An antimicrobial *Staphylococcus sciuri* with broad temperature and salt spectrum isolated from the surface of the African social spider, *Stegodyphus dumicola*

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Abstract

Some social arthropods engage in mutualistic symbiosis with antimicrobial compound-producing microorganisms that provide protection against pathogens. Social spiders live in communal nests and contain specific endosymbionts with unknown function. Bacteria are also found on the spiders' surface, including prevalent staphylococci, which may have protective potential. Here we present the genomic and phenotypic characterization of strain i1, isolated from the surface of the social spider Stegodyphus dumicola. Phylogenomic analysis identified i1 as novel strain of Staphylococcus sciuri within subgroup 2 of three newly defined genomic subgroups. Further phenotypic investigations showed that S. sciuri i1 is an extremophile that can grow at a broad range of temperatures (4°C-45°C), high salt concentrations (up to 27%), and has antimicrobial activity against closely related species. We identified a lactococcin 972-like bacteriocin gene cluster, likely responsible for the antimicrobial activity, and found it conserved in two of the three subgroups of S. sciuri. These features indicate that S. sciuri i1, though not a specific symbiont, is well-adapted to survive on the surface of social spiders and may gain a competitive advantage by inhibiting closely related species.

Introduction

Many social arthropods (e.g. ants, termites, wasps, and bees) engage in defensive mutualistic symbioses with antimicrobial-producing microbes (Currie et al., 1999; Hughes et al., 2008; Madden et al., 2013; Kaltenpoth and Engl, 2014). This symbiotic relationship is beneficial for the host, as the symbionts counteract some of the costs of social-living, namely the increased risk of spread of disease due to the dense concentration of closely-related individuals living and interacting with each-other (Fefferman et al., 2007; Liu et al., 2019). The association between hosts and their microbial symbionts can be stable and long-term, where symbionts are transmitted vertically from one generation to the next (Currie et al., 1999; Clay, 2014). Alternatively, protective symbionts can be recruited from the surrounding environment and maintained by the host providing the right growth conditions for symbiont proliferation (Chouvenc et al., 2013, 2018).

The African social spider *Stegodyphus dumicola* is found in subtropical deserts characterized by extreme daily fluctuation in temperature and overall low humidity (Bilde and Lubin, 2011). The spiders live in large communal nests that can persist for several generations and contain up to thousands of individuals (Smith et al., 2016). Within the nests, spiders cooperate in various activities such as building capture webs and capturing prey, maintaining the nest, and taking care of brood (Lubin and Bilde, 2007). A key characteristic of social spiders is their extremely low species-wide genetic diversity caused by a permanent inbreeding mating system, female-biased sex-ratio, and reproductive skew (Lubin and Bilde, 2007; Settepani et al., 2017). The inbreeding mating system of social spiders is thought to have short-term advantages and more fatal long-term consequences, leading to high risks of colony extinction due to a reduced ability to respond to pathogens and other environmental challenges (Agnarsson et al., 2013; Smith et al., 2016). Therefore, it is tempting to speculate that *S. dumicola*, like other social arthropods, might harbor defensive bacterial symbionts that could aid in protection against pathogens.

In a previous study, 16S rRNA gene amplicon sequencing of three social spider species revealed that the spider microbiomes were dominated by a few specific endosymbionts but amplicon sequence variants (ASVs) indicative of spider surface microbes were also detected (Busck et al., 2020). We therefore aimed at isolating and characterizing putative symbiotic bacteria from the surface of social spiders. One isolate, i1, had 100% 16S rRNA gene sequence identity to an ASV found in 35% of all spiders, mostly with low relative abundance (< 1%) but occasionally as high as 48%, and classified as *Staphylococcus* (Busck et al., 2020). Their broad distribution and sometimes high abundance in *Stegodyphus* spiders may indicate a (facultative) symbiotic relationship. Here we present genomic and phenotypic data to identify the isolate as *Staphylococcus sciuri* and provide indications for its success on social spiders.

Materials And Methods

Isolation and 16S rRNA gene sequencing

Strain i1 was isolated from an individual female of the social spider *Stegodyphus dumicola*. The spider was rubbed against 0.3% Tryptic soy broth agar plates (Schaurlau, Spain) supplemented with 0.5% Yeast extract (Merck, Spain) and 50 mg mL-1 Nystatin (Sigma Aldrich, Germany) using sterile forceps. The plates were incubated at 28°C for five days under aerobic conditions, and colonies were streaked to purity. The isolates' phylogenetic affiliation was quickly screened by colony-PCR of the 16S rRNA gene using primers EUB26F (Hicks, Amann and Stahl, 1992) and 1492R (Loy et al., 2002) and HotStar Taq Polymerase (QIAGEN) in a standard PCR with 57°C annealing. Purified PCR products (GenEluteTM PCR Clean-Up kit; Sigma-Aldrich, St.Louis, MO) were Sanger sequenced by Macrogen Europe and compared to the NCBI database (https://www.ncbi.nlm.nih.gov) using BLAST (Altschul et al., 1990).

