Functional Prediction of Biological Profile During Eutrophication in Marine Environment

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Yousra Sbaoui, Badreddine Nouadi, Abdelkarim Ezaouine, Mohamed Rida Salam, Mariame Elmessal, Faiza Bennis and Fatima Chegdani

Immunology and Biodiversity Laboratory, Faculty of Sciences Aïn Chock, Hassan II University of Casablanca, Casablanca, Morocco.

ABSTRACT: In the marine environment, coastal nutrient pollution and algal blooms are increasing in many coral reefs and surface waters around the world, leading to higher concentrations of dissolved organic carbon (DOC), nitrogen (N), phosphate (P), and sulfur (S) compounds. The adaptation of the marine microbiota to this stress involves evolutionary processes through mutations that can provide selective phenotypes. The aim of this in silico analysis is to elucidate the potential candidate hub proteins, biological processes, and key metabolic pathways involved in the pathogenicity of bacterioplankton during excess of nutrients. The analysis was carried out on the model organism *Escherichia coli K-12*, by adopting an analysis pipeline consisting of a set of packages from the Cystoscape platform. The results obtained show that the metabolism of carbon and sugars generally are the 2 driving mechanisms for the expression of virulence factors.

KEYWORDS: Differentially expressed genes, copiotrophic species, functional analysis virulence, metabolic pathways

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CORRESPONDING AUTHOR: Yousra Sbaoui, Immunology and Biodiversity Laboratory, Faculty of Sciences Aïn Chock, Hassan II University of Casablanca, Casablanca 13703, Morocco. Email: yousra.sbaoui@gmail.com

Introduction

In recent decades, the emergence of molecular methods, especially the omics approach, has facilitated the study of microbial communities to understand their activities, compositions, interactions between taxa, and the use of nutrients.[1] Transcriptomics has often been coupled with other methods to understand the response of microbes to ecological interactions, nutrient acquisition, membrane transport, and growth, generating a large number of results that require strong tools to derive useful information.[2,3]

The marine coastal areas are increasingly subjected to anthropogenic and natural pollutants that affect the growth of macroorganisms and microorganisms.[4] Bacterioplankton has been linked to several types of pollution including wastewater,[5,6] chemicals,[7] organic or biological products, and waste.[8] During nutrient pollution (NP) caused by excess of nutrients specifically in coastal areas, the biota is negatively affected by algal blooms, increased growth of macroalgae, increased sedimentation and oxygen consumption, oxygen depletion in lower water layers and, sometimes, mortality of benthic animals and fish.[9] Through these negative effects, the bacterioplankton also undergoes several types of stress that act directly and indirectly on the functioning of the ecosystem and the microbiota.[10] This stress is caused by the higher concentrations of dissolved organic carbon (DOC), Nitrogen (N), Phosphate (P), and Sulfur (S) compounds,[11,12] to which the adaptation of bacterioplankton depends on the community structure, the physiology of the organisms, the variety of environmental conditions, and their interactions.[13,14]

To survive changing environments, bacteria have evolved exquisite systems that not only sense stress but also trigger appropriate responses.[15] Their responses are related to an adaptation that involves a known resistance process especially in pathogenic bacteria such as the case of *Listeria monocytogenes* and a direction also of the expression of virulence genes at the appropriate time and place.[16,17] An appreciation of stress responses and their regulation is therefore essential to understand bacterial pathogenesis. Among the modules of understanding used is the analysis of changes at the molecular and cellular level regulated by highly complex signaling pathways.[18] The whole is modulated in the form of protein-protein interaction (PPI) networks and other resulting networks because the phenomenon of protection against stress strongly suggests the presence of central proteins that control the various responses to stress.[19]

The study of PPI networks requires several open source or integrated software packages that allow the integration of biomolecular interaction networks with high-throughput expression data and other molecular states in a unified conceptual framework.[20] Cytoscape is a powerful platform in this field, with its various plugins and its conjunction with large databases, it allows the extraction of central processes, central metabolic pathways (MPs), and hubs proteins during a particular stress in humans and model organisms.[21–23]

The investigation of interactomes in model organisms such as *Arabidopsis thaliana (L.)*,[24] *Saccharomyces cerevisiae* (Meyen), and *Escherichia coli K-12*[25] has been involved in predicting and improving the understanding of cellular processes and biological interactions in other organisms.[26,27] Furthermore, the power of Cytoscape plugins in the analysis of microbiota has been documented in several works and in different microbiomes including intestinal,[28] oral,[29] vaginal,[30] and marine.[31,32]

The study of the behavior of bacterioplankton during nutrient excess as one of the environmental parameters that affect its capacity of pathogenesis is not well documented and has never been analyzed in silico. In this work, we want to study this capacity during eutrophication and algal blooming in the model organism *Escherichia coli K12*, through the analysis of a profile of differentially overexpressed genes (DEGs) collected from several bibliographic sources to predict hubs proteins, biological processes (BPs), and MPs involved in the selection of copiotrophic species and the virulence of bacterioplankton.

Materials and Methods

Data set collection and the choice of the model organism

A list of 196 DEGs, during NP by excess of inorganic and organic nutrients in aquatic environments (NH^{4+}, NO_2, NO^{3-}, PO_4^{3-}, H_2PO_4, $HPO_4^{2-}{\cdot}O_4P^{3-}$, N_2O, $CCaO_3$ CO_3, CO_2, HCO^{3-}, CH_4, S, SO_3^{2-}, SO, SO_3), with a fold change values >1 and adjusted *P value* <.05 has been collected from scientific publications (Table 1). And to avoid disambiguation, their ID has been verified in the UniProt database (https://www.uniprot.org/) and *EcoCyc* (the Encyclopedia of *E coli K-12* genes and metabolism).

The predictive analysis has been performed using the bacterium strain *E coli K12* as model organism for the aquatic bacterioplankton; *E coli K12* serves as the best characterized and good leader model organism for bacterial genetics and molecular biology studies.

Cytoscape pipeline analysis

To start the analysis, the DEGs' profile was annotated in multiple Cytoscape packages (Version: 3.8.2 https://cytoscape.org/) following the pipeline (Figure 1). The raw list (196 DEGs) was queried in StringApp to obtain the PPI networks. The tab-delimited ranking list txt file generated from String was analyzed to generate a subnetwork with the hub proteins reflected by the network analyzer plugin to show a topological mapping. The subnetwork was analyzed by the ClueGO to identify BPs and MPs related to excess of nutrients.

