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Proteomic Studies of the Effects of Different Stress Conditions on Central Carbon Metabolism in Microorganisms

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Abstract

The ability of cells to respond rapidly to fluctuations in environmental conditions such as thermal stress, pH, hypoxia, exposure to radiation, chemicals and a number of other factors is important for competitive fitness, adaptation and cell survival. To understand the dynamics of the complex metabolic pathways involved, various approaches including genomics, transcriptomics, proteomics, metabolomics, kinetics and biochemical information are appropriate. Proteomics is useful for evaluating the expression profiles of proteins, their post-translational modification and interactions between molecules. This review summarizes recent proteomics data examining the effects of different stress conditions on central carbon metabolism (CCM) in microorganisms. Data, primarily on enzymes involved in glycolysis, the tricarboxylic acid cycle and pentose phosphate pathways, were extracted from various sources, analyzed and are presented herein. Many proteomics analyses show that particular stress conditions affect the expression of specific enzymes of CCM or their pathways. Taken together, proteomic studies provide both an opportunity and a challenge for the study of the global regulation of CCM protein expression, post-translation control of enzymes, and consequently the global regulation of enzymes under different stress conditions.

Keywords: Proteomics; Stress responses; Glycolysis; Pentose phosphate pathway; Tricarboxylic acid cycle

Abbreviations: AAB: Acetic Acid Bacteria; CCM: Central Carbon Metabolism; DIGE: Differential in Gel Electrophoresis; ER: Endoplasmic Reticulum; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; iTRAQ: Isobaric tag for Relative and Absolute Quantitation; LC-ESI-MS/MS: Liquid Chromatography Electrospray Ionization-Tandem Mass Spectrometry; MALDI-TOF-MS: matrix-assisted laser desorption/ionization time-of-Flight Mass Spectrometry; PP: Pentose Phosphate: TCA: Tricarboxylic Acid; 2-DE: Two-Dimensional Gel Electrophoresis

The Systems Biology Approach for the Study of Biological Pathways

The widespread availability of genome sequences and knowledge of the analysis methods in systems biology provide an opportunity to improve our understanding of complex biological systems. Systems biology integrates the results of different ~omics techniques such as transcriptomics, proteomics, metabolomics and interactomics [1,2]. Additionally, systems biology is an interdisciplinary field that focuses on complex interactions to study the function of biological systems, including gene function, molecular evolution, functional diversity and molecular adaptation [3,4]. The importance of using systems biology techniques is underscored by the fact that biological functions are often attributed to complex interactions between the cell's numerous constituents, like proteins, DNA, RNA and small molecules [5]. A particular challenge for systems biology is to understand the biological network such as biological pathways or protein-protein interaction networks to identify key regulatory proteins.

Intracellular biological pathways include metabolic pathways, regulatory networks and cell signaling networks [6]. The metabolic pathways, an important group of biological pathways, describe the transformation of different compounds through various reactions [7]. In each pathway, a primary metabolite is modified by a series of chemical reactions catalyzed by enzymes. Metabolomics is an essential approach to screen low molecular mass metabolites in biological samples and so an important tool for evaluation of metabolic pathways and their regulatory mechanisms [8-10]. However, it is very difficult to trace metabolites [11]. The majority of published papers in metabolomics describe the most abundant metabolites, meaning that only a limited number of metabolic pathways can be observed using these tools [12,13]. In contrast, transcriptomics analyses can assay entire genomes in a single experiment [14]. Therefore, microarray analysis can give indirect information about metabolic changes under different experimental conditions. However, mRNA expression, as the basis of microarray analysis, does not always reflect the change in protein expression level since gene expression is regulated at different steps in the synthetic pathway, from DNA to protein [15]. The regulation of gene expression includes many factors, such as how often a given gene is transcribed, how the RNA transcript is processed, which complete mRNAs in the cell nucleus are exported to the cytosol, which mRNAs in the cytoplasm are translated by ribosomes, and selectively degraded in the cytoplasm [16]. Therefore, to better understand the final product of translation, it is very important to determine the protein expression level. To determine the abundance of a protein in the cell, different relative and absolute quantitative proteomics techniques can be used. Several reviews describe a variety of techniques related to quantitative proteomics [17-19]. By using these approaches it is possible to determine the relative or absolute change of protein levels under the influence of different external and internal stimuli. Consequently, the variation in metabolic pathways under different conditions can be successively studied by using proteomic approaches.

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Central Carbon Metabolism (CCM)

Carbohydrates play crucial roles as primary carbon and energy sources in living organisms [20]. CCM uses a complex series of enzymatic steps to convert these carbohydrates into metabolic precursors that are then used to generate the entire biomass of the cell. The metabolism of carbohydrates requires the activity of enzymes of the central carbon metabolic network that include the glycolytic pathway, oxidative pentose phosphate (PP) pathway and the tricarboxylic acid (TCA) cycle (Figure 1). These three basic pathways provide the reduced compounds needed to fuel the electron transport chain and catalyze adenosine triphosphate (ATP) formation via oxidative phosphorylation.