Genome sequencing, assembly, and annotation

DNA was extracted from strain i1 using the DNeasy PowerLyzer PowerSoil DNA Isolation Kit (Qiagen); quality control, library preparation using Nextera XT DNA library preparation kit (Illumina), and 2 x 300 bp paired-end sequencing using the MiSeq System (Illumina) was performed in-house according to Illumina's standard protocol. The quality of the raw sequencing reads was verified using FastQC v0.11.4 (Wingett and Andrews, 2018) and trimmed using Trimmomatic v0.36 (Bolger et al., 2014) with a +33 Phred score, a quality cutoff of 20 in a 4-nucleotide window, and a minimum length of 100 bp. The trimmed reads were assembled using SPAdes v3.10.1 (Bankevich et al., 2012) with the default k-mers 21, 33, 55, 77, 99 and 127; the assembly was run with the option careful to reduce the number of mismatches and the cov-cutoff option for automatic calculation of the coverage cutoff threshold.

Scaffolds that most likely did not belong to the genome of interest were removed using a homemade Python script: first, the trimmed reads were mapped to the assembly using BBmap v.36.49 (https://sourceforge.net/projects/bbmap/) with default values to calculate per-scaffold coverage and GC-content. To determine the threshold for coverage and GC-content, respectively, the webtool Plotly v.2.0 (https://plot.ly/) was used to plot the calculated GC-content against coverage and scaffolds outside the coverage and GC threshold along with scaffolds shorter than 1000 bp were removed. CheckM v0.7.0

(Parks et al., 2015) and Quast v4.3 were used to assess the quality of the final assembly, along with a BLAST search against the SILVA SSU 132 16S rRNA gene reference database to identify 16S rRNA sequences in the genome (Gurevich et al., 2013). The decontaminated assembly was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016) and deposited in NCBI GenBank under accession number PRJNA412144. Protein-coding genes were classified into COG categories using the functional annotation tool EggNOG-mapper v.1, and the KEGG database was used to identify gene functions (Kanehisa et al., 2016; Huerta-Cepas et al., 2017).

Taxonomic analysis

To determine the taxonomy of the assembled genome, we used the web-based classification tool Microbial Genomes Atlas (Rodriguez-R et al., 2018) for whole genome comparison against the NCBI Genome database. Based on these results the average nucleotide identity (ANI) was calculated for the genomes of strain i1 and all 52 strains of *S. sciuri* available in NCBI using an online ANI matrix calculator (Rodriguez-R and Konstantinidis, 2016). Single copy orthologues common for these genomes were identified, and a concatenated protein alignment was produced using GTDB-TK v. 0.1.6 (Parks et al., 2018). Based on the alignment, a phylogenetic tree was built in IQ-Tree v.1.6.10, with the option testnew to find the best-fit substitution model, and with 100 bootstraps (Nguyen et al., 2015). The tree was visualized with FigTree v.1.4.4 (http://tree.bio.ed.ac.uk).

Mining for biosynthetic gene clusters (BGC)

The genome of strain i1 was analyzed with the bacteriocin genome mining web-tool BAGEL4 (Van Heel et al., 2018), and with AntiSMASH v. 5.0 (Blin et al., 2019) using the analysis parameters KnownClusterBlast, ActiveSiteFinder, SubClusterBlast and Clusterblast analysis. All genes identified in putative BGC were blasted against the NCBI database using BLASTp. In case the highest identity score was for a hypothetical protein, such as for genes SS2 and SS3, the amino acid sequences were uploaded to InterPro (https://www.ebi.ac.uk/interpro/) for further information about putative protein functions. Characterized precursor bacteriocins with similarity to the putative precursor bacteriocin identified in strain i1 were extracted from the BAGEL4 database. The amino acid sequences were aligned in Geneious v. 11 (Kearse et al., 2012) using the multiple sequence alignment tool MUSCLE with default settings and subsequently visualized using Jalview 2 (Waterhouse et al., 2009). The extended Clusterblast analysis was conducted using MultiGeneBlast, downloaded from (http://multigeneblast.sourceforge.net) (Medema et al., 2013). The BLAST database was constructed using five selected *S. sciuri* genomes per subgroup, i.e., those with the lowest number of contigs (< 200), retrieved from NCBI (see Figure 2a).

