Complete Genome Sequence and Comparative Analysis of Staphylococcus condimenti DSM 11674, a Potential Starter Culture Isolated from Soy Sauce Mash

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BACKGROUND

Coagulase-negative staphylococci (CNS) are key players in the majority of food fermentation ecosystems, which are commonly found in the production of fermented meat and milk products (Blaiotta et al., 2005; Resch et al., 2008). Strains of CNS have been implicated in exerting desirable effects as components of a fermentation flora, such as color formation, aroma development, and shelf-life enhancement, and may therefore have the potential for future application as starter cultures (Zell et al., 2008).

Staphylococcus condimenti is one of the most prominent species and has the potential for use in starter cultures for the production of fermented sausage and cured ham (Zell et al., 2008). S. condimenti DSM 11674 was originally isolated from fermenting soy sauce mash and suggested to be a new species in 1998 (Probst et al., 1998). However, S. condimenti has been found in a few clinical samples (Argemi et al., 2015; Misawa et al., 2015). Therefore, some concerns have been raised with regard to the safety of this species for use in food production (Zell et al., 2008; Seitter et al., 2011a,b).

To further understand the biochemical and genetic characteristics of DSM 11674 and advance the potential biotechnological applications of this strain, we constructed the complete genome sequence of S. condimenti DSM 11674.

MATERIALS AND METHODS

Bacterial Strain and Biochemical Characterization

Staphylococcus condimenti DSM 11674 (= JCM 6074 = CIP 105760) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. The isolate was identified by 16S rRNA sequencing. The sequence was then compared against NCBI database and EzTaxon-e database. To further explore its potential application in food fermentation, we calculated the nitrate reductase activity and catalase activity of S. condimenti DSM 11674 as described previously (Herrero et al., 1996; Miralles et al., 1996). Nitrite reductase activity was determined as described previously (Neubauer et al., 1999; Gotterup et al., 2007).

Minimum Inhibitory Concentrations (MICs) and DNA Purification

Minimum inhibitory concentrations were established by the Vitek 2 Compact system with AST-GP67 card (bioMérieux, France). The MICs were interpreted according to Clinical and Laboratory
Standards Institute (CLSI, 2016). Genomic DNA was extracted from 3-ml overnight cultures using a Gentra Puregene Yeast/Bact Kit (Qiagen, Hilden, Germany). Bacteria were treated with lysis buffer containing Proteinase K and RNaseA for 2 h at 65°C, and DNA purification was performed according to the manufacturer’s recommended protocols.

**Genome Sequencing and Assembly**

The genome of *S. condimenti* DSM 11674 was sequenced on the PacBio RS II single-molecule real-time (SMRT) system. Raw sequence data were *de novo* assembled using the hierarchical genome-assembly process (HGAP) protocol (Chin et al., 2013) and RS HGAP Assembly 2.1.

**Genome Annotation**

The genome was annotated using the Rapid Annotation using Subsystem Technology server (Aziz et al., 2008) and the NCBI Prokaryotic Genome Annotation Pipeline. Ribosomal RNAs were detected by RNAmmer (Lagesen et al., 2007) and transfer RNAs by tRNAscan-SE (Lowe and Eddy, 1997). CRISPRFinder was used to screen for the presence of CRISPR elements (Grissa et al., 2007). Coding sequences were analyzed to detect toxin genes by using VirulenceFinder2 and by comparing the protein sequences using BLASTP with sequences in virulence factor database (Chen et al., 2005). The Antibiotic Resistance Genes Database was applied to classify antibiotic resistance genes (Li and Pop, 2009).

**Comparative Genomic Analysis**

The core genome alignment module in the rapid large-scale prokaryotic pan genome analysis (Roary) pipeline was used to extract predicted coding regions from 21 complete *Staphylococcus* genome sequences (Page et al., 2015). Core genes were defined as those present in all isolates with default parameters. Common and unique orthologous groups identified among the genomes were defined as previously described (Zheng et al., 2014). Full genome alignments were performed using progressive MAUVE (Darling et al., 2010).

