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Staphylococcus brunensis sp. nov. isolated from human clinical specimens with a non-SCC genomic island outside of the *rlmH* gene bearing *ccrDE* recombinase gene complex

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INTRODUCTION

Over the last three decades, coagulase-negative staphylococcal species (CoNS) with the most significant being *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, have been recognized as opportunistic pathogens, especially in immunocompromised patients. The gene pool of substrate utilization pathways and resistance determinants enables CoNS to occupy various niches, providing favourable conditions for the emergence of multidrug-resistant CoNS and their subsequent spread in healthcare environments.

Only recently, molecular diagnostics approaches, mainly in-depth whole genome characterization, have assigned atypical *S. haemolyticus* strains isolated from clinical specimens into the new species *Staphylococcus borealis* (Pain et al., 2020) and *Staphylococcus taiwanensis* (Lin et al., 2022). The core genome phylogeny also led to the reclassification of the *S. petrasii* complex (Pantůček et al., 2013; Švec et al., 2015), which now consists of three species *S. petrasii*, *S. croceilyticus*, and *S. pragensis* (Madhaiyan et al., 2020).

HIGHLIGHTS

- **Based on the results of the polyphasic taxonomic approach, the species *Staphylococcus brunensis* sp. nov. is proposed**
- **A genomic island SbCI$_{ccrDE}$ integrated into the ribosomal protein L7 serine acetyltransferase gene *rimL* is described**
- **SbCI$_{ccrDE}$ harbors new *ccrDE* cassette chromosome recombinase gene complex**
- **The comparative genomics shows that the island accumulates virulence and drug resistance factors. Its spread into established pathogens would pose a threat to the healthcare system.**

RESULTS

1. Taxonomic description of *Staphylococcus brunensis* sp. nov.

The phylogenetic analysis of complete 16S rRNA gene sequences placed the five analyzed isolates in *S. haemolyticus* cluster group. The closest relatives and phenotypically the most similar taxa were *S. petrasii*, *S. pragensis* and *S. haemolyticus.*

The type strain is NRL/St 16/872^T (= CCM 9024^T = DSM 111349^T = LMG 31872^T). The species description is based on the characterization of five strains isolated from various human clinical materials, ear swabs, wounds and bile. The GenBank/ENA/DDBJ accession number for the 16S rRNA gene is OQ401401.

Table 1. Differentiation of *Staphylococcus brunensis* sp. nov. from closely related staphylococci occurring in human clinical material

Test	Result obtained for indicated type strain[a]/Result from species description						
	S. brunensis sp. nov.	*S. petrasii* CCM 8418^T	*S. croceilyticus* CCM 8421^T	*S. pragensis* CCM 8529^T	*S. haemolyticus* CCM 2737^T	*S. borealis* CCM 9145^T	*S. taiwanensis* CCM 9267^T
Arginin dihydrolase	+	+/+	+/+	-/-	+/+	+/+	+/+
Voges-Proskauer test	+	+/+	+/+	+/+	w/+	-/-	+/+
Urease	-	+/d	+/+	-/-	-/-	+/+	+/+
β-glucuronidase[a]	+	-/-	+/+	-/-	+/d	+/d	-/-
DNA hydrolysis	-	w/d	-/-	+/+	+/d	w/-	-/-
Acid from: lactose	+	-/d	-/-	-/-	-/d	-/-	-/-
galactose	+	-/d	-/-	-/-	+/d	-/nt	-/-
mannose	-	+/+	-/-	-/-	-/-	-/d	-/-
ribose	-	-/w	w/w	-/-	-/d	+/+	+/+
D-arabinose	-	-/-	+/+	-/-	-/-	-/-	-/-
N-acetylglucosamine	-	-/-	-/-	-/-	+/+	+/d	-/-
Pale yellow pigment	-	-/-	+/+	-/-	-/-	+/+	-/-

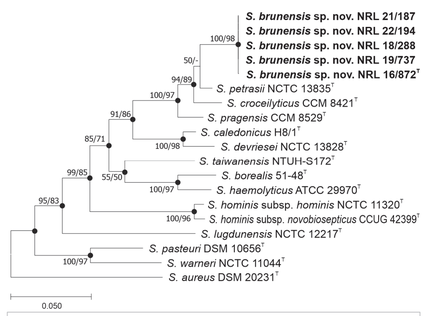

Fig. 1. Unrooted maximum likelihood tree based on multilocus sequence analysis of concatenated nucleotide sequences from six loci – *rpoB*, *hsp60*, *dnaJ*, *tufA*, *gap* and *sodA*. Filled circles indicate that the corresponding nodes were also identified from analysis by the neighbor-joining method.

2. Genome characterization of *Staphylococcus brunensis* sp. nov.

The *S. brunensis* sp. nov. genomes were 2.5–2.6 Mb long with GC content 33.3–33.4% encoding 2,500–2,700 CDS, 61–62 tRNA and 19 rRNA. The pangenome consists of 2,230 core and 416 accessory, and 569 unique genes in total. The sequenced genomes differ in variable genomic elements. The complete chromosome sequence of the type strain is available under GenBank accession number CP119327.

