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Microbial Proteomics: Microbial Stress Adaptation, Food Safety, Safety aspects of Genetically Modified Organisms [GMOs] and Identification of Foodborne Pathogens and Toxins

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Abstract:

The food sector is rife with the widespread malpractice of adulteration and mislabelling. There is deliberate mislabelling of foods that purport to be of animal origin that are actually made from plant ingredients. Because some people may be allergic to or sensitive to the substituted components, this sort of mislabelling raises serious concerns about food safety. Furthermore, foodborne bacteria are another serious concern when it comes to food safety. To address these and similar problems, powerful analytical tools are urgently required. Within this framework, proteomics emerges as an exciting method for documenting the aforementioned concerns. There are several areas of biology that have benefited greatly from advancements in the omics field, perhaps none more so than food science. All aspects of proteomics' present and future use in ensuring the quality, safety, and authenticity of food as well as its traceability are covered extensively in this review. There has also been extensive description of the other parts of proteomics. In addition, fresh insights on proteomics' application to food analysis can be provided by this review, which is a great plus. Protein profile differences also help in identifying food-borne infections. Proteinomics is one of the possible analytical methods for categorising changes in food commodities' safety and quality that occur during storage. Toxicology, adulterants, and food product authenticity can all be assessed using proteomics. Promptly estimating the items' quality and safety presents a number of obstacles. Finally, proteomics is a new field of study that has the potential to aid in the production of safe, high-quality food.

Keywords: Microbial Proteomics, Food Safety, GMOs, Foodborne Pathogens, Toxins

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Introduction

The term "proteomics" refers to the study of proteins in a single biological system through time-series analysis. Applying it to various analytical techniques makes it a highly valuable tool in food analysis. People throughout the world are becoming more health conscious and selective about the food they eat, which is leading to a dramatic shift in the food industry. As a result, understanding foodomics is becoming very prominent in this field. The term "foodomics" refers to research into the use of cutting-edge omics methods in the food industry [1,2]. To study food safety, traceability, and quality, as well as to discover novel bioactive components in food, foodomics incorporates genomics, epigenetics, transcriptomics, metabolomics, proteomics, peptidomics, and/or transcriptomics. Modern food science makes heavy use of omics, one of which is proteomics. These days, buyers consider more than just how a food item tastes before making a purchase. Foods that are minimally processed, nutrient-dense, functional, and additive-free are becoming more and more popular among consumers. Proteomics shows great promise in this area for establishing the trustworthiness and safety of food items. From the first ingredients to the final product, proteomics play a crucial role in product tracking. Proteomics also has a lot of promise for the food business when it comes to optimisation, safety, traceability, storage nutrition, and quality. Adopting such advanced technologies on an industrial scale, nevertheless, will require some time. Proteins reveal information about the food's provenance, characteristics, and preparation methods. In addition, proteomics can be utilised to assess the quality of food, which can be elevated by enhancing the procedures employed in food manufacturing. However, by substituting a less expensive substitute for the listed major ingredient, mislabelling occurs and fraudulent claims are made. A big point from a religious perspective is that different kinds of animals are utilised to create a certain product instead of the one that is listed on the label [3-5]. Because the substituted component could trigger adverse reactions in some people, this form of food fraud poses a risk to their health. Traditional approaches to specific authentication are laborious and ineffective at detecting product adulteration at levels below 5%. Here, proteomics shine as a powerful method for identifying food additives and fillers. Also, right down to the peptide level, proteomic analysis of food provides a thorough and rapid examination of food. Proteins serve as indicators of various dietary qualities, ingredients, and origins; hence, understanding proteomics is utilised for this objective. Protein profiling, product authenticity, and product traceability are all areas where proteomics expertise is put to use, particularly in the case of animal-based products (meat and dairy). In terms of people's well-being, food safety is paramount. Every year, a large number of individuals all over the world get sick from eating contaminated food. Conditions like hemolytic uremic syndrome, which can be caused by foodborne infections like E. coli O157:H7, can sometimes result in patient mortality due to neglect in food safety issues. Various types of food-borne infections can be more easily detected with the use of proteomic techniques, which aid in the identification of microbes by analysing their proteome changes. The database must also include proteomic assays that were utilised to identify the protein. There are a number of peptide fingerprint libraries on the market [7-9]. Among these libraries, there is a "spectra bank" that includes 120 species of food-related relevance and contains mass spectral fingerprints of pathogenic bacterial species and key spoilage-causing species in seafood. Toxins and allergens in food can be detected and identified using proteomic approaches such as HPLC and MS/LC-MS. Researching how various food production procedures affect protein structure, conformation, and posttranslational modifications (PTMs) allows for genetic enhancements and, ultimately, the creation of higher-quality foodstuffs. Tenderness, colour, and aroma are all aspects of meat that are influenced by a myriad of proteins. It is possible to improve food quality by identifying these proteins in food. Mass spectrometry (MALDI-TOF and electrospray ionisation), high-performance liquid chromatography (HPLC), and gel electrophoresis are some of the proteomics-based techniques that have been employed for food protein profiling, pathogen detection in food, and marker identification. In this review, we will go over some of the most cutting-edge proteomic food analysis methods and their uses in ensuring the authenticity, quality, and safety of food. The use of microbes in biotechnology and food technology dates back millennia. However, food poisoning can be caused by more than 250 different pathogens, the majority of which are microbes and the toxins they produce. Approximately 48 million cases of illness, 128,000 hospitalisations, and 3,000 fatalities have been reported in the US due to food poisoning [10-13]. The Third World countries likely have a somewhat greater figure, however it is either not recorded at all or only partially. The