String analysis. The input list of 196 DEGs was analyzed by StringApp (Version: 11.0 https://string-db.org/) for a fixed search parameter with a confidence score cutoff to 0.4 without additional interactors. The resulting networks were customized by the layout and visual style in the control panel.

Subselection and topological mapping of hub proteins analysis. Three networks obtained by StringApp were subselected based on degree and filtered to obtain the hub proteins. The highlighted hub proteins and their first neighbors obtained were filtered to select the most significant terms. The results were mapped by Network analyzer plugin "http://apps.cytoscape.org/media/networkanalyzer."

ClueGO analysis. The subnetwork resulting from the subselection has been analyzed by ClueGO (Version: 1.5 http://www.ici.upmc.fr/) to select representative GO processes and pathways and visualizing them in functionally organized networks. Statistical analysis of ClueGO enrichment was defined using a hypergeometric test with $P \leqslant .05$, corrected by the Benjamin-Hochberg method, and kappa scores ⩾0.4 as primary endpoint.

Results and Discussion

String results

The list of 196 collected proteins (Table 1) was imported and analyzed by the StringApp. This latter has mapped and annotated all the genes right away. The results were performed in the format of a network with different evidence indexes (Figure 2), and the PPI networks obtained have identified 7 associated networks with a total of 165 out of 196 nodes, 442 edges, and a *P value* $<10^{-16}$. The 165 annotated proteins in the principal network are linked either directly or indirectly through one or more interacting proteins, which enhances the existence of functional links between them. These results suggest that the proteins are at least partially biologically connected as a group, maybe participate together in the same process and have the same phenotype, which has given great importance to co-expression and high weight to genetic and protein interactions.

The obtained PPI network was accompanied by a global functional enrichment analysis where BP, hub proteins, and MPs were exported. The results of the most 5 representative terms are shown in Table 2, where GO terms are generation of precursor metabolites and energy (GO.0006091), monocarboxylic process (GO:0032787), nicotinamide and metabolic process (GO:0046496), antibiotic metabolic process (GO:0016999), and small molecule biosynthetic process (GO:0044283), and the most significant MP are carbon metabolism (eco01200), pyruvate metabolism (eco00620), glycolysis/gluconeogenesis (eco00010), pentose phosphate pathway (eco00030), and methane metabolism (eco00680). These BPs and MPs involve biochemical reactions and pathways that ultimately lead to the formation of precursor metabolites and substances from which energy is derived.[39-44] This energy production is essential for the regulation of nutrient content during stress, to persist long enough, continue its cycle, and invade a new host.[45]

Simultaneously, the 10 genes chosen as hub proteins (Table 3) based on their combined score and their connectivity in Figure 2, which shows a co-expression profile, neighborhood, and appearance links between them and between (*eno, ftsH, ravA, codA, hemN/yggW, puuD, codA, mngB, norV, can*) that encoded for virulence factors such as ferrochatalases, metalloenzymes, enolases, hydrolases, and cytotoxic chemotherapeutic agents. These factors are often linked to MPs for nutrients and toxins such as lipopolysaccharides, proteases (zinc metalloproteases), and virulence factors induced by sugar metabolism in bacteria.[46,47]

Table 1. List of genes differentially overexpressed during nutrient excess (log FC > 1), collected from several bibliographical sources.[33-38].

GENES SYMBOL	UNIPROT ACCESSION ID	PROTEIN NAME
AbfD	*P55792*	Vinylacetyl-CoA isomerase
AccA	*P0ABD5*	Acetyl-CoA carboxyltransferase subunit α
AccD	*P0A9Q5*	Acetyl-CoA carboxyltransferase subunit β
AcnB	*P36683*	Bifunctional aconitate hydratase B and 2-methylisocitrate dehydratase
AcnC	*P0ACI6*	DNA-binding transcriptional dual regulator
ACS	*P27550*	Acetyl-CoA synthetase
AdhE	*P0A9Q7*	Fused acetaldehyde-CoA dehydrogenase and iron-dependent alcohol dehydrogenase
Agaz	*P0C8K0*	Tagatose 6-phosphate aldolase 1, subunit Kbaz
AmoA	*Q04507/A0A5E9SRA6*	Ammonia monooxygenase alpha subunit
AppY	*P05052*	DLP12 prophage; DNA-binding transcriptional activator
AroD	*P05194*	3-dehydroquinate dehydratase
AstA	*P0AE37*	Arginine succinyltransferase
AtoB	*P76461*	Acetyl-CoA acetyltransferase
AtpF	*P0ABA0*	ATP synthase F0 complex—subunit b
Bcp	*P0AE52*	Thiol peroxidase, thioredoxin-dependent
BPSL3038	*Q63QI4*	Putative molybdopterin-containing oxidoreductase
BtuB	*P06129*	Cobalamin outer membrane transporter
Can	*P61517*	Carbonic anhydrase 2
ChaA	*P31801*	Sodium/calcium: proton antiporter (CaCA family)
CheY	*P0AE67*	Chemotaxis protein
CodA	*P25524*	Cytosine/isoguanine deaminase
CooS1	*P59934*	Carbon-monoxide dehydrogenase1
Cpc/ptrA	*C5P1W9/P05458*	Protease
CusR	*P0ACZ8*	DNA-binding transcriptional activator
CutA	*P69488*	Copper binding protein
CysH	*P17854*	Phosphoadenosine phosphosulfate reductase
DdpF	*P77622*	Putative dipeptide transport protein (ABC superfamily, atp_bind)
DgcZ	*P31129*	Enzyme diguanylate cyclase
DsbB	*P0A6M2*	Disulfide bond formation proteins (oxidoreductase) with quinone as electron acceptor, reoxidizes DsbA
DsrB	*P0AEG8*	Dissimilatory sulfate reductase
Edd	*P0ADF6*	Phosphogluconate dehydratase
Eno	*P0A6P9*	Enolase
EutC	*P19636*	Ethanolamine ammonia-lyase subunit β
EutG	*P76553*	Polypeptide putative alcohol dehydrogenase

(Continued)

Table 1. (Continued)

