains and *S. epidermidis* ATCC 12228. Sections of the *oriC* genes of *S. aureus* and *S. carnosus* illustrating the gene inversion are shown in Fig. 2. Similar to the case for the *S. epidermidis* RP62A genome, we set *rpmH* (Sca0001) as the start gene. The orientation of the *oriC* region is not species specific, as *S. epidermidis* strains RP62A and ATCC 12228 have *oriC* genes with different orientations.

**Gene content analysis and phylogeny.** We compared the *S. carnosus* genome with those of the following representatives of sequenced staphylococcal species: *S. aureus* N315, Mu50,

TABLE 1. General features of the *S. carnosus* TM300 chromosome in comparison with selected other staphylococcal species representatives

| Parameter | Value in: | | | | |
|---|---|---|---|---|---|
| | *Staphylococcus carnosus* TM300 | *Staphylococcus aureus* N315 | *Staphylococcus epidermidis* RP62A | *Staphylococcus haemolyticus* JCSC1435 | *Staphylococcus saprophyticus* ATCC15305 |
| Size (bp) | 2,566,428 | 2,814,816 | 2,616,530 | 2,685,015 | 2,516,575 |
| Coding density (%) | 86.0 | 83.4 | 83.9 | 86 | 83.5 |
| G+C content (%) | | | | | |
| Total | 34.6 | 32.8 | 32.1 | 32.8 | 33.2 |
| Coding sequences | 35.2 | 33.6 | 32.9 | 33.4 | 34.0 |
| No. of coding sequences | 2,474 | 2,593 | 2,553 | 2,678 | 2,446 |
| No. of RNAs | | | | | |
| 23S | 5 | 5 | 6 | 5 | 6 |
| 16S | 5 | 5 | 6 | 5 | 6 |
| 5S | 5 | 6 | 7 | 6 | 7 |
| tRNAs | 60 | 62 | 61 | 60 | 60 |
| tmRNAs | 1 | 1 | 1 | 1 | 1 |
| No. of mobile elements | | | | | |
| IS elements | | 20 | 23 (34 deg[a]) | 82 (60 deg) | 2 |
| Transposons | | 5 | 4 | 2 | |
| Transposases | (1 deg) | 33 (10 deg) | 5 (3 deg) | 92 | 9 |
| Genomic islands | 1 | 3 | 2 | 5 | 1 |
| Staphylococcal cassette chromosomes | | 1 | 1 | 1 | 2 |
| Prophages | 1 | 1 | 1 | 2 | 1 |
| Plasmids | | 1 | 1 | | 2 |

[a] deg, degenerated.

MW2, COL, MSSA476, MRSA252, and RF122; *S. epidermidis* RP62A and ATCC 12228; *S. haemolyticus* JCSC 1435; and *S. saprophyticus* ATCC 15305. The 12 staphylococcal genomes analyzed have a common set of 1,203 conserved gene products, which represents 46 to 50% of the individual proteome. In *S. carnosus*, the majority of these genes are localized between 0.1 and 2.0 Mbp (Fig. 1, orange circle). Full-genome alignments of the 12 staphylococcal strains show that this region is distinguished not only by a high degree of conserved gene order but also by highly conserved proteins. The fewest conserved genes are found in the last section of the genome (between 2.0 and 2.50 Mbp). Most of these genes represent species-specific genes and are found only in *S. carnosus* (Fig. 1, light blue circle).

A conservation plot of *S. carnosus* with *S. aureus* COL is shown in Fig. 3. The plot reveals that the few genes immediately flanking *oriC* (Sca0001 to -0006 and Sca2469 to -2474) are highly conserved. The two other highly conserved clusters correspond to ribosomal proteins and elongation factors (Sca0203 to -0209) and ribosomal proteins (Sca1708 to -1730), respectively. These genes have a high conservation score ranging from 0.83 to 0.93. There are also regions that have a very low score, which are marked by red circles.

An "*oriC* environ" is defined as a region in which typically fewer than 45% of the genes code for common staphylococcal proteins (69). Such an "*oriC* environ" is also present in the *S. carnosus* genome, comprising the poorly conserved genes Sca0019 to -0139 and Sca1942 to -2462. There are other poorly conserved clusters that comprise the ΦTM300-specific genes (Sca0463 to -0518) and a genomic island (Sca1511 to -1539) that we named *ν*SCA1. These genes have a score of only 0.26 to 0.44.

**Mobile genetic elements. (i) Prophage ΦTM300.** The *S. carnosus* genome contains a 45.7-kb prophage, termed ΦTM300, which is located at 0.5 Mbp (Fig. 1, green circle). According to its size, ΦTM300 belongs to the class II phages (38). The prophage encodes 56 proteins, of which 40 have no assigned function. The remaining ORFs code for typical phage functions such as integrase, holin, terminase subunits, structural proteins, and regulatory functions. One gene, Sca0505, coding for the N-terminal part of a tail tape measure protein, is truncated (see Table S1 in the supplemental material). ΦTM300 has no striking overall similarity to other staphylococcal prophages, but 18 proteins share more than 40% identical amino acid sequence with gene products from ΦCOL, a L54a-like phage that has been detected in *S. aureus* COL (20) and is the closest relative (Fig. 4). In dot plot alignments, ΦTM300 shows similarities with ΦCOL distributed over the whole genome length, while it reveals only partial similarities to phages ΦN315/ΦMu50 and ΦSH1 as well as to the 5′ and 3′ ends of phages ΦSLT and ΦETA. At the right end (downstream of Sca0518), the phage genome is flanked by two short sequences with similarity to part of the *attP* site of bacteriophage Φ11 (accession no. M20394), and at the 5′ end, immediately upstream of the integrase Sca0463, a stretch of 419 nucleotides has similarity to part of the *attB* site of bacteriophage ΦETA (accession no. AB046707). The prophage is inserted next to Sca0523, a truncated ORF that seems to be a remnant of a