development, persistence, and dissemination of food-borne diseases are all affected by environmental variables. An growth in the worldwide trade of foodstuffs, changes in the environment and ecology, new production procedures, evolving microorganism features, and new hazards are all contributing to this situation. Food safety regulations are also growing in importance. The worldwide spread of food-borne diseases and the interconnectedness of the world's food supply mean that we must take precautions to protect consumers from potentially harmful foods on a worldwide scale. It appears that food-borne illness outbreaks are on the increase once more in certain developed nations, this time affecting plant-based foods (vegetables, fruits, and grains), shellfish, and dry goods and ingredients rather than the traditionally contaminated animal-based foods (meat, eggs, milk, and dairy products). There have been several cases of food-borne pathogen contamination of both fresh and processed foods in recent years. In industrialised nations, the number of recorded cases of food poisoning has been on the decline, even though there have been many reports of food contamination with disease agents [14-17]. This is because of more stringent regulations meant to ensure the cleanliness and safety of food as well as better processing and storage techniques. The use of proteomic methods has opened up many new avenues for research into the production and health of plants and animals, as well as the quality and safety of foods derived from these sources. An accurate picture of what pathogens are up to during infection, disease outbreak, and recovery can be obtained from proteome analyses of contaminated food. Crucially, in vivo proteome data can efficiently direct additional functional research by prioritising identifying proteins with known functions. Quantification, post-translational modification identification, and inadequate coverage of secreted and low-abundance proteins are some of the technical constraints of the past. To get around most of these restrictions and difficulties, new genomic, proteomics, peptidomics, glycomic, and metabolomics approaches are appearing alongside more traditional genomic approaches. As a result, these methods will be an even more valuable tool for studying and fighting infectious diseases, and in vivo investigations will be even better. Approximately 3,705 genomes of microbes, including 1,750 of bacteria, 94 of archaea, and 119 of eukaryotic, have been fully sequenced. Genomes of sequenced microbes give ample data for high-throughput screening methods that are both quick and accurate [18-21]. The most pressing issues with fresh food right now are pathogenic germs like Escherichia coli, Salmonella, and Campylobacter, while the most pressing issues with processed items are contaminations caused by Listeria monocytogenes. The most potentially toxic eukaryotic food diseases are caused by the fungus Aspergillus, Fusarium, Penicillium, Alternaria, and Claviceps. Additional safety concerns have lately included prions and viruses.

Influences on the Prolongation and Persistence of Infectious Agents

The cellular physiology of an organism undergoes a number of basic modifications when it is exposed to stress. Because microbes live in environments that are always changing in terms of temperature, osmotic pressure, and nutritional availability, stress responses are very important to them. Several factors, including product type/combinations, storage temperature, mild processing methods (such as slicing, shredding, washing, and decontamination treatments), pressure, pulsed electric field processing, cold plasma, and advanced heating, package atmosphere, and competition from natural microflora on food-produce all play a role in how long pathogens can survive and grow on food-produce. Another key component that reduces the populations of bacteria on fresh produce is antimicrobial washing, which involves applying different disinfectants and adding natural antimicrobial agents. The current scenario surrounding food safety has been drastically altered by the recent consumer preference for less processed foods with lower concentrations of preservatives. In particular, the popular "ready to eat" vegetables that have undergone minimum processing. These foods still include a lot of the natural bacteria and other microbes in them, including some dangerous ones like Aeromonas and Yersinia. First, proteins are extracted. Then, they are separated and quantified. Then, they are identified. Finally, the data is analysed and understood. These are the four main phases that make up the working principle of proteomics. The analytical sample is treated with a protein extraction procedure. Partially purifying, selectively enriching, or depleting high abundance proteins are also possible in complicated samples. The two-dimensional gel electrophoresis technique is used for protein separation. The bottom-up proteomic methodology is used in both of these separation approaches. The second method is called the top-down strategy. It skips the digestion step and goes straight to mass spectrometry with the peptides from the broken proteins. Proteins are identified and quantified using mass spectrometric methods such as LC-MS, MALDI-TOF MS, or MS/MS after they have been separated and digested [21-25]. While MS/MS or LC-MS/MS can be utilised for protein characteriszation, quantification, and identification, MALDI-TOF MS is primarily employed for protein identification. Methods such as peptide mass fingerprinting and 2D electrophoresis are employed in this procedure,

which involves in-gel digestion of proteins and subsequent mass spectrometric analysis. Both methods involve running the target protein through a mass spectrometer and then matching it with an existing database entry. In the absence of a match in the database, the protein with the highest degree of homology is used for pairing.

