

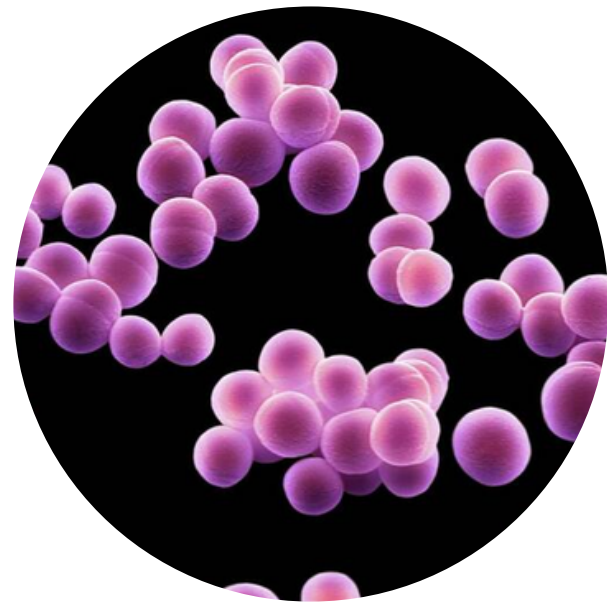


Exploring the Mechanisms behind *S. epidermidis* Resistance to Vancomycin using Bioinformatics Analysis

Laís Carvalho, Ângela França, Miguel Rocha



Coagulase-negative staphylococci (CoNS), particularly *Staphylococcus epidermidis*, are significant in healthcare due to their ability to form biofilms and colonize medical devices causing infections.



Staphylococcus epidermidis

This issue is exacerbated by its multidrug resistance, including resistance to vancomycin.

Premature infants, immunosuppressed patients, and those requiring long-term medical devices are especially at risk.



The aim of this project was to identify the main pathways enriched in the metabolism of *S. epidermidis* with vancomycin-induced resistance.



1

Data

Gene expression data in excel spreadsheet (xlsx)

2

ShinyGO

A web-based graphical tool for enriching gene sets

3

KEGG

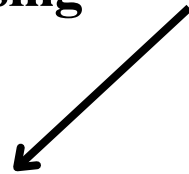
Bioinformatics database that integrates genomic, chemical and functional information, the information can be accessed by python packages and modules, such as **Bio.KEGG** and **bioservices**.

Methods



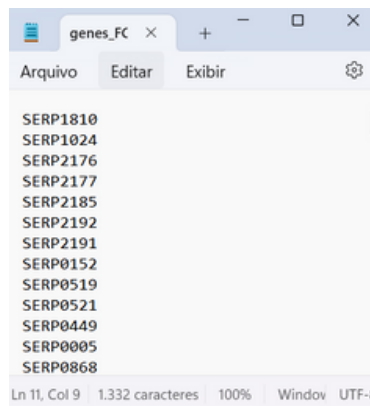
1

Data processing



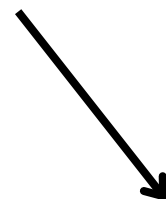
Feature ID	Experiment - Range (original values)	Experiment - IQR (original values)	Experiment - Difference (original values)	Experiment - Fold Change (original values)	Baggerley's test: IR VAN vs CT original values - Weighted proportions difference	Baggerley's test: IR VAN vs CT original values - Weighted proportions fold change
0	aacA 292.994173	228.882040	161.929852	1.628734	0.000173	1.715500
1	aap 39.799318	34.085116	23.779558	1.684861	0.000025	1.772160
2	abcA 92.503501	57.977391	-47.393972	-1.233737	-0.000036	-1.178618
3	accA 139.307849	126.905145	110.134086	1.457791	0.000119	1.525711
4	accB 187.444037	124.459192	113.418243	1.351291	0.000126	1.415937
...
2630	yycH 97.226507	40.392937	-49.531409	-1.400982	-0.000040	-1.330827
2631	yycI 127.615565	58.269470	-59.517179	-1.349253	-0.000048	-1.281687
2632	yycJ 27.050614	26.780691	-17.455786	-1.095749	-0.000009	-1.050033
2633	zwf-1 11.451203	9.420434	6.704356	1.788828	0.000007	1.875720

Data



2

Applying the gene list in ShinyGO



ShinyGO 0.80

Change species: *Staphylococcus epidermidis* *RP62A* STRINGdb

Demo genes:

Change the species if it is not human. Then just paste a list of genes and click Submit. Gene IDs can be NCBI, Ensembl, symbol, or other common types.

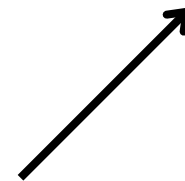
Background (recommended) ☐ Submit

FDR cutoff: # pathways to show:

Pathway size: Min. Max.

