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An immunoproteomic approach for characterization of dormancy within *Staphylococcus epidermidis* biofilms

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ABSTRACT

Virulence of *Staphylococcus epidermidis* is mainly attributed to surface colonization and biofilm formation in indwelling medical devices. Physiological heterogeneity of biofilms may influence host immune response and sensitivity to antibiotics. Dormant cells, among others, contribute to biofilm heterogeneity. The aim of this study was to identify immunogenic proteins of *S. epidermidis* biofilms associated with dormancy mechanism, by using two-dimensional electrophoresis (2-DE) immunoblotting and mass spectrometry (MS). A total of 19 bacterial proteins, recognized by human serum samples, were identified. These proteins were mainly involved in small molecule metabolic biological processes. Catalytic activity and ion binding were the most representative molecular functions. CodY and GpmA proteins were more reactive to sera when biofilm dormancy was induced, while FtnA and ClpP were more reactive when dormancy was prevented. This is the first work that identifies differences in immunoreactive proteins within bacterial biofilms with induced or prevented dormancy. Considering the importance of dormancy within biofilms, further evaluation of these proteins can provide insights into the mechanisms related to dormancy and help to improve current understanding on how dormancy affects the host immune response.

1. Introduction

Staphylococcus epidermidis is an important opportunistic bacterium that does not produce highly aggressive virulence determinants (Otto, 2009). Its main virulence factor is the ability to form biofilms in indwelling medical devices (Otto, 2014). Biofilms are a community of surface-attached bacteria surrounded by an extracellular polymeric matrix composed of substances such as DNA, polysaccharides and proteins (Costerton et al., 1999). The clinical implications of bacterial growth in a biofilm mode are higher tolerance to antibiotics (Cerca et al., 2005) and tolerance to the innate immune response (Gray et al., 1984; Yao et al., 2005; Cerca et al., 2014; Cerca et al., 2006). *S. epidermidis* biofilm evasion of the host immune response may be caused by the production of several molecules that provide protection to host defenses, such as proteins, exopolysaccharides and peptides with antimicrobial activity (Otto, 2012). Biofilm protection against components of the innate immune mechanisms (Vuong et al., 2004; Jesaitis et al., 2003; Leid et al., 2002), such as phagocytosis (Johnson et al., 1986) and activity of antimicrobial peptides (Kristian et al., 2008; Vuong et al., 2004), is mainly mediated by the extracellular polymeric matrix (Cerca et al., 2006). In *S. epidermidis* biofilms, polysaccharide intercellular adhesin (PIA), also named poly-N-acetylglucosamine (PNAG) is considered a major virulence factor in biomaterial associated infections (Rupp et al., 1999).

Nowadays, proteomic approaches are contributing to elucidate the immunological response to microorganisms (Fulton and Twine, 2013). Immunoproteomics allows the identification of immunogenic and immunoreactive proteins that may participate in host–pathogen interactions and in host immune response (Dennehy and McClean, 2012; Costa et al., 2013; Wang et al., 2013). Furthermore, immunoproteome analysis improves the understanding of pathogenesis and unravel novel therapeutics targets based on the repertoire of immunogens (Brady et al., 2006).

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Thus, plasma is one of the most relevant environmental factors in indwelling medical devices-related infections (Schuster et al., 2014). A few aspects of immune reaction to *S. epidermidis* infections were already elucidated (Sellman et al., 2005; Franca et al., 2014; Cheung et al., 2010; Hanke and Kielian, 2012; Scherr et al., 2014; Pourmand et al., 2006). By using serum from rabbits immunized with live *S. epidermidis*, or serum proteins eluted from the surface of bacteria grown in rabbit serum reactive against bacterial cell-surface extracts, immunogenic and serum binding proteins were identified by Western blotting (Sellman et al., 2005). Sellman and colleagues found 5 antigenic components candidates for the development of *S. epidermidis* vaccine, namely, acetyl-coenzyme A acetyltransferase (YqiL), Na^+/H^+ antiporter (SE1873), lipoate ligase (SE0360), cysteine synthase (CysK), and alanine dehydrogenase (Ald). Also, Pourmand et al. identified autolysin AtlE, lipase (GehD) and surface protein ScaB antigenic components with therapeutic potential since they had opsonic activity *in vitro* (Pourmand et al., 2006).