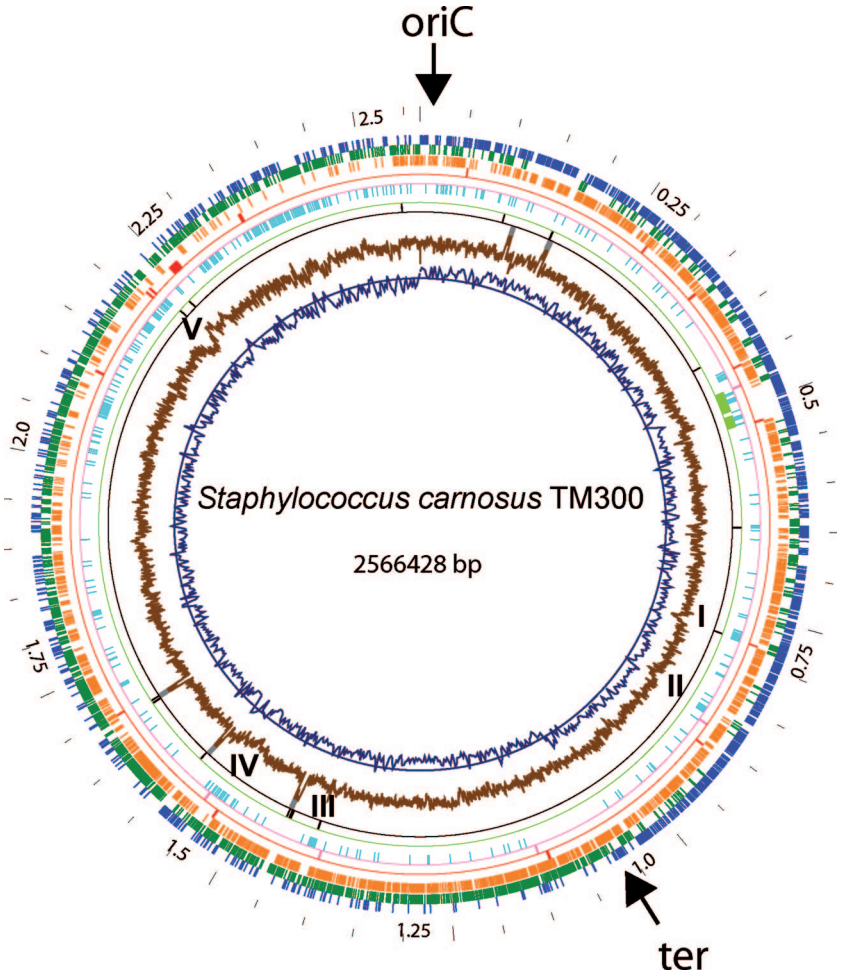


FIG. 1. Circular representation of the *S. carnosus* TM300 genome. The outermost wheel indicates the scale, with a resolution of 50 kb. Circles 2 (blue) and 3 (dark green) show predicted ORFs in the upper and lower strands, respectively. Circle 4 (orange) indicates gene products homologous to proteins encoded by the staphylococcal core genome. The fifth circle (red) shows positions of genes coding for homologs of virulence factors. Circle 6 (magenta) represents integrase-like genes. Circle 7 (light blue) shows the distribution of *S. carnosus*-specific genes. The location of the prophage ΦTM300 is indicated on circle 8 (light green). Circles 9 (black) and 10 (dark gray) represent tRNA and rRNA coding regions, respectively. The two innermost circles, 11 (brown) and 12 (dark blue), show the cumulative GC skew and the GC content, respectively. Positions of clusters of species-specific proteins are indicated by roman numbers (I to V).

gene that encoded a hemolysin-like protein. This resembles a situation that has been reported for the *S. aureus* phage ΦSa3, which is integrated into the β-hemolysin gene (*hlb*) of various *S. aureus* strains (20). It is very likely that ΦTM300 has lost its mobility, as it cannot be induced by mitomycin.

**(ii) Genomic islands.** In order to detect genome regions that have probably been acquired by horizontal gene transfer, we screened the genome for deviation of the average GC content by determining the cumulative GC plot or z′ curve as described by Zhang and Zhang (79). The *S. carnosus* genome displays five larger clusters of species-specific genes that are accompanied by a sudden decrease in the GC content (Fig. 1, regions I to V). Surprisingly, there are no clusters of genes with a markedly higher GC content; however, there are quite a number of single genes with an increased GC content. Clusters I and V are flanked by tRNA operons, and one tRNA operon is within cluster III. The presence of the gene clusters in the neighborhood of tRNA operons makes sense, as it is known that tRNAs are preferred integration sites for horizontal acquisitions. Most of the genes of clusters I to V encode hypothetical proteins (see Table S2 in the supplemental material). Cluster II encodes two components (Sca0875 and Sca0876) of an ABC transporter with unknown specificity. Cluster V harbors genes for hypothetical proteins, with the exception of a truncated gene for a putative glycosyl/glycerophosphate transferase.

Cluster IV (νSCA1) is the only element associated with an integrase-like gene (Sca1539). Most of the gene products in cluster IV are not classifiable in functional categories, except for a putative immunoglobulin G (IgG)-binding protein (Sca1511) that shows weak similarity (31% identity) to IgG-binding protein A of *S. aureus*. The mobile character of this genome region is indicated by the fact that four of the non-species-specific proteins that are encoded within this cluster show similarities to gene products of *S. aureus* pathogenicity island SA1 (Sca1522, -1524, -1531, and -1536; see Table S2 in the supplemental material). Sca1535 could originate from a