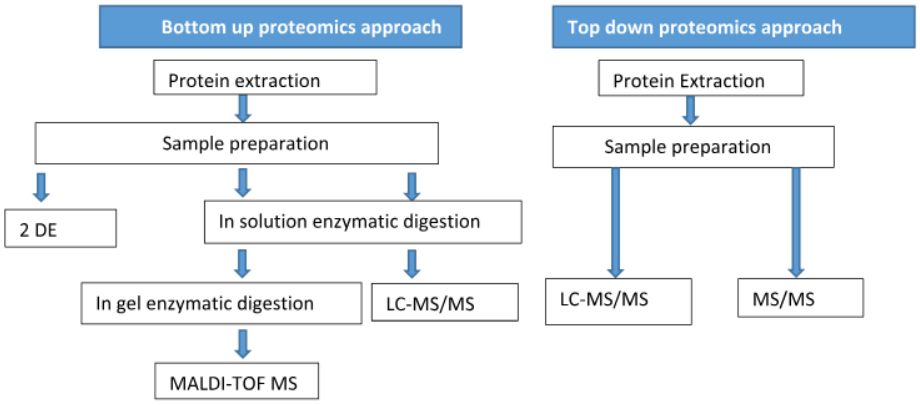

Figure 1. Proteomics workflows for bottom-up and top-down proteomics approaches.

Proteinomics in the Adaptation of Microbes to Stress

Bacterial adaptation to environmental and food-related stressors is an essential cellular defence mechanism. Quantitative correlations between adaptive stress and cellular indicators allow for the prediction of how different settings would affect the resistance and survival capabilities of bacteria. The preservation approach for cross-protection phenomena is an additional challenge in ensuring the sensory and nutritional quality, as well as the safety and stability of minimally processed food with respect to microbes. The correct set of barriers has the potential to reduce organoleptic changes in food, slow the growth of bacteria and pathogens, and prevent food deterioration. Heat, acid, salt, and oxidative agents are examples of moderate stress conditions that, when exposed to them for either long or short periods of time, can promote (cross-)protection against more dangerous stress conditions and may even influence the virulence of infections. Microbes' resistance-building capabilities in response to adaptive stress responses have several practical uses in industry, such as in the creation of stable probiotic strains and the selection of dependable starter cultures. At the levels of the transcriptome, proteome, and metabolome, Den Besten et al. (2010) discovered various biological indicators for the adaptive behaviour of bacteria under stress. The model microbe Bacillus cereus allowed for the identification of several possible biomarkers of stress response [26-29], including the transcriptional regulator sB, catalases that scavenge H2O2, chaperones, and ATP-dependent Clp proteases that repair and support proteins. To summarise, Abee et al. (2011) used a meta-transcriptome analysis of the RsbKY-controlled sB regulon and protease transcript levels and catalase to predict the robustness level of stress-adapted B. The study was based on two-component systems (TCS) using comparative genomics, and it integrated three separate research strategies to identify biomarkers important for robustness in Bacillus cereus. pressures that can kill cereus cells. B. continued the experiments by implementing a broad range of environmental adjustments. cereus, and their use was then expanded to other acteria such as Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Chronobacter turicensis, and many yeast species. Through the use of cellular biomarkers, this quantitative technique can anticipate how well microbes will perform in terms of detecting food-borne diseases early on and controlling their adaptive behaviour to make them more resistant.

Studying food-related protein-protein crosslinking using proteomic methods

Intermolecular covalent bonding between amino acid residues or intramolecular polypeptides is what's known as protein-protein crosslinking. Protein cross-links can be either naturally occurring in the raw material before processing or introduced artificially by enzymatic or chemical crosslinking reagents. Lastly, protein cross-links can be produced by processing or environmental disruptions like heat treatment, drying, pH changes, or ultraviolet light. Studying the structure and location of proteins expressed under particular conditions at a given moment is an important part of proteomics, which also includes characterising and detecting protein arrangements of research regions. Common uses include determining the protein's main structure, analysing proteins and peptides, quantifying proteins, and studying post-translational changes. Both bottom-up and bottom-down cross-links are problematic in proteomic approaches.

There are four main points that can be made to describe classical bottom-up proteomics. The first step in processing and extracting proteins from biological materials is to use procedures such as two-dimensional polyacrylamide and electrophoresis (2D-PAGE). Secondly, proteases break down proteins into smaller pieces, a process typically aided by trypsin. Thirdly, the complicated peptide mixture can be separated using a mix of liquid chromatography (LC) and 2D-PAGE, and then analysed using a mass spectrometer. Last but not least, MS/MS database search follows protein characteriszation and posttranslational modification analysis.