Mature biofilms encompass cells with different metabolic activity (Rani et al., 2007), including dormant cells (Cerca et al., 2011). Dormancy is defined by a physiological state where bacteria persist without division for extended periods (Kaprelyants et al., 1993; Lewis, 2007). Moreover, dormant bacteria are associated with higher tolerance to antibiotics (Williamson et al., 2012; Kim et al., 2009; Shapiro et al., 2011; Cerca et al., 2014) and may determine the inflammatory profile of a biofilm (Cerca et al., 2011, 2014). Previously, we developed an *in vitro* model to modulate dormancy within *S. epidermidis* biofilms (Cerca et al., 2011). We were able to show that *S. epidermidis* biofilms with higher proportions of dormant bacteria induced a lower activation of murine macrophages, since it reduced *in vitro* pro-inflammatory cytokine production and lead to decreased expression of surface activation markers *in vivo* (Cerca et al., 2011). More recently, we performed a global transcriptome analysis where we found that translation was downregulated in dormant biofilms and, oxidation–reduction processes were associated with dormancy (Carvalhais et al., 2014). We also performed a quantitative proteomic analysis, where the ribosome synthesis pathway was associated with prevented dormancy, and ion binding and catalytic activity were found overexpressed in dormancy (Carvalhais et al., 2015).

To determine the immunoproteomic pattern of *S. epidermidis* biofilms with prevented and induced dormancy, we resolved whole cell lysate by 2-dimensional gel electrophoresis (2-DE) and performed immunoblotting with human sera. We then identified the immunoreactive protein spots by MALDI-TOF/TOF. With this work we intend to define the reactive protein repertoire of *S. epidermidis* biofilms with different proportions of dormant bacteria to human serum and contribute to decipher the host immune differences to dormancy.

2. Materials and methods

2.1. Growth conditions

Growth culture condition was performed as previously described (Cerca et al., 2011). *S. epidermidis* strain 9142 (isolated from blood culture (Mack et al., 1992)) was used to establish biofilms with higher and lower ratios of dormant cells. Briefly, one colony of *S. epidermidis* was inoculated in Tryptic Soy Broth (TSB) (LiofilChem, Roseto Degli Abruzzi, Italy) and incubated at 37 °C in an orbital shaker at 120 rpm for 18 h. The overnight culture was adjusted to an optical density at 640 nm of 0.250 (±0.05) and 10 μL of the suspension was transferred into a 24-well plate (Orange Scientific, Braine-l'Alleud, Belgium) containing 1 mL of TSB supplemented with 0.4% glucose (v/v) (TSB 0.4% G) (Fisher Scientific, Waltham, MA, USA) or TSB 0.4% G enriched with 20 mM magnesium chloride ($MgCl_2$) (Merck, Darmstadt, Germany). The culture plates were then incubated at 37 °C in an orbital shaker at 120 rpm for 24 h. After this period, the culture medium was removed and replaced by fresh TSB supplemented with 1% glucose (v/v) (1% G) or TSB 1% G containing 20 mM $MgCl_2$ (1% G + Mg^{2+}). Biofilms were then allowed to grow in the same conditions for an additional 24 h. Next, biofilm culture medium was removed and biofilms were washed twice with phosphate buffered saline (PBS). Then, bacteria within the biofilms were resuspended in 1 mL of PBS. As previously described, biofilm dormancy was determined using the spread plate method in Trypticase Soy Agar (LiofilChem) through calculation of the number of CFU/mL in each biofilm growth condition (Cerca et al., 2011). A reduction of about one log difference is typically expected in similarly grown biofilms without Mg^{2+} (Cerca et al., 2011).

