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Comparative proteomic and transcriptomic profile of *Staphylococcus epidermidis* biofilms grown in glucose-enriched medium

Virginia Carvalhais [a,b,c], Angela França [a,c], Gerald B. Pier [c], Manuel Vilanova [d,e], Nuno Cerca [a], Rui Vitorino [b,*]

[a] CEB—Centre of Biological Engineering, LIBRO - Laboratory of Research in Biofilms Rosário Oliveira, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] QOPNA, Mass Spectrometry Center, Department of Chemistry, University of Aveiro, Aveiro, Portugal
[c] Division of Infectious diseases, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[d] ICBAS—Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal
[e] IBMC—Instituto de Biologia Molecular e Celular, Rua do Campo Alegre 83, Porto, Portugal

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ABSTRACT

Staphylococcus epidermidis is an important nosocomial agent among carriers of indwelling medical devices, due to its strong ability to form biofilms on inert surfaces. Contrary to some advances made in the transcriptomic field, proteome characterization of *S. epidermidis* biofilms is less developed. To highlight the relation between transcripts and proteins of *S. epidermidis* biofilms, we analyzed the proteomic profile obtained by two mechanical lysis methods (sonication and bead beating), associated with two distinct detergent extraction buffers, namely SDS and CHAPS. Based on gel electrophoresis-LC–MS/MS, we identified a total of 453 proteins. While lysis with glass beads provided greater amounts of protein, CHAPS extraction buffer allowed identification of a higher number of proteins compared to SDS. Our data shows the impact of different protein isolation methods in the characterization of the *S. epidermidis* biofilm proteome. Furthermore, the correlation between proteomic and transcriptomic profiles was evaluated. The results confirmed that proteomic and transcriptomic data should be analyzed simultaneously in order to have a comprehensive understanding of a specific microbiological condition.

1. Introduction

When *Staphylococcus epidermidis* establishes biofilms on indwelling medical devices, this may cause an early removal of the implant due to the lack of effective antibiotic therapeutics and risk of eventual systemic infection [1,2]. *S. epidermidis* biofilms grown in glucose-enriched medium were previously associated with an increased proportion of dormant cells within a biofilm [3]. Dormancy is a clinically relevant physiological state, since it has been associated with long-term bacterial survival, increased cellular tolerance to antibiotics [4–6] and evasion of the host immune system [3,7].

The availability of the complete genome of *S. epidermidis* strains RP62A (ATCC 35984) [8] and ATCC 12228 [9] has led to the development of proteomic studies, since it has been suggested that the genome sequence is not sufficient to elucidate the biological functions of an organism [10]. Although there have been major advances in the molecular characterization of the pathogenic mechanisms of *S. epidermidis* biofilms [1], much less is known regarding the proteome. A few proteomic studies, however, have been performed with some *Staphylococcus* spp. in order to identify specific features associated with the pathogenicity and physiology of these microorganisms [11–18]. In *S. epidermidis*, 2-Dimensional Electrophoresis (2-DE) analysis of proteomic patterns showed several differentially expressed proteins when comparing commensal and invasive strains [12]. To ensure high quality and reliable proteomic results, an appropriate sample preparation is fundamental [19,20]. Due to the complex structure of biofilms [21], it is necessary to develop an effective lysis method in order to obtain maximum coverage of the biofilm proteome and minimal protein losses, similar to the approach optimized for total RNA extraction from *S. epidermidis* biofilms [22]. Different protein extraction methods, including enzymatic, chemical, mechanical and other methods available via commercial extraction kits have been tested to obtain the highest number of proteins in *Staphylococcus* spp. [11,14]. Although the majority of these studies were performed with cell suspensions, relatively harsh techniques have been shown to be rapid and efficient to disrupt and lyse biofilms of Gram-positive bacteria, such as mechanical methods like bead beating with glass beads (FastPrep) or sonication [14,23,24]. Often, to optimize protein recovery, enzymes and detergents may be used in conjugation with mechanical lysis. Since lysostaphin efficiently cleaves staphylococcal cell wall peptidoglycan [25,26], it may

* Corresponding author.
E-mail address: rvitorino@ua.pt (R. Vitorino).