Concerns about the potential dangers of GMOs

There are concerns about the safety of food because to the spontaneous effects of transgenesis in GMOs. Consequently, the field of proteomics has also been utilised to verify genetically modified organisms. Food safety concerns and consumer knowledge have driven more than 40 nations to enact labelling laws for GMOs. As a result, it is crucial to have inspection methodologies for GM foods in order to offer accurate information. The term "substantual equivalence" is employed while evaluating the security of genetically modified foods. In order to analyse and evaluate GM food, we compare its features and attributes to those of traditional food. One of the main goals of genetically modified (GM) food safety testing is to establish that GM food poses no health risks to consumers. A combination of proteomics, LC-MS, and 2DE has been used to identify genetically modified (GM) and non-GM foods. Using a gel-free proteomic technique in conjunction with isobaric tags for relative and absolute quantification (iTRAQ) labelling, Luo et al. (2009) demonstrated that GM rice and wild-type rice expressed distinct proteins. Mora et al. (2013) demonstrated a label-free LC-MS approach for relative protein quantification in non-GM and GM tomato varieties. The proteome differences [30-33] between transgenic and nontransgenic maize were quantified in maize seeds using 2-DE and iTRAQ. Of the 148 proteins that showed differential expression, 42 were more abundant in genetically modified (GM) maize and 106 were more prevalent in non-GM maize. In addition, Liu et al. (2018) employed the iTRAQ method to compare the proteome profiles of non-GM and genetically modified soybeans. Researchers found that compared to GM soybeans, non-GM soybeans showed a higher and distinct differential in protein expressions. Therefore, omics- and proteomics-based methods can be useful in the cost-effective detection of genetically modified foods.

Temperature and Cold Adaptation

When cells are exposed to low temperatures, their ability to replicate, transcribe, and translate is compromised. In the aftermath of cold shock, mesophilic bacteria face a number of well-documented challenges, including low membrane fluidity, DNA with an excessively high superhelical density that prevents the double helix from opening, reduced enzyme activity and altered protein levels, ribosome function adaptation to low temperatures, and the start of RNA translation of secondary structures. In response to these stimuli, the right signal transduction pathways are set in motion, which in turn trigger the mobilisation of stress defence mechanisms via altering gene expression and action of proteins. Recent work using bacterial transcriptome and proteome stress analysis has shed light on how stress adaption responses mobilise changes in gene expression. Within the Gram-positive bacterial genus Bacillus, Staphylococcus, and Listeria, the transcriptional regulator sB plays a pivotal role in controlling the overall stress responses. A few of lactic acid bacteria lack this sigma factor, which may indicate that these bacteria's stress regulatory systems are distinct. Among B. cereus, another stressor that strongly activated sB was heat shock; nevertheless, other stresses (such as acid stress, osmotic shock, or ethanol shock) were also discovered to activate sB. In the food sector and in clinical settings, methods for pathogen management and inactivation could be developed with a mechanistic understanding of the -activation processes and evaluation of its regulons. E. coli was the subject of research into cold shock response and cold shock proteins. bacteria, both E. coli and B. use subtilis as a model organism. Budde et al. (2006) identified the members of B. chill-stress stimulon using a combination transcriptome and proteomic approach. subtilis over the entire genome. Approximately 14% of B's protein-coding potential is encoded by 580 genes. subtilis mutants showed temperature-dependent changes, including the activation of 279 genes and the repression of 301 genes [34-36]. An extensive list of proteins that were either up- or down-regulated in B cells cultured at low temperatures was identified when the transcriptional profiling was supplemented with a proteomic approach. subtilis cells for which this transcriptional analysis failed to detect differential expression of structural genes. The fact that this protein family is relatively big suggests that post-transcriptional regulatory events play a significant role in B's adaptability. subtilis to grow in cold environments. Elevated temperatures in E led to an accumulation of stress