Phenotypic characterization of strain i1.

Carbohydrate fermentation. The ability to degrade various types of carbohydrates (Table 1) was tested using a simple fermentation test. Briefly, phenol red broth contained 1% NaCl, 0.018% phenol red (Sigma-Aldrich, St.Louis, MO), and 1% trypticase (BD, Le Pont de Claix, France). Filter-sterilized carbohydrate solutions were added to the cooled phenol red broth to final concentrations of 1%. The tubes were

inoculated with 100 µL of an overnight culture of *S. sciuri* i1 and incubated at 35°C for two days without agitation.

Temperature range. Growth of strain i1 was tested at 4°C, 10°C, 15°C, 19°C, 23°C, 25°C, 25°C, 29°C, 30°C, 35°C, 37°C, 42°C, 45°C, 51°C, and 62.5°C on replicate nutrient broth (Scharlau, Spain) agar (NBA) plates. Plates were incubated under aerobic conditions for seven days.

Salt tolerance. Growth of strain i1 and the type strain *S. sciuri* SC116 under different salt concentrations was tested on replicate NBA plates supplemented with 2%, 4%, 6%, 8%, 10%, 12%, 15%, 17%, 20%, 22%, 25%, 27%, 30%, and 35% NaCl. Plates were incubated at 35°C under aerobic conditions for three days.

Antimicrobial testing. A modified version of the Kirby-Bauer Disc Diffusion Susceptibility Test protocol (Hudzicki, 2009) was used to determine the antimicrobial activity of strain i1 against the *S. sciuri* type strain (*S. sciuri* subsp. *sciuri* DSM20345), *S. epidermidis* ATCC 12228, *Bacillus subtilis* SN1 (isolated from an *S. dumicola* spider nest; Nazipi et al. submitted; NCBI genome accession PHIE01000000), *Escherichia coli* DSM022, and *Pseudomonas putida*. Briefly, the test isolates were suspended in 0.9% sterile saline to a final OD600 of 0.125 and an even lawn was spread onto NBA plates using sterile cotton swabs. The antimicrobial activity of i1 was tested with 10 µL cell-free supernatants from an overnight culture and from a 7-day-old culture, and with actively growing colonies point-inoculated directly onto the test plates. The plates were then incubated under aerobic conditions at 35°C and checked for inhibition zones after 24-48 hours.

Results And Discussion

Isolation

In total five distinct morphotypes appeared on plates after 2-3 days, with milky-white, circular colonies as the dominant type. Cells of these colonies were Gram-positive cocci, 0.7 to 1.2 µm in diameter, non-motile and non-sporeforming, and occurred singly or in pairs; their partial 16S rRNA gene sequence represented an ASV found in 35% of all sequenced spiders of a previous study (Busck et al., 2020). was 100% identical to *S. sciuri*.

Genome sequencing and assembly

We generated 6,642,442 raw paired-end reads that assembled into 45 scaffolds; the final, quality-filtered assembly of the i1 genome consisted of 8 scaffolds with a total length of 2.8 Mb, a coverage of 693x, and an N50 of 671,126 bp; the largest contig was 1,185,439 bp. Genome completeness was 98.56% with 6.30% contamination at the genus level; the GC-content was 32.42%, and one 1,533 bp-long 16S rRNA gene sequence was identified with 100% identity to *S. sciuri*. This species is typically host-associated and parasitic or pathogenic in diverse vertebrates but has also been found associated with various arthropods or plants (Kloos et al., 1976; Kloos et al., 1997; Stepanovi et al., 2001; Hauschild and Schwarz, 2003).