2.2. Preparation of protein extracts

Total protein extraction was performed from multiple biofilm replicates, as previously described (Carvalhais et al., 2015). Briefly, biofilms were directly scrapped and resuspended with detergent extraction buffer, consisting of 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). Then, mechanical lysis was performed in a bead beating using glass beads of 0.1 mm (Sigma–Aldrich) in a FastPrep® cell disruptor (BIO 101, ThermoElectron Corporation) (3 cycles of 30 s and a speed of 6.5 m/s). After lysis, cell debris was removed by centrifugation (15,000 × *g* for 15 min at 4 °C). Proteins were precipitated with 20% of trichloroacetic acid (TCA)-cold acetone. The lysates were mixed with 20% TCA (Sigma–Aldrich) and incubated for 60 min at −20 °C. Proteins were collected by centrifugation and washed three times with cold acetone. After drying, proteins were directly resuspended in 1% CHAPS, 8 M urea (Amersham Biosciences, Piscataway, NJ, USA), 2 M thiourea (Riedel-de Haën, Sigma–Aldrich) and 12 mM DTT (USB Corporation, Cleveland, OH, USA). Total protein was quantified using the RC-DC assay (Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions.

2.3. Two-dimensional electrophoresis (2-DE)

A total of 80 μg of protein was resuspended in rehydration sample buffer (8 M urea, 2 M thiourea, 1% CHAPS, 12 mM DTT, 0.5% IPG buffer). Then, immobilized pH gradient (IPG) 3–10 non-linear strips, 7 cm, (Immobiline™ pH Gradient, GE Healthcare) were in-gel rehydrated overnight for the first dimension isoelectric focusing (IEF), performed on a horizontal Ettan™ IPGPhor (Amersham Biosciences, USA). Isoelectric separation was performed using the following focusing program: 12 h at 50 mW (rehydration), 1 h at 150 V (gradient), 1 h at 500 V (gradient), 1 h at 1000 V (gradient) and 90 min at 5000 V ("step-and-hold"). After IEF, IPG strips were equilibrated with equilibration buffer (2% (w/v) SDS, 6 M urea, 30% glycerol, 0.05 M Tris–HCl pH 8.8 and 20 mg/ml DTT) for 30 min at room temperature. Strips were then placed on the top of a 12% SDS-PAGE gel for the second dimension separation and ran at a constant voltage. Gels were stained with colloidal Coomassie G-250 or gels were transferred onto a nitrocellulose membrane. Proteins were blotted on a nitrocellulose membrane (Whatman®, Protan) in transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3 and 20% methanol) during 2 h at 200 mA. Stained gels were analyzed by using Melanie analysis software v.7.0 (GeneBio, Switzerland). Protein separations by 2-DE was carried out three independent times. The signal intensities of proteins spots were compared among both conditions and scored by fold-change.

Table 1
Immunoreactive proteins identified by 2DE-MALDI-TOF/TOF.