GENES SYMBOL	UNIPROT ACCESSION ID	PROTEIN NAME
FAZ83_23975	*A0A6D2XMK9*	Branched-chain amino acid ABC transporter permease
FbaA	*P0AB71*	Fructose-1,6-bisphosphate aldolase
Fbp	*P0A993*	Fructose-1,6-bisphosphatase
FccA	*W1IBJ7*	Flavocytochrome c sulfide dehydrogenase
FdhF	*P07658*	Formate dehydrogenase H
Fic	*P20605*	Possible cell filamentation protein, induced in stationary phase
FlhA	*P76298*	Flagellar biosynthesis protein
FocA	*P0AC23*	Formate transport protein (formate channel 1) (FNT family)
FolC	*P08192*	Bifunctional folylpolyglutamate synthase/dihydrofolate synthase
FolD	*P24186*	Methylene-tetrahydrofolate dehydrogenase
FolP1/Sul1	*Q4GY13*	Dihydropteroate synthase
FrpC	*P55127*	Iron-regulated protein
FtsH	*P0AAI3*	ATP-dependent zinc metalloprotease
FucR	*P0ACK8*	DNA-binding transcriptional activator
FumA	*P0AC33*	Fumarate hydratase class I
FumC	*P05042*	Fumarate hydratase class II
GapA	*P0A9B2*	Glyceraldehyde-3-phosphate dehydrogenase
GatC	*P69831*	Galactitol-specific PTS enzyme IIC component
GcX	*P39366*	Polypeptide KpLE2 phage-like element; putative endoglucanase with Zn-dependent exopeptidase domain
GlpX	*P0A9C9*	Fructose-1,6-bisphosphatase 1 class 2
GltA	*P0ABH7*	Citrate synthase
GpmB	*P0A7A2*	Putative phosphoglyceromutase 2
GuaC	*P60560*	GMP reductase
HcaF	*Q47140*	Putative 3-phenylpropionate/cinnamate dioxygenase subunit β
Hcp	*P75825*	Hydroxylamine oxidoreductase-like protein
HemH	*P23871*	Ferrochelatase
HemW	*P52062*	Heme chaperone
Hha	*P0ACE3*	Hemolysin expression-modulating protein
HlpA	*P0AEU7*	Periplasmic molecular chaperone for outer membrane proteins
HlyB	*P15492*	Alpha-hemolysin translocation ATP-binding protein
HlyE	*P77335*	Hemolysin E, chromosomal
Hns	*P0ACF8*	DNA-binding protein
HpcD	*Q05354*	5-carboxymethyl-2-hydroxymuconate Delta-isomerase

(Continued)

Table 1. (Continued)

GENES SYMBOL	UNIPROT ACCESSION ID	PROTEIN NAME
HtgA	*P28697*	Transcriptional activator for sigma H (sigma 32) promoters, permitting growth at high temperature
HybF	*P0A703*	Hydrogenase maturation protein
IbpA	*P0C054*	Small heat shock protein
IdnD	*P39346*	L-idonate 5-dehydrogenase
KorB	*P07674*	Transcriptional repressor protein
LtxA	*P16462*	Leukotoxin
LtxB	*A0A2G8ZPA1*	RTX toxin hemolysin A
MaeA	*P26616*	NAD-dependent malic enzyme
Mdh	*P61889*	Malate dehydrogenase
Mfd	*P30958*	Transcription-repair ATP-dependent coupling factor
MhpT	*P77589*	3-(3-Hydroxy-phenyl) propionate transporter
MoaD	*P30749*	Molybdopterin-containing oxidoreductase
Nac	*Q47005*	Nitrogen assimilation transcription factor
NapA	*P33938*	Nitrate reductase, periplasmic, large subunit
NapF	*P0AAL0*	Polypeptide ferredoxin-type protein
Nar	*P11350*	Nitrate reductase
NemA	*P77258*	N-ethylmaleimide reductase
NfsA	*P17117*	Oxygen-insensitive NADPH nitroreductase, also anaerobic azo reductase
NifH	*P00459*	Nitrogenase iron protein
NirB	*P08201*	Nitrite reductase (NADH) large subunit
NirD	*P0A9I8*	Nitrite reductase (NADH) small subunit
NirK	*P38501*	Copper containing nitrite reductase
NirS	*P24474*	Nitrite reductase
NmpC	*P21420*	DLP12 prophage; putative outer membrane porin
NorV	*Q46877*	Nitric oxide reductase
NosZ	*P19573*	Nitrous oxide reductase
NuoH	*P0AFD4*	NADH: quinone oxidoreductase subunit H
NuoJ	*P0AFE0*	NADH: quinone oxidoreductase subunit J
PaaZ	*P77455*	Crotonyl-CoA hydratase
ParC	*P0AFI2*	Dimer of DNA topoisomerase IV subunit A
PckA	*P22259*	Phosphoenolpyruvate carboxykinase
PfkA	*P0A796*	6-Phosphofructokinase
Pgi	*P0A6T1*	Glucose-6-phosphate isomerase
Pgk	*P0A799*	3-phosphoglycerate kinase
PheA	*P0A9J8*	Bifunctional: chorismate mutase P (N-terminal); prephenate dehydratase (C-terminal)

(Continued)

Table 1. (Continued)

GENES SYMBOL	UNIPROT ACCESSION ID	PROTEIN NAME
PheL	*P0AD72*	Phe operon leader peptide
PheT	*P07395*	Phenylalanine tRNA synthetase, beta-subunit
PhoU	*P0A9K7*	Phosphate-specific transport system accessory protein
PKS	*B2HIL7*	Polyketide synthase
PotH	*P31135*	Putrescine transport protein (ABC superfamily, membrane)
PpsA	*P23538*	Phosphoenolpyruvate synthase
Ppx	*P0AFL6*	Exopolyphosphatase
PRK1	*A0A4S5AZM1*	Phosphoribulokinase
ProA	*P07004*	Gamma-glutamyl phosphate reductase
PurL	*P15254*	Phosphoribosylformylglycinamidine synthase II
PurU	*P37051*	Formyltetrahydrofolate synthetase
PuuA	*P78061*	Gamma-glutamylputrescine synthetase
PuuD	*P76038*	Gamma-glutamyl-gamma-aminobutyrate hydrolase
Pyc	*Q58626*	Pyruvate carboxylase
PykA	*P21599*	Pyruvate kinase II
RavA	*P31473*	Regulatory ATPase
RbcL	*A0A2J1D642*	Ribulose bisphosphate carboxylase
Rbn	*P0A8V0*	Ribonuclease BN
RbsR	*P0ACQ0*	DNA-binding transcriptional dual regulator
RfbD	*P37760*	TDP-rhamnose synthetase, NAD(P)-binding
Rpe	*P0AG07*	Ribulose-5-phosphate 3-epimerase
RpiA	*P0A7Z0*	Ribose 5-phosphate isomerase
RpmJ	*P0A7Q6*	50S ribosomal subunit protein L36
RpoN	*P24255*	RNA polymerase, sigma 54 (sigma N) factor
RseP	*P0AEH1*	Intramembrane zinc metalloprotease
RtxA	*A0A3L0W7I6*	Multifunctional-autoprocessing repeats-in-toxin
ScpA	*P27253*	Methylmalonyl-coa epimerase
SdhA	*P0AC41*	Succinate dehydrogenase
SdhA	*P0AC41*	Succinate: quinone oxidoreductase, FAD binding protein
SeqA	*P0AFY8*	Negative modulator of initiation of replication
SoxR	*P0ACS2*	DNA-binding transcriptional dual regulator
Sqr	*P0AC41*	Sulfide: quinone reductase
StfR	*P76072*	Rac prophage; putative tail fiber protein
Suc	*P0AGE9*	Succinyl-CoA synthetase
SucA	*P0AFG3*	2-Oxoglutarate dehydrogenase E1 component
SulA	*P0AFZ5*	Suppressor of ion; inhibitor of cell division and FtsZ ring formation on DNA damage/inhibition

