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Analysis of the gene expression profile of *Staphylococcus aureus* treated with nisin

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The bacterium *Staphylococcus aureus* is most often recognized as a common cause of food poisoning. *S. aureus* colonization in humans can cause serious infections, toxinoses and life-threatening diseases. Nisin is a bacteriocin that has been extensively used as a natural preservative in the food industry, but the overall transcriptional response of *S. aureus* to nisin has not been well characterized. We used whole-genome DNA microarrays of *S. aureus* to determine the global transcription profile triggered by nisin treatment in *S. aureus*. In response to nisin treatment, a total of 601 genes were differentially regulated; transcription of 327 genes was up-regulated, and transcription of 274 genes was down-regulated. Various transporter genes showed elevated transcription, including genes involved in capsule polysaccharide (CP) synthesis, cell-wall synthesis, nucleic-acid and nucleotide metabolism as well as genes encoding urease, transport/binding proteins, and lipoproteins. To our knowledge, this transcriptome analysis provides the first insights into the transcriptional response of *S. aureus* to nisin challenge and enables exploration of the mechanisms of nisin antimicrobial activity toward *S. aureus*. The findings suggest that nisin regulates multiple desirable targets which could be further explored in the development of new food preservatives to avoid the contamination of *S. aureus* in food processing and storage.
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1. Introduction

Staphylococcus aureus is widely distributed in the environment and therefore has many routes of entry and contamination of human food. *S. aureus* is one of the most commonly identified causes of food poisoning due to the ability of enterotoxigenic strains to preform staphylococcal enterotoxins (SEs) in food (Di Giannatale, Prencipe, Tonelli, Marfoglia, & Migliorati, 2011; Fetsch et al., 2014). *S. aureus* colonization in humans can cause serious infections, toxinoses and life-threatening diseases, such as skin and soft tissue infections, toxic shock syndrome and septicemia (Wattinger, Stephan, Layer, & Johler, 2012). Due to these serious threats, safe and natural food preservatives that inhibit bacterial and fungal growth have become a major priority in the past decades (Badaoui Najjar, Kashtanov, & Chikindas, 2007). The bacteriocin nisin is one example of a natural preservative that has been extensively used in the food industry.

In recent years, considerable effort has been directed toward finding natural antimicrobials that improve the quality and shelf life of food. Nisin is an antimicrobial peptide produced by strains of *Lactococcus lactis* subsp. *lactis* and has been recognized as safe for food applications by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives. Nisin is especially widely used in pasteurized and processed cheese spreads in over 48 countries (Kindrachuk et al., 2012). Nisin exhibits antimicrobial activity toward a wide range of food-borne Gram-positive pathogenic bacteria, including *Listeria monocytogenes*, *S. aureus*, *Enterococcus* species, *Clostridium* and *Bacillus* spp (Rodriguez, 1996; Thomas et al., 2002). It was demonstrated that *in vitro*, nisin inhibited bacterial cell-wall biosynthesis (Reisinger, Seidel, Tschesche, & Hammes, 1980). Subsequently, it was shown that nisin kills bacterial cells by interfering with basic energy transduction at the cytoplasmic membrane (Kordel & Sahl, 1986; Ruhr & Sahl, 1985). It was found that pores

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formed in the membrane by the nisin molecules allowed the diffusion of small compounds, as no transport system for ATP has been reported (Abee, Rombouts, Hugenholtz, Guihard, & Letellier, 1994). The increase in membrane permeability results in the collapse of the proton motive force (PMF); in the case of nisin, both the ΔpH and the Δψ are dissipated, leading to rapid cessation of all biosynthetic processes (Bruno, Kaiser, & Montville, 1992; Bruno & Montville, 1993; Okereke & Montville, 1992). Furthermore, recent work has shown that the activity of nisin is dependent on the concentration of the peptidoglycan precursor molecule lipid II (undecaprenyl-pyrophosphoryl-MurNAc-(pentapeptide)-GlcNAc) in the membrane of sensitive cells (Breukink et al., 1999).

In a recent study, Kramer et al. used whole-genome DNA microarrays of *L. lactis* IL1403 to identify the factors underlying acquired nisin resistance (Kramer, van Hijum, Knol, Kok, & Kuipers, 2006). However, little information exists regarding the inhibitory mechanisms of nisin toward *S. aureus* on a molecular level. Transcriptional profiles generated with Affymetrix GeneChips have been used to identify *S. aureus* genetic transcripts that are induced in response to antibiotics such as vancomycin, oxacillin, D-cycloserine, and bacitracin (McAleese et al., 2006; Utaida et al., 2003). Bacterial transcriptional profiles generated by GeneChip analysis provide an effective tool to investigate differential gene expression as a starting point toward the exploration of antimicrobial mechanisms (Smith et al., 2010).

However, to our knowledge, no studies have used a whole-genome approach to elucidate the mechanism of nisin as a potential antibacterial agent. In this report, we use DNA microarray analysis to identify differentially expressed genes in *S. aureus* in response to treatment with nisin. Our study offers insight into the mechanisms of nisin effects on cells and organisms.