proteins, such as disaggregation chaperones (DnaK and ClpB), enolase, polynucleotide phosphorylase (PNP), and components of the RNA degradation complex. c. diff. Lethanh et al. (2005) found that at 42°C, there was an increase in alpha-glucosidase activity and an over-expression of the inclusion body-associated proteins A and B (ibpAB). According to these writers, heat-shock proteins play a crucial role in cellular defence in extreme conditions, and this role is mostly dependent on the ratio of IbpA to IbpB. To avoid food poisoning and spoilage [39-41], it is important to learn more about how food-borne microorganisms react to cold shock. The hemorrhagic enterococci (E. Cold stress resistance was much higher in E. coli O157 (EHEC) strain (B-1) than in non-pathogenic E. Eugenia coli K-12 DH5a. By comparing the proteomes of Hypo-thermally adapted E. Vidovic et al. (2011, 2012) found that cold temperature differentially expressed proteins in coli O157 wild-type and rpoS mutant strains. These proteins included RpoS, a 37.8-kDa protein that controls the expression of proteins involved in homeoviscous adaptation during cold shock, and several proteins involved in the central metabolic pathways of this food-borne pathogen. The suggested membrane location of peptidyl glutamyl peptide hydrolase (PgpH) is consistent with the idea that the thermosensing activities of bacteria originate from the membrane. One possible modulator of cold signalling in L. is the PgpH multidrug resistance protein, according to Liu et al. (2006). the bacteria. Adapted L. monocytogenes cells showed changes in the expression of many gene transcripts and proteins. The proteome alterations that underlie L. thermoregulatory response to cold adaptation were examined by Cacace et al. (2010). monocytogenes that can survive and multiply in cold environments. Cells cultured at 4°C exhibited increased levels of adaptation mechanisms involving proteins involved in energy metabolism pathways like glycolysis and the Pta-AckA pathway. In cold-exposed L., the DNA and RNA chaperone activities are provided by increased production and activity of cold-shock proteins (Csp) at low temperatures. isolates of monocytogenes cells to aid with the resolution of nucleic acid structural obstacles. This bacterium cannot withstand cold or osmotic [42-45] stress effectively without these proteins. In reaction to cold stress, CspA is more important than CspD and CspB, whereas in response to NaCl salt osmotic stress, CspD is more important than CspA and CspB, as demonstrated by Schmid et al. (2009). Furthermore, CspA homologues play a role in a wide variety of processes, including spore heat tolerance, osmotic stress, hunger, antibiotic biosynthesis, resistance to antimicrobial pep-tides, and sensitivity to ultraviolet light. These proteins are peptidases, periplasmic serine proteases, complex-forming ATPase, cytoplasmic proteases, and zinc metalloproteases that are bound to the cell membrane. This bacterium's adaptive resilience was examined in a subsequent work that employed the quantitative proteomics approach of Carranza et al. (2010) to examine it in various temperature environments. This study provides evidence that this opportunistic pathogen responds to low growth temperatures by changing its protein expression profile in complex ways and by increasing the production of virulence components when exposed to higher temperatures. Given that food is often heated to around 12°C, it stands to reason that Staphylococcus aureus will adapt its metabolism to the lower temperature range before thriving in this environment. In vivo proteomic comparisons of two molecular methods for S. aureus cold acclimatisation were carried out by Sánchez et al. (2010). Cold acclimation is accompanied by two distinct protein patterns, one involving glycolytic proteins (pattern A) and the other involving general stress and regulatory proteins (pattern B). The virulence factors of Campylobacter jejuni have been identified to include PEB1, CadF, and CDT, which stands for cytolethal distending toxin B. A total of fifteen and twenty proteins were identified for C that showed differential expression. The jejuni bacteria were cultivated on agar at 37°C and in broth at 42°C. Some of these proteins may aid in host adaptationand/or pathogenicity in the human intestines; they are best expressed at 37°C. The survival of a rpoN lacking mutant was compared to wild-type C in a study conducted by Hwang et al. (2011). mussels in a variety of stressful environments. Osmotic stress (0.8% w/v NaCl), acid stress (pH 5), hydrogen peroxide, heat, cold, and antimicrobials all had a greater impact on the rpoN mutant than on the wild type. It is suggested by the authors that RpoN is involved in C. jejuni's defence mechanisms against a variety of threats that this bacterium may face when infecting humans or during transmission. Cells that have been cold-shocked before freezing display better cryotolerance. Thus, by directly freezing the food, food-spoilage microorganisms can be rendered susceptible to the damaging effects of cold temperatures [46-49]. To reduce economic losses caused by lower starter culture activity/viability owing to extreme temperature changes in the fermentation processes, it is possible to increase the cryotolerance of commercially significant microorganisms such lactic acid bacteria starter cultures. Energy production through carbohydrate and lipid metabolism, metabolite transport, respiration, redox balance, and ROS detoxification, cell wall modification, DNA damage repair, and protein chaperones utilised for refolding and degradation are among the processes whose genes are upregulated under heat

stress. Görner et al. (2002) demonstrate that in response to glucose repletion, the cytoplasmic re-localization of the Msn2p transcription factor is regulated by protein kinase A (PKA) phosphorylation of the nuclear localisation signal. However, changes in nuclear export may mainly regulate the re-localization of Msn2p in reaction to other circumstances, which is distinct from this domain. The response to heat stress involves the transcription factors Msn2 and Msn4. Until the heat-shock factor Hsf1 remains unchanged, the mRNA levels of genes associated with translation tend to decline. Saccharomyces cerevisiae's Hsf1 and Msn2/Msn4 transcription factors activate gene expression in reaction to a variety of stressors, such as heat shock, oxidative stress, and nutritional deprivation, and hence contribute significantly to cellular homeostasis. An important function of Yak1 kinase is to mediate the inhibition of Hsf1 and Msn2/Msn4 by PKA. This information will shed light on the incredible resilience of many organisms, including yeast, to withstand extreme environmental changes. Aspergillus fumigatus, a type of mould, can withstand temperatures more than 55 degrees Celsius, or around 70 degrees Fahrenheit. When it comes to heat, A. The unique characteristics of Aspergillus fumigatus, including its potential elevated levels of heat-shock proteins (Hsp), may enhance its pathogenicity. Proteins Hsp30/Hsp42 and Hsp90 exhibited the greatest increase in abundance during the heat-shock reaction. Adapting to new environmental conditions, food-borne viruses have developed distinct regulatory systems to survive processing stress. Bacterial, yeast, and fungal cells undergo a wide variety of post-translational modifications in response to cold- and heat-stress factors; these modifications control essential processes that contribute to the pathogenicity of the organisms in question.