(Continued)

Table 1. (Continued)

GENES SYMBOL	UNIPROT ACCESSION ID	PROTEIN NAME
TktA	*P27302*	Transketolase 1
TorC	*P33226*	Cytochrome c-type protein in TMAO respiration; with TorA, also negative regulator of tor operon
TpiA	*P0A858*	Triosephosphate isomerase
Tsr	*P02942*	Protein methyl-accepting chemotaxis protein—serine-sensing
UgpA	*P10905*	Sn-glycerol-3-phosphate transport system permease protein
UmuD	*P0AG11*	Component of DNA polymerase V, signal peptidase with UmuC
Ung	*P12295*	Uracil-DNA-glycosylase
UraA	*P0AGM7*	Uracil: H+ symporter
UspD	*P0AAB8*	Universal stress protein D
XanP	*P0AGM9*	Xanthine: H+ symporter
XdhA	*Q46799*	Putative xanthine dehydrogenase molybdenum-binding subunit
YacG	*P0A8H8*	DNA gyrase inhibitor
YadC	*P31058*	Uncharacterized fimbrial-like adhesion protein
YafO	*Q47157*	mRNA interferase toxin YafO
YagZ	*P0AAA3*	Common pilus major subunit
YaiL	*A0A376JCL2*	Nucleoprotein/polynucleotide-associated enzyme
YbcJ	*P0AAS7*	Putative RNA-binding protein
YbdO	*P77746*	Putative LysR family DNA-binding transcriptional regulator
YbeV	*P77359*	Putative chaperone with DnaJ-like domain
YbgG	*P54746*	Putative sugar hydrolase with alpha-mannosidase domain
YcaP	*P75839*	DUF421 domain-containing protein
YcdG	*P75892*	Putative uracil transport protein (NCS2 family)
YcfJ	*P0AB35*	Putative membrane protein
YcgR	*P76010*	Flagellar brake protein
YdeP	*P77561*	Putative formate dehydrogenase, related to acid resistance with formate dehydrogenase/DMSO reductase
YdgF	*P69212*	Multidrug/spermidine efflux pump membrane subunit
YdiJ	*P77748*	Putative FAD-linked oxidoreductase
YdiL	*P76196*	Conserved hypothetical protein
YdjX	*P76219*	DedA family protein
YehT	*P0AFT5*	DNA-binding transcriptional dual regulator
yfaL	*P45508*	Serine protease autotransporter
YfbQ,	*P0A959*	Glutamate—pyruvate aminotransferase

(Continued)

Table 1. (Continued)

GENES SYMBOL	UNIPROT ACCESSION ID	PROTEIN NAME
YfdM	*P76509*	Prophage; putative methyltransferase
YfiF	*P0AGJ5*	Putative methyltransferase
YfjR	*P52133*	CP4-57 prophage; putative DNA-binding transcriptional regulator
YgeF	*Q46786*	Conserved hypothetical protein
YgeP	*Q46796*	Unknown CDS
YhbH	*P0AFX0*	Putative sigma N (sigma 54) modulator
YhbP	*P67762*	Putative FMN binding protein
YhcE	*P45421*	Putative uncharacterized protein
YheO	*P64624*	DNA-binding transcriptional regulator
YhjB	*P37640*	Putative response regulator in 2-component regulatory system
YhjC	*P37641*	Putative DNA-binding transcriptional regulator
YiaK	*P37672*	2,3-Diketo-L-gulonate reductase
YidE	*P60872*	Putative transport protein
YieM	*P0ADN0*	Conserved protein with Integrin A (or I) domain
YjaG	*P0A9V5*	Uncharacterized HTH-type transcriptional regulator
YjcE	*P32703*	Putative transporter
YjeK	*P39280*	Lysine 2,3-aminomutase
YjiD	*P39375*	Anti-adapter protein
YjiH	*P39379*	Gate family protein
YkfF	*P75677*	CP4-6 prophage; protein
YmdC	*P75919*	Putative synthase with phospholipase D/nuclease domain
YnbE	*P64448*	Lipoprotein
YnfD	*P76172*	DUF1161 domain-containing protein
YtfJ	*P39187*	Conserved hypothetical protein

Kennelly and Potts (1996) have stated that during stress conditions, microorganisms develop signal transduction systems from the outside to the inside of the cell.[48] These signals include degradative enzymes such as proteases, lipases, and substrate capture enzymes such as glutamine synthetase and alkaline phosphatase to detect environmental stresses and to control the coordinated expression of genes involved in cellular defense mechanisms.[49-51] Their response to these signals will enable their survival; enhance their resistance to a number of environmental stresses such as low pH, heat, and oxidative stress;[52,53] and/or enhance their virulence.