2. Materials and methods

2.1. Bacterial strains and materials

S. aureus ATCC 29213 was obtained from the China Medical Culture Collection (CMCC) Center. Twenty clinical strains of *S. aureus* were isolated from the First Hospital of Jilin University. Mueller-Hinton broth II (MHB II) and Mueller-Hinton agar (MHA) were purchased from BD (Biosciences, Inc., Sparks, MD). FDA-approved nisin (USFDA, 2001) was purchased from Sigma–Aldrich (N5764, containing 2.5% pure nisin, balance sodium chloride, and denatured milk solids, activity of 1×10^6 IU/g, according to the manufacturer). The procedure in preparing the nisin stock solution (5000 IU/g) was adopted from the Compendium of Food Additive Specifications (FAO, 2008). A nisin stock solution was prepared by dissolving 10 mg of nisin in 2-mL sterile 0.02 N HCl. Nisin used in the following studies was diluted by MHB, and the equal 0.02 N HCl in MHB was used as a negative control.

2.2. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of nisin against the above-mentioned *S. aureus* strains were determined in triplicate by a broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The MICs were defined as the lowest antimicrobial concentration that inhibited >90% of growth by visual reading.

2.3. Growth curves

S. aureus strain ATCC 29213 was grown to an optical density (OD) of 0.3 at 600 nm in MHB II and was distributed as 100-mL volumes into five 250-mL Erlenmeyer flasks. The cultures were supplemented with nisin at concentrations of 0, 1/4 × MIC (2 μg/mL), 1/2 × MIC (4 μg/mL), 1 × MIC (8 μg/mL), and 2 × MIC (16 μg/mL). The cultures were grown at 37 °C with constant shaking under aerobic conditions, and cell growth was spectrophotometrically monitored by recording the OD at 600 nm at regular time intervals (Xing et al., 2012).

2.4. GeneChip hybridization and analysis of nisin-treated S. aureus

The *S. aureus* strain ATCC 29213 was treated with nisin as previously described (Xing et al., 2012). Briefly, *S. aureus* planktonic cells were treated with nisin for 60 min at final concentrations of 1/2 × MIC. The procedures used for construction of a genome-wide DNA microarray for *S. aureus*, RNA preparation, cDNA labeling, GeneChip hybridization and microarray data processing were performed as described previously (Hutter et al., 2004). The GeneChip *S. aureus* genome array (antisense) was provided by the Bioassay Laboratory of CapitalBio Corporation (http://www.capitalbio.com), a service provider authorized by Affymetrix Inc. (Santa Clara, CA). The GeneChip includes N315, Mu50, NCTC 8325, and COL. The array contains probe sets for over 3300 *S. aureus* ORFs and over 4800 intergenic regions. Labeled cDNA from independent RNA preparations were hybridized to six separate GeneChips. A total of 1.5 μg of labeled material was hybridized to each GeneChip for 16 h at 45 °C. After hybridization, the washing and staining of arrays were performed using the GeneChips® Fluidics Station 450, followed by scanning with the Affymetrix GeneChip Scanner 3000 according to the manufacturer's instructions for antisense prokaryotic arrays (Affymetrix, Inc.). To select the differentially expressed genes, we used threshold values of ≥1.5-fold and ≤ −1.5-fold changes between nisin-treated samples and the three controls. The false discovery rate (FDR) significance level was set to <5%.

2.5. Quantitative real-time RT-PCR

Reverse transcription (RT)-PCR was used to verify the microarray results. The same nisin-treated and control RNA preparation samples used in the microarray experiments were also used for RT-

Table 1
Primers used in real-time RT-PCR.

Primer	N315 ORF(a)	Sequence
16S rRNAfor	SArRNA01	CGTGCTACAATGGACAATACAAA
16S rRNArev	SArRNA01	ATCTACGATTACTAGCGATTCCA
isdA for	SA0977	AAGTACATATTGTCGTGCCACA
isdA rev	SA0977	CTTGTTTAGGCGTTTCGTTATG
SA0302for	SA0302	GAATGGAAAAACAGGAAAAC
SA0302rev	SA0302	GCAAACACATAGCCAATAAG
sspAfor	SA0901	CGATCGTCACCAAATCACAGA
sspArev	SA0901	TGCGTAGCATCTACGACGTGT
ribAfor	SA1587	GCTTACATTCTGCGTGCCTTAC
ribArev	SA1587	ACAATCCTATGCCACGACCT
ebpSfor	SA1312	CAAAATGAGGCTGGCACA
ebpSrev	SA1312	TGGTTTAGGTTGCTGAGATTG
capC for	SA0146	CATCCAGAGCGGAATAAAGC
capC rev	SA0146	GTGTTATGCGCATCTGAACC
lytMfor	SA0265	ACGGTGTCGACTATGCAATGC
lytMrev	SA0265	TACTTGATTGCCGCCACCA
ureBfor	SA2083	TTTTGGTTTTCGTGGTATGG
ureBrev	SA2083	ACCGTTATCTCCGAATACACC
glpTfor	SA0325	CGACTTTGCTACAAGCGATAA
glpTrev	SA0325	CGCCCAATCAAGTACACCA
msmXfor	SA0206	CATTTGGGCTAAAGCTACG
msmXrev	SA0206	GACGCTGTCCACCAGATAA
pyrRfor	SA1041	TTGATGATGTGCTGTATACTGG
pyrRfor frev	SA1041	CGAATTGGTAACTCACGATGT

ORF, open reading frame.

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