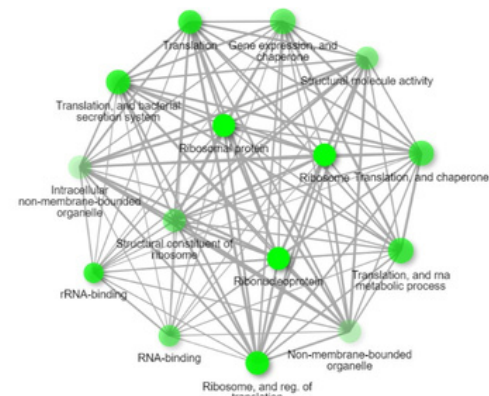
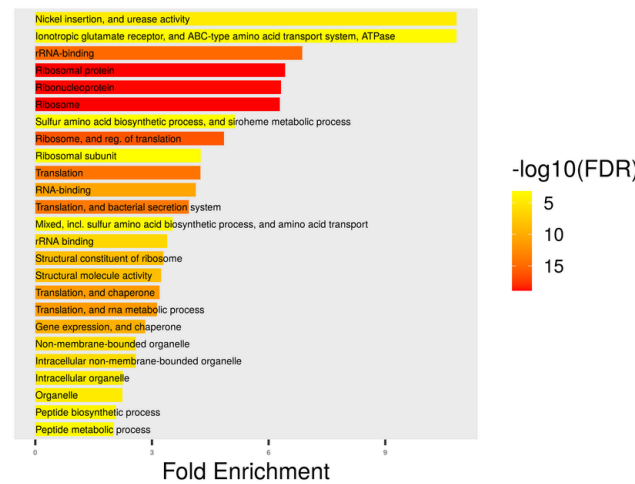
☒ Remove redundancy ☒ Abbreviate pathways

☐ Use pathway DB for gene counts ☐ Show pathway IDs



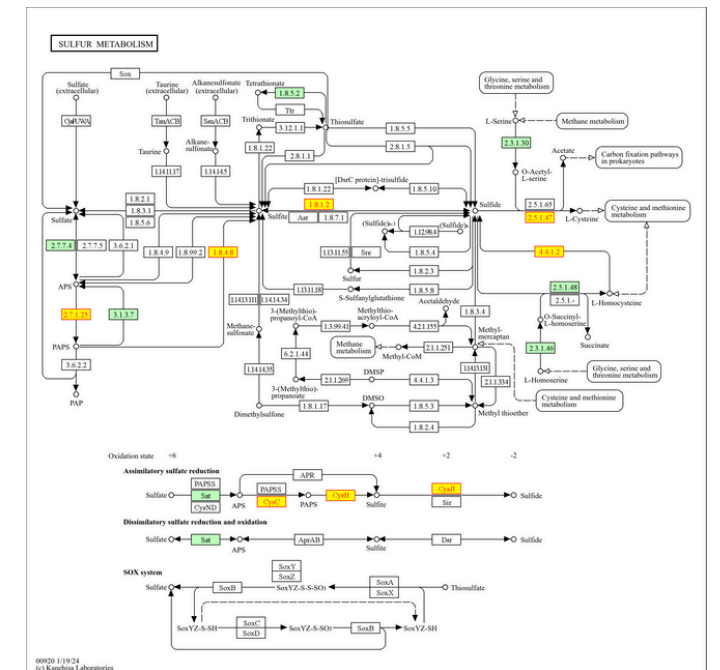
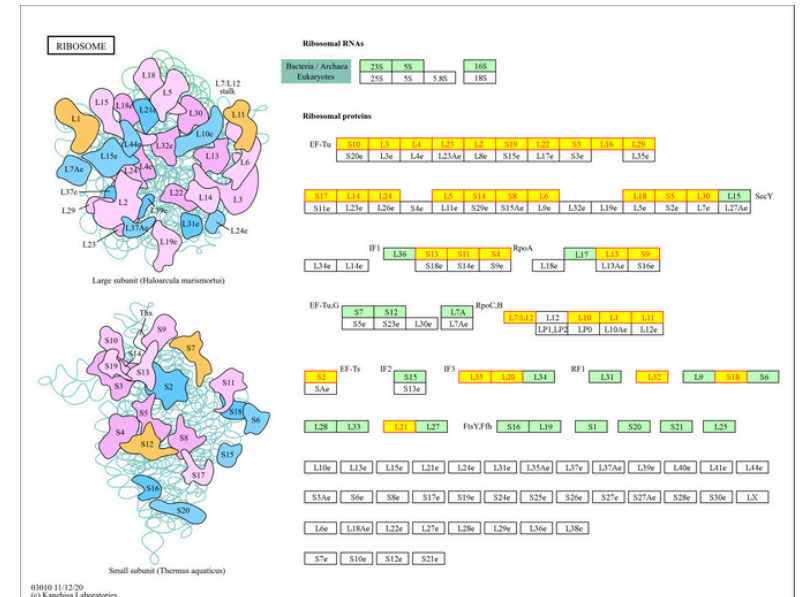
Results