This is relatively true because Gram-positive bacteria especially *Actinobacteria* and *Firmicutes* present a diverse collection of regulatory proteins (*CcpA, CodY, and Rex*) of central metabolic capacities and virulence, which have been shaped by reductive evolution.[45,54,55] Among these Gram-positive bacteria is *Staphylococcus aureus* (*S aureus*), a strain indigenous to aquatic environments and thus transferred by discharges. In the presence of excess carbon, the regulatory protein *CcpA* stimulates transcription of *ilvB*operon, making *CodY* more active as a repressor of many pathways that remove intermediates from glycolysis and gluconeogenesis to be fully pathogenic.[45] And in Gram-negative bacteria, regulation is stimulated by *FNR* which is influenced by the histone-like protein *H-NS*; nevertheless, *FNR* has been shown to be important for virulence and survival of *Salmonella*.[15,56]

In the light of the above discussed results, we suggest that the metabolic behavior and central BPs are highly correlated

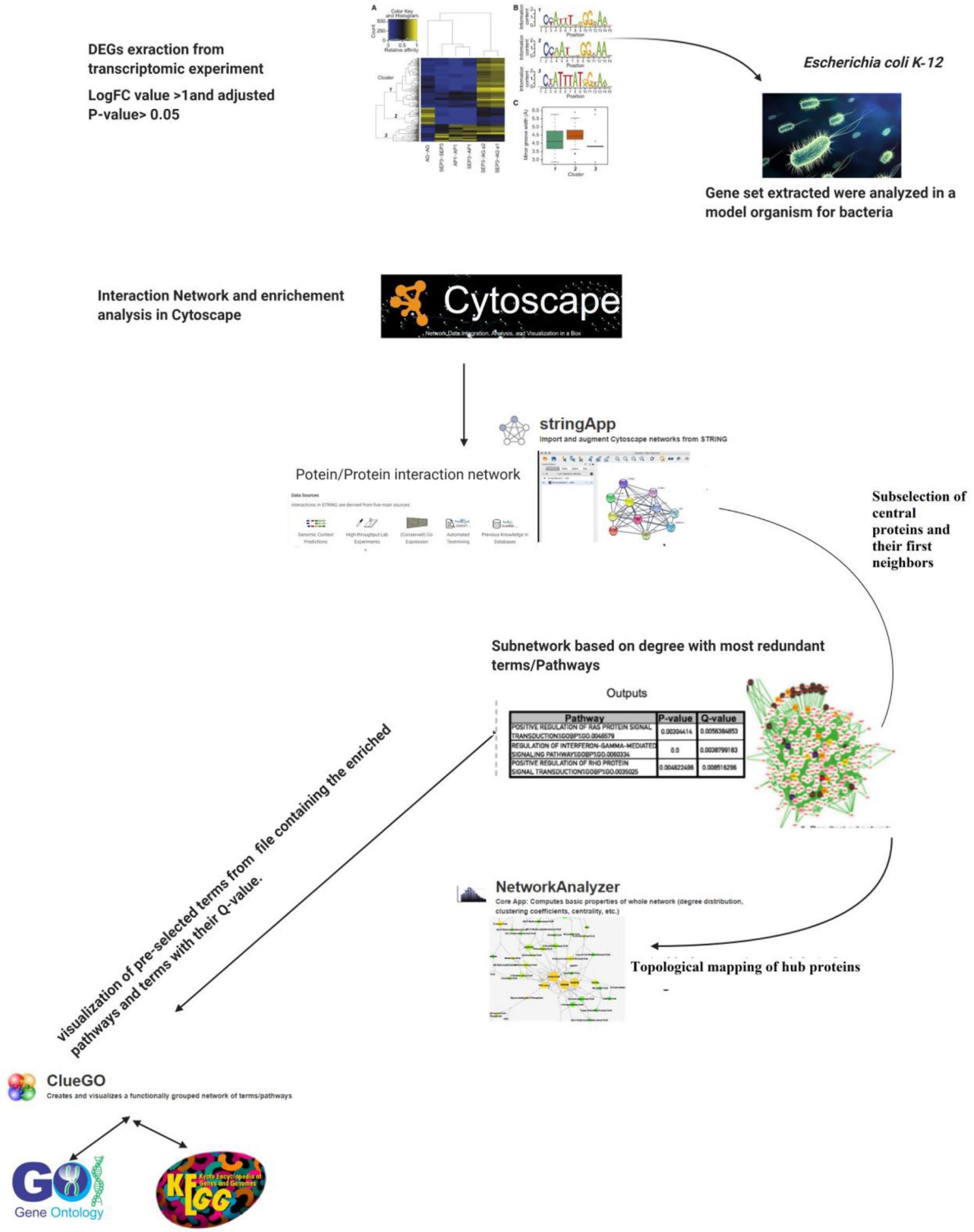

Figure 1. Graphical abstract of the analysis pipeline (step by step), from data collection to hub proteins and metabolic pathway identification with the various packages and databases used.

with nutrient metabolism, contributing toward the progression of complications that can affect cell behavior and bacterioplankton phenotype, because as it has been mentioned, the growth of microorganisms in a non-optimal environment suggests evolutionary adaptations through specific mutations responsible for a physical form.[57] In addition, the involvement of hub proteins related to carbohydrate metabolism, proteins, nucleic acids, and membrane transport have been reported in the selection of copiotrophic and pathogenic species,[34,58] but these results require further studies because the existing research to date has not thoroughly evaluated the 4 nutrients (C, N, P, and S) together.