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be used to disrupt staphylococcal biofilms [27]. However, due to high costs of lysostaphin, detergents are frequently used to enhance protein isolation and solubilisation [28].

In an attempt to determine the relation between protein and mRNA levels, several studies have shown that often the correlation is surprisingly low, and differs widely among organisms [29]. Correlation coefficients were found to vary from 0.09 to 0.46 in multi-cellular organisms, from 0.34 to 0.87 in yeasts, whereas in bacteria the correlations ranged from 0.20 to 0.47 (reviewed in [30]). Up to now, no correlation analysis between the transcriptome and proteome of *S. epidermidis* biofilms has been conducted.

Hence, herein, since distinct lysis and extraction methods may yield different protein recoveries, we assessed different lysis methods to obtain proteins from *S. epidermidis* biofilms grown in glucose-enriched medium. Then, we compared the proteomic profile with the gene expression profile obtained by RNA sequencing (RNA-seq) technology., We undertook a gel-based method to determine protein isolation efficiency, using total protein extracted with a detergent-based extraction (SDS or CHAPS) coupled with mechanical lysis (sonication or bead beating). A detailed analysis of proteomic data was performed in each condition. Label-free relative protein abundance index (emPAI) was used for the relative quantitation of the proteome and was compared to transcriptomic profile. The overall goal was to characterize and correlate both proteomic and transcriptomic profiles of *S. epidermidis* biofilm-grown cells.

2. Materials and methods

2.1. Growth conditions

Biofilm forming *S. epidermidis* 9142 strain was used as a model [31]. One colony was inoculated into 1 mL of Tryptic Soy Broth (TSB) (Liofilchem, Roseto degli Abruzzi, Italy) and incubated at 37 °C at 120 rpm for 18 h. The overnight culture was adjusted to an optical density at 640 nm of 0.250 (± 0.05) with TSB and 10 μL of the suspension was transferred into a 24-well plate (Thermo Fisher Scientific, Waltham, MA, USA) containing 1 mL of TSB supplemented with 0.4% glucose (v/v) (TSB 0.4% G) (Thermo Fisher Scientific). The culture plate was then incubated at 37 °C at 120 rpm for 24 h. After this period, the culture medium covering the biofilm was removed and replaced by fresh TSB supplemented with 1% glucose (v/v) (TSB 1% G). Biofilms were allowed to grow in these same conditions for 24 additional hours. Thereafter, biofilm culture medium was removed and biofilms were washed twice with phosphate buffered saline (PBS).

2.2. Protein preparation

The same number of biofilms (12 biofilms for each condition) were directly scraped and suspended in detergent extraction buffers: 25 mM Tris–HCl (pH=7.2) (Pharmacia Biotech, Uppsala, Sweden), 10 mM CHAPS (Sigma-Aldrich, St. Louis, MO, USA, 0.5 M NaCl (VWR, Radnor, PA, USA), 5% glycerol (Sigma-Aldrich) and 1 mM PMSF (Sigma-Aldrich) or 60 mM Tris–HCl (pH=6.8), 10% glycerol, 5% SDS (USB Corporation, Cleveland, OH, USA) and 1 mM PMSF. Mechanical lyses were performed in a sonicator (Cole-Parmer® 750-Watt Ultrasonic Homogenizer, Cole Parmer, Vernon Hills, IL, USA) (10 min, 30 s running, 10 s pause, 40% amplitude) or by bead beating, using glass beads of 0.1 mm (Sigma-Aldrich) in a FastPrep® cell disruptor (BIO 101, ThermoElectron Corporation, Thermo Fisher Scientific) (3 cycles of 30 s and 6.5 m/s). After lyses, cell debris were removed by centrifugation (15,000g for 15 min at 4 °C) and proteins precipitated with cold acetone [32]. Then, protein quantification was performed using the RC-DC assay (Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions.