Genome-based identification and phylogeny

From the whole genome comparison of the assembled genome to other related genomes, strain i1 belongs to the genus *Staphylococcus* (p-value: 0.0027) and the species *S. sciuri* (p-value: 0.039). Based on pairwise ANI, all 52 *S. sciuri* genomes and strain i1 were highly similar (96-100%) except for *S. sciuri* subsp. *rodentium* that showed only 76-78% ANI to all other *S. sciuri* strains (Table S3). The ANI matrix indicated that *S. sciuri* can be divided into three distinct genomic groups, with more than 98% ANI within each group (Figure 1, Table S1). Strain i1 belongs to Group 2, together with only five other *S. sciuri* strains, i.e., strain P879, BL01, P575, NS202, and SAP15-1 (Figure 1, Table S1). This group was confirmed by our phylogenomic analysis based on concatenated single copy orthologues, which grouped strain i1 with high confidence (bootstrap values >80%) together with the same five strains. Likewise, Group 1 was recovered with high confidence (Figure 1). In contrast, the strains of the third group did not form a coherent genomic cluster and most branchings were only poorly supported (bootstrap values <50%), possibly due to the poor genome quality in this group. Taken together, our combined ANI and phylogenomic results indicate that the species *S. sciuri* can be genomically subdivided into three distinct groups, which are represented by *S. sciuri* subsp. *sciuri* DSM20345 (Group 1), our novel isolate *S. sciuri* i1 (Group 2), and *S. sciuri* subp. *carnaticus* CCUG 39509 (Group 3), respectively.

Genomic and phenotypic characterization

The genome of strain i1 contained 2,876 genes, including 72 RNA genes (54 tRNAs, 14 rRNAs) and 24 pseudogenes. Of the 2,780 protein-coding genes, 87%were assigned to COG categories, with the largest COG category encoding unknown proteins (20.3%) (Table S2). The most represented classes of proteins with known function were involved in metabolism and transport of amino acids (8.3%) and carbohydrates (7.9%), and in transcription (7.6%) (Table S2). Using the KEGG-database, 1,660 genes (59%) were assigned a putative function, indicating that strain i1 has the genetic potential for glycolysis, gluconeogenesis, the citric acid cycle, both oxidative and non-oxidative pentose phosphate pathway, and fermentation of pyruvate to acetate (Table S3). *S. sciuri* i1 furthermore encodes both aerobic respiration and anaerobic respiration by dissimilative reduction of nitrate to ammonium, and the biosynthesis of most building blocks required for growth, such as amino acids, fatty acids, and nucleotides (Table S3). The genome encodes the conserved Sec-SRP secretion system, 16 complete ABC transporters and 15 phosphotransferase systems (PTS) (Table S3). *S. sciuri* i1 and other Group 2 strains encode all genes necessary for degradation of seven out of the 15 tested carbohydrates (Table 1). In contrast, *S. sciuri* i1 was able to degrade all 15 carbohydrates except for arabinose (Table 1), which could indicate that the transporters for lactose, xylose, maltose, fucose, raffinose, galactose, and glycerol have not been identified in the annotation or (less likely) have been missed during sequencing and assembly.

The genomic grouping of the species *S. sciuri* (Figure 1) is reflected in the phenotypes of representative strains of the three groups (Table 1): the carbohydrate degradation pattern was distinct for *S. sciuri* subsp. sciuri DSM20345 (Kloos et al., 1976; Group 1), *S. sciuri* i1 (Group 2), and *S. sciuri* subp. carnaticus CCUG 39509 (Kloos et al., 1997; Group 3), with i1 as the only strain to form acid from raffinose but otherwise most similar to Group 1, even though this had not be inferred from the genomic analysis (Table 1).

S. sciuri can generally grow at high salt concentrations and high temperatures (Table 1), and strain i1 is no exception: it grew in the presence of up to 27% NaCl, classifying it as extremely halotolerant (Larsen, 1986), and over a broad temperature spectrum (4°C-45°C; Table 1). This is in agreement with the genetic potential for a glycine betaine/proline ABC transporter responsible for the import of osmoprotectants (Table S3). The accumulation of osmoprotectants such as glycine betaine, proline, and choline in response to osmotic stress is known in e.g., *S. aureus* (Amin, Lash and Wilkinson, 1995; Peddle et al., 1999). In addition, the branched-chain amino acid transporter BrnQ confers salt tolerance in *S. aureus* (Vijaranakul et al., 1997; Lee, Heo and Jeong, 2018). The brnQ gene is also found in the *S. sciuri* i1 and the predicted protein shows 97.98% sequence similarity to that of *S. aureus* (BlastP analysis). The ability to grow over a broad range of temperature combined with the ability to tolerate high salt concentrations and the described genomic imprints suggest that *S. sciuri* i1 is well-adapted to life on the surface of spiders, whose natural habitat are desert environments with extreme temperature fluctuations and aridity (Bilde and Lubin, 2011).

Antimicrobial activity of *S. sciuri* i1 is restricted to closely related species.

S. sciuri i1 inhibited the growth of the closely related species, *S. epidermidis* ATCC 12228, but only when in direct contact with the test strain. No inhibition was detected against a strain of the same species (*S. sciuri* subsp. *sciuri* DMS20345) or against other genera, and cell-free supernatants of strain i1 were never inhibitory (Table S4).