How to Adapt to Osmotic Stress

Because it greatly increases storage time by decreasing water activity, salt (NaCl) is a widely used compound for food preservation. In environments with little water activity and high salt levels, germs can survive and even thrive due to osmotic stress. Strategies and potential cross-protection mechanisms based on transcriptome and phenotypic traits can be better understood through cellular stress adaption. In order to find out how cells react when exposed to mild salt stress (2.5% NaCl,w/v) and severe salt stress (5% NaCl, w/v), we examined the transcriptome profiles for the two salt situations. An overlap in the transcriptome response was found in the whole-genome expression analysis of Bacillus cereus cells that were weakly and highly salt-stressed. Extensive research has been conducted at the protein or gene level to understand the physiological response of Bacillus subtilis to changes in osmolality. Many membrane proteins, particularly those that transport ions and solutes that are compatible, are recognised to play an important role in B adaptability. stress caused by salt. To better understand the dynamic alterations taking place in B. subtilis under salt stress, transcriptomic and proteomic methods can be employed. research on subtilis cultures offer a neutral perspective on cell replication in high salinity environments. In addition to providing protection from heat and cold, salt stress also stimulated the transcription of osmoprotectant transporters. For quantitative proteomic analysis, Hahne et al. (2010) used metabolic labelling with 14 N- and 15 N-labeled medium in conjunction with whole genome microarray technology. They found that during an osmotic upshift, a considerable portion of the SigB, SigW, SigM, and SigX regulons (genes regulated by the same regulatory protein) are involved in a coordinated induction of gene expression. In the realm of 500 osmotic upregulated genes found in B. Finding subtilis genes provides a wealth of information for studying B. subtilis's physiological and genetic responses in greater detail. hypoxia in Bacillus subtilis. High salt levels, a wide temperature range, and acidity are just a few of the environmental challenges that Listeria monocytogenes can withstand. Because of this high level of bacterial resistance, controlling this food-borne disease is challenging. Food processing and preservation-related L. monocytogenes resistance is another issue. Glycine betaine and carnitine accumulate through transporters encoded by different operons: the betL gene for glycine betaine and the gbu operon for carnitine. In hyperosmotic environments, the macromolecular structure of cells is maintained through the increased uptake of glycine betaine and carnitine osmolytes through betL, gbu, and opuC encoded transporters, which prevent internal water loss by counteracting external osmolarity. Duché et al. (2002a, b) investigated the influence of NaCl (with a concentration ranging from 3% to 6% NaCl, w/v) on the protein expression of L. in order to have a better grasp of how salt stress affects food processing and preservation. the bacteria. 2-D electrophoresis, followed by mass spectrometry (MS) or N-terminal sequence analysis and database searching, was used to identify salt-shock and salt-acclimation proteins. Presence of salt promotes the production of phospho-glycerate mutase (Pgm), glyceraldehyde-3-phosphate dehydrogenase (Gap), and pyruvate dehydrogenase A (PdhA). Enzymes involved in catabolic metabolism could be represented by these proteins. L. pneumoniae also contains other proteins that are damaged by salt. monocytogenes seemed to serve a variety of purposes as well [50]. The salt-stress response is

directly related to one protein, GbuA, which Duché et al. (2002a) found to be overexpressed when salt is present. Since two Gsp proteins—general stress protein (Ctc) and heat-shock protein (DnaK) were elevated in response to salt stress, there is a connection between this response and the general stress response. It appears that some of the mechanisms that combat osmotic and cold stress may be common in L. These transporter systems were also found to be expressed under hyperosmotic stress settings. the bacteria. Understanding the mechanisms of action of salt-shock proteins (Ssp) and stress acclimation proteins (Sap) is a challenging task, as revealed by these observations.

Resistance to Stress at High Hydrostatic Pressures (HHP)