**RESULTS AND DISCUSSION**

**Biochemical and Antimicrobial Characteristics**

In our study, the strain of *S. condimenti* DSM 11674 has the highest capacity to reduce nitrate (13.67 mM nitrate reduced to nitrite per milligram of dry weight) and exhibits a high catalase activity compared to *Staphylococcus aureus* ATCC 25923 and the clinical isolate of *S. condimenti* CJ1628 (Figure S1A in Supplementary Material). Moreover, the strain of *S. condimenti* DSM 11674 exhibited the enhanced nitrite reductase activity when cultured with nitrite (2 mM) and nitrate (20 mM) under anaerobic condition (Table S1 in Supplementary Material). Antimicrobial susceptibility tests show that *S. condimenti* DSM 11674 is susceptible to all antibiotics tested, including amikacin, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, ciprofloxacin, erythromycin, gentamicin, imipenem, levofloxacin, tobramycin, and trimethoprim/sulfamethoxazole. These data are consistent with that of traditional starter culture *Staphylococcus carnosus* (Landeta et al., 2013) and indicate that *S. condimenti* is suitable as fermented meat starter.

**Genome Features**

The complete circular chromosome was 2,659,676 bp with a G + C content of 34.7%. A total of 2,516 protein coding genes, 18 rRNA genes, 58 tRNA genes, 46 pseudogenes, and 2 CRISPR arrays were identified in the genome (Table S2 and Figure S2 in Supplementary Material).

**Comparison of Staphylococci Genomes**

A total of 28,680 gene clusters and 22 core genes were defined, and a phylogenetic tree was constructed based on the core gene alignment (Figure 1A; Table S3 in Supplementary Material). On this tree, *Staphylococcus saprophyticus* ATCC 15305, three *Staphylococcus xylosus* isolates, *S. carnosus* TM300, *Staphylococcus hyicus* ATCC11249, *Staphylococcus schleiferi* 1360-13, and *S. condimenti* DSM 11674 formed a monophyletic branch, providing strong evidence for the taxonomic relatedness of these isolates (Figure 1A). Of note, *S. condimenti* DSM 11674 has the closest relationship with *S. carnosus* TM300. On the basis of this, we further identified unique and shared gene content in *S. condimenti*, with commercial meat starter culture bacteria *S. carnosus* TM300 (Rosenstein et al., 2009) and *S. xylosus* SMG-121 (El Haddad et al., 2014), which are widely used in the food industry. A Venn diagram of the unique/shared gene content was generated with a custom R script using the VennDiagram package (Figure 1B). These three strains share 1,743 CDS in their genome. In addition, a noticeable overlap between DSM 11674 and TM300 became evident, and these two strains shared 493 orthologous CDS. Moreover, 280 CDS from the DSM 11674 genome were classified as unique. The MAUVE analysis revealed a significant portion of the genetic information has been conserved among DSM 11674 and TM300, as the majority of the local collinear blocks are shared by these two strains (Figure S3 in Supplementary Material).

**Fermentative Activity-Associated Genes**

*In silico* analyses revealed that complete pathways involved in the reduction of nitrate to nitrite (nitrate reductase, WP_047131530) and further to ammonia (nitrite reductase, WP_047131535) were found in the genome of DSM 11674. Two catalases (WP_047130958, WP_047132101) were also identified in genome. These data are in agreement with our enzyme activity results and provide clues to explain the production of both nitrate reductase and catalase in DSM 11674. In addition, two l-lactate dehydrogenase (WP_047131934, WP_047132743) and two d-lactate dehydrogenase (WP_047132560, WP_047131604) were encoded, which match with the phenotypic trait that both l-lactate and d-lactate are produced in this strain (Probst et al., 1998).