Two biosynthetic gene clusters were identified by antiSMASH, a bacteriocin cluster and a siderophore cluster with no similarity to known BGCs found in the MIBiG repository (Figure S1). Bacteriocins are antibacterial proteins with antibacterial activity, often with narrow specificity against closely related species and inducible by high cell densities and co-culture (Jack, Tagg and Ray, 1995; Nes and Holo, 2000; Eijsink et al. 2002).

Since the inhibitory activity of *S. sciuri* i1 was only detected during co-cultivation and only against the close relative *S. epidermidis* ATCC 12228, we suspect that the active compound was a bacteriocin and will now focus on describing the bacteriocin gene cluster.

Characterization of the BGC encoding the *S. sciuri* i1 lactococcin 972-like bacteriocin

The biosynthesis of *S. sciuri* i1 bacteriocin was predicted to involve 11 genes with the core biosynthetic gene identified as a lactococcin 972 family bacteriocin (Figure S1). Lactococcins are classified as subclass IId bacteriocins characterized as single-peptide, linear, and non-pediocin-like bacteriocins that do not undergo post-translational modifications (Yang et al., 2014). Lactococcin is initially synthesized as an inactive pre-form before being processed and secreted by either specific membrane-bound ABC transporters or via the sec-dependent pathway (Stoddard et al., 1992; Nes et al., 1996; Martínez, Rodríguez and Suárez, 2000). In general, the lactococcin gene cluster consists of four genes, 1) precursor bacteriocin, 2) immunity gene(s), 3) ABC-type transporter, and 4) transport accessory genes (Alvarez-Sieiro et al., 2016; Daba et al., 2017). Lactococcin 972 has previously been predicted to be synthesized by

two genes, lclA encoding the 91-residue precursor lactococcin 972, and lclB encoding a 563-residue putative immunity protein (Martínez et al., 1999). More recently, it was suggested that four genes are associated with the biosynthesis of lactococcin 972-like bacteriocins in *Staphylococcus* (Lundström, 2012). Based on this information and the fact that the BGC is well-defined and highly conserved (Figure 2a, S2), we propose that the BGC of the *S. sciuri* i1 bacteriocin is composed of four genes: SS1, SS2, SS3, and SS4 (Figure 2b).

Gene SS1 encodes a 97-amino acid precursor bacteriocin with low identity to characterized lactococcin 972-like bacteriocins retrieved from the BAGEL4 database, i.e., 25% identity with *Lactococcus lactis* lactococcin 972, 27% identity with *Thermobifida fusca* XY lactococcin, and 35% identity with *S. aureus* bacteriocins, respectively (Figure 2c). It contains signal sequences for excretion via the ABC-transporter pathway and sec-dependent pathway (Figure S3).

Gene SS2 encodes a 661-residue transmembrane protein with low similarity (46 % amino acid identity) to a transmembrane lactococcin 972-family protein associated with immunity (InterPro entry: IPR006541; (Martínez, Rodríguez and Suárez, 2000). The function of the immunity genes is to protect the bacterium from the bactericidal effect of the secreted bacteriocin (Jack, Tagg and Ray, 1995; Eijsink et al. 2002). The lack of antimicrobial activity against a strain from the same species (*S. sciuri* subsp. sciuri DSM20345), which encodes the entire BGC including the putative immunity gene (Figure 2a), and the absence of SS2-like immunity genes in the sensitive *S. epidermidis* ATCC 12228 further supports our prediction of SS2 as immunity gene in the bacteriocin cluster.

Gene SS3 encodes a small protein with 100% amino acid identity to an YxeA family protein of *S. sciuri*. YxeA family proteins are exclusively found in Gram-positive bacteria and encoded near ABC-transporters (InterPro accession number: IPR006541). Even though very little is known about the function of the YxeA protein family, some evidence of YxeA as an accessory protein is found in *B. subtilis*. In *B. subtilis*, YxeA is co-transcribed with an ABC-transporter and conveys immunity against cationic antimicrobial peptides (Joseph et al., 2004; Pietiäinen et al., 2005). In another study, the accessory protein (LcnD) and ABC-transporter (LcnC) were found to be essential for the transportation and maturation of lactococcin A (Stoddard et al., 1992; Franke et al., 1996). Given the position and homology of SS3 to known accessory proteins, we propose that SS3 in *S. sciuri* i1 functions as an accessory transport protein.