Spot	Protein	Accession number	Protein name	M_w (KDa)	p*I*	Function	PSORTb localization	Cello localization	Number of epitopes
1	FtnA	Q5HN41	Ferritin	19.58	4.55	Iron-storage protein	Cytoplasmic	Cytoplasmic	7
2	ClpP	Q5HQW0	ATP-dependent Clp protease proteolytic subunit	21.38	5	Cleaves peptides in various proteins in a process that requires ATP hydrolysis. Has a chymotrypsin-like activity. Plays a major role in the degradation of misfolded proteins	Cytoplasmic	Cytoplasmic	7
3	Pgk	Q5HQV3	Phosphoglycerate kinase	42.74	4.76	Catalyzes the transference of a phosphate group from 3-phospho-D-glycerate to ADP	Cytoplasmic	Cytoplasmic	14
4	SsaA	Q5HLV2	Staphylococcal secretory antigen SsaA	27.91	8.4	Not known; immunogenic protein expressed during sepsis and particularly during episodes of infective endocarditis	Extracellular	Extracellular	16
5	EF-TU	Q5HRK4	Elongation factor Tu	43.16	4.7	This protein promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis	Cytoplasmic	Cytoplasmic	22
6	Fda	Q5HL21	Fructose-bisphosphate aldolase class 1	32.99	4.89	Glycolytic enzyme that catalyses D-fructose 1,6-bisphosphate into glycerone phosphate and D-glyceraldehyde 3-phosphate	Unknown	Cytoplasmic	8
7	TpiA	Q5HQV2	Triosephosphate isomerase	27.37	4.9	Catalyses the interconversion of D-glyceraldehyde 3-phosphate and glycerone phosphate	Cytoplasmic	Cytoplasmic	13
8	GpmA	Q5HLI0	2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase	26.7	6.46	Catalyzes the interconversion of 2-phosphoglycerate and 3-phosphoglycerate	Cytoplasmic	Cytoplasmic	10
9	CodY	Q5HPT7	GTP-sensing transcriptional pleiotropic repressor CodY	28.75	5.61	It is a GTP-binding protein that senses the intracellular GTP concentration as an indicator of nutritional limitations. At low GTP concentration it no longer binds GTP and stop to act as a transcriptional repressor	Cytoplasmic	Cytoplasmic	15
10	Ldh	Q5HL31	L-lactate dehydrogenase	34.1	4.93	Catalyzes the reduction of pyruvate into lactate	Cytoplasmic	Cytoplasmic	9
11	GpmI	Q5HQV1	2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	56.36	4.8	Catalyzes the interconversion of 2-phosphoglycerate and 3-phosphoglycerate	Cytoplasmic	Cytoplasmic	18
12	FusA	Q5HRK5	Elongation factor G	76.88	4.8	This protein promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome	Cytoplasmic	Cytoplasmic	34
13	DnaK	Q5HNW6	Chaperone protein DnaK	66.15	4.57	Acts as a chaperone	Cytoplasmic	Cytoplasmic	28
14	GroEL	Q5HMZ1	60 kDa chaperonin	57.75	4.59	Prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions	Cytoplasmic	Cytoplasmic	26
15	ButA	Q5HKG6	Diacetyl reductase [(S)-acetoin forming]	27.91	4.66	Catalyzes the irreversible reduction of 2,3-butanediol to (S)-acetoin in the presence of NADH	Cytoplasmic	Cytoplasmic	12
16	PfkA	Q5HNK6	6-phosphofructokinase	34.88	5.34	Catalyzes the reaction of D-fructose 6-phosphate into D-fructose 1,6-bisphosphate	Cytoplasmic	Cytoplasmic	12
17	Asp23	Q5HM47	Alkaline shock protein 23	19	4.92	May play a key role in alkaline pH tolerance	Unknown	Cytoplasmic	7
18	Ald	Q5HNJ6	Alanine dehydrogenase	40.2	5.03	May play a role in cell wall synthesis as L-alanine is an important constituent of the peptidoglycan layer	Cytoplasmic	Cytoplasmic	14
19	RpsA	Q5HP69	30S ribosomal protein S1	43.37	4.46	Binds mRNA; thus facilitating recognition of the initiation point. It is needed to translate mRNA with a short Shine-Dalgarno (SD) purine-rich sequence	Cytoplasmic	Cytoplasmic	15

2.4. Immunoblotting

Sera samples were obtained from three volunteers after informed consent was obtained. The experiment was approved by the Ethics Committee of Instituto Ciências Biomédicas Abel Salazar (document number 081/2014).

Nitrocellulose membranes were blocked in 5% BSA (Sigma–Aldrich) in TBS-T (Tris-Buffered Saline-Tween 20) for 2 h. Then, membranes were incubated with human serum (1:200) for 2 h at room temperature. Following this incubation, membranes were washed with TBS-T for 10 min. Membrane washing step was repeated three times. Membranes were incubated with a secondary anti-human Imunoglobulin G (IgG, A0170, Sigma–Aldrich) (1:1000) during 1 h at room temperature. After washing the membranes, immunodetection was performed with enhanced chemiluminescence ECL (Amersham Pharmacia Biotech) according to the manufacturer's procedure and the images were recorded using X-ray films (GE Healthcare). The films and the gels were scanned in Molecular Imager Gel Doc XR + System (Bio-Rad) and analyzed with QuantityOne software (v. 4.6.9 Bio-Rad).