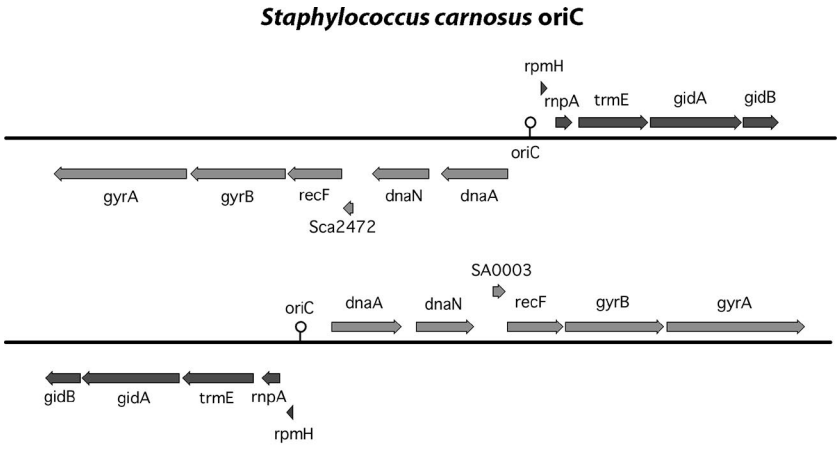


FIG. 2. Comparison of the orders of the genes that surround the chromosomal replication origins of *S. carnosus* and *S. aureus* N315. Sections of the *oriC* regions of *S. aureus* and *S. carnosus* illustrating the gene inversion observed in the staphylococcal genomes are shown. Due to the different orientations of *oriC*, the *S. aureus* genome map starts with gene *dnaA* while in *S. carnosus*, *rpmH* is the first gene.

phage, as it shows 54% identity to a putative prophage antirepressor of *S. saprophyticus*. Furthermore, the genome region comprising the genes of cluster IV shows a clear drop of the conservation score in comparison with other staphylococcal species (Fig. 3). All features of cluster IV indicate that it represents a genomic island, which we named νSCA1. The G+C content of νSCA1 is only 30.4%, which is well below the average value of 34.6%, suggesting that the island was derived from a species with a much lower GC value, such as *S. epidermidis* (Table 1). Its gene organization together with annotated genes is shown in Fig. S1 in the supplemental material.

**(iii) Integrases/recombinases.** We found 14 genes in the *S. carnosus* genome encoding recombinases. Besides the housekeeping recombinases with general functions in DNA replication and DNA repair, such as RecA, RecF, RecG, RecN, RecO, RecQ, RecR, XerC, and XerD, there are also a number of site-specific

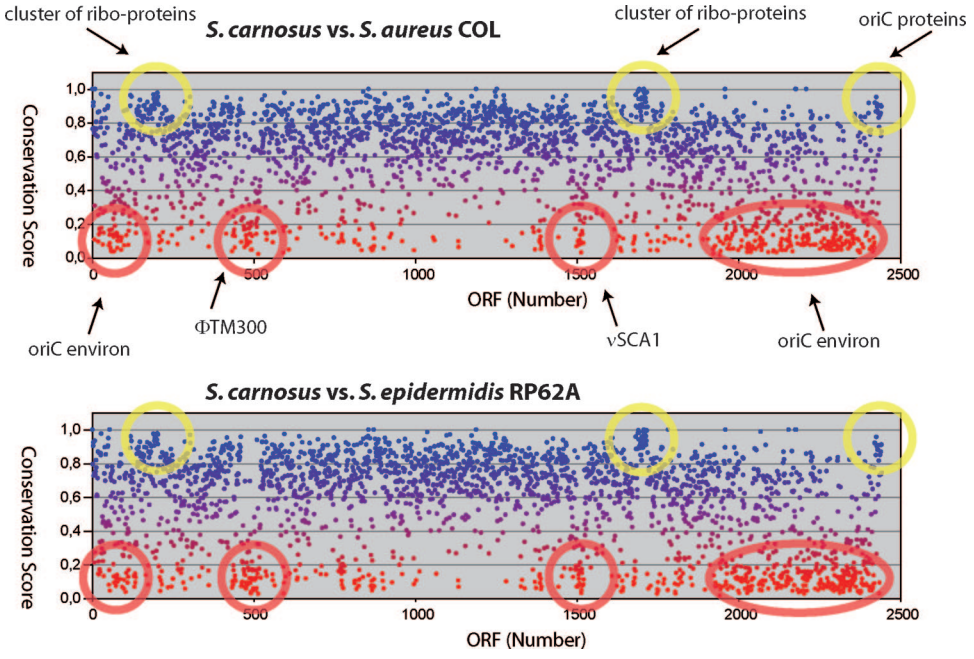


FIG. 3. Conservation scores for *S. carnosus* proteins. Plots of conservation scores (BLAST score of best hit in the compared species divided by the BLAST score of the analyzed *S. carnosus* protein aligned with itself) for *S. carnosus* proteins compared with those of *S. aureus* COL (upper panel) and *S. epidermidis* RP62A (lower panel), respectively. Yellow circles highlight clusters of proteins with high conservation scores, and red circles indicate those that show a significant drop of the normalized score. As indicated, the highly conserved *S. carnosus* proteins correspond to clusters of ribosomal proteins and the *oriC* proteins, whereas the weakly conserved proteins are clustered in the so-called "*oriC* environ" and in prophage ΦTM300 and genomic island νSCA1.

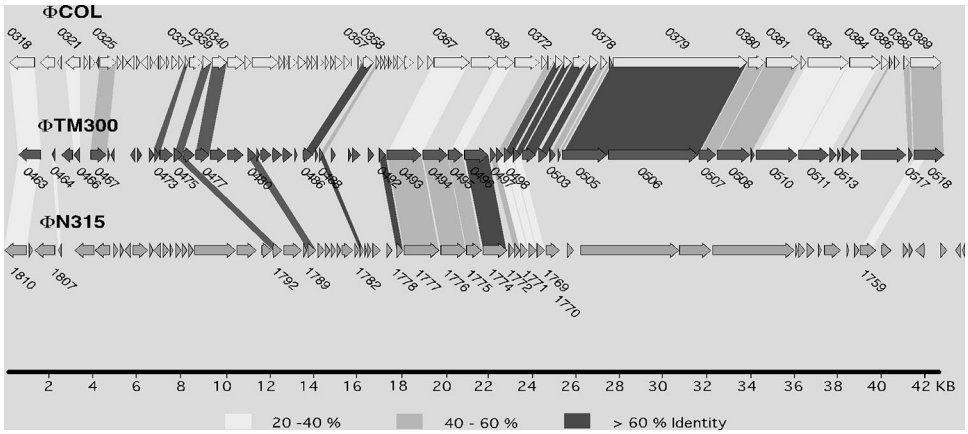


FIG. 4. Comparison of *S. carnosus* prophage ΦTM300 with ΦCOL and ΦN315. Shaded fields indicate similar gene products. The level of amino acid identity corresponds to the different shading densities as indicated below the genome maps. Similar proteins are labeled with the corresponding gene identifications for *S. carnosus*, *S. aureus* COL, and *S. aureus* N315, respectively.