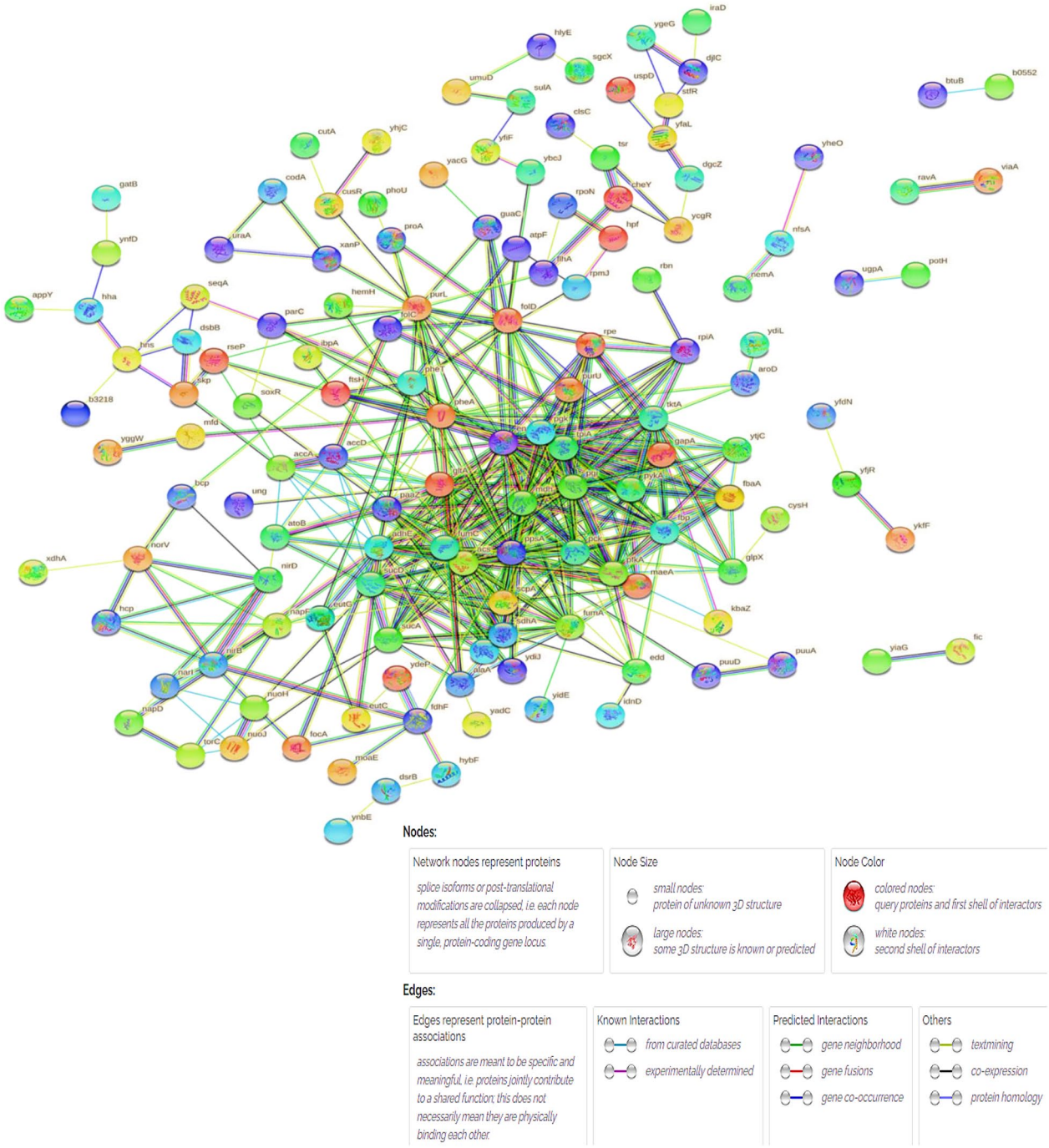

Figure 2. Predicted protein-protein interaction networks. Parameters: Score (0.4), no additional nodes; interaction sources used: experimentation, databases, co-expression, co-occurrence, gene fusion, and neighborhood. In the interaction networks, separate lines of different colors are used to show the type of evidence that supports each interaction.

Subselection and network analyzer results

The network generated by string software was imported as a pre-existing unformatted array in Cytoscape software. The network analyzer plugin function was used for providing network filtration and customization. The principal subnetwork obtained (Figure 3) provides 72/165 nodes with a confidence score of 0.8 and a PPI enrichment *P value* $<10^{-16}$. The list of 72 genes was filtered and 10 hub proteins were subselected (Table 3). All of these genes exhibit the highest interactions between them to regulate some cellular functions. Indeed, several studies have demonstrated the key role of these enzymes in microbial metabolism such as glycolysis/gluconeogenesis,[59] pyruvate metabolism,[60] secondary metabolite biosynthesis, carbon metabolism,[61] and other fundamental intracellular processes. These results would be linked to the virulence of bacteria in the presence of an excess of nutrient.[59] According to this work, other studies have suggested that these enzymes are

Table 2. Most representative GO terms of biological processes and their associated pathways.

CATEGORY	GO TERM	DESCRIPTION	FDR VALUE	NUMBER OF GENES
GO Process	GO:0036091	Generation of precursor metabolites and energy	9.48E-36	44
	GO:0032787	Monocarboxylic process	3.12E-21	34
	GO:0046496	Nicotinamide and metabolic process	1.1E-20	21
	GO:0016999	Antibiotic metabolic process	9.2E-20	23
	GO:0044283	Small molecule biosynthetic process	8.82E-15	32
	GO:0036006	Glucose metabolic process	3.45E-14	14
KEGG Pathway	eco01200	Carbon metabolism	1.14E-57	51
	eco00620	Pyruvate metabolism	3.57E-26	25
	eco00010	Glycolysis/gluconeogenesis	1.16E-20	20
	eco00030	Pentose phosphate pathway	1.52E-18	17
	eco00680	Methane metabolism	1.79E-16	15
	eco00020	Citrate cycle (TCA cycle)	2.48E-16	15

Table 3. List of top 10 hub proteins with their betweenness centrality (BC) and degree values.

ROW	GENE NAME	PROTEIN NAME	BC	DEGREE
1	*pfo*	Probable pyruvate-flavodoxin oxidoreductase	0.16	36
2	*pykF*	Pyruvate kinase I (formerly F)	0.03	29
3	*gltA*	Citrate synthase	0.05	28
4	*glcB*	Malate synthase G	0.02	27
5	*pgi*	Glucose-6-phosphate isomerase	0.04	26
6	*maeB*	NADP-dependent malic enzyme	0.02	25
7	*aceE*	Pyruvate dehydrogenase E1 component	0.02	23
8	*ptA*	Phosphate acetyltransferase	0.02	22
9	*tktB*	Transketolase 2	0.02	21
10	*aceF*	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	0.008	20

considered moonlight proteins and are involved in microbial virulence.[46,47,62,63]

ClueGO results

ClueGOapp was launched by an ontological and metabolic analyses to evaluate over-represented GO terms and MP by annotating subselected proteins and their first neighbors in biological terms hierarchically (parent-child relation) and to assign them to functional MP pathways. The results are presented as a pie chart (Figure 4) for BP and a functionally grouped network (Figure 5) for MP, and 80 terms were associated with the 72 proteins. The major representative terms for GO processes are the metabolic process of small molecules, the catabolic process of organic substances, the metabolic process of carbohydrates, the metabolic process of alpha-amino acids, and the positive regulation of biological process; the major representative terms for MP are glycolysis/glycogenesis, pyruvate metabolism, the 2-component system, purine metabolism, and oxidative phosphorylation for MPs.