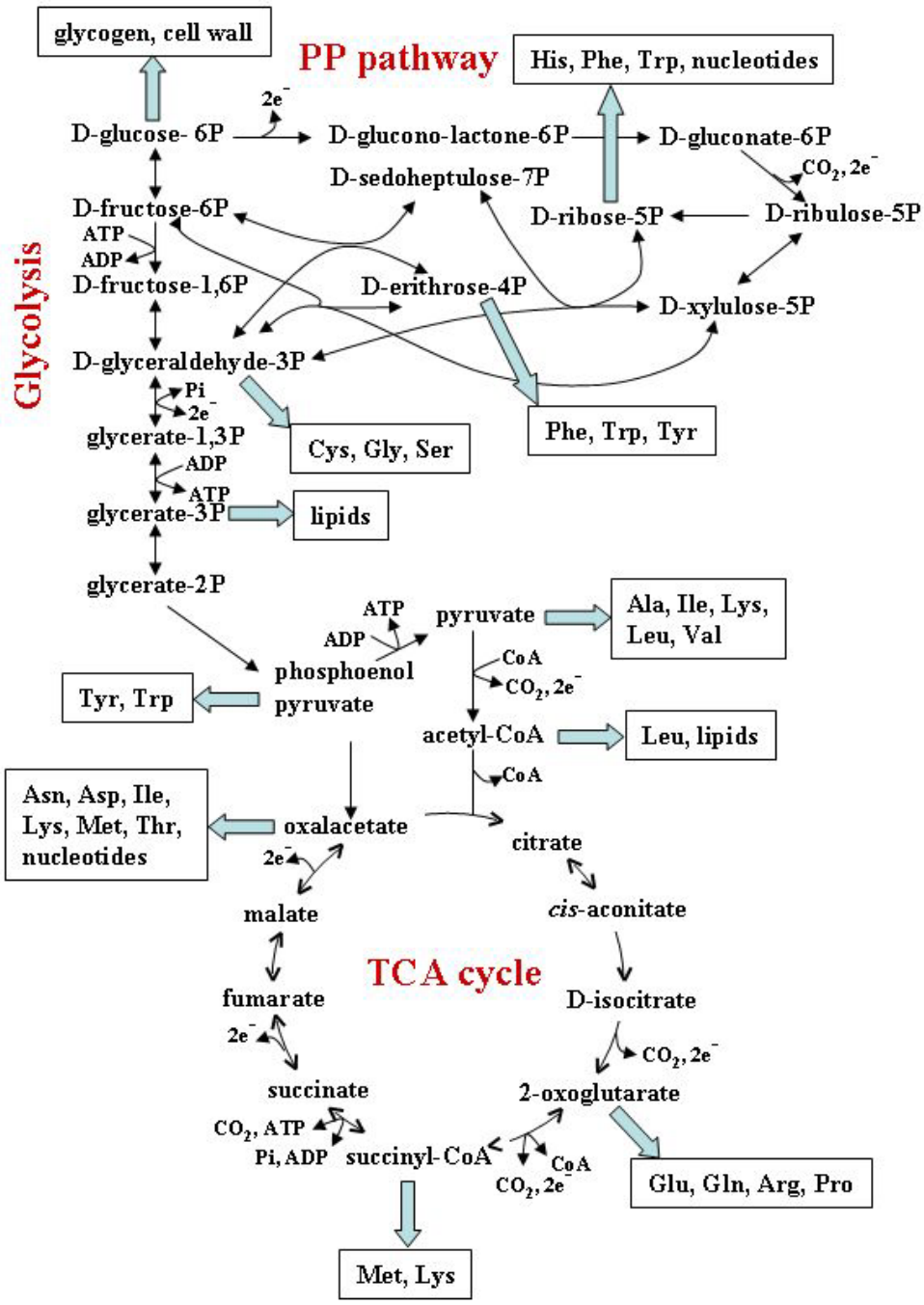

Figure 1: Simplified reactions of central carbon metabolism. Shown are glycolysis, the pentose phosphate pathway and tricarboxylic acid cycle. Arrows refer to precursors provided by central carbon metabolism to biosynthetic pathways for a variety of metabolites (metabolite names are shown in the boxes).

Glycolysis and the oxidative PP pathway convert carbohydrates into pyruvate and malate. The glycolytic pathway is comprised of ten sequential steps, which convert glucose into two molecules of pyruvic acid. Glycolysis consists from two main phases [21]: the first involving energy investment in which two molecules of ATP are converted to two molecules of ADP, and the second energy generation phase in which four molecules of ADP are converted to ATP and two molecules of NAD^+ are converted to two NADH. In addition to glucose, other sugars such as fructose, galactose, and mannose can be converted to glycolytic intermediates and then further processed through the glycolytic pathway. Glycolysis is almost ubiquitous, albeit with variations, across organisms, both aerobic and anaerobic, and it appears to be one of the most ancient metabolic pathways [22].

The PP pathway is a major pathway for the recycling of $NADP^+$ to NADPH and for the production of ribose-5-phosphate and erythrose-4-phosphate used in the synthesis of nucleotides, nucleic acids and aromatic amino acids. An additional role of the PP pathway is to protect the cell against oxidants, for example during oxidative stress CCM is reconfigured and the metabolic flux reroutes from glycolysis into the PP pathway, allowing the cell to generate more of the antioxidant cofactor NADPH [23]. Both glycolysis and the PP pathway are executed in the cytosol.

The end product from glycolysis, pyruvate, is transported into the mitochondria by a specific carrier and is decarboxylated and oxidized into acetyl-CoA by pyruvate dehydrogenase, a large complex (bigger than a ribosome), linking glycolysis to the TCA cycle. Acetyl-CoA then reacts with oxaloacetate, a TCA intermediate, to form citrate. The TCA cycle is involved in cell respiration, producing NADH and $FADH_2$ for the electron transport chain, with eight reactions that process incoming molecules of acetyl CoA that leave the cycle in the form of carbon dioxide. Each glucose metabolized fuels two turns of the TCA cycle, producing two molecules of GTP (or ATP), six NADH, two ubiquinone (QH_2) (or $FADH_2$) and four CO_2. The TCA cycle is followed by oxidative phosphorylation during which the energy contained within reduced cofactors, NADH and QH_2, is used to fuel the proton gradient that ultimately provide energy for the production of ATP, the energy currency of the cell.

Insight into Biological Systems Under Stress

All living organisms are fundamentally well adapted to certain environmental conditions and natural variations. Therefore, the Earth's biosphere is home to a diverse array of living organisms and environmental conditions. Some organisms are well adapted to an environment that for others can be considered extreme. For example, the environment of deep sea hydrothermal vents is characterized by high temperature, high pressure, chemical toxicity, acidic pH and limited light, yet a variety of microorganisms and many animal species are specifically adapted to this hostile environment [24-26]. Each particular species can adapt and grow optimally under a very specific set of environmental conditions [27]. Further, most species have mechanisms that allow them tolerate extreme situations for a limited period of time. However, excessive alteration of certain habitats can result in a stress that is too great, causing extinction of entire species. For this reason, the ability to efficiently respond to stress is very important for species survival and their successful adaptation to the challenges faced in suboptimal environments.

Stress from a diverse array of exogenous and endogenous sources is sometimes controlled by changes in enzyme kinetics that often depend on the concentration of cellular metabolites, the action of specific metabolites (i.e. feedback regulation) or post-translational modifications to enzymes themselves [28]. In some cases, responses and adaptations to stress and environmental constraints involve reprogramming of gene expression and therefore metabolism, precisely coordinated by cellular signaling [29]. Therefore, stress can induce changes in mRNA levels and consequently induce substantial changes in cell metabolic pathways and metabolite levels by adjusting protein levels. Since proteomics offers information about protein expression levels, it can speak to the response of metabolic pathways to stress.