recombinases present that are very likely part of mobile elements (see Table S3 in the supplemental material). Sca1387 shows the highest similarity to the transposase of the insertion sequence (IS) element ISSha1 of *S. haemolyticus* JCSC 1435. Database comparisons indicate that Sca1387 is truncated. Sca1539 has 74% identity to integrases from several staphylococcal species, including those of various prophages. Sca0463 is associated with the prophage sequence in the *S. carnosus* genome and is very similar to integrases of other staphylococcal phages such as Φ55, Φ71, Φ11, and ΦEta. Sca1656 is a putative transposase that does not have any other orthologs in staphylococcal genomes but does in *Clostridium perfringens*. In addition to the phage-like integrase Sca0463, the putative integrase Sca1539 may also be involved in the integration of foreign DNA into the *S. carnosus* genome. Sca1539 is localized within one of the *S. carnosus*-specific gene clusters. Sca0886 and Sca1123 are highly similar to the site-specific recombinases XerC and XerD of *S. epidermidis and S. saprophyticus*, respectively, which play a crucial role in the termination of replication.

**(iv) The *S. carnosus* genome is poor in mobile elements.** Besides the genomic island *ν*SCA1 and the prophage ΦTM300, no further mobile genetic elements were detectable in the *S. carnosus* genome. Thus, compared to other staphylococcal genomes, *S. carnosus* represents the species with the lowest overall content of mobile elements (Table 2). The *S. carnosus* genome also displays a comparatively low number of repetitive DNA sequences (inverted, direct, and palindrome repeats, which facilitate genomic diversification due to recombinational events [3]) (see Table S4 in the supplemental material). In contrast to the case for *S. aureus* and *S. epidermidis* (20), no GC-rich repeats corresponding to STAR elements (16) were found in *S. carnosus*. These factors support our observation that *S. carnosus* has comparatively few gene rearrangements and genetic instabilities.

TABLE 2. Mobile elements

| Species and strain | No. of: | | | | | Reference |
|---|---|---|---|---|---|---|
| | IS elements | Transposons | Prophages | Staphylococcal cassette chromosomes | Genomic islands | |
| *S. aureus* | | | | | | |
| N315 | 20 | 5 | 1 | 1 | 3 | 35a |
| Mu50 | 23 | 3 | 2 | 1 | 4 | 35a |
| MW2 | 6 | 0 | 2 | 1 | 4 | 20 |
| COL | 6 | 0 | 1 | 1 | 5 | 4a |
| MRSA252 | 22 | 3 | 2 | 1 | 3 | 29a |
| MSSA476 | 1 | 0 | 2 | 1 | 2 | 29a |
| *S. epidermidis* | | | | | | |
| RP62A | 23 | 4 | 1 | 1 | 2 | 20 |
| ATCC 12228 | 21 | 6 | 0 | 0 | 4 | 80 |
| *S. saprophyticus* ATCC 15305 | 2 | 0 | 0 | 2 | 1 | 35b |
| *S. haemolyticus* JCSC 1435 | 82 | 2 | 2 | 1 | 5 | 69 |
| *S. carnosus* TM300 | 0 | 0 | 1 | 0 | 1 | This work |

TABLE 3. Homologs of global regulators in *S. carnosus*

| Gene | Locus | Gene product | Best hit (strain/accession no.) | Identity (%)[a] |
|---|---|---|---|---|
| Sca1545 | *agr* | AgrB | *S. saprophyticus* ATCC 15305/YP_306932 | 55 |
| Sca1546 | | AgrD | *S. simulans*/AAL65845 | 75 |
| Sca1547 | | AgrC′ | *S. haemolyticus* JCSC 1435/BAED4303 | 45 |
| Sca1548 | | AgrC″ | *S. aureus* RF122/YP_417381 | 68 |
| Sca1549 | | AgrA | *S. epidermidis* ATCC 12228/AA005237 | 78 |
| Sca0352 | *sae* | SaeR′ | *S. aureus* COL/AAW37825 | 66 |
| Sca0352a | | SaeR″ | *S. aureus* COL/AAW37825 | 69 |
| Sca0353 | | SaeS | *S. aureus* MRSA252/YP_040185 | 60 |
| Sca1924 | *lyt* | LytR | *S. saprophyticus* ATCC 15305/YP_300553 | 62 |
| Sca1923 | | LytS | *S. epidermidis* RP62A/AAW52842 | 63 |
| Sca1060 | *arl* | ArlR | *S. epidermidis* RP62A/AAW54343 | 82 |
| Sca1059 | | ArlS | *S. saprophyticus* ATCC 15305/YP_301414 | 62 |
| Sca1458 | *vra* | VraR | *S. saprophyticus* ATCC 15305/YP_300999 | 92 |
| Sca0302 | *gra* | GraR | *S. aureus* MRSA252/YP_040111 | 69 |
| Sca0303 | | GraS | *S. haemolyticus*/YP_254149 | 52 |
| Sca1459 | | VraS | *S. aureus* MRSA252/CAG40962 | 84 |
| Sca0266 | *sar* | SarA | *S. haemolyticus* JCSC1435/BAE05590 | 78 |
| Sca1787 | | SarR | *S. epidermidis* RP62A/AAW55256 | 29 |
| Sca1118 | *srr* | SrrA | *S. saprophyticus* ATCC 15305/YP_301350 | 86 |
| Sca1117 | | SrrB | *S. saprophyticus* ATCC 15305/YP_301351 | 67 |
| Sca0333 | *mgrA* | MgrA | *S. saprophyticus* ATCC 15305/YP_302122 | 82 |
| Sca2456 | *yyc* | YycF | *S. epidermidis* RP62A/AAW53342 | 94 |
| Sca2455 | | YycG | *S. aureus* COL/AAW37408 | 85 |
| Sca1570 | Other | SigB | *S. aureus* MRSA252/YP_041514 | 89 |
| Sca1859 | | TcaR | *S. epidermidis* RP62A/AAW52831 | 48 |
| Sca0597 | | Spx | *S. saprophyticus* ATCC 15305/Q49WC6 | 97 |
| Sca2433 | | SvrA | *S. aureus* N315/BAB41547 | 89 |

[a] Percentage of identical amino acids in BlastP alignments.