2.5. In-gel protein digestion and protein identification by mass spectrometry

Reactive protein spots were selected and manually excised from stained gels. An *in-gel* digestion of gel spots, with trypsin TPCK treated (ABSciex), was performed as previously described (Carvalhais et al., 2015). Briefly, peptides extraction was made with 10% formic acid/acetonitrile acid. Dried peptides were dissolved in 5% acetonitrile (VWR), 0.1% formic acid (Fluka Analytical, Sigma–Aldrich) and 0.1% trifluoroacetic acid (Sigma–Aldrich). Peptides were directly deposited onto 384-well MALDI plates (Applied Biosystems, Foster City, CA, USA) with α-CHCA matrix solution. α-CHCA matrix solution was prepared by diluting 2.5 mg/mL of α-CHCA in ACN 70%/TFA 0.3%. Experiments were carried out in technical duplicates. Mass spectra were obtained on a matrix assisted laser desorption/ionization-time of flight MALDI-TOF/TOF mass spectrometer (4800 Proteomics Analyzer, Applied Biosystems) in the positive ion reflector mode and obtained in 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 Global Protein Server (GPS) Workstation (Applied Biosystems). Searches were performed against the SwissProt (release date 06022013) protein database for *Firmicutes*. The database search parameters were: mass tolerance of 40 ppm for precursor ions and 0.4 Da for fragment ions; trypsin digestion with two missed cleavages. Protein identity was accepted at the 95% confidence level.

2.6. Bioinformatic analyses

Gene ontology (GO) (Ashburner et al., 2000) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (Kanehisa et al., 2004) analysis were performed to determine the function of