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathway	URL	Genes
0	4.530005e-21	26	41	10.745976		SERP0046 SERP0178 SERP0179 SERP0180 SERP1211 S...
1	1.256079e-26	35	59	10.052462		SERP0046 SERP0178 SERP0179 SERP0180 SERP0181 S...
2	1.430888e-26	35	60	9.884921		SERP0046 SERP0178 SERP0179 SERP0180 SERP0181 S...
3	4.719116e-26	35	62	9.566052		SERP0046 SERP0178 SERP0179 SERP0180 SERP0181 S...
4	1.103149e-05	9	19	8.026853		SERP0036 SERP0094 SERP0046 SERP0152 SERP1166 S...
5	1.480076e-23	38	87	7.401517		SERP0046 SERP0178 SERP0179 SERP0180 SERP0181 S...
6	5.612674e-06	11	28	6.657191	http://amigo.geneontology.org/amigo/term/GO:00...	SERP0179 SERP0180 SERP0181 SERP0223 SERP1284 S...
7	1.199628e-20	38	102	6.313059		SERP0046 SERP0178 SERP0179 SERP0180 SERP0181 S...



3

Get the metabolic pathways





Feature ID	Experiment - Range (original values)	Experiment - IQR (original values)	Experiment - Difference (original values)	Experiment - Fold Change (original values)	Baggerley's test: IR VAN vs CT original values - Weighted proportions difference	Baggerley's test: IR VAN vs CT original values - Weighted proportions fold change	Baggerley's test: IR VAN vs CT original values - Test statistic	Baggerley's test: IR VAN vs CT original values - P-value
aacA	292.994173	228.882040	161.929852	1.628734	0.000173	1.715500	1.487221	0.136957
aap	39.799318	34.085116	23.779558	1.684861	0.000025	1.772160	1.648674	0.099215
abcA	92.503501	57.977391	-47.393972	-1.233737	-0.000036	-1.178618	-1.005391	0.314709
accA	139.307849	126.905145	110.134086	1.457791	0.000119	1.525711	3.404836	0.000662
accB	187.444037	124.459192	113.418243	1.351291	0.000126	1.415937	1.959460	0.050059
...
yycH	97.226507	40.392937	-49.531409	-1.400982	-0.000040	-1.330827	-1.087926	0.276628
yycI	127.615565	58.269470	-59.517179	-1.349253	-0.000048	-1.281687	-0.906014	0.364929
yycJ	27.050614	26.780691	-17.455786	-1.095749	-0.000009	-1.050033	-0.674502	0.499992
zwf-1	11.451203	9.420434	6.704356	1.788828	0.000007	1.875720	1.591939	0.111399
zwf-2	232.208325	230.292300	-147.181758	-1.402003	-0.000123	-1.342866	-1.575461	0.115150

Data

The data was filtered to keep only the genes with FDR p-values < 0.05 and a Fold Change > 2 .

The list of genes generated to be applied in ShinyGO had to have the names of the genes changed to match the identifiers used in the STRING.

All genes (2 genes) corresponding to tRNA or pseudogenes were eliminated.



ShinyGO 0.80

Change species

Staphylococcus epidermidis
RP62A STRINGdb

Demo genes

Reset

SERP2079
SERP2081
SERP2115
SERP2117
SERP2130
SERP2131
SERP2138
SERP2178

Background (recommended)

Submit

Pathway database:

All available gene sets

FDR cutoff

0,05

pathways to show

25

Pathway size: Min.

2

Max.

5000



☒ Remove redundancy

☐ Abbreviate pathways













The input to this tool is the list of genes of interest, and it is also necessary to select the species of the study organisms and the number of pathways you want to observe.

The other parameters have been left by default (FDR cutoff, maximum and minimum pathway size and search databases).



 jupyter Bioinf_project Last Checkpoint: há 12 minutos (autosaved)  Logout

File Edit View Insert Cell Kernel Widgets Help Not Trusted Python 3 (ipykernel)

           Markdown 

Obtain the graphic representation of the enriched pathways

The ShinyGO tool allows graphical visualization of the enriched pathways. However, limitations such as the difficulty in integrating data from different databases, the lack of annotations on the organism studied or the outdated nature of the online tool can interfere with the results and make it impossible to visualize the pathways graphically.