2.3. One-dimensional gel electrophoresis (SDS-PAGE) in gel protein digestion and protein identification

Forty μg of protein were incubated with SDS 10% (w/v), 0.5 M Tris–HCl (pH 6.8), glycerol, mercaptoethanol and bromophenol blue (w/v) for 5 min at 100 °C. Then, samples were loaded on Novex NuPAGE® 4–12% Bis-Tris gel (Life Tecnologies, Grand Island, NY, USA) and proteins were separated at a constant voltage (200 V). The gel was stained with colloidal Coomassie G-250 and all the lanes were manually excised into 16 gel slices for in-gel digestion with trypsin TPCK (AB Sciex, Framingham, MA, USA). Peptide extraction was made with 10% formic acid/ 50% acetonitrile. Dried peptides were dissolved in 5% acetonitrile (VWR), 0.1% formic acid (Sigma-Aldrich) and 0.1% trifluoroacetic acid (Sigma-Aldrich). Separation of tryptic peptides by nano-HPLC was performed on the module separation Ultimate 3000 (Dionex, Thermo Fisher Scientific) using a capillary column (Pepmap100 C18; 3 μm particle size, 0.75 μm internal diameter, 15 cm in length). A gradient of solvent A, (water/acetonitrile/trifluoroacetic acid (98:2:0.05, v/v/v)) to solvent B (water/acetonitrile/trifluoroacetic acid (10:90:0.045, v/v/v)) was used. The separation of 2 μg/μL sample was performed using a linear gradient (5–50 % B for 30 min, 50–70% B for 10 min and 70–5% A for 5 min) with a flow rate of 0.3 μL/min. The eluted peptides were mixed with a continuous flow of CHCA matrix solution (270 nL/min, 2 mg/mL in 70% ACN/0.1% TFA and internal standard Glu-Fib at 15 ftmol) and applied directly on a MALDI plate in 20 s fractions using an automatic fraction collector Probot (Dionex, Thermo Fisher Scientific). Mass spectra were obtained on a matrix assisted laser desorption/ionization–time of flight MALDI-TOF/TOF mass spectrometer (4800 Proteomics Analyzer, Applied Biosystems, Foster City, CA, USA) in the positive ion reflector mode and obtained over the mass range from 700 to 4500 Da with 900 laser shots. A fragmentation voltage of 2 kV was used throughout the automated runs. The spectra were processed and analyzed by the T2S (v1.0, Matrix Science Ltd, London, U.K) and submitted in Mascot software (v.2.3.0.2, Matrix Science Ltd) for protein/ peptide identification based on MS/MS data using the following criteria: trypsin as enzyme; a maximum of two missed cleavages; mass tolerances of 40 ppm for peptide precursors, mass tolerance of 0.6 Da was set for fragment ions. Simultaneously, phosphorylation of threonine (T), serine (S) and tyrosine (Y) were searched as variable modifications. The local false discovery rate (FDR) was calculated by searching the spectra against SwissProt (Firmicutes, release date 06022013) decoy (random) database. Protein identification was considered reliable when the individual ion score for each peptide had a minimum individual score of 95% and a minimum sequence tag of four aminoacids. Relative quantitation was performed using emPAI. Gene ontology [33] analysis was performed with proteins identified in each extraction condition using STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) (version 9.1) [34] (statistically significant specific GO terms, FDR adjust $p < 0.05$). Two independent experiments were performed with pools of *S. epidermidis* biofilms.

2.4. RNA sequencing

RNA extraction from *S. epidermidis* biofilms, cDNA library preparation and RNA-seq, were performed as previously described, with some modifications [35]. Briefly, total RNA was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA, USA). To remove genomic DNA, Ambion® TURBO DNA-free™ kit (Life Technologies) was used followed by acid–phenol:chloroform precipitation (Ambion®, Life