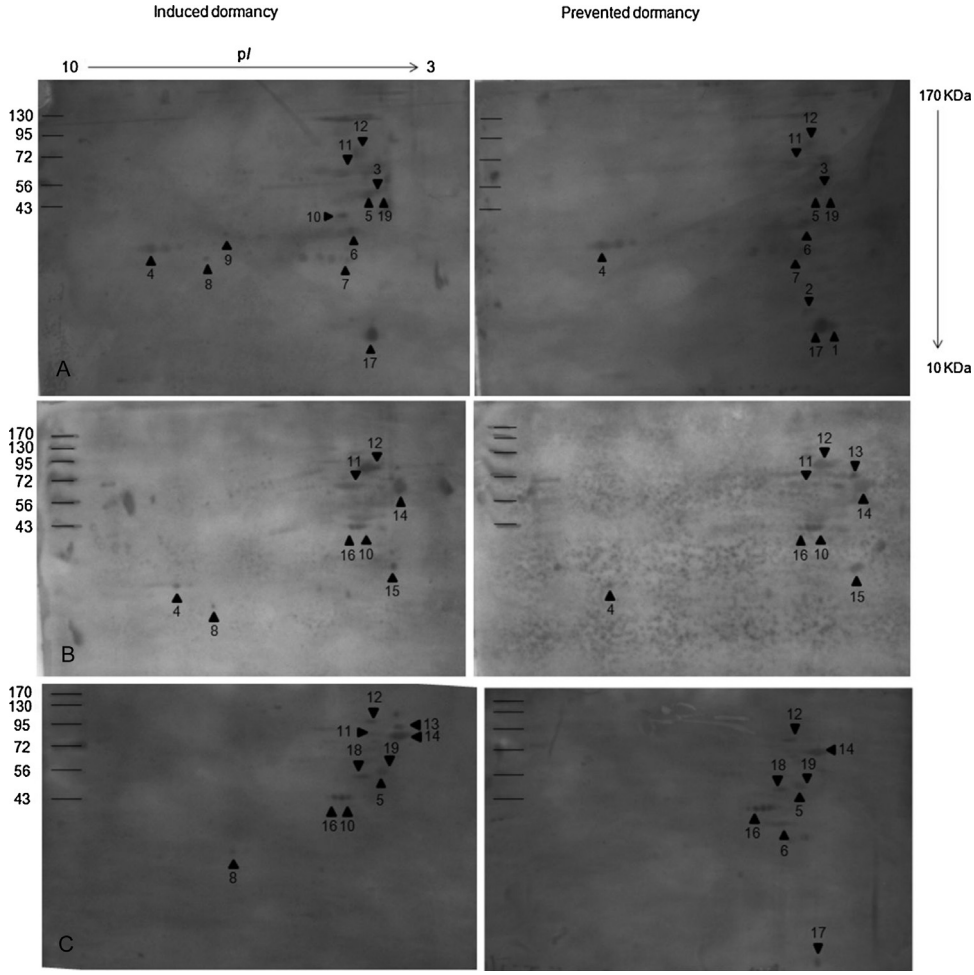

Fig. 1. Immunoblotting profile of whole proteins of *S. epidermidis* biofilms with induced and prevented dormancy. The immunoblotting analysis was performed with different human sera. (A) donor 1, (B) donor 2, and (C) donor 3. Protein spot identification is mentioned in Table 1.

identified proteins, using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (version 9.1) (at 28112014) (Franceschini et al., 2013). STRING was also used to construct the protein network. Cellular localization of proteins were predicted or identified by PSORTb program v.3.0.2 (Yu et al., 2010) and by Cello v.2.5 (Yu et al., 2004). Immunoreactive proteins were query in ABCPred to predict B cells epitopes *in silico* (Saha and Raghava, 2006).

3. Results and discussion

Biofilm formation is considered the major virulence factor of *S. epidermidis* (Otto, 2009). It has been described that bacteria antigen profile is affected by the mode of bacterial growth (Sanchez et al., 2011), such as planktonic or biofilm cells, which may have potentially meaningful implications in host recognition and consequent immune response. Here, to identify the repertoire of immunoreactive proteins in dormant condition, we characterized the immunoproteome of *S. epidermidis* biofilms with induced and prevented dormancy (see Supplementary Fig. 1), using human serum as a probe. Analysis of the 2-DE separation of whole biofilm cell protein extract from both conditions showed more than 120 protein spots in induced and prevented dormancy (Supplementary Fig. 2). A 2.0 fold-change protein intensity was set as a significant threshold between the two conditions. Apparently, up to 5 spots in each experiment had a fold-change higher than 2.0. The employment of 2-DE methodology in immunoproteomic studies is a powerful tool to identify antigens when combined with Western blotting (Fulton and Twine, 2013). Interestingly, a distinct immunoreactive protein profile of *S. epidermidis* biofilms with prevented and induced dormancy was found in all tested sera (Fig. 1). Our results showed a total of 19 immunoreactive protein spots identified by MALDI-TOF/TOF (Table 1). Most of the immunoreactive proteins spots were located in the pH range of 4–5 and in the molecular weight range of 20–40 kDa (Table 1).

Only GpmI and FusA proteins were reactive to all tested sera, independently of biofilm dormancy. Both are related to ion binding molecular function, but no more evident connections are known, since GpmI is associated with glycolysis and FusA with protein biosynthesis. On the other hand, GpmA protein seemed to be more immunoreactive in induced dormancy, in all tested sera. Other proteins, such as Pgk, TpiA and Ald, were recognized by only one serum sample. The reactive pattern diversity found among sera samples may be due to differences on immune response of donors or previous exposure to *S. epidermidis*, since it is a commensal microorganism of skin and mucosae (Otto, 2012). Interestingly, the immunoblot pattern included a set of reactive proteins which seemed to be only immunoreactive in biofilms with induced dormancy, such as CodY and GpmA. Conversely, FtnA and ClpP proteins were reactive in biofilms with prevented dormancy condition. Interestingly, CodY is a GTP-binding protein which responds to GTP and senses nutrient availability, controlling the expression of genes involved in the biosynthesis and transport of amino acids in several Gram-positive species (Sonenshein, 2005). Typically, CodY is repressed during rapid growth and induced when cells experience nutrient deprivation (Sonenshein, 2005), which may be related with dormancy.

