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Genome Note

# Draft genome sequence of *Staphylococcus agnetis* 3682, the producing strain of the broad-spectrum lantibiotic agneticin 3682

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ABSTRACT

*Objectives:* Here we report the draft genome sequence of *Staphylococcus agnetis* 3682, a strain producing agneticin 3682, a broad-spectrum lantibiotic with potential medical applications. The inhibitory activity of *S. agnetis* 3682 against multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) isolates involved in human infections was also investigated.

*Methods:* A sequencing library was constructed using a Nextera XT DNA Library Preparation Kit. An Illumina MiSeq system was used to perform whole-genome shotgun sequencing. De novo genome assembly was performed using the A5-miseq pipeline. *Staphylococcus agnetis* 3628 genome annotation was performed by the RAST server, and BAGEL4 and antiSMASH v.4.0 platforms were used for mining bacteriocin gene clusters. The inhibitory activity of *S. agnetis* 3682 against 20 multidrug-resistant MRSA strains involved in human infections in two Brazilian hospitals was determined by the deferred antagonism assay on brain–heart infusion (BHI) agar plates.

*Results:* The total scaffold size was determined to be 2 502 817 bp with a G + C content of 35.6%. Genome analyses revealed 2437 coding sequences, 76 RNA genes, 27 genes involved in drug resistance and 2 bacteriocinogenic gene clusters (for agneticin 3682 and hyicin 4244). *Staphylococcus agnetis* 3682 inhibited 80% of the MRSA isolates tested.

*Conclusion:* This study describes the main features of the draft genome of *S. agnetis* 3682, a strain producing the first bacteriocin (agneticin 3682) reported in this species. A second gene cluster encoding a sactipeptide was also found in the bacterial chromosome. Agneticin 3682 shows a new potential application against clinical MRSA isolates.



## 1. Introduction

Bacteriocins are bacterial ribosomally-synthesised peptides with antimicrobial activity generally against other bacteria [1]. *Staphylococcus* spp. are Gram-positive bacteria with medical, veterinary and food importance, however they have also been shown to produce bacteriocins [2]. *Staphylococcus agnetis* 3682, previously identified as *Staphylococcus hyicus* based on biochemical tests and 16S rDNA sequencing, was isolated from bovine mastitic milk and was reported to produce a lantibiotic named hyicin 3682 in 2011 [3]. Hyicin 3682, now designated agneticin 3682 according to the correct species identification resulting from this genome sequencing study, was the first bacteriocin described in this staphylococcal species. Its gene cluster is located on a large (ca. 54 kb) plasmid named pRJ109 [3,4]. The broad-spectrum activity of this 22-residue peptide includes food-spoilage microorganisms and food-associated pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* [4]. Its activity against animal pathogens, such as staphylococci and streptococci, has also been reported [4]. The potential applications of agneticin 3682 in food biopreservation and veterinary medicine encouraged us to sequence the genome of *S. agnetis* 3682. Moreover, analysis of the inhibitory activity of this strain was extended to include human multidrug-resistant methicillin-resistant *S. aureus* (MRSA) isolates.

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## 2. Methods

Genomic DNA of *S. agnetis* 3682 was extracted using a GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer's recommendations. A DNA library was constructed using a Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA, USA). An Illumina MiSeq system was used to perform whole-genome shotgun sequencing. De novo genome assembly was performed using the A5-miseq pipeline (https://sourceforge.net/projects/ngopt/). Genome annotation was performed by the Rapid Annotation using Subsystem Technology (RAST) server (http://rast.nmpdr.org), and BAGEL4 (http://bagel.molgenrug.nl) and antiSMASH v.4.0 (https://antismash.secondarymetabolites.org) platforms were used to mine antimicrobial peptide (AMP) gene clusters. The BLASTp program was employed to search for sequence similarities between proteins. The PHAST server (http://phast.wishartlab.com) was used for putative prophage identification in the genome. The inhibitory activity of *S. agnetis* 3682 against 20 MRSA strains isolated from human anatomical sites in two Brazilian hospitals was tested using the deferred antagonism assay on brain–heart infusion (BHI) agar plates [5]. Briefly, 10 μL of a bacterial suspension containing 9-log CFU/mL of the producing strain *S. agnetis* 3682 were used to prepare spots on the surface of a BHI agar plate (containing 20 mL of medium), which was overlaid with 6-log CFU/mL of the target strain in 3 mL of BHI soft agar. Following incubation at 37 °C for 24 h, the diameter of the zone of inhibition was measured in mm. Experiments were performed in triplicate.