The nonthermal technique of high-pressure processing (HHP) is employed to destroy harmful and spoilage microbes in food. One of the newer ways to preserve food is with the use of HHP technology. Cell membrane and morphological modifications, effects on proteins (including enzymes), and changes to genetic pathways were among the many changes generated by pressure treatment in microorganisms. Nevertheless, our understanding of how HHP inactivates microbes remains incomplete. Some identified variably expressed proteins showed qualitative alterations in the proteome of Bacillus cereus that Martínez-Gomariz et al. (2009) examined during the HHP treatment. Different microbes react differently under pressure. When compared to Gram-negative and cocci bacteria, Gram-positive bacteria often exhibit greater pressure resistance. Escherichia coli O157:H7, three strains of Listeria monocytogenes, and other bacteria and strains can be quite resistant to pressure. The food matrices and microorganisms determine the range of HHP treatments, which can be anywhere from 5 to 30 minutes and use pressures from 200 to 800 MPa. Toxic mould growth's response to pressure is poorly understood. It was common practice to mix shock with other processes, including post-heat shock, to render them inactive. The pressure levels employed during food HPP usually range from 300 to 800 MPa, which is significantly above E. coli's physiological tolerance limits. Cell maintenance and repair are thought to entail numerous cellular mechanisms. Through the use of a selective enrichment strategy, Vanlint et al. (2012) investigated and contrasted the inherent capacity for HHP resistance development among various strains of Yersinia enterocolitica, Salmonella enteritidis, Escherichia coli, Shigella flexneri, Salmonella typhimurium, Aeromonas hydrophila, Pseudomonas aeruginosa, and Listeria innocua. Some E. coli strains were reported to have HHP resistance, suggesting that resistance development [51] may be influenced by a specific genetic predisposition. In the absence of HHP exposure, HHP resistance was found to be a relatively stable characteristic that was maintained for over 80 generations. The authors imply that, on a molecular level, there is no correlation between HHP resistance and either the derepression of heat-shock genes or the persistence phenomenon. The pre-induction of stress response regulators like SigB, which can activate many protective genes under stressful conditions, may impact L. monocytogenes survival when exposed to HHP. Low temperature and high heat treatments have been found to significantly increase the expression of cold-shock proteins in Listeria monocytogene. In contrast to the reactions of other bacterial species to this stress, C. jejuni exhibited a unique reaction to HHP treatment. When we looked at how C. jejuni reacted to HHP shock, we found that we could only activate oxidative stress-specific proteins. This may clarify why C. jejuni is relatively more susceptible to this antibiotic than other strains of Gram-negative bacteria.

Different Sources of Stress

Surfaces that come into touch with food are frequently cleaned and disinfected using antimicrobial agents. Chemicals are a typical source of oxidative stress. It is not known, however, how different disinfectants affect the cellular response of bacteria. Examining the transcriptome responses of B. cereus ATCC 14579 to different disinfectant treatments, including benzalkonium chloride (BC), sodium hypochlorite (SH), hydrogen peroxide (HP), and peracetic acid (PAA), Ceragioli et al. (2010) employ a genome-wide comparative transcriptional approach. Researchers found that BC increased expression of genes related to fatty acid metabolism. The overexpression of quaternary ammonium compounds (QACs) resistance membrane-bound proteins, which can promote bacterial tolerance towards other antibacterial chemicals, was another effect of BC and SH treatments on B. cereus cells. The SOS response and other DNA damage repair genes were upregulated in B. cereus ATCC 14579 after exposure to HP and PAA. These findings have the potential to impact B. cereus's recontamination capacity and, by extension, the quality and safety of food.

The distinct mechanism that leads to the activation of sB in B. cereus as compared to other Gram-positive bacteria is demonstrated by the central function of RsbY in controlling sB activity. An essential defensive mechanism of bacteria

against H2 O2 is the synthesis and activation of catalase. Catalases are produced by the majority of bacteria as a result of their aerobic growth process [52]. A thermolabile catalase is found in vegetative cells of Bacillus cereus, while a thermostable catalase is found in spores. The sB deletion mutant of B. cereus ATCC 14579 was studied for its hyper-resistance to H2O2 by van Schaik et al. (2005c). This deletion mutant bacteria exhibited hyper-resistance to H2O2, which was attributed in large part to the elevated transcription of the katA gene, which encodes the primary vegetative cell catalase. Using three transcriptome samples and clustered transcriptoma data, Abee et al. (2011) expose stressors such as detergents (benzalkonium chloride, hydrogen peroxide, peracetic acid, sodium hypochlorite), ethanol, heat, acid (HCl, lactic acid, acetic acid), and salt stress at varying concentrations and time intervals. For disinfection and preservation, QACs like cetrimide and benzalkonium chloride (BC) are commonly utilised. Multiple investigations have established a connection between the inherent or natural resistance of Gram-negative bacteria to tenside-based disinfectants like QACs and either a low outer membrane permeability or broad-spectrum efflux mechanisms. Most research on how Escherichia coli acquire resistance has focused on alterations to the membrane's lipopolysaccharide (LPS) and fatty acid composition. Biocides impact numerous cellular components, in contrast to antibiotics that target highly particular areas of the bacterial cell. When exposed to hypochlorite, YjiE, a hypochlorite-responsive transcription factor, regulates a vast array of genes. YjiE makes E. coli cells resistant to hypochlorite. Adapted strains, like E. coli K-12, exhibited enhanced resistance to BC, which includes responses typically associated with the Mar regulon and safeguards against oxidative stress. A protein phosphatase 2C (PP2C)-type phosphatase called RsbU is required for the activation of sB, which in turn controls a vast regulon in Staphylococcus aureus. After Staphylococcus aureus was exposed to alkaline stress, Pané-Farré et al. (2009) looked at how the stress signalling protein phosphatase RsbU controlled sB activity. In contrast to B. subtilis, S. aureus was able to promote Sb-dependent transcription with only the increased expression of RsbU. Based on the data we have, it seems like B. subtilis regulates RsbU and sB activity differently than S. aureus is. Salmonella enteritidis proteomes are stressed by prolonged exposure to high concentrations of propionate (PA) in food processing systems and in infected hosts' guts.