Proteomics Investigation about Enzymes of the Central Carbon Metabolic Pathways Under Stress Conditions

The central carbon metabolic network plays essential roles in the cell, providing energy metabolism and precursors for many biosynthetic reactions (Figure 1). Under stress conditions such as temperature, salt shock, pH, irradiation, exposure to toxic compounds (organic solvents or antibiotics) and other stress-inducing molecules, the central carbon and other metabolic pathways can be altered. This aspect of metabolic changes has been studied by using different approaches [30-35]. This review summarizes current knowledge about enzymes of the central carbon metabolic pathways that are changed by different stress conditions and are investigated by proteomics studies. A detailed description of enzymes of the central carbon metabolic pathways (glycolysis, PP pathway and TCA cycle) that are changed by different stress conditions is given in Table 1.

Stress responses in prokaryotic microorganisms

Cisplatin is one of the most effective anticancer agents widely used in the treatment of tumors. In order to identify the impact of this anticancer drug on *Escherichia coli* [36]. Proteomic analysis showed that proteins affected by cisplatin are involved not only in cellular stress responses but also in energy metabolism, from glycolysis to the TCA cycle. The majority of enzymes that catalyze reactions of the central metabolic pathway such as glycolysis (fructose-bisphosphate aldolase class 2, phosphoglycerate kinase, 2-oxoglutarate dehydrogenase E1 component) and the TCA cycle (citrate synthase, aconitate hydratase 1 and 2) were down-regulated. Aconitate hydratase 1 is a key regulator of the TCA cycle which affects several other pathways and consequently the inhibition of the central metabolism that blocks energy supply.

A differential proteome has been applied to investigate the response of *E. coli* K12 BW25113 to chlortetracycline stress using isobaric tags for relative and absolute quantitation labeling quantitative proteomics technology [37]. Chlortetracycline is an antibiotic used in treating many bacterial and rickettsial infections, obtained from the bacterium *Streptomyces aureofaciens*. A total of 723 proteins were identified by liquid chromatography matrix assisted laser desorption ionization mass spectrometry with 184 decreasing and 147 increasing in abundance. The majority of identified proteins (51.3%) were involved in metabolic pathways such as pyruvate metabolism, pyrimidine metabolism, the TCA cycle and butanoate metabolism, 82% decreased and only 18% were augmented in abundance. These results indicate that the fluctuation of metabolic pathways, including the TCA cycle, may represent an antibiotic-resistant mechanism.

Acinetobacter baumannii has emerged over several decades as a problematic human pathogen, the major cause of healthcare-associated infections including bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection [38,39]. The carbapenems are a class of β-lactam antibiotics most commonly prescribed to treat infection, but *A. baumannii* has developed resistance leading to significant health

Reference	Organism	Stress condition	Enzyme/Regulation#		
			Glycolysis	**PP pathway**	**TCA cycle**
	Prokaryote				
[36]	*Escherichia coli*	Cisplatin	-Fructose-bisphosphate aldolase class 2 ↓ -phosphoglycerate kinase ↓ -2-Oxoglutarate dehydrogenase E1 component ↓		-Citrate synthase ↓ -Aconitate hydratase 1 and 2 ↓
[37]	*Escherichia coli*	Chlortetracycline	-6-Phosphofructokinase isozyme 1 ↓ -2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase ↓ -Fructose-bisphosphate aldolase class 1 ↓ -Glyceraldehyde-3-phosphate dehydrogenase A ↓ -Pyruvate kinase ↓ -6-Phosphofructokinase isozyme 2 ↓ -Fructose-bisphosphate aldolase class 2 ↓ -Phosphoglycerate kinase ↓ -Enolase ↓	- Phosphoglucomutase ↓	-Fumarate reductase flavoprotein subunit ↓ -Pyruvate dehydrogenase E1 component ↓ -Succinyl-CoA ligase [ADP-forming] subunit alpha ↓ -Aconitate hydratase 2 ↓ -Citrate synthase ↓ -Isocitrate dehydrogenase ↓ -Fumarate reductase iron-sulfur subunit ↓ -Succinate dehydrogenase iron-sulfur subunit ↓ -Fumarate hydratase class I ↓ -Malate dehydrogenase ↓
[39]	*Acinetobacter baumannii* (resistant strain RS307 vs ATCC 19606)	Carbapenem			-Dihydrolipoamide dehydrogenase ↑ -Malate dehydrogenase ↑ -2-Oxoglutarate dehydrogenase complex ↑
[40]	*Escherichia coli*	Ultraviolet A	-Enolase ↓ -Fructose-bisphosphate aldolase class 2 ↓ -Pyruvate kinase II ↓	-Transketolase 1 ↓ -Transketolase 2 ↓	-2-Oxoglutarate dehydrogenase E1 component ↓ -Pyruvate dehydrogenase E1 component ↓ -Aconitate hydratase 2 ↓ -Succinate dehydrogenase flavoprotein subunit ↓ -Dihydrolipoyl dehydrogenase ↓ -Acetyl-coenzyme A synthetase -Citrate synthase -Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex ↓ -Succinyl-CoA synthetase beta chain ↓ -Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex ↓
[43]	*Deinococcus radiodurans* R1	6 kGy Irradiation			-Citrate synthase ↓ -Aconitate hydratase ↓
[45]	*Lactobacillus brevis* NCL912	Acid stress	-Glyceraldehyde-3-phosphate dehydrogenase ↑		
[46]	*Streptococcus sobrinus*	Acid stress	-Fructose-1,6-biphosphate adolase ↑ -6-Phosphofructokinase ↑ -Pyruvate kinase ↑ -Phosphoglycerate kinase ↑ -Glycerehyde-3-phosphate dehydrogenase ↑	- L-Ribulose 5-phosphate 4-epimerase ↑	-Aconitate hydratase aconitase ↑ -Citrate synthase ↓
[48]	*Acetobacter pasteurianus*	Acid stress (acetic acid)	-Glyceraldehyde-3-phosphate dehydrogenase ↑		-2-Oxoglutarate dehydrogenase E2 component ↑ -Aconitate hydratase ↑
[49]	*Listeria monocytogenes*	Low temperature	-Hypothetical protein lmo2457 similar to triosephosphate isomerase ↑ -Glyceraldehyde-3-phosphate dehydrogenase ↑ -Phosphoglycerate kinase ↑ -Hypothetical protein lmo0907 similar to phosphoglycerate mutase family protein ↑ -Enolase ↑		-Hypothetical protein lmo1052 similar to pyruvate dehydrogenase complex, E1 component ↑ -Pyruvate dehydrogenase alpha subunit ↑ -Hypothetical protein lmo1053 similar to pyruvate dehydrogenase E1 component subunit beta ↑
[50]	*Thermus* sp. GH5	Cold shock	-Fructose-1,6-bisphosphate aldolase, class II ↓	-Transaldolase ↑ -Ribose-5-phosphate isomerase ↑	
[51]	*Bifidobacterium longum*	Bile	-Pyruvate kinase ↑ -Pphosphoglycerate kinase ↑ -Glyceraldehyde-3-phosphate dehydrogenase C ↑ -Phosphoglycerate mutase ↑	-6-Phosphogluconate dehydrogenase decarboxylatin II ↑ -Fructose 6-phosphate phosphoketolas ↑ -Transketolase ↑	