## 3. Results

Agneticin 3682, a lantibiotic produced by *S. agnetis* 3682, has a broad spectrum of activity with an attractive biotechnological potential, justifying genome sequencing of *S. agnetis* 3682. The resulting draft genome exceeded 350-fold coverage, comprising 34 scaffolds ranging from 641 bp to 381 232 bp. The genome consists of a single chromosome and a single plasmid (pRJ109). A genetic study regarding pRJ109 is in progress. The $N_{50}$ value was estimated at 129 563 bp. The total scaffold size was determined to be 2 502 817 bp with a G + C content of 35.6%. Genome annotation identified 2437 coding sequences and 76 RNA sequences. Most genes were related to amino acid, protein and RNA biosynthesis as well as carbohydrate metabolism. Genes involved in vitamin biosynthesis, such as biotin and thiamine, were also found. Genome annotation reported 27 genes involved in drug resistance, including genes related to heavy metal (mercury and cadmium) and antibiotic resistance (fluoroquinolones and teicoplanin). One complete *S. aureus* prophage, named JS01 (41 kb), was also found. A second AMP gene cluster was mined from the chromosome of *S. agnetis* 3682 (from scaffold 4). This bacteriocinogenic gene cluster is identical to that of hyicin 4244, a sactibiotic recently reported in *S. hyicus* 4244 [6]. It comprises the same eight genes (*hycSABC-DEFG*) coding for identical proteins.

The antimicrobial activity of *S. agnetis* 3682 was tested against clinical MRSA strains (Table 1). This is the first report of its action against human isolates, inhibiting the majority (80%; 16/20) of strains tested with zones of inhibition ranging from 11.6–35.5 mm.

## 4. Discussion

The occurrence of infections caused by multidrug-resistant *S. aureus* is still of great clinical concern, leading to reduced treatment options [8]. *Staphylococcus agnetis* 3682 was shown to exhibit broad-spectrum activity against several bacterial genera from distinct sources, including staphylococci [3,4].

When tested against several MRSA strains involved in human infections in the present study, *S. agnetis* 3682 proved to inhibit most strains (80%) with zones of inhibition ranging from ca. 12–35 mm. Various factors can influence the size of the inhibition zone, such as the amount of bacteriocin produced and secreted by the producer strain, the molecular mass of the peptide and the susceptibility of the target strain, among others. Agneticin 3682 is a small peptide (2117 Da) produced and secreted by its producer strain in large amounts (10 240 BU/mL) [5]. The bacteriocin begins to be produced during the early log-phase, therefore the peptide can accumulate at high concentration around the producer strain grown on solid medium and rapidly diffuse through the medium giving rise to large inhibition zones. Some MRSA strains, such as

**Table 1**
Antimicrobial activity of *Staphylococcus agnetis* 3682 against human multidrug-resistant methicillin-resistant *Staphylococcus aureus* isolates from two Brazilian hospitals.