Toxin and pathogen identification in food

It is possible to detect, identify, and quantify food-borne pathogens using proteomic methods. Food poisoning can be caused by about 250 different pathogens, most of which are bacteria and the toxins they produce. Proteomic technologies are being utilised to aid in the identification of food-spoiled diseases and microorganisms, in addition to morphological, biochemical, and DNA-based approaches to microorganism classification and identification. To achieve this goal, classical and genetically-based identification methods are supplemented with modern technologies that can identify and categorise microbes quickly and accurately, such as MS-based proteomics tools. Proteomics methods have been routinely employed for bacterial identification in fields such as clinical microbiology, biodefense, and environmental science. Classifying food-borne microbes and pathogens responsible for food deterioration has received minimal attention in the subject of microbiological food MS. Use of MALDI-TOF MS on intact bacterial cells allowed for the detection of 24 distinct food spoilage bacteria and foodborne pathogens, including genera like Staphylococcus, Yersinia, Proteus, Escherichia, Lactococcus, Listeria, Pseudomonas, Morganella, and Salmonella. The use of matrix-assisted laser desorption ionization-time of flight (MALDI-TOF MS) for the detection of bacteria and other foodborne pathogens has been documented in multiple investigations. This method is both efficient and economical, and it can detect and identify a variety of foodborne pathogens, including listeria and Escherichia coli monocytogens (Singhal et al., 2015). In order to provide a unique fingerprint for the examined microbes at that particular moment and physiological state, MALDI-TOF MS profiles the entire bacterial proteome. Since the fingerprints produced by these microbes are unique to them, they have numerous potential uses, such as in subspecies, strain, and serovar characteriszation. Using tandem mass spectrometry and MALDI-TOF, Fagerquist et al. (2014) identified Shiga toxin-producing Escherichia coli (STEC) as a causative agent in significant outbreaks of food poisoning. Within thirty hours, MALDI-TOF MS can detect as few as one colony-forming unit (CFU) of L. monocytogens per millilitre. Nearly 185,000 individuals in the United States get sick every year from Staphylococcus aureus. Callahan et al. (2006) characterised staphylococcal enterotoxin B, one of its toxins, using a mass spectrometry-based technique. Food processing methods commonly used today cannot eliminate toxins. Detection of mycotoxins and aflatoxins in food has also made use of proteomic approaches such as LS-MS/MS. The utilisation of proteomics in toxin discovery was examined by Martinović et al. (2016).

CONCLUSION

In an effort to reduce the frequency of food poisoning incidents, new methods and tools have been developed. Yet, new dangers have emerged as a result of food-borne viruses' ability to adapt and establish novel survival mechanisms. Many bacteria and fungi that cause food poisoning are able to build biofilms as a defence mechanism against the host's reaction and microbicidal agents. To further understand the role of biofilm matrix proteins, a proteomic study of the biofilm matrix of the Gram-negative bacterium Vibrio cholerae was conducted. We used MS/MS to examine the protein blends that were made. To forecast where proteins will be found within cells, we use an in silico approach. According to the proteomic research, NusA is a transcription elongation factor that is expected to be secreted. Bacterial appendages like the flagellum and mannose-sensitive hemagglutinin type IV pilus (MshA) were also identified as secreted proteins. RbmA and RbmC, which were co-regulated with the genes for VPS synthesis, were also included. Not only that, but three proteins—a hemolysin (HlyA), a chitinase, and the hemagglutinin/protease (HAP)—were also discovered to be involved in biofilms. Seventeen of these proteins were found in the periplasm, while twenty-six were found in the outer membrane (OM). It was not possible to determine with absolute accuracy where 18 proteins were located. Researchers discovered that while the uniformly dispersed RbmA protein in the biofilm enhances connections between cells, the communally synthesised Bap1 protein was shown to congregate at the biofilm-surface interface and stabilise the association between the biofilm and the surface. In 2009, Villena et al. looked into the first intracellular proteome profiling of biofilm cultures of Aspergillus niger ATCC 10864 grown on polyester fabric. We compared the proteome of A. niger ATCC 10864 to that of traditionally cultivated free-living submerged cultures using 2D-PAGE and MS-TOF analysis. Proteomic maps revealed distinct expression patterns in the two culture systems, with differentially expressed proteins in each. In the case of biofilm cultures, for example, 19% and 32% of the selected protein locations were overexpressed, respectively. Proteomes of A. niger biofilm and free-living mycelia differ significantly, according to these data. According to these findings, surface adhesion fermentation relies on cell adhesion as the primary stimulus for biofilm growth. A better understanding of the factors contributing to the rise of biofilm-associated microbial resistance has been aided by proteomics. There hasn't been nearly enough research on proteomics, even though the field has seen tremendous advancements in the last several years. This highlights the need for more robust, cutting-edge, high-throughput proteomic investigations on a worldwide scale, especially for biofilms derived from in vivo clinical sources and for food safety concerns. A comprehensive understanding of these issues may be possible through the integration of proteomics with genomes and other multidimensional technologies.

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