The ClueGO results are consistent with those provided by StringApp, which also involve biochemical reactions and pathways that ultimately lead to the formation of precursor metabolites and substances from which energy is derived and most of them refers to the MPs of the purine and citrate cycle (tricarboxylic acid [TCA] cycle). The metabolic process of purine

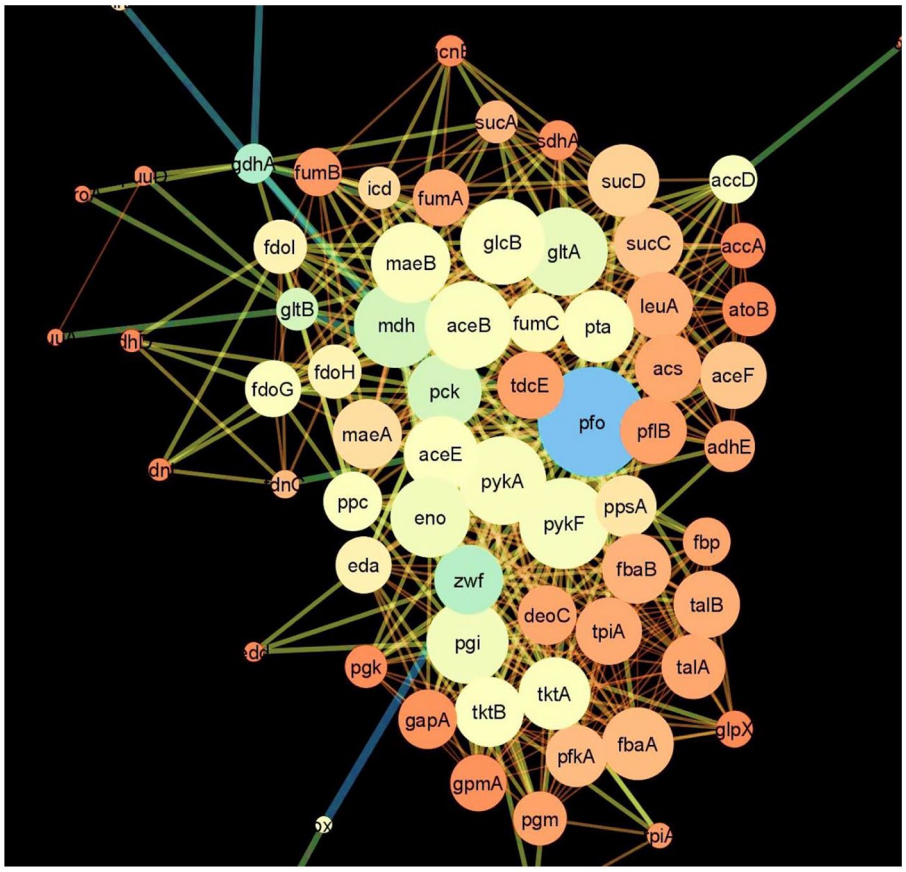

Figure 3. Topological mapping of the hub proteins obtained in the subselection analysis based on the cutoff value BC <0.02 and node degree >20. The larger circles correspond to the higher degrees and brown to blue color refers to increment of betweenness; the thickness of the lines represents the confidence score of the associations and different colors are used to show the type of evidence that supports each interaction .

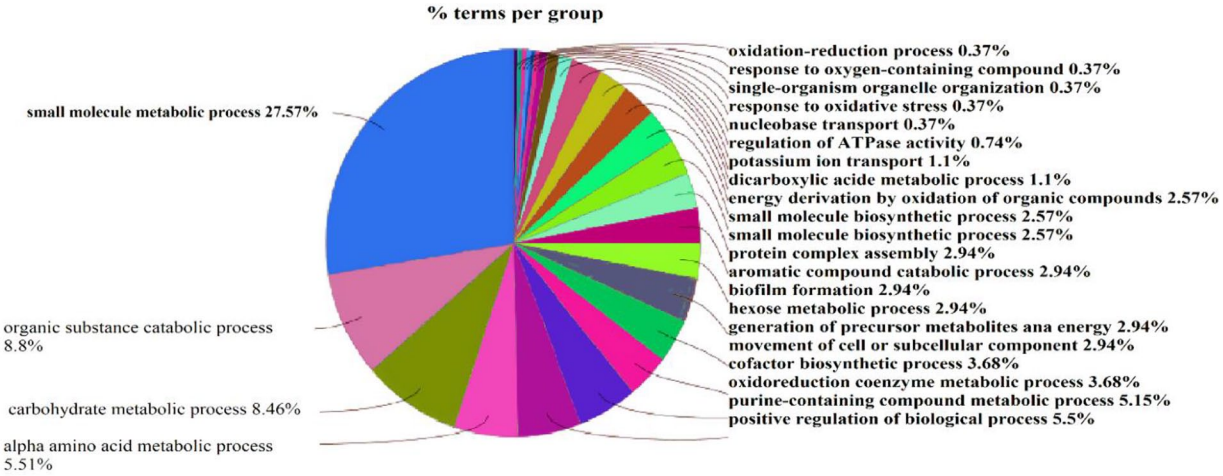

Figure 4. Predicted functional enrichment pie chart for the GO BPs by ClueGO.

seems to be a widespread phenomenon.[64] It has been found to be a key modulator in virulence of pathogens.[65] The TCA cycle, also known as the citric acid cycle or Krebs cycle, produces energy by the complete oxidation of acetate, derived from carbohydrates, fats and proteins, to carbon dioxide.[66]

In Table 2, 51 out of 165 proteins were assigned to carbon metabolism, which suggests it as the central metabolic process and the main nutrient during eutrophication. Deutscher et al and Görke and Stülke reported the binding of carbon catabolism to microbial virulence.[67,68] Excessive carbon sources and DOC were documented as enhancers of bacterial growth, oxygen removal, and selector for copiotrophs and opportunistic pathogens in both seawater and coral holobiota[69,70] using their preferred carbon substrate through ATP-binding cassette