To obtain the graphs of the enriched paths, the following functions were developed:

```
In [1]: # Useful python packages
import pandas as pd
from Bio.KEGG import REST
import re
from bioservices import KEGG
```

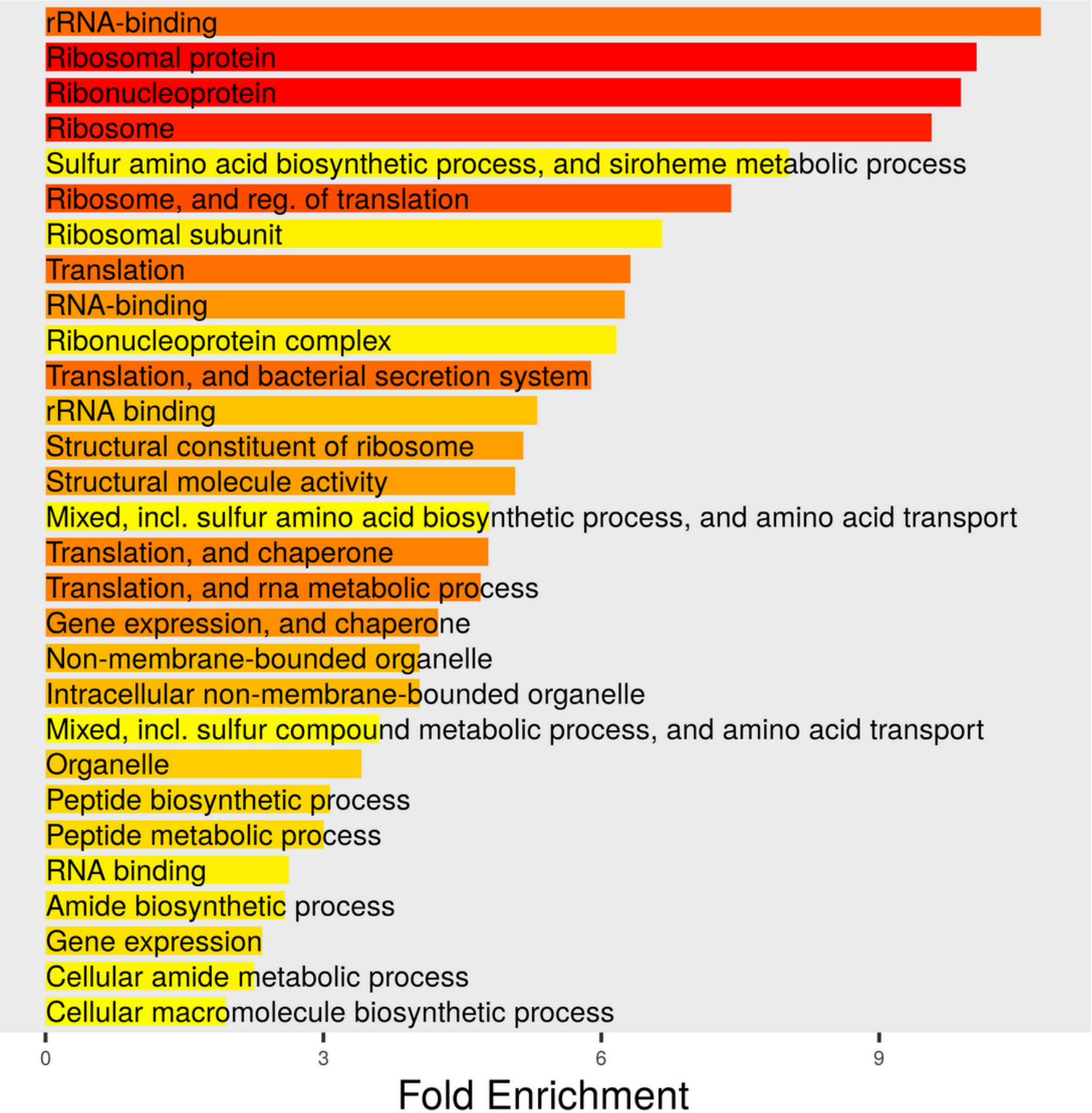
```
In [2]: def gene_name_format(gene_list, term):
        """Function that receives a list of genes and adds the specified term (usually the identification term of the study organism)
        gene_list_form = []
        for gene in gene_list:
            gene_list_form.append(term + gene)
        return gene_list_form

def get_pathways_shinyGO(file_path):
    """It uploads the table (result) obtained to ShinyGO and formulates a dictionary that stores the name of the enriched pathway
    df_ShinyGO = pd.read_csv(file_path)
    pathway_dict = {}
    for index, row in df_ShinyGO.iterrows():
        pathway = row['Pathway']
        gene = row['Genes']

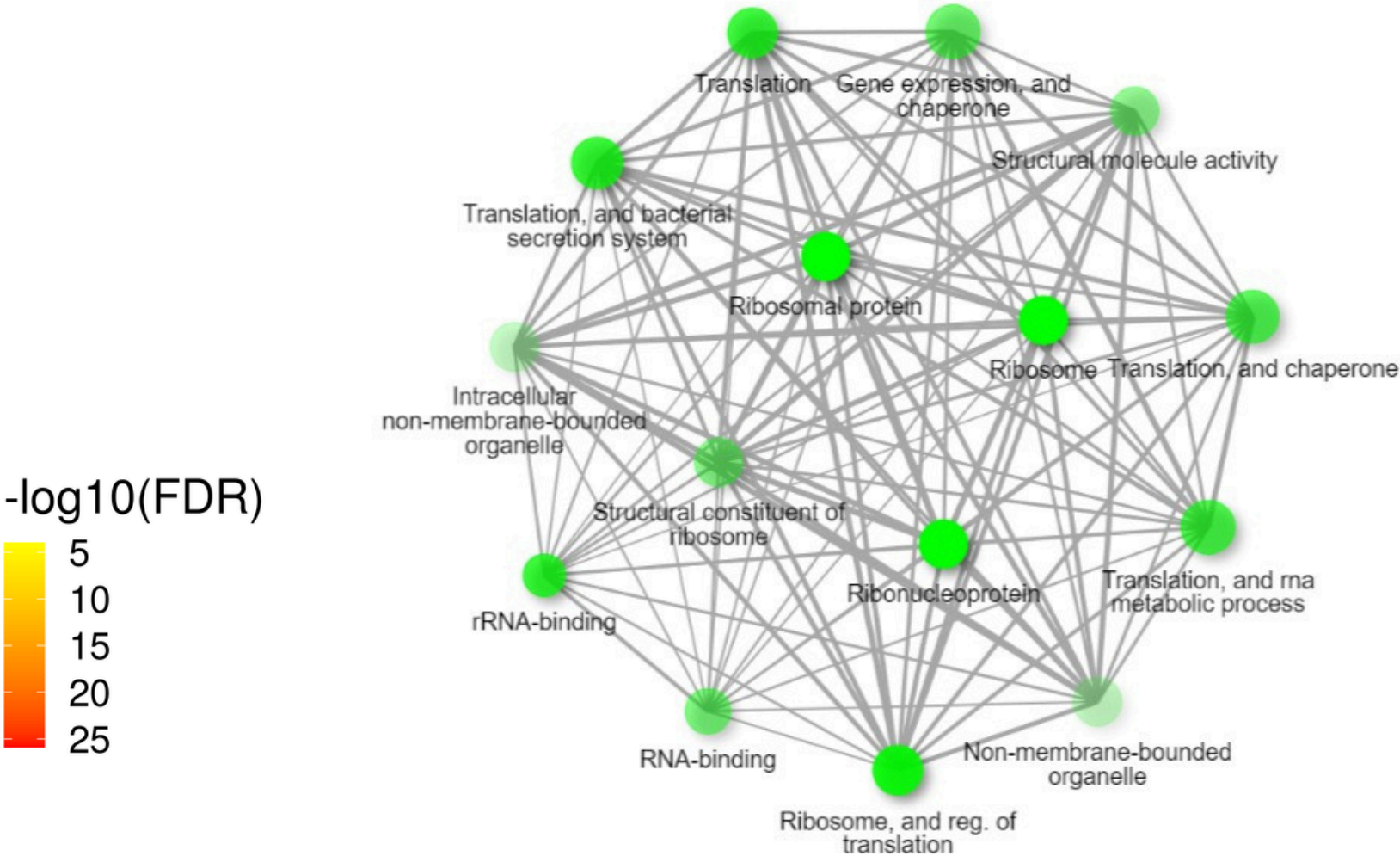
        if pathway not in pathway_dict:
            pathway_dict[pathway] = []
```