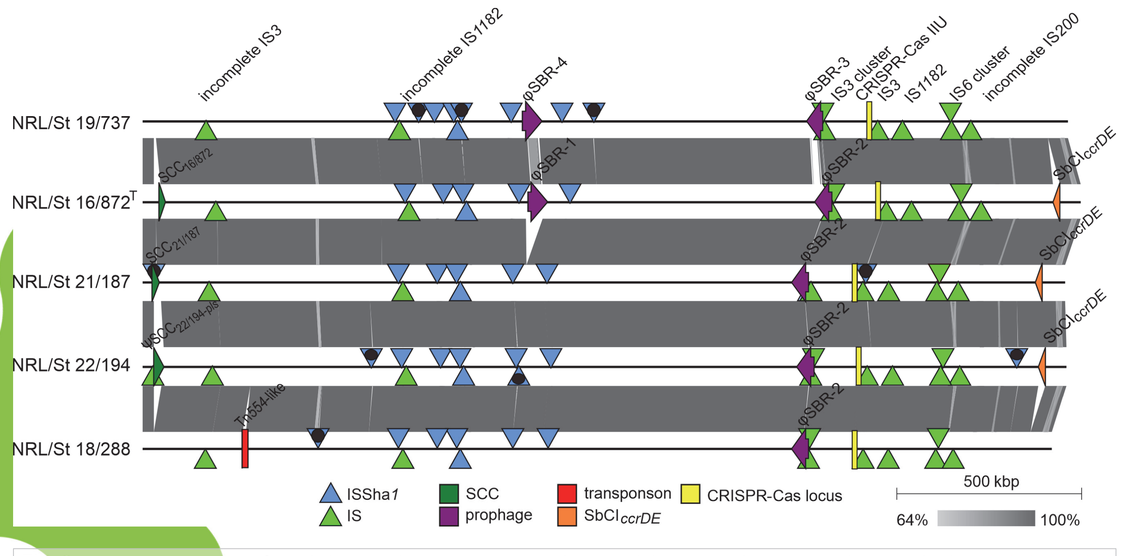

Fig. 2. Genome comparison of *Staphylococcus brunensis* sp. nov. isolates. Mobile genetic elements are shown and colour coded as in the legend. The ISSha1 loci that are divergent for the respective strains are marked with black circles. Only nucleotide blast hits above 64% identity and longer than 2 kb are shown.

3. Novel genomic island harbouring *ccrDE g*enes

The strains NRL/St 16/872T, NRL/St 21/187 and NRL/St 22/194 harbour a mobile element of size 18.8 kb designated SbCI$_{ccrDE}$ with cassette chromosome recombinase genes, integrated in the *rimJ*/*rimL* gene orthologous to the ribosomal-protein-serine acetyltransferase gene. The island harbours another homologue of *rimL* with 70% nt identity to the original *rimL*.

SbCI$_{ccrDE}$ possesses a cluster of genes homologous to the *ccr* gene complex from SCC elements. The core of the *ccr* complex consists of two genes, similar to SCC elements type IV or II. Additional genes in the *ccr* complex, i. e. the putative primase *polA*, cassette chromosome helicase *cch2*, four short hypothetical genes were homologous to those in SCC*mec* type V.

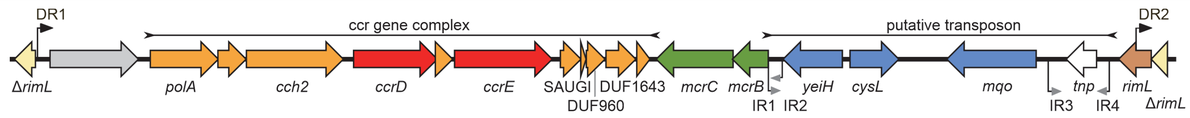

Fig. 3. Annotated map of genes and possible functions in genomic island SbCI$_{ccrDE}$ harboured by *Staphylococcus brunensis* sp. nov. Genes are labelled according to known or putative function. Sequnce of direct repeats DR1, DR2: 5'-ATTCCACAATGAAATCCAT-3'; sequence of inverted repeats IR1–IR4: 5'-TGGTTCTGTTGCAAAGT-3'.

4. Reclassification of *ccrA8B9* to *ccrDE*

SbCI$_{ccrDE}$ *ccr* genes share more than 98% DNA sequence identity to *ccrA8B9* recombinases discovered recently in the *S. haemolyticus* genome (Xiao et al., 2023). The values of nucleotide identity to currently known *ccrA1-7*, *ccrB1-8* and *ccrC1-2* genes from SCCs range from 38.0 to 53.3%. Although it is slightly above the threshold of 50.0% for the definition of a gene variant according to the rules of IWG-SCC (2009), the amino acid (aa) identity level of SbCI$_{ccrDE}$ recombinases to known CcrA, CcrB, and CcrC reaches a maximum of 39.9%, which is substantially lower than aa identity among Ccr allotypes, which range from 50.9 to 92.3%. Therefore, based on the borderline nucleotide identities and low protein identities to CcrA, CcrB, and CcrC allotypes, we propose designating the SbCIccr recombinases as new allelic types *ccrD* and *ccrE.* The reclassification is supported by the distinct integration site of SbCI$_{ccrDE}$ that differs from the canonical methyltransferase gene *rlmH* (*orfX*) exploited by SCCs.