The last gene of the BGC, SS4, was predicted as an ABC transporter ATP-binding protein (100% amino acid identity). The ATP-binding protein makes up the cytoplasmic domain of ABC-transporters (Havarstein, Diep and Nes, 1995; Nes et al., 1996). For lactococcin 972-like bacteriocins, it has been proposed that the ATP-binding protein (SS4), together with the transmembrane immunity protein (SS2), constitutes the complete ABC-transporter system involved in immunity and secretion for these bacteriocins (Lundström 2012). We suggest that a similar mechanism is utilized by *S. sciuri* i1 for its bacteriocin secretion and immunity.

The lactococcin 972-like BGC was detected in group 1 and 2 but not group 3 *S. sciuri*.

The initial ClusterBlast analysis from antiSMASH revealed that the Lactococcin 972-like BGC was highly conserved in *S. sciuri* but also found in some other *Staphylococcus* species (Figure S2). Motivated by this result, we extended the ClusterBlast to include representatives of all three *S. sciuri* subgroups. Only strains belonging to Group 1 and 2 contained the lactococcin 972-like BGC (Figure 2a), while it was never detected in Group 3. This finding corroborates the ANI-based grouping and phylogenetic clustering of *S. sciuri*, with Groups 1 and 2 more similar compared to Group 3.

In conclusion, we have isolated a novel *S. sciuri* strain from the surface of the social spider *Stegodyphus dumicola*. The phenotypic assays, together with the genomic insight, indicate that *S. sciuri* i1 is not a specific, protective symbiont of social spiders, as it shares similar characteristics with other *S. sciuri* strains isolated from many different animal hosts. However, with its halotolerance, broad temperature range, and its antibacterial activity against closely related species, *S. sciuri* appears well-adapted to the extreme niche on the body surface of social spiders in the African desert. Furthermore, we have characterized the lactococcin 972-like bacteriocin gene cluster, which is widely conserved in two of the three subgroups of *S. sciuri* and may be involved in a competitive advantage of *S. sciuri* i1 and related strains against other staphylococci. Whether this could also be beneficial to the spider (or other) hosts remains currently unresolved.

Declarations

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Conflicts of interest/Competing interests. None.

Ethics approval, Consent to participate, Consent for publication. Not applicable

Availability of data and material. The genome sequence of *S. sciuri* i1 has been deposited in NCBI GenBank under accession number PRJNA412144. The strain is available from the authors on request.

Code availability. All software used is publicly available as stated in the methods section.

Authors' contributions. SN, SV, TB, AS conceived and designed the study; SN, SV, DKL performed research; SN, SV, MMB, IM, MBL, AS analyzed data; SN, SV, AS wrote the paper.

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Table

Table 1. Phenotypic and genomic traits of *Staphylococcus sciuri* Group 1, 2, and 3 strains

	Genomic characterization[a]			Phenotypic characterization		
	Group 1 (n = 5)	Group 2 (n = 5)	Group 3 (n = 5)	*S. sciuri* subsp. *sciuri* DSM20345[b] (Group 1)	*S. sciuri* i1 (Group 2)	*S. sciuri* subsp. *carnaticus* CCUG 39509[c] (Group 3)
Carbohydrate degradation						
D-(+)-lactose	n.d.[d]	n.d.	n.d.	+	+	-
D-(+)-glucose	100	100	100	+	+	+
D-(+)-mannose	100	100	100	+	+	+
D-(-)-fructose	100	100	100	+	+	+
D-(+)-cellobiose	100	100	100	+	+	-
D-(+)-xylose	n.d.	n.d.	n.d.	-	+	+
D-sorbitol	100	100	100	+	+	+
D-(+)-maltose	n.d.	n.d.	n.d.	+	+	+
D-trehalose	100	100	100	+	+	-
D-(-)-arabinose	0	0	0	+	-	-
Sucrose	100	100	80	+	+	+
D-(+)-fucose	n.d.	n.d.	n.d.	+	+	n.d.
Raffinose	n.d.	n.d.	n.d.	-	+	-
D-(+)-galactose	n.d.	n.d.	n.d.	+	+	n.d.
Glycerol	n.d.	n.d.	n.d.	+	+	n.d.
Temperature range						
Growth range ()	NA[e]	NA	NA	15 - 45	4-45	n.d.
Optimum ()	NA	NA	NA	n.d.	15-45	n.d.
Salt tolerance						
NaCl range (%)	NA	NA	NA	2-27	2-27	n.d.
Optimum (%)	NA	NA	NA	2-15	2-15	n.d.

[a] Genotypic characterization is based on results from *in silico* comparative genomic analysis from this study and expressed as the percentage of strains encoding the necessary genes for complete carbohydrate degradation.

