Exploring the Mechanisms behind *S. epidermidis* Resistance to Vancomycin using Bioinformatics Analysis

Laís Carvalho[1,2 [0009-0006-4769-139X]], Ângela França[1,3 [0000-0001-7117-6837]] and Miguel Rocha[2,3[0000-0001-8439-8172]]

1 LIBRO-Laboratório de Investigação em Biofilmes Rosário Oliveira, Centre of Biological Engineering, University of Minho, Braga, Portugal; 2 Bioinformatics Laboratory, Centre of Biological Engineering, University of Minho, Braga, Portugal; 3 LABBELS-Associate Laboratory, Braga/Guimarães, Portugal.

1 Introduction

The *Staphylococcus* genus is composed of bacteria that can be found as a natural constituent of human skin and mucous membranes and is divided into two groups: coagulase-positive and coagulase-negative (CoNS) [1], which are differentiated by their ability to produce free coagulase [2]. CoNS group, which is represented by heterogeneous and opportunistic bacteria, are a recurring subject with regard to human health [3], due to their ability to colonize medical devices (such as vascular catheters, prosthetic joints, vascular grafts, heart valve prostheses and peritoneal catheters), putting the success of medical procedures at risk [3, 4]. Within this group, the *Staphylococcus epidermidis* species is one of the most investigated, as it is the main bacterium isolated from medical-device related infections [5], and because of its multidrug-resistance that complicates the treatment these infections [6].

To develop more effective therapeutic strategies against the infections caused by *S. epidermidis*, it is necessary to understand how the bacterium respond, for instance, in the presence of antibiotics. In that sense, omics sciences, which enable a comprehensive understanding of the biological systems, have been pivotal in creating new solutions to medical and scientific problems.

Herein, the aim of this study is to use transcriptomics to characterize the response of *S. epidermidis* cells sensitive and with induced-resistance to vancomycin to analyze differentially expressed genes and subsequently identify genetic networks and metabolic pathways involved in the resistance to vancomycin. This will provide an orientation for future research focused on the development of new therapeutic approaches.

2 *S. epidermidis* and its implications

2.1 The bacteria

S. epidermidis, is the bacterium most frequently isolated from the human epithelium, colonizing the armpits, head and nasal openings. The unfavorable conditions of this bacterium's natural habitat are overcome by its genetic makeup, which interestingly also gives it advantage as an opportunist pathogen [7]. In addition to this, this bacterium

has other mechanisms that increase resistance and/or virulence namely: (i) the ability to form biofilms and (ii) horizontal transfer of genes and elements related to antibiotic resistance [8].

2.2 Biofilm formation

Biofilms are aggregates of microorganisms adhered to biotic and abiotic surfaces and embedded in a matrix composed of extracellular molecules. These constitute a protection strategy against natural or artificial antibiotics, bacteriophages and phagocytic cells [9]. *S. epidermidis*, for example, can form biofilms quickly on medical implants resulting in symptoms of infection, with the removal of the implant being the most efficient form of treatment [10], which has important socioeconomic implications.

Biofilm lifecycle in *S. epidermidis*, is determined by three phases: (i) adhesion, which can be on biotic or abiotic surfaces; in the case of abiotic surfaces coated with host molecules, the adhesion occurs like on biotic surfaces, being facilitated by ligand-receptor interactions and adhesins of the bacterial surface; (ii) maturation, which is the stage at which bacterial cells begin to divide, connect with each other and, thus, form a protective matrix composed of polysaccharides, proteins, nucleic acids and molecules from the environment; and, finally,(iii) the dispersal, where bacterial cells leave the biofilm and potentially colonize new surfaces; this, is an important stage for the survival of the bacterium and the spread of infection [8, 11].

Biofilm is the main virulence factor of CoNS bacteria, including *S. epidermidis*, as it serves as physical protection against antimicrobial agents and has a well-established relationship with antibiotic resistance [12, 13].

2.3 Antibiotic resistance

The introduction of antibiotics in human and veterinary medicines in the mid-1940s favored the development of antibiotic-resistant bacteria. According to the World Health Organization, antimicrobial resistance is one of the main global threats to public health and development, resulting in 1.27 million global deaths in 2019 [19]. The beginning of the 2020s was marked by review articles aimed at highlighting the clinical emergence of CoNS bacteria [20-22], as well as original articles characterizing CoNS, in particular *S. epidermidis* strains resistant to different antibiotics, as a way of understanding the mechanisms that give this group of bacteria this phenotype [23-27].

The number of *S. epidermidis* resistant to penicillin, oxacillin/methicillin, ciprofloxacin, clindamycin and erythromycin has increased dramatically [14]. β-Lactam antibiotics, such as methicillin, interfere in the biosynthesis of the bacterial cell wall, but their action is less effective due to the presence of the *blaZ* gene, located in plasmids and producing penicillinases, and the *mecA* gene present in the SCCmec chromosomal cassette, in which its phenotype is responsible for inactivating the activity of most β-lactams [8].