**The *Staphylococcus* core set of conserved genes.** Based on BlastP analysis, we compared the derived gene products of *S. carnosus* with the proteins encoded by *S. aureus* strains N315, Mu50, MW2, COL, MSSA476, MRSA252, and RF122; *S. epidermidis* strains RP62A and ATCC 12228; *S. haemolyticus* JCSC 1435; and *S. saprophyticus* ATCC 15305. We applied the same parameters for this comparison as described for a previous comparative analysis of six staphylococcal genomes (20). Based on these parameters, the staphylococcal core set of conserved genes comprises 1,203 genes corresponding to 46 to 50% of the encoded proteins in an individual genome. Approximately 14% of the conserved proteins in staphylococci cannot be classified in COG categories or assigned a putative function. Of the remaining 86% of encoded proteins, the majority fall into categories comprising proteins involved in basic metabolic pathways, i.e., transport and metabolism of substrates, coenzymes, energy production, transcription, translation, or replication.

**Regulatory systems.** Various global regulation systems for staphylococci are known to control extracellular and cell wall-bound proteins, including virulence factors such as exotoxins and surface adhesions involved in colonization, immune evasion, and tissue degradation. Typical global regulators identified in staphylococci are the two-component regulatory elements such as *agr* (59), *sae* (21), *lyt* (10), *arl* (18), *vra* (35), *sar* (11), and *srr* (76) and other factors such as *graRS* (8), *mgrA* (31), *tcaR* (9), *spx* (56), *svrA* (19), *rot* (47), and *sigB* (75). With the exceptions of *rot*, *sarS*, and *sarT*, orthologs exist for all of these systems in *S. carnosus* (Table 3). The *agr* system is very likely to be nonfunctional, as *agrC* carries a stop codon within the coding sequence and *saeR*, which coordinates environmental signals with the *agr* quorum-sensing system (54), carries a frameshift mutation.

**Metabolic pathways encoded in the *S. carnosus* TM300 genome. (i) Glycolysis and sugar fermentation.** *S. carnosus* possesses the genetic potential for uptake of glucose by both the phosphoenolpyruvate-dependent PTS and a permease (GlcU). GlcA (Sca0999) and GlcB (Sca1000) (factor IIA and factor IIB of the glucose-specific PTS) share 67% identity with each other. Only *S. aureus* has homologs to each of these proteins, while the other staphylococcal species encode only one protein with similarity to both factor IIA and factor IIB. The genome also contains all genes necessary for the glycolytic and gluconeogenetic pathway (see Table S5.1 in the supplemental

material). Two lactate dehydrogenase (LDH) homologs (Sca0343 and Sca1369) are encoded in the *S. carnosus* genome (see Table S5.1 in the supplemental material). Sca1369 shows the highest similarity with the LDH1 and LDH2 genes of *S. saprophyticus* and other staphylococci, while Sca0343 is also most similar to the genes of *S. saprophyticus*; however, the next similarities are to the LDHs of *Bacillus*, *Propionibacterium*, and *Clostridium*. The two LDHs of *S. carnosus* show only weak similarity to each other (29% identity according to Bl2seq).

Genes for the utilization of fructose, mannose, mannitol, sorbitol, ribose, and glycerol are also present (see Table S5.2 in the supplemental material); however, those for maltose and sucrose utilization are absent, which is in line with data from fermentation studies (64). Lactose, mannitol, and trehalose are also taken up via a PTS; however, the putative trehalose-specific IIBC component is truncated. *S. haemolyticus* and *S. saprophyticus* have no fructose-specific PTS. The PTS-dependent uptake of sorbitol is present exclusively in *S. carnosus* and was not found in any other sequenced staphylococcal genome. *S. aureus* COL has no fructose-specific permease, but all other *S. aureus* strains do. *S. carnosus*, *S. epidermidis*, and *S. aureus* have two mannose-6-phosphate isomerases. There are two ribose permease genes that are missing in the *S. haemolyticus* and *S. saprophyticus* genomes.

Interestingly, *S. carnosus* can transport lactose either by a lactose-specific permease or by a lactose-specific PTS system. In contrast, the other staphylococcal genomes encode only one of these alternative lactose uptake systems (PTS in *S. aureus*, *S. epidermidis*, and *S. haemolyticus* and lactose permease in *S. saprophyticus*). Also, in contrast to the other species, *S. carnosus* contains two pathways for lactose catabolism: the Leloir and the tagatose-6-phosphate pathway (see Table S5.3 in the supplemental material). *S. aureus*, *S. epidermidis*, and *S. hominis* utilize lactose only via the tagatose-6-phosphate pathway (23), and *S. intermedius*, *S. saprophyticus*, and *S. xylosus* utilize galactose only via the Leloir pathway (65). *S. carnosus* contains both the *lacRPH* operon (Sca1967, Sca1968, and Sca1966) and genes involved in the uptake and degradation of lactose to glyceraldehyde-3-phosphate, including a lactose-specific PTS system (LacF [Sca0667] and LacE [Sca0668]), a β-galactosidase (LacG [Sca0669]), and homologs of LacR, -A, -B, -C, and -D (Sca0676 to Sca0672). Another unique characteristic of *S. carnosus* is that there are two other putative UDP-glucose-4-epimerases (Sca1770 and Sca2065) present besides GalE (Sca1770), of which one (Sca2065) has a homolog in *S. aureus* COL while the other is present only in *S. carnosus*. The *lac* genes are organized in two inversely orientated transcription units, *lacF* and *-G* and *lacR* to *-D*, which are separated by genes coding for homologs of the arsenate reductase ArsC (Sca0670) and the arsenic efflux pump ArsB (Sca0671) (63). It appears that the typical plasmid-carried arsenate/arsenite resistance genes were integrated within the *lac* operon without inactivating the *lac* genes (see Fig. S2 in the supplemental material).