[52]	*Staphylococcus aureus*	Fluid shear rates (50, 100, 500 and 1000 s^{-1}	Glyceraldehyde 3-phosphate dehydrogenase ↓		-Malate dehydrogenase ↑ -Succinyl CoA synthetase ↓
[53]	*Staphylococcus warneri* SG1	Butanol	-Glyceraldehyde 3-phosphate dehydrogenase 2 ↑ -6-Phosphofructokinase ↑ -Phosphoglycerate mutase ↑ -Fructose-1,6-bisphosphate aldolase ↑ -Pyruvate kinase ↑ -Aldose 1-epimerase ↑ -Glyceraldehyde-3-phosphate dehydrogenase ↓ -Triosephosphate isomerase ↓	-Ribose-5-phosphate isomerase A ↑ -Phosphopentomutase ↑ -Putative transaldolase ↑ -Transketolase ↑	-Succinyl-CoA synthetase subunit alpha ↑ -Succinyl-CoA synthetase subunit beta ↑ -2-Oxoglutarate ferredoxin oxidoreductase ↑
[54]	*Pseudomonas putida* P8	Benzoate		-Transaldolase ↑	-Isocitrate dehydrogenase ↑ -Succinyl-CoA synthetase ↑ -Aconitate hydratase AcnB ↑
[55]	*Pseudomonas fluorescens*	Reactive nitrogen species			-Isocitrate dehydrogenase ↓ -Malate dehydrogenase ↓ -Aconitase ↓
[57]	*Anabaena* sp. PCC7120	Arsenic	-Fructose bis phosphate aldolase II ↓ -Phosphoglycerate kinase ↓ -Fructose 1,6 bisphosphatase ↓	-Transketolase ↓	
	Eukaryote				
[60]	*Aspergillus fumigatus*	Hypoxia	-Glyceraldehyde 3-phosphate-dehydrogenase ↑ -Phosphoglycerate kinase ↑ -Enolase/allergen Asp F 22 ↑ -Fructose-bisphosphate aldolase class II ↑	-6-Phosphogluconate-dehydrogenase ↑ -Transaldolase ↑	-Dihydrolipoamide succinyltransferase ↑ -Aconitate hydratase ↑ -Succinyl-CoA synthetase ↑ -Succinate dehydrogenase subunit Sdh1 ↓ -Pyruvate- dehydrogenase complex component Pdx1
[61]	*Pichia pastoris*	Hypoxia	-6-Phosphofructokinase gamma-subunit ↑ -Glyceraldehyde-3-phosphate dehydrogenase ↑ -Pyruvate kinase ↑ -Phosphoglucose isomeras ↑ -Enolase ↑ -Fructose 1,6-bisphosphate aldolase ↑ -Phosphoglycerate mutase ↑		-Aconitase ↓ -Fumarase ↓ -Mitochondrial malate dehydrogenase ↓
[64]	*Debaryomyces hansenii*	NaCl	-Enolase ↓ -Phosphoglycerate mutase ↓ -Aldolase ↑ -Triosephosphate isomerase ↑		-Pyruvate dehydrogenase β-subunit ↓
[65]	*Boletus edulis*	NaCl	-glyceraldehyde-3-phosphate dehydrogenase ↑		
[66]	*Saccharomyces cerevisiae*	Vacuum fermentation	-Glucokinase ↑ -Glucose-6-phosphate isomeras ↑ -Fructose-bisphosphate aldolase ↑ -Triosephosphate isomerase ↑ -Glyceraldehyde-3-phosphate dehydrogenase ↑ -Phosphoglycerate kinase ↑ -Enolase 2 ↑ -Enolase 1 ↑	-Glucose-6-phosphate dehydrogenase ↑ -Transketolase ↑	-Fumarate reductase ↑
[67]	*Fusarium oxysporum*	Crocidolite	-Glyceraldehyde 3-phosphate dehydrogenase ↓	-6-Phosphogluconate dehydrogenase ↑	
[69]	*Kluyveromyces lactis*	H_2O_2	WT: -3-Phosphoglycerate kinase ↑ -Pyruvate kinase ↓ -3-Phosphoglycerate kinase ↓ -Fructose-1,6-bisphosphate aldolase ↓ -Triosephosphate isomerase ↓ Δ*glr1*: -Pyruvate kinase ↑ -Hexokinase isoenzyme 2 ↑ -Enolase ↑ -Glyceraldehyde-3-phosphate dehydrogenase 2 ↓	Δ*glr1*: -6-Phosphogluconate dehydrogenase ↑	Δ*glr1*: -Aconitase ↓ -Fumarase ↑ -Citrate synthase ↑ -Malate dehydrogenase ↑
[70]	*Alternaria brassicicola*	Phytoalexin brassinin	0.5 mM brassinin: -Triosephosphate isomerase ↑ -Pyruvate kinase ↑ -Glucose-6-phosphate isomerase ↑ -Similar to glucose-6-phosphate 1-dehydrogenase ↑	0.5 mM brassinin: -Transaldolase 1 ↑ -Putative 6-phospho-gluconolactonase ↑	0.1 mM brassinin: -Aconitate hydratase ↑ -Malate dehydrogenase ↑ -ATP citrate synthase ↑
[71]	*Alternaria brassicicola*	Phytoalexin camalexin (0.1 mM)	-Putative glucokinase ↓ -Hexokinase ↓ -2,3-Bisphosphoglycerate-independent phosphoglycerate mutase ↓ -Pyruvate kinase ↓ -Phosphoglycerate kinase ↓	-Putative 6-phospho-gluconolactonase ↑ -6-Phosphogluconate dehydrogenase 1 ↑ -Phosphoglucomutase ↓	-Aconitate hydratase ↑ -ATP-citrate synthase ↓ -Putative aconitate hydratase ↓ -Putative isocitrate dehydrogenase subunit 2 ↓