Due to the multi-resistance shown by CoNS, the number of alternative drugs for treating infections caused by this group has been reduced, now one of the last-line agents is vancomycin, and the development of resistance to this antibiotic is a scientific

medical concern.[15]. Vancomycin is an antibiotic from the glycopeptide group, widely used to treat infections caused by methicillin-resistant staphylococci. With the research carried out on the subject, several plasmid-mediated genes (such as *vanA*, *vanB*, *vanB1*, *vanB2*, *vanC1*, *vanC2*, *vanC3*, *vanD*, *vanE*, and *vanG*) have been associated with vancomycin resistance and were initially acquired through contact with *Enterococcus faecalis*, which is naturally resistant to this antibiotic [14]. In 1999, the first case of *S. epidermidis* less susceptible to the action of vancomycin was recorded in the United States [16], but before this, in 1979 and 1983, the first cases of CoNS resistant to this antibiotic were recorded, in studies of the synergy of different drugs for the treatment of infections caused mainly by *S. epidermidis* [17, 18].

3 Bioinformatics resources and tools in research on *S. epidermidis*

As one of the many approaches to understanding the mechanisms of resistance of different bacteria to antibiotics, omics data such as the transcriptome and metabolome are used to determine the genes or pathways under- or over-expressed in resistant bacteria [19]. The high volume of data generated in omics experiments requires storage locations, which are biological databases. These online libraries are essential for searching for scientific information [20], and for carrying out bioinformatics analysis. Many bioinformatics analyses are dependent on the presence of data and the quality of biological annotations to guarantee the robustness and significance of the results. Taking this into account, different databases aggregate information on S. epidermidis and can serve as safe resources for downloading information, datasets and contextualizing the results obtained.

3.1 Genomic and proteomic databases

Universal Protein Resource (UniProt)

UniProt (https://www.uniprot.org/) is a protein database, featuring amino acid sequences, annotations of functions, structures, interactions, sub-cellular localization, and gene expression, among others. UniProt Knowledgebase (UniProtKB) is the central resource that combines Swiss-Prot, which contains sequences annotated by literature review or peer review, and TrEMBL, which contains sequences annotated automatically by computational predictions [22].

Serving mainly as a data server, it also features analysis tools capable of aligning sequences and generating phylogenetic trees between different organisms. Featuring more than 2400 protein records from different strains of *S. epidermidis* in Swiss-Prot, this database is commonly used in different studies with this opportunistic bacterium, such as in studies to discover non-human homologous targets in the bacterium and the development of new drugs, serving as a reliable source for downloading sequences from different strains [23, 24], and as a tool to elucidate the differences in enzymatic activity between two different growth states, biofilm and planktonic [25].

Bacterial and Viral Bioinformatics Resource Center (BV-BRC)
The BV-BRC (https://www.bv-brc.org/) is the result of combining the resources of the BCR, the Pathosystems Resource Integration Center (PATRIC), the Influenza Research Database (IRD) and the Virus Pathogen Database and Analysis Resource (ViPR). The most well-known and commonly used acronym for the BV-BRC is PATRIC, a platform for searching and analyzing pathosystem data, encompassing genomic information, annotations, metadata and non-genomic data, such as domains and motifs [26].

With PATRIC it is possible to perform gene expression analyses, predict metabolic pathways, analyze antibiotic resistance, phylogenetics, genome comparisons and analyze pathogenicity. Featuring different *S. epidermidis* strains, this database and its tools can be used to identify *S. epidermidis* defense mechanisms and mobile elements from the available annotations [6], or for assessing genome diversity and identifying virulence elements [27]. And it can also be used for comparative genomics between *S. aureus* and *S. epidermidis* to identify homologous or unique genes of each species [28].

3.2 Database of metabolic pathways and functional annotations

Kyoto Encyclopedia of Genes and Genomes
KEGG (https://www.genome.jp/kegg/) is a database and software based on understanding and simulating the functional behavior of cells or organisms through their protein information [29]. This database contains different information, such as genomes, metabolic pathways, protein interactions, gene expression, modules and signaling pathways [30]. With wide applicability, it can be used to analyze the metabolic pathways of *S. epidermidis*, being functional in determining proteins involved in pathogenic pathways for the development of possible new drugs [31]. Another way of applying the functionalities of KEGG is through the identification of enriched metabolic pathways resulting from protein modifications [32].

BioCyc
This database (https://biocyc.org/) contains genomic and metabolic pathway data, integrates information from other databases and uses computational tools to predict the organism's metabolic network [33]. It features software, Pathway Tools, which allows you to obtain data from regulatory networks, metabolic modelling and ways to analyze omics data [34]. Linked to KEGG, this database has records of use with *S. epidermidis* to search for additional metabolic reactions in metabolic modelling studies [35], or to confirm the accuracy of the metabolic model created for this bacterium [36].