Despite no direct involvement with dormancy has been previously described (Amato et al., 2014), genes encoding proteins here identified, have been linked to particular circumstances in biofilms. For example, the expression of *ftnA* was affected by the presence of iron and manganese ions in the biofilm growth conditions (Morrissey et al., 2004). Also, the deletion of *clpP* was associated with a reduced ability to form *S. epidermidis* biofilms and with reduced virulence in a rat model of biofilm-associated infection

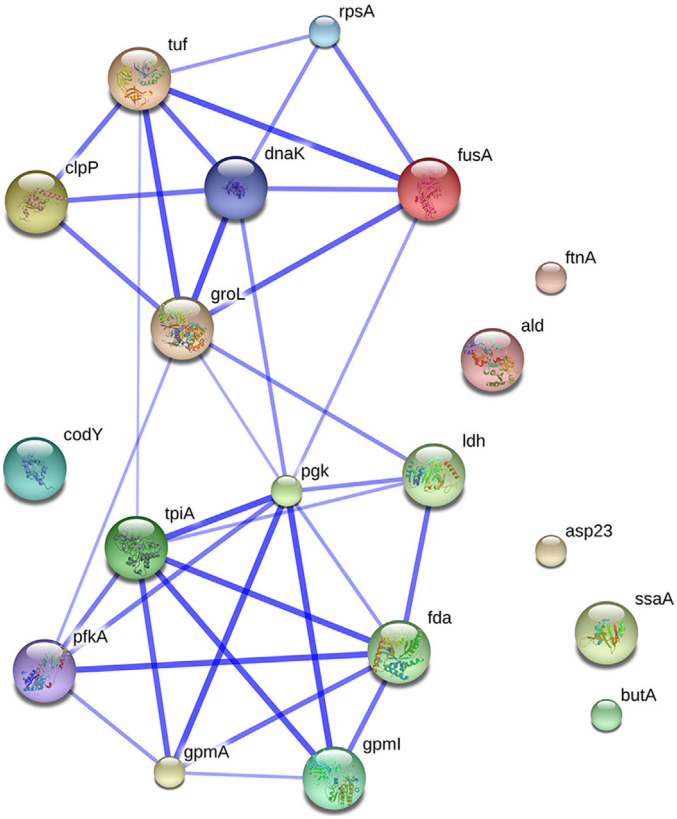

Fig. 2. STRING network generated with immunoreactive proteins identified by 2DE-MALDI-TOF/TOF.

(Wang et al., 2007). Interestingly, among the immunoreactive proteins, 3 had previously been reported to be immunoreactive with sera from rabbits infected with live *S. epidermidis* (Sellman et al., 2005), namely EF-TU, Fda and Ald. Additionally, surface proteins are known to be crucial determinants for host colonization (Scott and Barnett, 2006), such as SsaA, which is a well-known immunogenic protein (Lang et al., 2000). Similarly, in *Chlamydia trachomatis*, proteins like DnaK, EF-TU, GroEL and RpsA were also immunoreactive (Sanchez-Campillo et al., 1999). Since EF-TU, DnaK and GroEL are highly conserved genes (Craig, 1985), they are frequently found as immunogens in several species (Sanchez-Campillo et al., 1999; Mariappan et al., 2010; Shinoy et al., 2013; Yang et al., 2011). Additionally, EF-TU, DnaK and GroEL are among the proteins here identified, with a higher number of antigenic determinants to B-cell (Van Regenmortel, 2009) (Table 1), which may be the reason for been highly reactive.