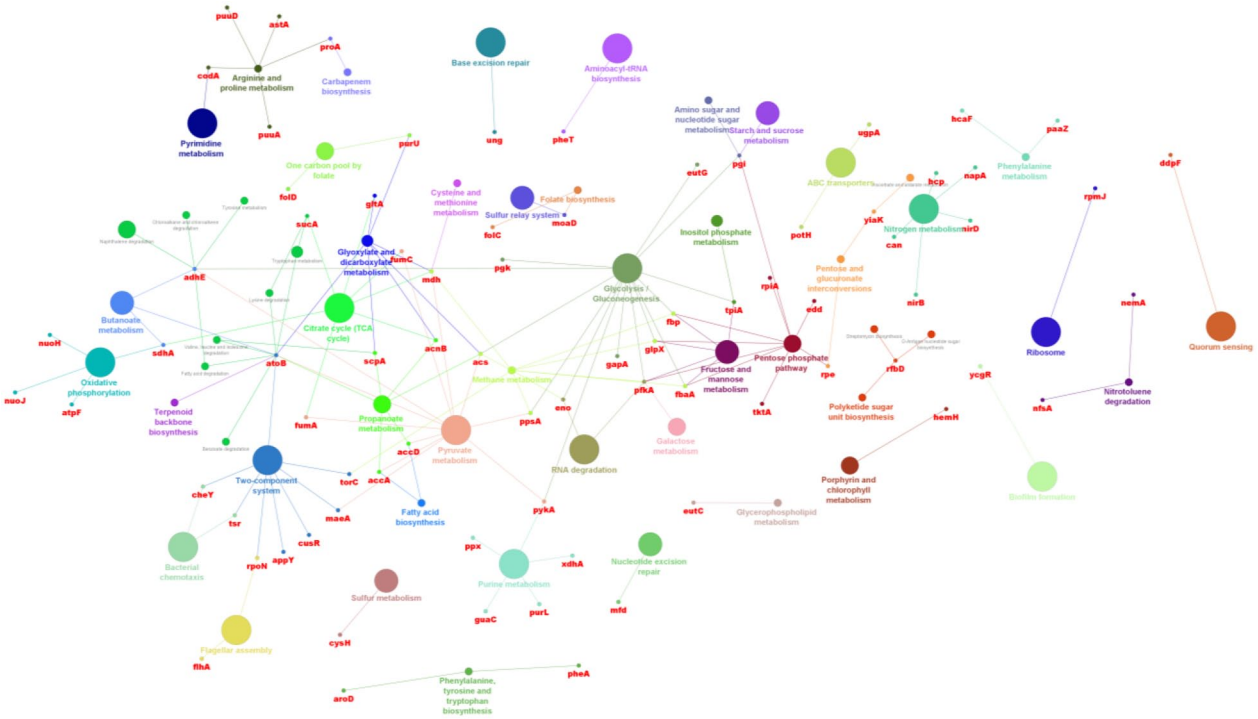

Figure 5. In ClueGO, metabolic pathways were predicted from KEGG as a network with the terms of the enriched pathways visualized using Cytoscape's ClueGo/CluePedia plugin where several proteins share common functions. The size of the nodes corresponds to the importance of the metabolic pathway.

(ABC) transporters.[71,72] The ABC transporters were reported in studies involving genes related to virulence and symbiotic interactions[73] and highly reported in copiotrophs to the opposites of oligotrophs.[74] Haas et al[75] reported the abundance of *Gammaproteobacteria* and *Alphaproteobacteria* in enriched and algal-dominated waters in contrast to coral-dominated oligotrophic waters, and this suggests the possible adaptation of the studied bacterioplankton in case of existence in such an environment, but all this needs further study and discussion to draw strong conclusions.

In Figure 5, many proteins are multitasking and provide at least 2 MPs, which reminds us of moonlighting proteins. The existence of moonlighting proteins in microorganisms is a known, but still poorly understood phenomenon.[76] Most of these proteins exercise their role in the cytoplasm and outside the cell. Their existence has been linked to virulence and they are often domestic enzymes, especially those of the glycolytic pathway, such as enolase, aldolase, dehydrogenase, heat shock proteins, and transcription factors, and they may perform non-catalytic roles with different functions depending on their cellular localization and the concentration of substrates.[62] In the analyzed differential gene expression (DGE) profile, pyruvate metabolism,[60] carbon metabolism,[61] and glycolysis/gluconeogenesis[59] (Figure 5) are central glycolytic MP that involved moonlight proteins and are related to virulence in bacteria. Taken together, the analyses of BP and MP (Figures 4 and 5) reveal that the interconnected proteins during the nutrient excess and the bloom proliferation phase in the model organism *E Coli K12* are involved in chemical reactions and cellular metabolism involving carbohydrates and organic acids. Thus, several studies have reported the relationship between moonlight proteins, carbon catabolism, and microbial virulence factors.[67,68] In addition, the involvement of hub proteins related to carbohydrate metabolism, proteins, nucleic acids, and membrane transport has been reported in the selection of copiotrophic and pathogenic species.[34,58]

Conclusions

Transcriptomic data are increasingly numerous and varied, facilitating data mining at a system level. A large number of approaches/tools have been developed to detect pathways and processes that are significantly altered between different experimental conditions during stress by pollutants or other substances. The objective of this work is to study the capacity of bacterioplankton during eutrophication and algal blooms in the model organism *E coli K12*, through the analysis of a profile of DEGs collected from several bibliographic sources to predict hub proteins, BP and MP involved in copiotrophic species selection, and bacterioplankton virulence.

The obtained results suggested that the metabolic behavior and central BPs are strongly correlated with carbon and carbohydrate metabolism, contributing to the progression of complications that can affect the cellular behavior and phenotype of bacterioplankton. The involvement of hub proteins related to carbohydrate, protein, nucleic acid metabolism, and membrane transport has been reported in the selection of copiotrophic

and pathogenic species during excess of nutrients, but these findings require further study.

The bacterial stress adaptation of *E coli* to excess nutrients and the possibility of increased virulence associated with stress need to be studied in more detail to prevent potential risks of host-microbiota interactions. This is important because understanding the mechanisms and regulation of bacterioplankton stress adaptation will provide information for pathogen control and enhance the effective design of new control methods. Furthermore, the identification of moonlight proteins is clearly not an easy process as most of the currently identified bacterial moonlight proteins were discovered by chance.

Today, researchers are using antimicrobial susceptibility testing to address the problem of multidrug resistance by Gram-positive and Gram-negative commensal and pathogenic bacteria. But questions arise as to their use in the treatment of pathogenesis in aquatic habitats. In aquatic environments, the use of such strategy has often been associated with aquaculture. Moreover, with the mechanisms of microbial evolution, their adaptations, the poor practices of treatment, and discharge of microbes in some laboratories in developing countries and the discharge of wastewater into aquatic environments, such a process suggests the development and diffusion of resistance genes to biomolecules (phenolic compounds) through horizontal and vertical transfers while creating a new problem to be solved but in the long term.

Author Contributions

YS, BN, AE, RS, MEM, FB, and CF contributed to conceptualization; YS, BN, AE, and RS contributed to data curation; YS contributed to formal analysis; FB and CF contributed to validation; YS, MEM, FB, and CF contributed to supervision of this study; YS contributed to writing original draft, reviewing, and editing; YS, BN, AE, RS, MEM, FB, and CF contributed to visualization.

ORCID iDs

Yousra Sbaoui https://orcid.org/0000-0002-6919-4929
Badreddine Nouadi https://orcid.org/0000-0001-5175-4601

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