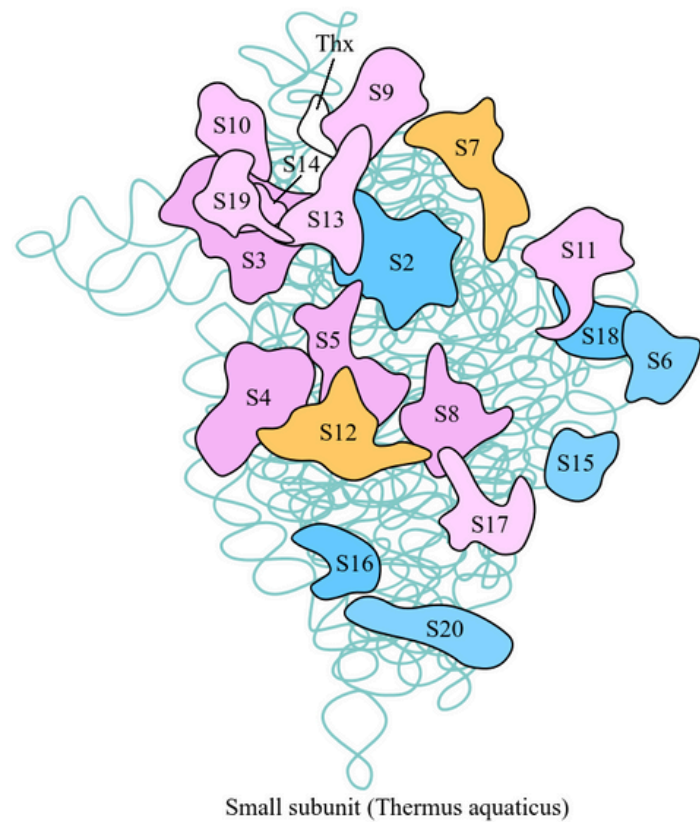



Barplot of enriched metabolic pathways



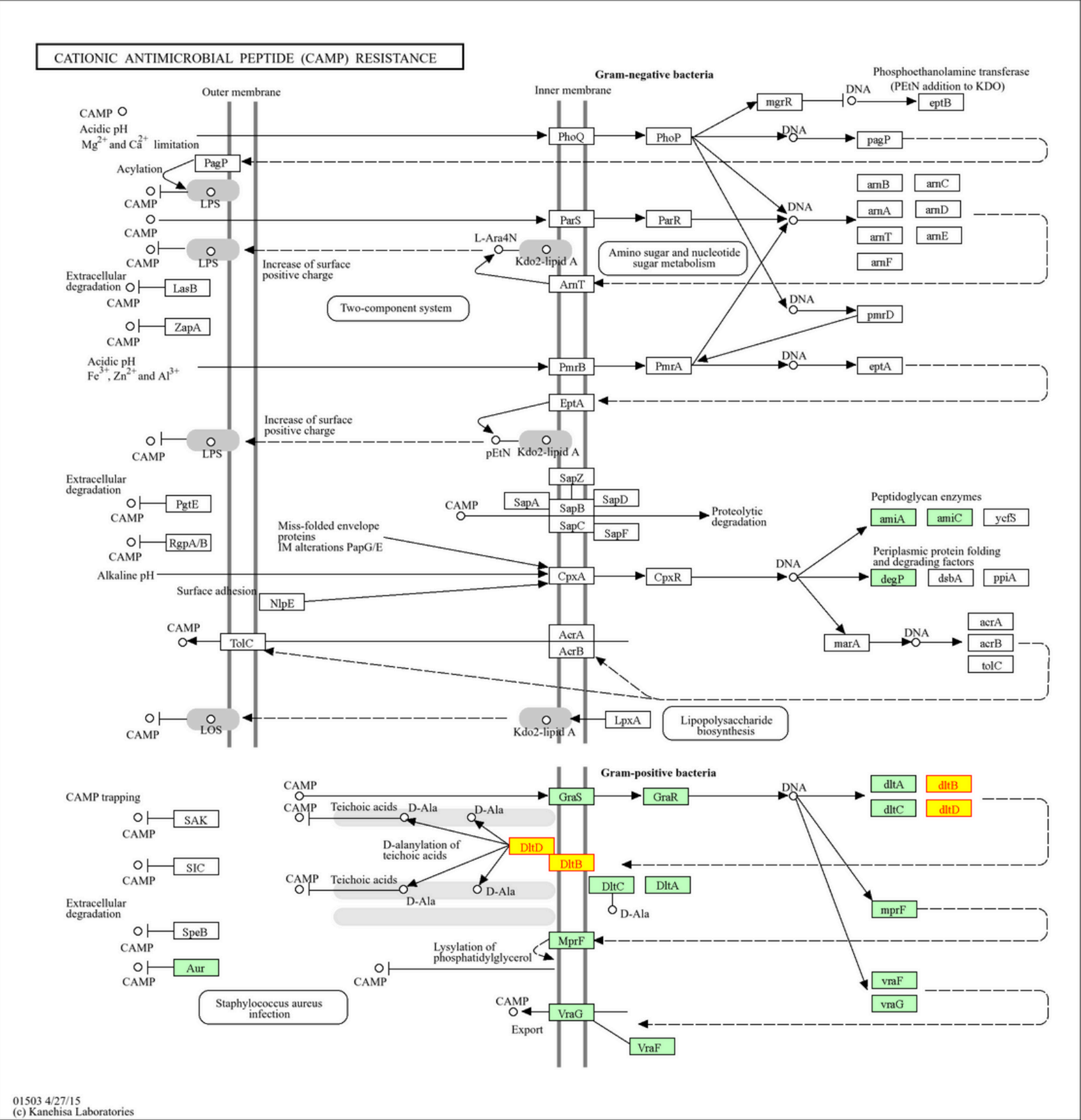
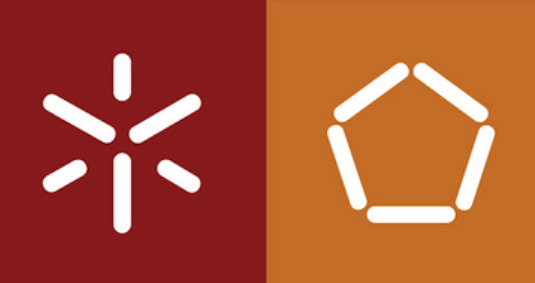
Network of enriched pathways





Ribosome

The main enriched metabolic pathway. The genes highlighted in yellow are those present in the set of genes studied (fold change > 2).