[73]	*Candida glabrata*	Different pH pH 7.4/8.0 vs pH 4.0	-Glyceraldehyde-3-phosphate dehydrogenase ↓ -Pyruvate kinase ↓ -Fructose-bisphosphate aldolase ↓ -Glucose-6-phosphate isomerase ↓ -6-Phosphofructokinase, alpha subunit ↑ -6-Phosphofructokinase, beta subunit ↑	-Transketolase ↓ -Transaldolase ↑	-Aconitate hydratase ↓ -Malate dehydrogenase ↓ -Succinate-CoA ligase beta subunit ↓

Table 1: An overview of the proteomic analysis of differentially expressed enzymes from central carbon metabolic pathways that change under different stress conditions. # Up-regulation of enzymes are indicated by ↑ and down-regulation of enzymes are indicated by ↓.

problems, high morbidity and mortality. Membrane and cytoplasmic proteins from the *A. baumannii* strain ATCC 17978 extracted and separated by 2-DE followed by MALDI-TOF/TOF analysis revealed a versatile and robust metabolism capable of utilizing a wide range of nutrient sources [38]. Proteomic analysis of the inner-membrane fraction of a carbapenem-resistant strain of *A. baumannii*, using differential in-gel electrophoresis (DIGE) with DeCyder, Progenesis and LC-MS/MS, identified 19 over-expressed and 4 down-regulated proteins in the resistant strain (RS307) compared to the reference (ATCC 19606) [39]. Proteins up-regulated in the resistant strain are associated with β-lactamase, protein synthesis and chaperonins, seven of which have a role in metabolism and energy production. Energy production through the TCA cycle included the following enzymes: dihydrolipoamide dehydrogenase, malate dehydrogenase and 2-oxoglutarate dehydrogenase complex. These results suggest that antibiotic resistance, at least for *A. baumannii*, requires additional energy for which the TCA cycle plays an important role.

A proteome analysis of *E. coli* cells irradiated with ultraviolet A (UVA) light has been evaluated [40]. A variety of proteins important for cell function were targeted by UVA irradiation, such as the transcription and translation apparatus, transport systems, amino acid synthesis and degradation, respiration, ATP synthesis, glycolysis, the TCA cycle, chaperone functions and catalase. The protein damage pattern caused by UVA light strongly resembles the pattern caused by reactive oxygen species and so likely accelerates cell senescence and leads to cell death.

Deinococcus radiodurans exhibits unusual resistance to ionizing radiation, desiccation, and other stresses [41-43]. The molecular basis of post-irradiation recovery has been the subject of intense study, but remains inadequately understood. The kinetics of proteomic changes in *D. radiodurans* strain R1 following exposure to 6 kGy irradiation [43] showed selective degradation of proteins, such as chaperones, TCA cycle enzymes, stress proteins, and several hypothetical proteins. Most of these degraded proteins were resynthesized and restored, for instance enzymes of TCA cycle, citrate synthase and aconitate hydratase, were restored after 6 h. The resynthesis of these enzymes may help cells to restore energy status following irradiation.

Lactobacilli, which play a crucial role in the production of a large variety of fermented foods, are generally considered to be of low virulence, rarely causing infection in humans [44]. Lactobacilli are able to induce a series of acid stress responses to survive and grow in acidic environments. Proteomic analyses of differential protein expression in *Lactobacillus brevis* NCL912 under acid stress revealed twenty-five proteins with altered expression levels [45]. Among them, eight protein spots were identified, of which seven were up-regulated and one was down-regulated. Up-regulated proteins were categorized under stress response, DNA repair, protein synthesis, glycolysis and one of its key enzymes, glyceraldehyde-3-phosphate dehydrogenase, in particular implies that acid stress induces early perturbations in glycolysis.

Dental caries is an infectious transmissible biofilm disease that results from interactions of acidogenic/aciduric bacteria colonizing the tooth surface of the oral environment [46]. *Streptococcus sobrinus* is considered the primary organism responsible for human dental caries which is able to generate acid and adapt to low pH conditions. Transcriptomic and proteomic integrative analyses were used to characterize the acid adaptive mechanisms of this organism [46], revealing that among other proteins many glycolytic enzymes such as fructose-1,6-biphosphate adolase, 6-phosphofructokinase, pyruvate kinase, phosphoglycerate kinase and glycerehyde-3-phosphate dehydrogenase were up-regulated. Taken together with a previous report [47], this suggests that glucose transport and glycolysis are enhanced in cells grown at low pH.

Acetic acid bacteria (AAB) are bacteria that obtain their energy from the oxidation of ethanol to acetic acid during fermentation. These strictly aerobic microorganismw are characterized by a unique resistance to ethanol and acetic acid. A member of AAB, *Acetobacter pasteurianus*, has been studied using the differentially expressed proteome during the transformation of ethanol into acetic acid (oxidative fermentation) [48]. As a result of the oxidative fermentation process 112 protein spots were differentially expressed, and among them 70 were up-regulated, and 42 were down-regulated. During oxidative fermentation at the beginning of the stationary growth phase, enzymes associated with the TCA cycle, such as 2-oxoglutarate, aconitate hydratase (aconitase) or isocitrate were up-regulated, so as a consequence of complete ethanol consumption acetic acid will be assimilated through the TCA cycle.

One of the major food-related pathogens, *Listeria monocytogenes* has adapted to survive in unique conditions such as high salt, a wide pH range, low humidity, and temperatures ranging from -0.4 to 45°C [49]. Expression proteomics analyses of *L. monocytogenes* under refrigeration revealed that the adaptation processes affect several biochemical pathways, including proteins involved in energy production pathways such as glycolysis and the phosphotransacetylase-acetate kinase pathway [49]. The increased expression of enzymes involved in glycolysis suggests that cell growth under cold conditions involves the production of high energy intermediates such as glyceraldehyde-3-phosphate and glycerone-phosphate.

Thermus sp. GH5 is an aerobic thermophilic bacterium with optimal growth between 70–75°C [48]. Based on the industrial and biotechnological applications of thermophils, it is important to understand their adaptive mechanisms. Proteomics has been used to examine the response of *Thermus* sp. GH5 to different cold shock conditions [50]. After a sudden decrease in temperature, several proteins involved in the degradation of carbon/energy sources molecules, synthesis of amino acids and nucleotides are up-regulated. An increase in the accumulation of transaldolase and ribose-5-phosphate isomerase from the PP pathway in late cold shock suggests a reconfiguration of metabolism towards the production of intermediates targeted to alternate metabolic pathways and NADPH biosynthesis.