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1https://github.com/PacificBiosciences/SMRT-Analysis.
2http://cge.cbs.dtu.dk/services/VirulenceFinder/.
FIGURE 1 | Genomic comparison of the Staphylococcus condimenti DSM 11674 with other staphylococci. (A) Phylogenetic tree based on all core gene sequences of 21 staphylococci complete genomes. Multiple sequence alignments of concatenated core gene sequences were calculated within Roary pipeline. The branch of the members of the S. condimenti is delimited by a red dash line. The isolates used in this study include Staphylococcus schleiferi 1360-13 (CP009470), Staphylococcus epidermidis RP62A (CP000029), S. epidermidis ATCC 12228 (AE015929), Staphylococcus capitis AYP1020 (CP007601), Staphylococcus warneri SG1 (CP003668), Staphylococcus pasteurii SP1 (CP004014), Staphylococcus haemolyticus S167 (CP013911), S. haemolyticus JCSC1435 (AP006716), Staphylococcus lugdunensis HKU09-01 (CP001837), Staphylococcus hyicus (CP008747), S. condimenti DSM 11674 (CP015114), Staphylococcus carnosus TM300 (AM295250), Staphylococcus xylosus SMQ-121 (CP008724), S. xylosus C2a (LN54884), S. xylosus HKUQPL8 (CP007208.1), Staphylococcus saprophyticus ATCC 15305 (AP008934), Staphylococcus aureus RF122 (AJ938182), S. aureus COL (CP000046), S. aureus EDH (CP001781), S. aureus JH1 (CP000736), and S. aureus JH9 (CP000703). (B) Core genome analysis of S. condimenti DSM 11674, S. carnosus TM300, and S. xylosus SMQ-121. Numbers inside the Venn diagrams indicate the number of genes found to be shared among the indicated genomes.
Interestingly, lactate dehydrogenase has been reported to play a role in the improvement of starter fermentative activity (Cheng et al., 2014). Therefore, these results indicated that DSM 11674 has strong potential for use as a novel starter culture.

**Salt-Dependent and Salt Acclimation Genes**

During soy sauce mash fermentation, the DSM 11674 strain experiences significant osmotic stress. To explain the genetic determinants involved in the acclimation of this strain to high salt conditions, we identified several genes known to be important for survival under saline stress (Table 1). The strain contains six Na\(^+\)/H\(^+\) antiporter subunits and seven monovalent cation/H\(^+\) antiporter subunits, which are homologs of the antiporter genes of S. carnosus TM300. Furthermore, we identified 15 additional salt-dependent and salt acclimation genes in the DSM 11674 strain. This high content of osmoprotective factors in the genome is consistent well with the ability of this species to grow readily in the presence of 15% NaCl (Probst et al., 1998).

| Gene name | Protein product | Length | Function of gene product |
|-----------|----------------|--------|--------------------------|
| nhaA      | WP_047132913   | 805    | Na\(^+\)/H\(^+\) antiporter subunit A |
| nhaD      | WP_047132917   | 498    | Na\(^+\)/H\(^+\) antiporter subunit D |
| nhaG      | WP_047132908   | 122    | Na\(^+\)/H\(^+\) antiporter subunit G |
| nhaA      | WP_047132066   | 511    | Na\(^+\)/H\(^+\) antiporter subunit A |
| nhaE      | WP_047132563   | 162    | Na\(^+\)/H\(^+\) antiporter subunit E |
| nhaC      | WP_047131754   | 472    | Na\(^+\)/H\(^+\) antiporter subunit C |
| prk12573  | WP_047132912   | 142    | Monovalent cation/H\(^+\) antiporter subunit B |
| prk12651  | WP_047132910   | 159    | Monovalent cation/H\(^+\) antiporter subunit E |
| prk12600  | WP_047132909   | 98     | Monovalent cation/H\(^+\) antiporter subunit F |
| prk12646  | WP_047132567   | 824    | Monovalent cation/H\(^+\) antiporter subunit A |
| prk12574  | WP_047132566   | 141    | Monovalent cation/H\(^+\) antiporter subunit B |
| prk12663  | WP_047132564   | 498    | Monovalent cation/H\(^+\) antiporter subunit D |
| prk12657  | WP_047132562   | 98     | Monovalent cation/H\(^+\) antiporter subunit F |
| prk10429  | WP_047131598   | 477    | Melibiose/sodium symporter |
| sdf       | WP_047132975   | 426    | Na\(^+\)/dicarboxylate symporter |
| alsT      | WP_047131152   | 487    | Na\(^+\)/alanine symporter |
| alsT      | WP_047131695   | 548    | Na\(^+\)/alanine symporter |
| yufF      | WP_047132905   | 438    | Na\(^+\)/proton antiporter |
| nhaP      | WP_047131353   | 679    | Na\(^+\)/proton antiporter |
| tyy1      | WP_047132939   | 443    | Sodium-dependent transporter |
| tcyP      | WP_047132269   | 461    | Na\(^+\)/dicarboxylate symporter |
| putP      | WP_047133075   | 518    | Na\(^+\)/alanine symporter |
| putP      | WP_047132835   | 513    | Na\(^+\)/proline cotransporter PutP |
| arsB      | WP_047133077   | 497    | Anion permease ArsB/NhaD |
| yhaQ      | WP_047131485   | 299    | Sodium ABC transporter ATP-binding protein |
| natB      | WP_047131484   | 409    | Sodium ABC transporter permease |
| nhaC      | WP_047131472   | 437    | Sodium/proton antiporter |
| ccmA      | WP_047132811   | 296    | Sodium ABC transporter ATP-binding protein |