[b] Data from Kloos et al., 1976 & Kloos et al., 1997, except for salt tolerance (this study).

[c] Data from Kloos et al., 1997.

[d] n.d., no data available

[e] NA, not applicable.

Figures

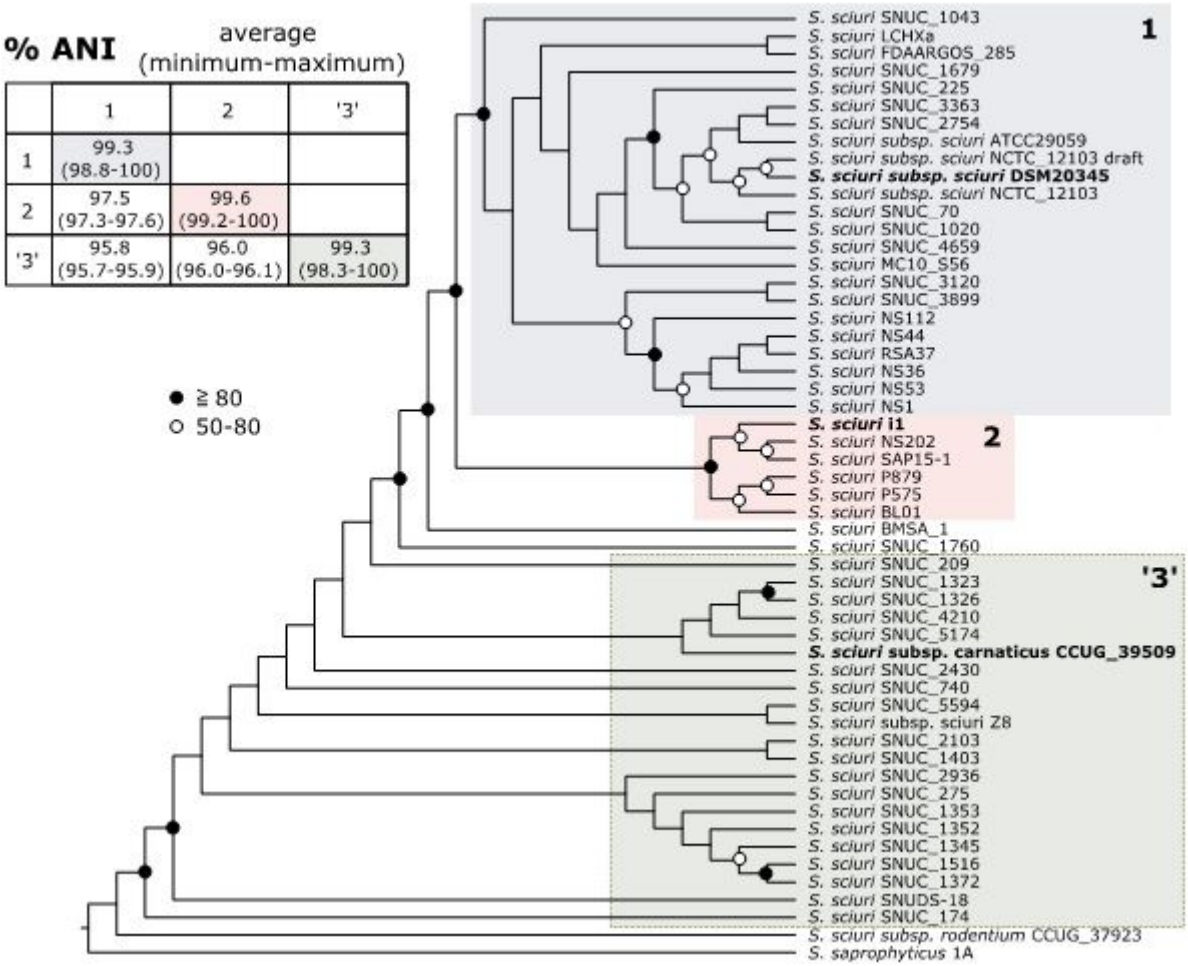

Figure 1

Phylogeny of S. sciuri i1 and grouping of S. sciuri strains. The concatenated protein alignment tree is based on 52 S. sciuri genomes obtained from NCBI with S. saprophyticus 1A as the outgroup. The bootstrap values > 50% are indicated by circles at each node; nodes without circles have bootstrap values <50%. The coloured boxes highlight the grouping of S. sciuri strains into three phylogenetic groups based on % ANI similarity (inset).