Adaptation to and tolerance of bile stress are important limiting

factors to ensure survival of bifidobacteria in the intestinal environment of humans. The effect of bile salts on protein expression patterns of *Bifidobacterium longum* has been examined by using proteomic analyses [51] with the identification of 34 differentially regulated proteins, the majority of which were induced after both a minor (0.6 g/1) and a major (1.2 g/l) exposure to bile. In particular, proteins of general stress response pathways and several enzymes of glycolysis (pyruvate kinase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate mutase) and pyruvate catabolism were identified. The up-regulation of several glycolytic enzymes in the presence of bile salts, suggests a global activation of the glycolytic pathway, which is important for enhancing production of energy-rich intermediates and reducing equivalents.

The biofilm forming bacterium *Staphylococcus aureus* frequently found in the human respiratory tract and on the skin is responsible for maladies ranging from severe skin infection to diseases such as bacteremia, endocarditis and osteomyelitis. Proteomics of the membrane and cytosolic fractions of *S. aureus* biofilms grown under four physiologically relevant fluid shear rates (50, 100, 500 and 1000 s^{-1}) [52] revealed an altered expression of sixteen proteins in the membrane-enriched fraction and eight proteins in the cytosolic fraction. These 24 proteins were identified using nano-LC-ESI-MS/MS and found to be associated with various metabolic functions, protein synthesis and stress tolerance. Most of the proteins with altered expression could be related to carbohydrate metabolism, in particular glycolysis and the TCA cycle. Glyceraldehyde 3-phosphate dehydrogenase, the enzyme that catalyzes the sixth step of glycolysis, was significantly reduced as a function of increased fluid shear rates, demonstrating a slowing of metabolic activity under these conditions.

Staphylococcus warneri is a solvent tolerant Gram-positive bacterium that constitutes part of the human skin flora. This bacterium, specifically the SG1 strain, is tolerant to alkanes, short-chain alcohols, esters, cyclic aromatic compounds and 1-butanol, making it an excellent candidate for biofuel production [53]. The molecular mechanisms of *S. warneri* SG1 in response to butanol studied with two-dimensional liquid chromatography tandem mass spectrometric proteomics [53] identified 1585 proteins (representing 65% of the predicted open reading frames), covering all major metabolic pathways. Among them 967 proteins were quantified and found to be involved in energy metabolism, lipid and cell envelope biogenesis, and chaperone functions. The proteomic data showed up-regulation of enzymes responsible for critical energy-producing reactions in the TCA cycle, while metabolite profiling identified a significant increase in the α-ketoglutarate: isocitrate and oxaloacetate: malate ratios under butanol challenge. These results show how the key role played by the TCA cycle plays in butanol tolerance of *S. warneri* SG1.

Pseudomonas putida P8 was originally isolated from phenol-contaminated wastewater and this non-pathogenic Gram-negative bacterium has attracted considerable interest for studying the biodegradation of toxic aromatic compounds such as phenol [54]. Proteomic analyses examining the cellular responses of *Pseudomonas putida* P8 during growth on benzoate [54] identified differentially expressed proteins involved in detoxification, the stress response, carbohydrate, amino acid/protein and energy metabolism, cell envelope biogenesis and cell division. Enzymes of the TCA cycle, isocitrate dehydrogenase, succinyl-CoA synthetase and aconitate hydratase AcnB were up-regulated when *P. putida* cells were grown on benzoate, along with increases to cellular levels of succinate semialdehyde dehydrogenase. The latter enzyme in *Pseudomonas* species catalyzes the conversion of succinate semialdehyde to succinate with NAD^+ as a cofactor, producing NADH [54]. The up-regulation of NADH-production can be involved in maintaining the cellular energy levels under benzoate stress.

Nitrosative stress arises when the production of reactive nitrogen species outmatches an organism's ability to neutralize and dispose of those species [55], which are capable of damaging nucleic acids, lipids and amino acids. Evaluation of the metabolic responses of *Pseudomonas fluorescens* to nitrosative stress revealed that this soil bacterium reprograms its metabolic networks to survive this toxic challenge [55]. The effect of reactive nitrogen species on the TCA cycle and oxidative phosphorylation was mitigated by the up-regulation of citrate lyase, an enzyme that degrades citrate into oxaloacetate and acetate and two additional enzymes, phosphoenolpyruvate carboxylase and pyruvate phosphate dikinase that convert oxaloacetate into pyruvate and ATP. Thus, metabolic changes associated with the generation of pyruvate and ATP ensures the survival of *P. fluorescens* under exposure to reactive nitrogen species.

The increasing groundwater contamination by arsenic and its use for irrigation as well as excessive use of chemical fertilizers and metal-containing pesticides, especially in South East Asian countries, can affect the survival of microbial communities including cyanobacteria in the soil [56]. Proteomics in combination with morphological, physiological and biochemical studies have shed light on the survival of the nitrogen fixing cyanobacterium *Anabaena* sp. PCC7120 under arsenic stress [57]. Proteomic analyses revealed that some enzymes such as phosphoglycerate kinase, fructose bisphosphate aldolase II, fructose 1,6 bisphosphatase, transketolase, and ATP synthase were down-regulated on day 1 but then significantly recovered on the 15th day. Thus, to survive arsenic exposure, the organism presumably maintained glycolysis, the PP pathway and turnover rate of the Calvin cycle. Transketolase is an amphibolic universally required enzyme of the PP pathway, for NADPH production, and the Calvin cycle and its down-regulation may relate to accumulated H_2O_2 [58,59]. Recovery of transketolase after 1 d, when H_2O_2 levels have decreased, creates increased metabolic flux through the PP pathway generating glyceraldehyde 3-phosphate that can be utilized in the glycolytic pathway for the generation of ATP.

Stress responses in eukaryotic microorganisms

The mold *Aspergillus fumigatus* is the most important *Aspergillus* species causing human infections, and its adaptation to hypoxia represents an important virulence factor. Analysis of the *A. fumigatus* proteome in response to hypoxia led to the identification of 117 proteins with an altered abundance [60] for which increased activity was associated with the glycolytic pathway (glyceraldehyde 3-phosphate-dehydrogenase, phosphoglycerate kinase, pyruvate-dehydrogenase complex-dihydrolipoamide acetyltransferase component, pyruvate-dehydrogenase E1-component alpha subunit, enolase/allergen Asp F 22, fructose-bisphosphate aldolase class II and pyruvate- dehydrogenase complex component Pdx1), enzymes of the TCA-cycle (dihydrolipoamide succinyltransferase, fumarate reductase, succinate-semialdehyde-dehydrogenase, aconitate hydratase mitochondrial, succinyl-CoA synthetase alpha subunit and succinate dehydrogenase subunit Sdh1) and the pentose phosphate pathway (6-phosphogluconate- dehydrogenase Gnd1 and transaldolase).