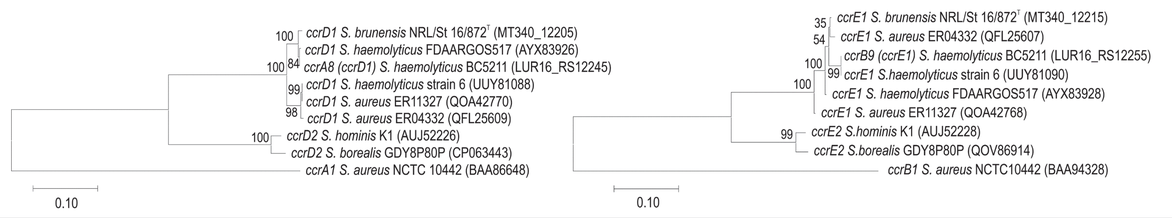

Fig. 4. Maximum likelihood trees of nucleotide sequences of *ccrD and ccrA8* recombinases with *ccrA1* as an outgroup, and *ccrE and ccrB9* recombinases with *ccrB1* as an outgroup, respectively.

5. Comparative genomic analysis of SbCI$_{ccrDE}$ related islands

We surveyed the GenBank database for sequences resembling SbCI$_{ccrDE}$. In addition to *S. haemolyticus*, we found related genomic islands with *ccrDE* in the genomes of *S. hominis*, *S. borealis*, and in *S. aureus.* The island is consistently inserted in the *rimL* gene, in a locus downstream of the conserved *metE* gene which is 17-56 kb counterclockwise from the replication origin in CoNS species.

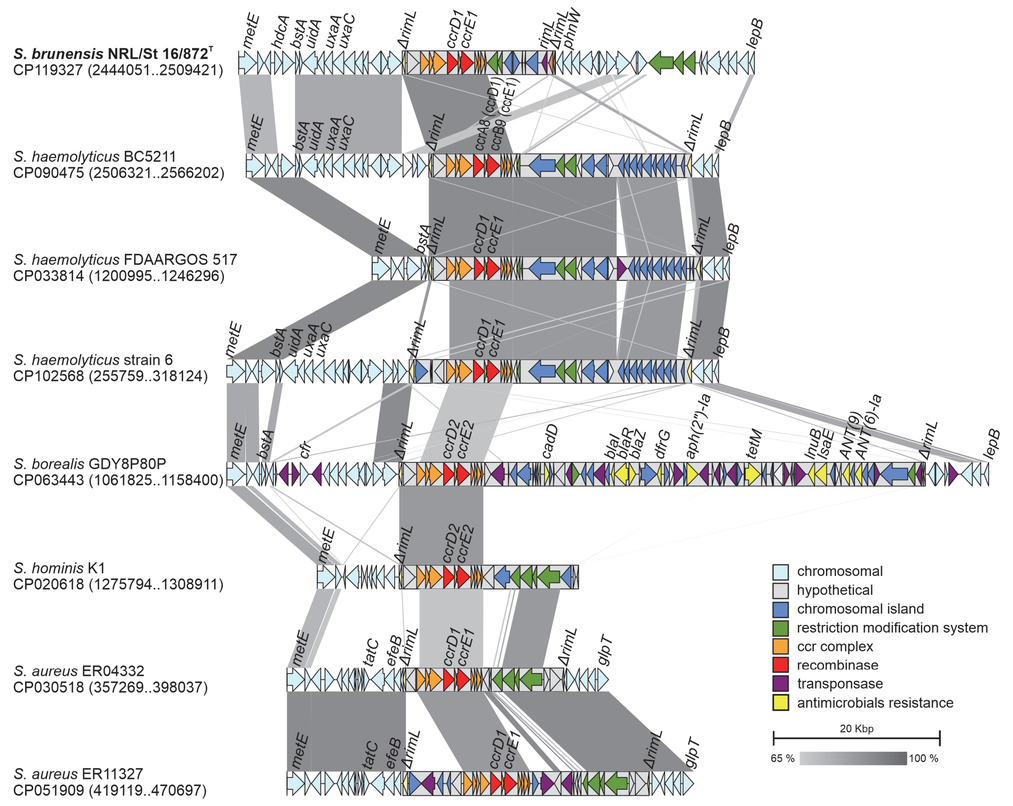

Fig. 5. Comparative analysis of chromosomal islands harbouring *ccrDE* cassette chromosome recombinases (CI$_{ccrDE}$). **(A)** Comparison CI$_{ccrDE}$ and flanking regions from different staphylococcal species. The genomic island CI$_{ccrDE}$ is highlighted with a grey background. Genes are labelled according to known or putative functions, as shown in the legend. Only nucleotide blast hits above 65% identity and longer than 2 kb are shown.

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