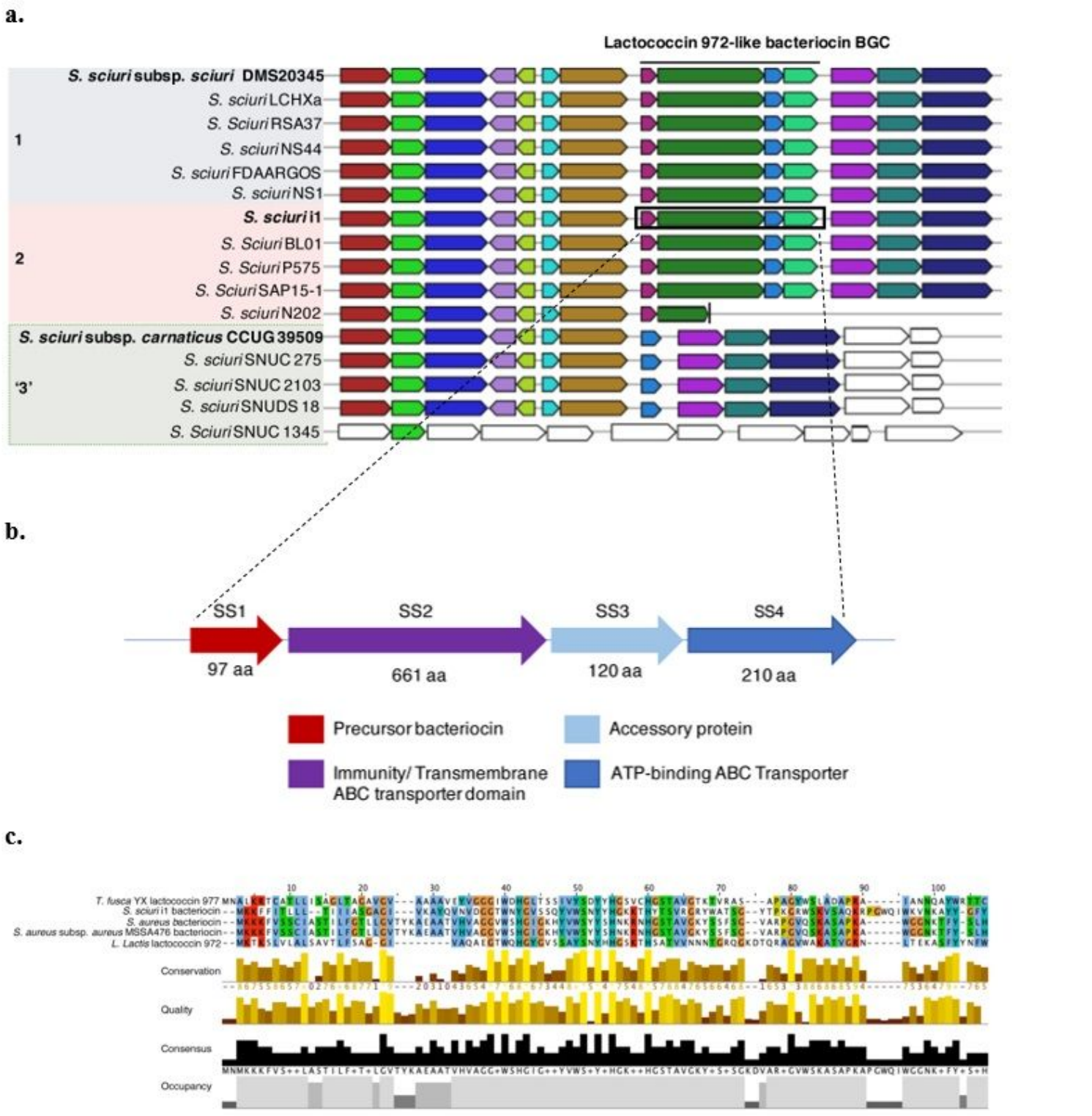

Figure 2

The putative lactococcin 972-like bacteriocin gene cluster of S. sciuri i1. (a) MultiGeneBlast analysis of genomes representative of group 1 (blue), group 2 (pink) and group 3 (green) S. sciuri. Type strains and S. sciuri i1 are highlighted in bold. (b) Detailed information of the putative S. sciuri i1 lactococcin 972-like bacteriocin gene cluster including gene name, size, and gene annotation. (c) Multiple alignment of S.

sciuri i1 precursor bacteriocin (SS1) based on the MUSCLE algorithm against known subclass IId precursor bacteriocins retrieved from the BAGEL4 database.

Supplementary Files

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