**(ii) Pentose phosphate pathway.** Most genes involved in the oxidative and nonoxidative pentose phosphate pathways are present (see Table S5.4 in the supplemental material). However, staphylococci do not have a gluconolactonase and therefore obviously cannot form 6-phosphogluconate via 6-phosphogluconolactone. In the presence of ribose, *S. carnosus* could feed via the pentose phosphate pathway by uptake and phosphorylation of ribose, as a ribose permease and ribokinase are present in *S. carnosus* but not in *S. saprophyticus* and *S. haemolyticus*.

**(iii) Tricarboxylic acid cycle and respiratory chain.** All enzymes involved in the tricarboxylic acid cycle are present in *S. carnosus* (see Table S5.5 in the supplemental material). Interestingly, malate dehydrogenase (Sca0338) has no homologs in *S. aureus* and *S. haemolyticus*. *S. carnosus* carries all components of the respiratory chain complex I (NADH dehydrogenase), II (succinate dehydrogenase), IV cytochrome *c* oxidase (E; B/A), and $cbb_3$ type and the components responsible for cyanide/pyocyanin-insensitive respiration, CydAB. We have shown that nonpathogenic staphylococcal species generally have a cyanide/pyocyanin-insensitive CydAB quinol oxidase, while in the pathogenic species such as *S. aureus* and *S. epidermidis*, CydAB quinol oxidase homologs are cyanide/pyocyanin sensitive (72). The B subunit determines whether the chinol oxidase is sensitive or insensitive and is much less conserved than the A subunit.

**(iv) Catalase, SOD, and peroxidase.** The *S. carnosus* genome is well equipped to deal with reactive oxygen species (see Table S5.6 in the supplemental material). There are one SOD, two catalases, and various peroxidases. While KatA (Sca2336) is highly conserved among the staphylococci, the putative catalase Sca2210 has no homologs in the known staphylococcal genomes.

**(v) Nitrate/nitrite reduction.** The genes involved in nitrate and nitrite reduction in *S. carnosus* (17) have been assigned to the genes Sca1887 to Sca1900. In contrast to the case in the other staphylococcal species, no homologs of the Nir or Nar proteins are present in *S. saprophyticus*. Interestingly, the siroheme synthase SirA of *S. carnosus* does not have closely related relatives in the other staphylococci (see Table S5.7 in the supplemental material).

**(vi) Mevalonate and fatty acid biosynthesis.** *S. carnosus* shows a complete mevalonate pathway leading from 3-hydroxy-3-methylglutaryl-coenzyme A to isopentenyl-pyrophosphate (see Table S5.8 in the supplemental material). Concerning carotinoid biosynthesis, *S. carnosus* encodes only a CrtM (squalene synthase) homolog and none of the other enzymes involved in the biosynthesis of staphyloxanthin starting from farnesyl-pyrophosphate (58). This is in agreement with the whitish colony color. Staphyloxanthin appears to be more advantageous for *S. aureus*, where it plays a role in coping with oxidative stress (13). There are no obvious differences between *S. carnosus* and the other staphylococci with regard to the fatty acid biosynthesis pathway. However, *S. carnosus* 3-oxoacyl-[acyl-carrier protein] reductase is truncated by 20 amino acids at the C terminus (see Table S5.9 and S5.10 in the supplemental material).

**(vii) Amino acid biosynthesis.** We determined the essential amino acids necessary for growth of *S. carnosus* and found that TM300 is auxotrophic for proline, arginine, valine, leucine, glutamate, threonine, serine, and cysteine (49). This suggests that the corresponding metabolic pathways might be defective in *S. carnosus*. Based on the genomic analysis of the amino acid biosynthetic genes, only the auxotrophy for leucine can be explained, because the corresponding aminoacyl-tRNA synthetase is interrupted. The remaining genes involved in the amino acid biosynthetic pathways do not reveal any obvious

defects (see Tables S6.1 to S6.5 in the supplemental material). Therefore, the discrepancy between the genomic data and the observed auxotrophies might be due to disturbed regulation of the biosynthetic pathways.

**(viii) Biogenic amines.** Since *S. carnosus* is used as a starter culture in food fermentation, its potential to synthesize biogenic amines is of particular interest, as they are toxic in large doses. Indeed, *S. carnosus* encodes an ornithine decarboxylase (Sca0122) that could account for the synthesis of putrescine from ornithine or cadaverine from lysine. Biogenic amines such as putrescine are produced by *S. carnosus* and also by a group of lactic acid bacteria known as carnobacteria, such as *Lactobacillus buchneri*, *L. curvatus*, *L. reuteri*, *L. alimentarius*, *L. brevis*, *L. bavaricus*, and *L. delbrueckii* subsp. *lactis* (68).

**(ix) Iron uptake systems.** We classified 127 *S. carnosus* proteins according to COG category as being involved in "inorganic ion transport and metabolism." This group contains 15 iron transport systems, of which 6 are multicomponent systems. Two systems, coding for an ABC-type $Fe^{3+}$ transporter, are not represented in the other staphylococcal genomes (see Table S7 in the supplemental material). On the other hand, there are several iron transport systems present in *S. aureus* COL and in some cases also in *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus* that have no homolog in *S. carnosus* TM300, such as heme or ferritin transporters. The *isd* (iron-responsive surface determinant) locus is present exclusively in *S. aureus*. The surface proteins IsdA, IsdB, IsdC, and IsdH and the ATP-binding cassette transporter IsdDEF comprise the machinery for acquiring heme as a preferred iron source (42, 46). Previous studies have shown that heme iron is the preferred iron source during the initiation of infection in *S. aureus*. The lack of *isd* homologs and transferring transporters could be among various reasons why *S. carnosus* is apathogenic. However, the total number of iron transport systems in *S. carnosus* is in the same range as in the pathogenic staphylococcal species.