Oxygen limitation strongly affects the core metabolism of anaerobic yeasts by causing energy deprivation, which can affect the production of many pharmaceutical proteins and industrial enzymes

[61]. A systems biology approach integrating 2D DIGE proteomics, DNA microarrays and metabolic flux analyses has been used to investigate the physiological adaptation of *Pichia pastoris* to oxygen availability [59]. Proteomic analyses showed that glycolytic enzymes were strongly induced, while enzymes of TCA cycle were in very low abundance under hypoxic conditions (Table 1). Further, there was a strong transcriptional induction of enzymes involved in glycolysis and the non-oxidative PP pathways, and the down-regulation of enzymes of the TCA cycle under hypoxic conditions. Such transcriptional changes have been correlated with proteomic and metabolic flux data. In contrast to *P. pastoris*, there is no such correlation in *S. cerevisiae* for glycolysis and the PP pathways under hypoxic conditions [62,63]. Therefore, it seems plausible that the increased production of specific heterologous proteins in response to hypoxia may at least partially result from increased transcriptional levels of glycolytic genes.

The yeast *Debaryomyces hansenii* has been isolated from different environments and is a yeast of biotechnological importance with interesting genetic and biochemical properties [64]. The influence of NaCl on the *D. hansenii* proteome shows a differential expression of several proteins [64]. Salt-induced enzymes are involved in glycerol synthesis/dissimilation and the first half of glycolysis (aldolase and triosephosphate isomerase), whereas repressed proteins were enzymes involved in second half of glycolysis (enolase and phosphoglycerate mutase) and the reaction leading directly from glycolysis to the TCA cycle (pyruvate dehydrogenase β-subunit). Another study evaluated changes of an ectomycorrhizal fungus, *Boletus edulis* in the presence of 4% NaCl using proteomics to identify twenty-two protein spots with differential expression, of which 14 were up-regulated and 8 were down-regulated, [65]. One glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase, was up-regulated. These observations indicate that NaCl triggers a switch in energy metabolism towards higher glycerol production.

Vacuum fermentation was invented to effectively eliminate the inhibitory effects of the volatile products (e.g., ethanol and acetic acid) on cells and improve ethanol production by continuously removing ethanol from the fermentation broth [66]. The protein expression profiles of industrial *Saccharomyces cerevisiae* under vacuum fermentation studied by two-dimensional gel electrophoresis (2-DE) combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) [66] show differentially expressed proteins to be involved in metabolism, signaling pathways and stress responses. Seventeen proteins were associated with carbohydrate metabolism, in particular the up-regulation of proteins involved in glycolysis, trehalose biosynthesis, and the PP pathway, (glucokinase, fructose-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, mitochondrial aldehyde dehydrogenase, enolase 2, enolase 1, trehalose-6-phosphate synthase, glucose-6-phosphate dehydrogenase, transketolase, and glycerol-3-phosphate dehydrogenase). These results suggest that a vacuum-induced rearrangement of the metabolic flux gives rise to reorganization of glycolysis, trehalose biosynthesis, and the PP pathway.

Asbestos is a group of fibrous hydrated silicate minerals that have been extensively mined and processed for industrial and commercial applications in the past centuries. Exposure to airborne asbestos fibrils can cause severe pneumoconiosis (asbestosis) and malignancies such as bronchogenic carcinoma and pleural mesothelioma [67]. Prior studies have shown that crocidolite asbestos fibers pretreated with *Fusarium oxysporum* had a significant reduction in surface reactivity and caused significantly less oxidative damage to naked DNA than control fibres [68]. A combined proteomic approach (2-DE, shotgun and quantitative iTRAQ) investigating cellular responses of the soil fungus *F. oxysporum* to crocidolite, an iron-rich type of asbestos [67] demonstrate how the airborne asbestos fibrils caused up-regulation of metabolic pathways involved in protection from oxidative stress. In response to asbestos exposure, *F. oxysporum* also seems to redirect carbohydrate flux from glycolysis to the PP pathway to counteract perturbations in the cytoplasmic redox state. As such, glyceraldehyde 3-phosphate dehydrogenase, an enzyme that catalyzes a crucial step of glycolysis, was down-regulated. On the other hand, a key enzyme involved in the PP pathway, 6-phosphogluconate dehydrogenase that catalyzes the first NADPH-producing reaction was up-regulated. These results suggest that cellular exposure to oxidants requires an increase in the antioxidant cofactor NADPH, synthesized in the cytosol through the PP pathway, for the proper function of NADPH-dependent glycolytic enzymes. Therefore, the NADPH generated in the PP pathway plays an essential role in the defense against reactive oxygen species (ROS).

ROS are formed as a natural byproduct of aerobic metabolism, but in excess they can chemically modify various macromolecules and cause cellular damage. Proteomic analysis of the oxidative stress response has been carried out in a wild-type and glutathione reductase deletion mutant in *Kluyveromyces lactis* [69]. In response to H_2O_2 treatment, there were a large number of proteins showing differential expression between the wild-type and Δ*glr1* strains, including a variety of antioxidant enzymes, chaperones, and oxidoreductases related to oxidative stress defense and damage repair. In addition, many enzymes of carbohydrate metabolism were differentially expressed, indicating a sensitivity of these metabolic pathways to H_2O_2 and/or loss of glutathione reductase. H_2O_2 exposure caused down-regulation of glycolytic and TCA enzymes in the wild-type, whereas the same treatment of the Δ*glr1* strain caused up-regulation of glycolysis, the TCA cycle, and oxidative PP pathways. Taken together, these results suggest that redox imbalance might control glucose metabolic fluxes in *K. lactis.*

Phytoalexins are antimicrobial compounds produced by plants as a response to biotic and abiotic stresses. Proteomic analyses of mycelial cultures of *Alternaria brassicicola* following treatment with the cruciferous phytoalexin brassinin (0.50 and 0.10 mM) [70] identified many proteins involved in primary metabolism, particularly in the TCA cycle and PP pathway, to be altered. Brassinin at 0.50 mM and 0.1 mM induced expression of enzymes in the PP pathway (glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase and glucose-6-phosphate isomerase) and those in the TCA cycle (aconitate hydratase, ATP citrate synthase and malate dehydrogenase), respectively. Thus, *A. brassicicola* under brassinin stress redirects metabolic flux to affect the TCA cycle and PP pathway, both important for producing energy in the form of NADPH and ATP. Similar studies with 0.1 mM camalexin [71] identified a total of 158 proteins exhibiting significant changes in expression, of which 45 were up-regulated and 113 down-regulated relative to controls. The majority of differentially expressed proteins are those involved in metabolism, protein processing, genetic information processing, protein folding (chaperones) and cellular processes. Most proteins in carbohydrate metabolism are down-regulated and are related to metabolic pathways such as the TCA cycle (aconitate hydratase, ATP-citrate synthase), glycolysis (2,3-bisphosphoglycerate-independent phosphoglycerate mutase, pyruvate decarboxylase, pyruvate kinase and phosphoglycerate kinase) and the PP pathway (putative 6-phospho-gluconolactonase, phosphoglucomutase and 6-phosphogluconate dehydrogenase 1). These studies highlight how

structurally different phytoalexins, such as brassinin and camalexin, have very different effects protein expression patterns.