**Stress Response and Antimicrobial Resistance Genes**

The DSM 11674 genome also possesses genes encoding an ATP synthase complex (WP_047132344-WP_047132350), which enables regulation of the internal pH and could confer the ability to adapt to stressful conditions (Cotter and Hill, 2003). Moreover, we identified two cold-shock protein-encoding (CspA and CspC) genes in the chromosome, which are involved in stress responses (Katzif et al., 2003). Also, the heat-shock regulon (hrcA-grpE-dnaK-dnaJ and groESL) (Singh et al., 2007; Rossi et al., 2017) and several other heat-shock protein encoding genes were found in DSM 11674. Finally, the screening of antimicrobial resistance genes revealed a putative β-lactamase encoding gene; this was consistent with susceptibility testing results. Thus, DSM 11674 strain shows technological characteristics that makes it a good candidate for biotechnological application.

In summary, this study reports the complete genome sequence of S. condimenti, a bacterial strain that is potentially useful in a variety of food preparation applications. Genomics-based analysis of this functional staphylococci starter culture candidate revealed important insights into its metabolic capacities and niche adaptations. This is also the first comparative genome sequence analysis of staphylococci starter culture strains, revealing their core genome and pan genome. Finally, the biochemical and genetic characteristics of S. condimenti DSM 11674 revealed in this study are essential to generate further insights into the functional role of staphylococci in general and S. condimenti in particular during the food fermentation process.

**ETHICS STATEMENT**

This article does not contain any studies with human participants or animals performed by any of the authors.

**DATA ACCESS**

The complete genome sequence of Staphylococcus condimenti DSM 11674 has been deposited at DDBJ/EMBL/GenBank under the accession number CP015114.

**AUTHOR CONTRIBUTIONS**

BZ conceived and designed the research; HD and JC performed experiments and analyzed data; LG and AH analyzed data; BZ, HD, and AH wrote the manuscript; and all authors commented on the manuscript and approved the contents.

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activity of S. condimenti DSM 11674. Enzyme activity was measured as spectrophotometric methods. Staphylococcus aureus ATCC 25923 and clinical isolate S. condimenti CJ1628 were used for comparison.

FIGURE S2 | Genome atlas of Staphylococcus condimenti DSM 11674. The circles represent (from the outside to the inside): circle 1, reverse CDS (cyan); circle 2, forward CDS (yellow); circle 3, rRNA (blue); circle 4, rRNA (red); circle 5, GC plot; and circle 6, GC skew.

FIGURE S3 | Genomic comparison of the Staphylococcus condimenti DSM 11674 with starter culture strains Staphylococcus carnosus TM500 and Staphylococcus xylosus SM2121 by Mauve. Alignment is represented as local collinear blocks (LCBs) filled with a similarity plot. LCBs of conserved sequences among the strains are represented by rectangles of the same color. Connecting lines can be used to visualize synteny or rearrangement. LCBs positioned above or under the chromosome (black line) correspond to the forward and reverse orientation, respectively. The level of conservation is equivalent to the level of vertical color filling within the LCBs. Sequences not placed within an LCB are unique for the particular strain.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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