Gene Ontology (GO)
The GO (http://geneontology.org) is a vast database that presents functional data, such as gene functions, cellular components and biological processes [37], used as a source of information in research with *S. epidermidis*, is a safe resource for obtaining useful annotations to differentiate proteomic and transcriptomic profiles of bacterial biofilms grown under certain conditions [38]. It is linked to other databases or bioinformatics

tools, enabling enrichment analyses, identification of metabolic pathways and functional annotation of genes.

4 Gene Set Enrichment Analysis

Gene set enrichment analysis (GSEA) is a method used in bioinformatic analysis of experimental expression data and offers powerful biological insights. This method is used to determine whether a group of genes is differentially expressed in different phenotypes [39], and is a way of interpreting gene expression data and identifying relevant patterns. The method is based on: (i) obtaining expression data for the different phenotypic conditions of study and processing this data; (ii) carrying out differential expression analysis for individual genes using the most viable method, such as fold change, t-statistics, ANOVA coefficient, and others, (iii) defining a list of genes of interest; (iv) selecting a group of reference genes, this part is associated with databases such as KEEG, GO, and others; (v) carrying out the statistical method, which would be the application of GSEA or variant methods [40, 41].

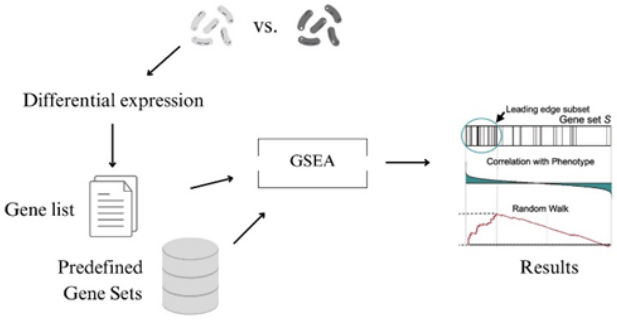

Figure 1. Workflow for gene set enrichment analysis [40–42].

There are different tools for carrying out this type of analysis that only differ in the statistical method used, such as: Protein Analysis Through Evolutionary Relationships (PANTHER) tool, which is interconnected with GO; GSEA; DAVID; PAGE; topGO; Enrichr and ShinyGO [43–45]. Other ways of carrying out GSEA is by using packages in R or Python after determining the reference genes. Some of the packages used in R are: "fgsea" [46], GAGE [47] and “pathfindR” [48]. And in Python they are: GSEAp [49], or Enrichr, which provides a Python API [45] and GeneSetAnalysis.

Some research has applied this type of analysis to determine the metabolic pathways enriched in states of dormancy among *S. epidermidis* biofilms using the Cytoscape v2.8.3 software [50], others have applied methods similar to GSEA, GAGE, to assess the plasticity of *S. epidermidis* under a range of stresses and nutrient-limited conditions [51]. Some studies use several databases to search for and build the annotations needed to carry out the enrichment analysis of a group of genes, as was done by [52], who used

KEGG, GO and BioCyc to search for annotations and "fgsea" in conjunction with GSEA to understand the response of *S. epidermidis* to heat shock and medically relevant glucose levels.

4.1 ShinyGO

ShinyGO is a web-based functional enrichment tool designed for analysis with animals and plants, but it also extends its applications to other types of organisms, such as bacteria and archeal. It is a Shiny-based implementation for analyzing enriched GO terms. It contains an annotation base derived from Ensembl and STRING-db and allows graphical visualization of enrichment results. The main input for carrying out the analysis is uploading the list of genes and selecting the species you want to investigate. With an easy-to-use interface, it is a valuable tool for exploring biological functions [42].

5 Project plan

The project aims to investigate, by enrichment analysis of a group of differentially expressed genes, the mechanisms underlying the resistance of *S. epidermidis* to vancomycin. To reach these conclusions, it is planned to:

(1) Generate the list of differentially expressed genes from previous differential analyses, on RNA-Seq reads of the transcriptome of *S. epidermidis* RP62A bacteria before and after induction of vancomycin resistance[1];

(2) To evaluate different tools for performing enrichment analysis of a group of genes, considering the presence of annotations for the *S. epidermidis* species. The intention is to focus on the ShinyGO tool;

(3) Execution of the analysis and graphically visualizing and interpreting the results obtained;

As a topic of great importance to health, the project aims to gain relevant insights into *S. epidermidis* defense strategies against antibiotics, in particular vancomycin, which will serve as a starting point for future studies aiming to develop new and more effective therapeutic strategies.

[1] As mentioned above, the transcriptomic data was obtained previously. In the references [38, 53] you can find information on the processing and obtaining of the transcriptomic data, and in the references [54–58], you can understand some of the methodologies used to define the protocol for inducing *S. epidermidis* resistance to vancomycin.

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