| Source/strain | Antimicrobial resistance profile[a] | Diameter of ZOI (mm)[b] |
|---|---|---|
| Hospital Universitário Clementino Fraga Filho | | |
| Sa10 | CEF, ERY, GEN, NOR, OXA, PEN | 24.6 ± 3.5 |
| Sa12 | CEF, CHL, ERY, GEN, NOR, OXA, PEN | 18.0 ± 0 |
| Sa13 | CEF, CHL, ERY, GEN, NOR, OXA, PEN, TET | 13.0 ± 3.6 |
| Sa128 | CEF, CHL, ERY, GEN, NOR, OXA, PEN | 11.6 ± 2.0 |
| Sa132 | CEF, CHL, ERY, GEN, NOR, OXA, PEN | –[c] |
| Sa133 | CEF, ERY, GEN, NOR, OXA, PEN | 23.5 ± 2.8 |
| Sa134 | CEF, CHL, ERY, GEN, NOR, OXA, PEN | – |
| Sa135 | CEF, CHL, ERY, GEN, NOR, OXA, PEN | – |
| Sa136 | CEF, CHL, ERY, GEN, NOR, OXA, PEN | 16.5 ± 3.5 |
| Sa137 | CEF, ERY, GEN, NOR, OXA, PEN | – |
| Sa138 | CEF, ERY, GEN, NOR, OXA, PEN | 18.5 ± 3.8 |
| Hospital Estadual Pedro Ernesto | | |
| Sa456 | CEF, CHL, ERY, GEN, NOR, OXA, PEN | 15.6 ± 0.5 |
| Sa458 | CEF, CHL, ERY, NOR, OXA, PEN, TET | 31.0 ± 0.6 |
| Sa460 | CEF, CHL, ERY, NOR, OXA, PEN, TET | 31.0 ± 4.2 |
| Sa461 | CEF, CHL, ERY, NOR, OXA, PEN, TET | 35.5 ± 5.8 |
| Sa462 | CEF, CHL, ERY, GEN, NOR, OXA, PEN, TET | 33.0 ± 0 |
| Sa463 | CEF, CHL, ERY, NOR, OXA, PEN, TET | 35.0 ± 5.6 |
| Sa465 | CEF, CHL, ERY, GEN, NOR, OXA, PEN, TET | 33.0 ± 3.4 |
| Sa466 | CEF, CHL, ERY, NOR, OXA, PEN, TET | 31.3 ± 2.1 |
| Sa468 | CEF, CHL, ERY, NOR, OXA, PEN, TET | 30.3 ± 4.9 |

ZOI, zone of inhibition; CEF, cefalotin; ERY, erythromycin; GEN, gentamicin; NOR, norfloxacin; OXA, oxacillin; PEN, penicillin; CHL, chloramphenicol; TET, tetracycline.
[a] Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines [7].
[b] Data are the mean ± standard deviation from three independent experiments.
[c] –, no inhibition observed.

Sa13 and Sa128, proved to be less susceptible to the bacteriocin, exhibiting smaller inhibition zones. However, it is well known that the spectrum of antimicrobial activity of a given bacteriocin can also vary depending on the target bacterial genus and species, and even among different strains of a given species [9].

The biotechnological potential of the AMP produced by *S. agnetis* 3682 encouraged us to further investigate the genome organisation of this strain, which proved to belong to a staphylococcal species described only in 2012 [10]. This species has been associated with animal infections [10]. Surprisingly, an additional AMP gene cluster was found on its chromosome that was shown to be identical to that encoding the sactipeptide hyicin 4244 [6]. However, a peptide with the molecular mass of hyicin 4244 (3274 Da) has never been found in the purified preparations of agneticin 3682 (2117 Da) [4]. One step employed for purification of agneticin 3682 uses cation-exchange chromatography [3], a method considered inappropriate for purification of hyicin 4244, which is expected to be anionic (pI of 4.03) [6]. Moreover, despite many attempts, hyicin 4244 purification has never been achieved, which has been attributed to its presence in the culture supernatant at very low levels [6]. Such findings led us to assume that the antimicrobial activity exhibited by *S. agnetis* 3682 against several target strains investigated so far, including the MRSA strains tested in this study, is solely due to agneticin 3682 activity. However, future RT-PCR and gene knockout experiments are required to completely rule out the production and export of hyicin 4244 by *S. agnetis* 3682.

## 5. Nucleotide sequence accession number

This whole-genome shotgun project has been deposited at DBJ/ENA/GenBank under the accession no. **VKCY00000000**. The version described in this paper is the first version (**VKCY00000000**).

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## Competing interests

None declared.

## Ethical approval

Not required.

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