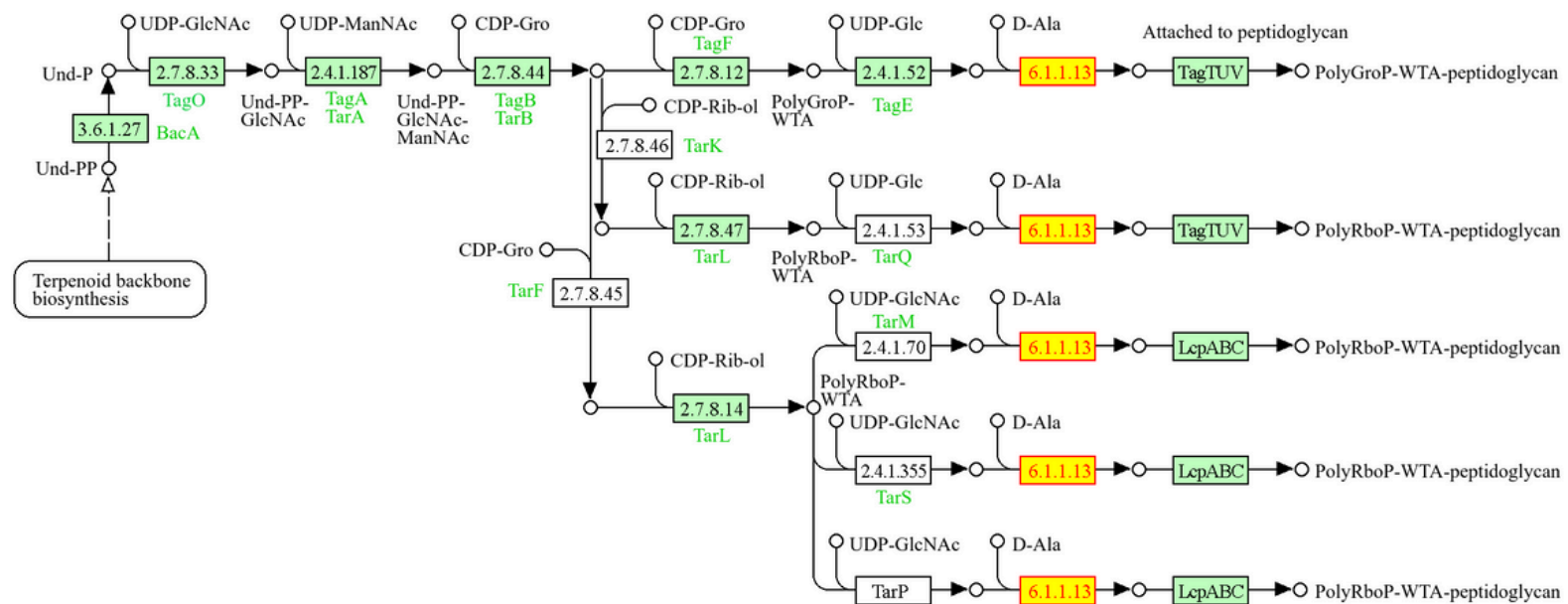
This pathway is related to mechanisms to overcome the antimicrobial action of cationic proteins of the host's innate immune system, and may include modification of the bacteria's surface charge what can influence the interaction of vancomycin with the cell wall and, thus, vancomycin efficacy.

Cationic antimicrobial peptide (CAMP) resistance

Image of the metabolic pathway obtained from KEGG. The genes highlighted in yellow are those present in the set of genes studied (fold change > 2).