Candida glabrata is currently the second most common cause of systemic and disseminated candidiasis in humans [72], for which adaptation to varying pH is crucial for its survival and virulence within the host. Proteomic analysis (2-DE) of *C. glabrata* in response to changes in ambient pH (4.0, 7.4 or 8.0) [73] reveal a statistically significant change in protein expression level. Peptide mass fingerprinting identified proteins involved in glucose metabolism, the TCA cycle, respiration and protein synthesis to have decreased expression during growth at pH 7.4 and/or 4.0, whereas proteins involved in stress responses and protein catabolism were preferentially expressed under alkaline (pH 8.0) conditions. Enzymes involved in the TCA cycle such as aconitate hydratase, the succinate-CoA ligase beta subunit and malate dehydrogenase were down-regulated at pH 8.0 but had increased in expression at pH 4.0. Therefore, glycolytic enzymes and those of the TCA cycle had higher expression levels in *C. glabrata* cells growing at pH 4.0, meaning that increased glucose catabolism might be required to generate energy for the maintenance of intracellular pH homeostasis under acidic conditions.

Conclusions

Various environmental variations can result in molecular adaptation and consequently affect cellular functions in microorganisms. Studying the relationship between stress conditions and the regulation of enzyme kinetics, metabolite concentrations and gene (protein) expression is important for understanding microorganism physiology, genetics, and ecology. Stress responses under various specific circumstances, such as fluctuations in environmental conditions and experimental perturbations, are usually controlled by changes in their metabolic composition [28,74]. To understand the observed metabolic adaptations it is necessary to identify and quantify the metabolome of cells in different states. Although there have been significant improvements in metabolomics technology, a significant number of metabolites cannot be measured experimentally [28,74]. There are several obstacles to metabolic identification and quantification that include mixture complexity, small concentrations, molecular sizes, lipophilicity, volatility, or other physicochemical properties. For this reason, other approaches to elucidate unidentified metabolic systems are required, including transcriptomics and proteomics, for the global analyses of biological systems in response to environmental changes. The advantage of these approaches is that, in addition to identifying missing pieces of the puzzle for metabolic systems, it is possible to elucidate global regulatory systems that control the simultaneous expression of a large number of genes in response to a variety of stress conditions [75,76]. The proteomics approach can further provide information related to posttranslational modification of proteins and interactions between macromolecules [77-80]. Proteomics technology thus becomes the technology of choice for quantifying proteins and their modifications in relation to different metabolic pathways [81].

Molecular mechanisms of adaptive responses require extra energy and the CCM plays a crucial role in generating that energy. Proteomic analyses demonstrate how particular stress conditions affect the expression of specific enzymes of CCM, such that up-regulation of these proteins or pathways can enhance energy production. Further, the CCM is important for providing synthetic precursors for other primary and secondary metabolites, such that increases or decreases in CCM metabolites can alter global metabolic flux. For this reason, the regulation of CCM in microorganisms is important for the regulation and coordination of a wide range of metabolic reactions and pathways [82]. Taken together, it is clear that the control of CCM enzymes under a variety of stress conditions plays important roles in maintaining optimal metabolite concentrations and eventually global metabolic homeostasis.

Interestingly, the differential expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been observed under a variety of stress conditions in most investigations. This enzyme is responsible for the inter-conversion of 1,3-diphosphoglycerate and glyceraldehyde-3-phosphate, a central step in glycolysis which produces NADH while executing a phosphorylation reaction. This enzyme is very important source of NADH during glycolysis, and is also involved in the regulation of mRNA stability, intracellular membrane trafficking, iron uptake and transport, heme metabolism, the maintenance of genomic integrity, the regulation of gene expression and nuclear tRNA export [83]. GAPDH is also subject to multiple post-translational modifications including acetylation, phosphorylation, and nitrosylation through modification with O-linked N-acetyl glucosamine or by oxidation. Moreover, this enzyme is localized in multiple cellular compartments including the cytosol, the membrane, the nucleus, polysomes, the ER and the Golgi [84]. Taken together with proteomic data under stress conditions, the multifunctional enzyme GAPDH seems to play an important role in the regulation of CCM.

Protein post-translational modification has been shown to play significant roles in the regulation of enzymatic activity. For example, phosphorylation is known to be involved in the regulation of enzymatic activity in yeast central carbon and storage metabolism [85-87]. A recent study using combined flux analysis with proteomics and phosphoproteomics led to the identification of new phosphorylated enzymes that are involved in yeast central metabolism [88]. Proteomics approaches can offer an unprecedented opportunity to study the control of metabolic flux by phosphorylation under various stress conditions, can help establish the cellular localization of proteins and determine protein interactions [89].

One of the interesting outstanding tasks is to predict the mechanism of enzyme regulation in the CCM under a variety of stress conditions. The metabolic network is very complex, for example in *Escherichia coli* it is interconnected through a large number of biochemical and regulatory reactions [90]. Comparative studies of the effect of stress conditions on CCM using different approaches such as metabolomics, transcriptomics, proteomics and bioinformatics could approve an excellent strategy to unravel the mechanisms by which a stress conditions govern CCM. Such information may contribute to the development of metabolic pathway models that describe how the cell works under different stress conditions, which may be useful to biotechnology [91]. Various engineered microorganisms are currently used to produce products such as formate, acetate, acetone, propionate, propanol, propanodiol, butyrate, butanol, bioelectricity, and many others [92]. Altered environmental conditions are capable of changing metabolic flux, and consequently under controlled conditions some reaction pathways could be used to enhance production of desired products or induce alternate pathways not normally activated.

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