In parallel, STRING tool was used to construct the interaction network with immunoreactive proteins. Generated network was enriched in protein interactions as shown in Fig. 2. The three most representative classes of these proteins for biological processes, molecular functions and KEGG pathways are shown in Fig. 3. The immunogenic proteins represented a broad range of biological functions, including small molecule metabolic process (GO:0044281), catabolic processes (GO:0009056), carbohydrate metabolic processes (GO:0005975), generation of precursor metabolites and energy (GO:0006091) and biosynthetic processes (GO: 0009058). These proteins are mainly involved in metabolic pathways, such as glycolysis/gluconeogenesis, microbial metabolism in diverse environments, biosynthesis of secondary metabolites, fructose and mannose metabolism and methane metabolism. The main molecular functions found were related to catalytic activity (GO:0003824), ion binding (GO:0043167) and oxidoreductase activity (GO:0016491). Bioinformatic analyses were

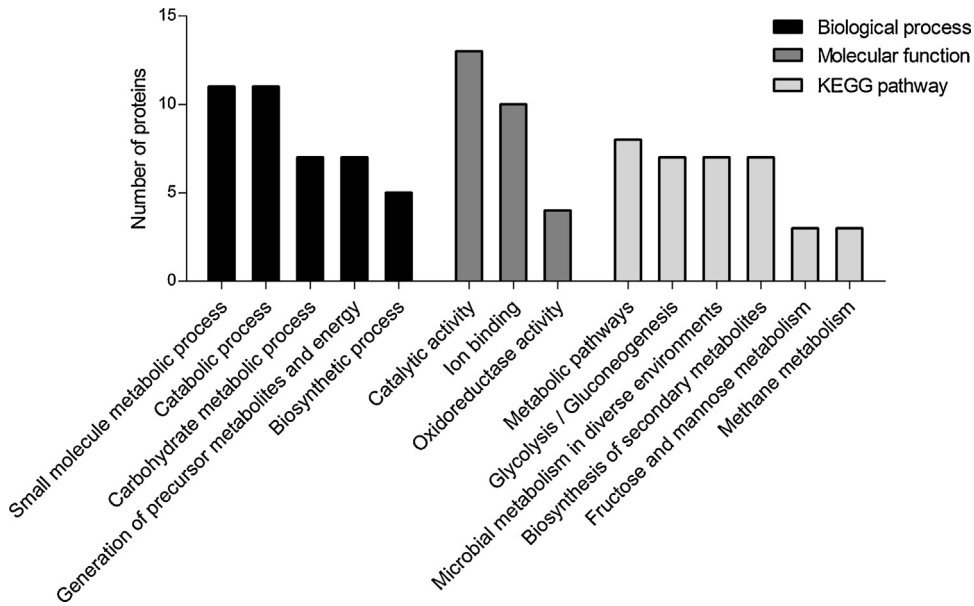

Fig. 3. The most representative GO terms of biological processes, molecular functions and KEGG pathways of immunoreactive proteins.

used to predict subcellular localization of proteins. Results showed one protein with extracellular localization. Remaining proteins were predicted to have cytoplasmic localization (Table 1). These results suggest that ion binding function may influence the host immune response, since previously, we identified this class of proteins with altered expression between *S. epidermidis* biofilms with induced and prevented dormancy (Carvalhais et al., 2015).

4. Conclusion

Taken together, our results showed that proteins from *S. epidermidis* biofilms with prevented and induced dormancy had different reactivity to human serum, providing the first evidences of dormancy impact in the human–bacteria immune interaction. Nevertheless, differences in the reactivity pattern were mainly observed in intracellular proteins, which can present difficult access to immune system. Despite individual host factors, we found differences in the immunoreactive protein pattern between *S. epidermidis* biofilms with different proportion of dormant bacteria. The immunoreactive proteins made part of a diverse group of proteins, ranging from proteins with proteolysis activity (ClpP), through proteins involved in iron transport (FtnA), proteins associated with glycolysis (GpmA) or proteins with transcription regulation biological function (CodY). In general, it is most likely that immunoreactive proteins are involved in small molecule metabolic processes or catabolic process, with capacity to interact with ion or charged atoms and catalytic activity.

Characterization of dormancy within biofilms using immunoproteomics provided new insights into the protein expression that may determine *S. epidermidis* contact with the host. Moreover, these proteins are promising candidates as biofilm markers allowing the discrimination of physiological condition displayed by biofilm bacteria and will be worth to consider in further studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molimm.2015.02.024.

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