**(x) Osmoprotection.** The natural habitat of *S. carnosus* is unknown. However, strains can be consistently isolated from fermented fish, sausage, or soy (78). Osmoprotection appears to play a crucial role in this habitat (see Table S8 in the supplemental material). Therefore, it is not surprising that *S. carnosus* has several osmoprotection pathways and is at least as well equipped as *S. aureus* and *S. epidermidis* (20). *S. carnosus* reveals nine osmoprotection systems; four systems are involved in proline transport, of which two are also thought to be responsible for glycine betaine transport. The other transporters include three OpuD-like glycine betaine transporters; one multicomponent transport system for choline, glycine betaine, and carnitine; and one system for the uptake and dehydrogenation of choline.

In addition, *S. carnosus* has five sodium ion/proton antiporter systems and homologs to the mechano-sensitive ion channels MscL and MscS. This high content of osmoprotective factors in the *S. carnosus* genome correlates well with the ability of this species to grow readily in the presence of 15% NaCl, which has been used in the phenotypic separation of *S. carnosus* from other staphylococcal species (64). One can assume that *S. carnosus* originates from a habitat that exerts osmotic stress on the organisms, for example, during raw-sausage fermentation. Indeed, it has been found that strains of *S. carnosus* were more salt tolerant than strains of *S. equorum* and *S. xylosus*, especially at high pH and temperature (67).

**Indication of horizontal gene transfer.** The *S. carnosus* genome encodes 345 proteins (14% of the total proteins) that are specific for this species. Almost 50% of the genes are located in a segment (2.0 to 2.55 Mbp) counterclockwise of *oriC*; the residual genes are spotted around the genome circle (Fig. 1, light blue circle). A large proportion (41%) of the species-specific proteins have unknown function. The high content of unknown proteins in the other species could be due to inconsistent annotation. Quite a number of the encoded proteins in TM300 share the highest homology not to staphylococcal sequences but to proteins of other genera (see Table S9.1 in the supplemental material). The individual genes and the most homologous species/genus are listed in Table 9.2. in the supplemental material. Some examples are given. Sca1656 has similarity to a transposase that is associated with various IS elements in *Clostridium perfringens*. Sca0567 is similar to an amidohydrolase of a *Burkholderia* sp., and Sca1521 is similar to a phage-associated protein from *Mycoplasma mycoides*. The genome also has remnants of metabolic pathways that have obviously been acquired via an intergenus gene transfer, such as an incomplete pathway for uptake and degradation of allantoin (Sca2053 to Sca2063).

The species-specific gene products of TM300 also comprise proteins required for the PTS-dependent uptake of sorbitol as well as a putative regulator and a sorbitol-6-phosphate 2-dehydrogenase (Sca2317 to Sca2322). Some metabolic pathways seem to be catalyzed by a mixture of TM300-specific proteins and those that have orthologs in other staphylococcal species. An example is the pathway for the biosynthesis of UMP (Sca0812 to Sca821), where the subunits of the dihydroorotate dehydrogenases PyrK (Sca0818) and PyrD (Sca0819) have the closest homologs in *Geobacillus* and *Fusobacterium*, respectively, while the remaining enzymes have equivalents in other staphylococcal genomes. Furthermore, two of the genes involved in nitrite reduction (51), the putative siroheme biosynthesis protein SirA (Sca1898) and the putative regulator NirR (Sca1899), are TM300 specific, while NirB, -C, and -D and SirB are not. This may account for the fact that *S. carnosus* has a very high level of dissimilatory nitrate respiration (50).

**Truncated genes.** We identified 55 ORFs in the *S. carnosus* chromosome that were truncated or interrupted (see Table S10 in the supplemental material). This is a relatively high number compared to those in other published staphylococcal genomes. Two of the truncated genes stem from global regulation systems involved in the expression of exoproteins. *agrC*, encoding the sensor histidine kinase (Sca1547; Sca1548) is truncated due to a UAA stop codon; the first part of the gene was given the name Sca1547, and the second part was given Sca1548. *saeR*, encoding a global response regulator, is truncated by a frameshift mutation; the first part was given the name Sca0352 and the second part was given Sca0352a. We confirmed the presence of the *agrC* truncation also for the *S. carnosus* type strain, DSM 20501 (unpublished results). A frameshift mutation is also within the signal recognition particle-encoding gene *ffh* (Sca0861), while the corresponding 4.5 S RNA is intact. Ffh is involved in the secretion of membrane proteins and a subset of the secreted proteins in *Bacillus* (77). The inactivation of

TABLE 4. Homologs of toxins, binding proteins, and exoenzymes in *S. carnosus*

| Gene | Similar product | Best hit | Identity (%) | Accession no. |
|---|---|---|---|---|
| Sca0349 | Putative hemolysin | *S. aureus* COL | 79 | NC_002951 |
| Sca0523 | Hemolysin-related protein (truncated) | *S. epidermidis* ATCC 12228 | 64 | NP_764168 |
| Sca0785 | Antibacterial protein (phenol-soluble modulin) | *S. aureus* COL | 70 | AAW36566 |
| Sca0784 | Antibacterial protein (phenol-soluble modulin) | *S. aureus* MRSA252 | 60 | YP_040561 |
| Sca1670 | Hemolysin III, putative | *S. epidermidis* RP62A | 68 | YP_189333 |
| Sca0436 | Exotoxin | *S. aureus* MRSA252 | 43 | YP_040552 |
| Sca0905 | Exotoxin 3, putative | *S. aureus* COL | 39 | AAW37599 |
| Sca1112 | Putative elastin-binding protein | *S. saprophyticus* ATCC 15305 | 38 | YP_301356 |
| Sca0054 | Fibrinogen-binding protein precursor | *S. aureus* Mu50 | 30 | NP_371683 |
| Sca2185 | Intercellular adhesion protein C | *S. aureus* MRSA252 | 30 | YP_042088 |
| Sca2182 | Intercellular adhesion protein C | *S. aureus* Mu50 | 30 | NP_373193 |
| Sca2283 | Clumping factor B | *S. aureus* Newman | 29 | CAA12115 |
| Sca1511 | IgG-binding protein SBI | *S. aureus* COL | 27 | AAW37242 |
| Sca2092 | IgG-binding protein A precursor | *S. aureus* MSSA476 | 26 | CAG41852 |
| Sca0273 | Lipoprotein SitC, streptococcal adhesin PsaA homolog, Fe acquisition | *S. aureus* N315 | 78 | BAB41819 |
| Sca2202a, Sca2202, Sca2207 | Hypothetical protein SH0326, similar to cell wall surface anchor family protein[a] | *S. haemolyticus* JCSC 1435 | 51 | YP_252241 |

[a] Sca2202a, Sca2202, and Sca2207 are truncation fragments of an ancestor gene. The listed data refer to a protein that represents a fusion of all three fragments.