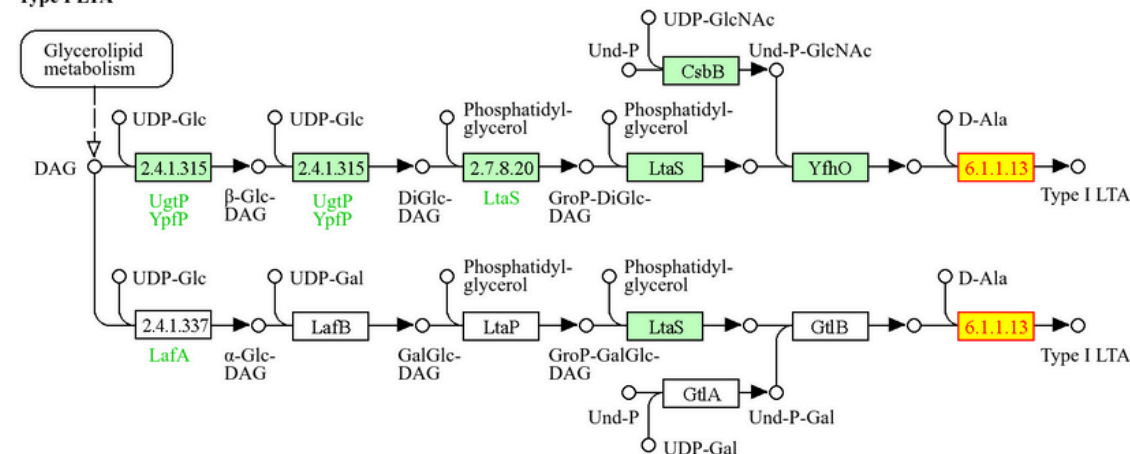
TEICHOIC ACID BIOSYNTHESIS

Wall teichoic acid (WTA)

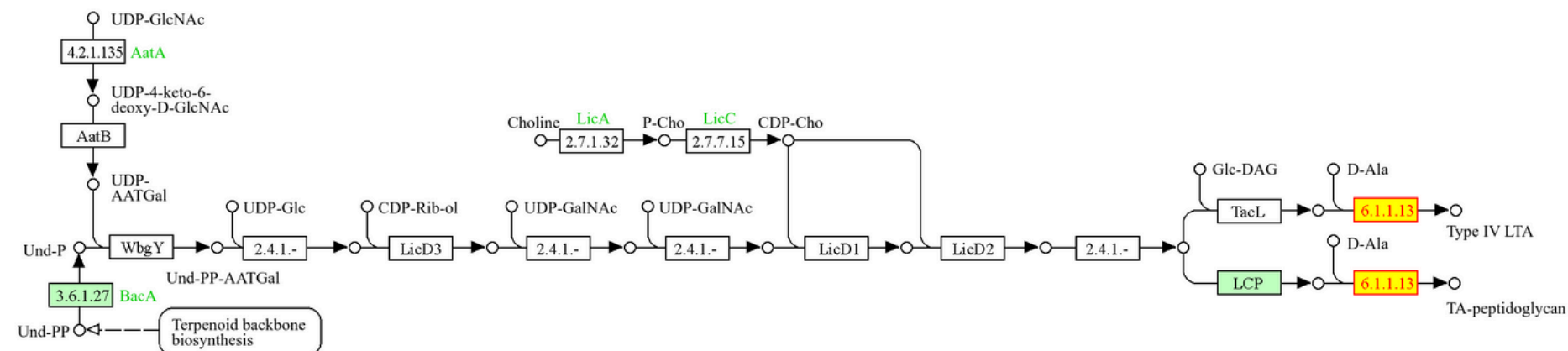


Lipoteichoic acid (LTA)

Type I LTA



Type IV LTA and TA-peptidoglycan



Results

The synthesis of teichoic acid is an essential process in the formation of the cell wall in *S. epidermidis* and other Gram-positive bacteria.

Teichoic acid biosynthesis

Image of the metabolic pathway obtained from KEGG. The genes highlighted in yellow are those present in the set of genes studied (fold change > 2).



Final considerations

Highly expressed genes are present in pathways that may explain the resistance of *S. epidermidis* to vancomycin;

Directing laboratory research to resistance mechanisms.

Next steps

Further explore the additional pathways acquired;

Rerun the analysis for another strain;

Rerun the analysis for under-expressed genes.



- França, A., Gaio, V., Lopes, N., Melo, L.D.R.: Virulence Factors in Coagulase-Negative Staphylococci. *Pathogens*. 10, 170 (2021). <https://doi.org/10.3390/pathogens10020170>.
- Rogers, K.L., Fey, P.D., Rupp, M.E.: Coagulase-Negative Staphylococcal Infections. *Infect. Dis. Clin. North Am.* 23, 73–98 (2009). <https://doi.org/10.1016/j.idc.2008.10.001>.
- Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., Hattori, M.: The KEGG resource for deciphering the genome. *Nucleic Acids Res.* 32, D277–D280 (2004). <https://doi.org/10.1093/nar/gkh063>.
- Ge, S.X., Jung, D., Yao, R.: ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics*. 36, 2628–2629 (2020). <https://doi.org/10.1093/bioinformatics/btz931>.
- Loureiro, Rui João, Roque, Fátima, Rodrigues, António Teixeira, Herdeiro, Maria Teresa, & Ramalheira, Elmano. (2016). O uso de antibióticos e as resistências bacterianas: breves notas sobre a sua evolução. *Revista Portuguesa de Saúde Pública*, 34(1), 77-84. <https://doi.org/10.1016/j.rpsp.2015.11.003>



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

Laís Carvalho, Ângela França, Miguel Rocha