PROTEOMICS

Supporting Information for Proteomics
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Chi Hai Vu, Julia Kolata, Sebastian Stentzel, Anica Beyer, Manuela Gesell Salazar, Leif Steil, Jan Pané-Farré, Vanessa Rühmling, Susanne Engelmann, Friedrich Götz, Jan Maarten van Dijl, Michael Hecker, Ulrike Mäder, Frank Schmidt, Uwe Völker and Barbara M. Bröker

Adaptive immune response to lipoproteins of Staphylococcus aureus in healthy subjects
SUPPORTING INFORMATION METHODS

Quantitative protein profiling

Four micrograms of protein extracts were digested into peptides overnight by 160 ng sequencing-grade modified trypsin (Promega GmbH, Mannheim, Germany) per sample dissolved in 20 mM aqueous ammonium bicarbonate. The digestion was stopped with acetic acid at a final concentration of 1%. Tryptic peptides were purified using ZipTipµ-C18 pipette tips (Merck Millipore, Billerica, MA, USA).

Nano-LC-MS/MS analysis of peptides was performed by reverse phase peptide separation on a Dionex UltiMate 3000 nano-LC system (Dionex/Thermo Fisher Scientific, Idstein, Germany). For MS data generation a Q Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a TriVersa NanoMate (Advion, Ltd., Harlow, UK) was used. Peptide solution was separated on a 25 cm Acclaim PepMap RSLC analytical column (2 μm C18 particles, Thermo Scientific) with a linear gradient ranging from 2 to 25% buffer b (0.1% (v/v) acetic acid in acetonitrile) in 120 min at a flow rate of 300 nL/min.

MS raw data were imported into Genedata Expressionist Refiner MS version 9.1 (Genedata AG, Basel, Switzerland). After loading the data, first the chemical noise and the general background were subtracted. Next, each individual dataset was independently aligned in retention time direction by the Pairwise Alignment Based tree scheme. Subsequently, Chromatogram Peak Detection, Chromatogram Isotope Clustering, MS/MS Consolidation and Chromatogram Singleton Filter were performed. Processed tandem-MS spectra were searched against a S. aureus COL proteome FASTA database and peptide identification was performed using the Mascot search engine (Matrix Science, version 2.5, Ltd, London, GB) with a precursor mass tolerance of 5 ppm and a fragment tolerance of 0.01 Da. Since the quantitation was restricted to the three highest peptides per protein, no missed cleavages and no modification were applied. Protein quantitation based on MS1 intensities (area under the curve) was performed according
to the Hi3-method provided by Genedata Expressionist® Refiner MS (Genedata AG, Basel, Switzerland) with a minimum of two peptides per protein. The maximum intensities of the Hi3 peptides were further used for protein quantitation.

Subsequently, protein intensities were median normalized using the central tendency normalization option provided by the Genedata Expressionist Analyst® version 9.1 (Genedata AG). To predict the subcellular localization of the proteins Psort [http://www.psort.org/] as well as LocateP [http://www.cmbi.ru.nl/locatep-db/cgi-bin/locatepdb.py] were used.

Analysis of gene expression level

The condition-dependent transcriptome of *S. aureus* HG001, a derivative of strain NCTC 8325, was analyzed by strand-specific tiling array hybridizations [3].

References

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