AgrC, SaeR, and Ffh could explain the comparatively low exoprotein production in TM300.

Some missing enzymatic activities can be directly attributed to the inactivation of the corresponding genes. For example, defective (truncated) genes were identified for a lipase (Sca0131/0132), a DNase (Sca0372/0373), a thiol protease (Sca0601/602), a putative peptidase (Sca1224), and an amidase (Sca2264). Also, two genes involved in the adaptation to high-salt conditions were inactivated by point mutations: a putative cardiolipin synthetase gene (Sca0960) and a gene coding for a glycine betaine/choline-binding lipoprotein (Sca2238).

Furthermore, Sca2207, Sca2202a, and Sca2202 are disrupted by a frameshift mutation. Their translation products can be joined to a protein of 3,686 amino acids that is exceptionally serine rich (1,498 residues corresponding to 41% of all amino acids). These fragments are probably remnants of a cell wall-anchored protein that contains a typical signal peptide and a sorting sequence with a LPXTG sorting motif. The database hit with the highest similarity is a hypothetical protein with a length of 3,608 amino acids from *S. haemolyticus* JCSC 1435. The next best hits are from nonstaphylococcal species, with the length of the corresponding proteins differing significantly.

TM300 has another large protein with a typical signal peptide and a sorting sequence. Sca2192 codes for a protein with 4,244 amino acids. The size of this protein is reminiscent of that of the giant Ebh protein (approximately 10,000 amino acids) (12), but the sequences share no relationship. This protein is distinguished by high glycine and threonine residue contents (about 10% each). Interestingly, no protein with a similar length and a significant identity can be found in the databases; therefore, this protein appears to be *S. carnosus* specific. The unusually high GC content suggests that the gene was probably obtained by horizontal gene transfer.

**The *S. carnosus* genome essentially lacks the typical *S. aureus*-associated virulence factors.** If we consider only real virulence factors (toxins, host-specific binding proteins, and antibiotic resistance genes), there are only a few potential virulence factors detectable in *S. carnosus* (Table 4; see Table S11 in the supplemental material). The annotation of the orthologs of these potential virulence factors in different staphylococcal genomes is not consistent, and therefore the real function of the proteins remains unclear. For example, there are three putative hemolysin and two exotoxin genes present; however, *S. carnosus* shows no hemolysis activity. There are also two phenol-soluble modulin genes present, with unclear function. The *S. carnosus* TM300 genome lacks the typical toxin genes such as those for α-hemolysin (*hla*), γ-hemolysin components (*hlgA*, *hlgC*, and *hlgB*), Panton-Valentine leukocidin (*lukS-PV* and *lukF-PV*), leucocidins (*lukD*, *lukE*, and *lukM*), or exfoliative toxins A and B (*eta* and *etb*), which are responsible for staphylococcal scalded skin syndrome. Additionally, TM300 lacks the known superantigens such as toxic shock syndrome toxin 1 (*tst*) and enterotoxins (*sea* to *sep*).

There are a few binding proteins with weak (≤30%) protein identity present. Although there are genes encoding proteins with low similarity to IgG-binding protein SBI and IgG-binding protein A precursor, we could not detect an IgG-binding activity in a Western blot with labeled IgG either in culture supernatants or in the lysostaphin-treated cell wall fraction (unpublished results). Some annotated genes show similarity to proteases and lipase genes. In contrast to many *S. aureus* strains, TM300 shows no proteolytic activity on casein agar in the supernatant. However, we detected a cell wall-bound protease activity; a lipase activity is not detectable. In contrast, the annotated thermonuclease shows activity (unpublished results). Unlike many clinical isolates of *S. epidermidis* and *S. aureus*, TM300 is biofilm negative and lacks the *ica* (*i*ntercellular *a*dhesin) operon (15, 29) involved in polysaccharide intercellular adhesin (44) biosynthesis.

**Conclusion.** The *S. carnosus* TM300 genome sequence reveals a number of features that set this species representative apart from the known staphylococcal genomes. It has a small size, the GC content averages about 2% higher than in other species, and quite a number of proteins (193) have the highest similarities to proteins of non-*Staphylococcus* species, suggesting that horizontal gene transfer occurred. On the other hand,

the genome contains the lowest number of mobile elements as well as forward and palindromic repeats, indicating a low genome plasticity. A high number of ORFs (55) are truncated, which might reflect the nutrient-rich environment in which some of the functions are dispensable. *S. carnosus* fulfills the requirements for a meat starter culture. All of the genes for dissimilatory nitrate/nitrite respiration are present. There are also two catalase genes, which are necessary to reduce hydrogen peroxide produced by the catalase-negative lactobacilli that were frequently used in combination with *S. carnosus*. Unlike the other staphylococcal genomes, *S. carnosus* has the genetic potential to degrade lactose and galactose by both the Leloir- and the tagatose-6-phosphate pathways. On the other hand, the genome contains an ornithine decarboxylase gene that accounts for the synthesis of the biogenic amines putrescine and cadaverine, and elimination of this gene could improve the starter quality. *S. carnosus* is regarded as a nonpathogenic species, and indeed the genome lacks all the *S. aureus* and *S. epidermidis* specific toxins and superantigens, many binding proteins, and polysaccharide intercellular adhesin-mediated biofilm formation. The low genome plasticity in combination with lack of virulence factors emphasizes the role of mobile elements in the pathogenicity of *Staphylococcus*. Our results show that TM300 is an interesting candidate to study those factors and for use as a cloning host in biotechnology.

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