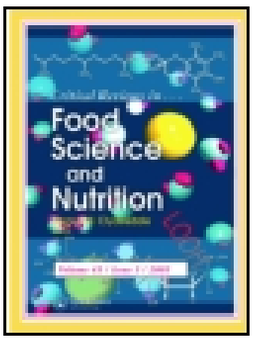

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REVIEW ARTICLE

Integrating omics to unravel the stress response mechanisms in probiotic bacteria: approaches, challenges, and prospects

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ABSTRACT

Identifying the stress response mechanism of probiotic bacteria has always captivated the interest of the food producers. It is crucial to identify probiotic bacteria that have increased stress tolerance to survive during production, processing, and storage of food products. However, in order to achieve high resistance to environmental factors, there is a need to better understand the stress induced responses and adaptive mechanisms. With the advances in bacterial genomics, there has been an upsurge in application of other omics platforms such as transcriptomics, proteomics, metabolomics, and some more recent ones such as interactomics, fluxomics, and phenomics. These omics technologies have revolutionized the functional genomics and their application. There have been several studies implementing the various omics technologies to investigate the stress responses of probiotic bacteria. Integrated omics has the potential to provide in-depth information about the mechanisms of stress-induced responses in bacteria.

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However, there still remain challenges in integrating the information from different omics platforms. This review discusses the current omics techniques and the challenges faced in integrating various omics platforms with focus on their use in stress response studies.

KEYWORDS

bacteria, stress, proteomics, transcriptomics, metabolomics

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INTRODUCTION

Increasing awareness about the food-health relationship has given tremendous impetus to the research and development of functional food. Food is no more considered as a mere source of energy and body-forming substances, but more attention and interest is now being drawn to the biologically active components in food, and to the health benefits which they impart. Probiotic foods have gained much popularity during the last decade and have a huge potential for research and development of more new probiotic food. While some of these health benefits of probiotics have been well established, most of them need to be confirmed by human studies (Shah, 2007). The most critical aspect of probiotic food products is to maintain the viability of the probiotic bacteria, during production, processing and storage. In this aspect, the various environmental stressors encountered by the bacteria during production process and in the food matrix pose a major challenge. These stress factors such as exposure to acid and heat, osmotic imbalance, and oxidative stress pose several technological challenges; however, for the bacteria to confer their functionality as probiotics, it is important to maintain their viability in the product during production, processing, and storage (Shah, 2000). The probiotic bacteria must therefore demonstrate resilience to hostile conditions encountered during the production, processing, and storage of food products. The ability of the bacteria to respond and adapt to stress is strain dependent and is essential for their survival. Understanding the stress responses of these bacteria provides insights to their mechanisms, which in turn has led to increasing attempts to enhance their robustness.

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While conventional molecular techniques provide an outline, the whole genome sequencing technology supplemented with the more recent functional genomic analysis provides a deeper understanding of the complex bacterial system. Whole genome sequencing of bacteria has the potential to significantly advance the existing knowledge on stress response and adaptation to environmental stress factors; however, it has proven to be not comprehensive enough for gaining complete mechanistic information about the complex bacterial system. Whole genome sequencing provides enormous amount of information on the bacterial genetic makeup, which can be then applied to other omics platforms for detailed functional analysis. For instance, proteomics and transcriptomics technologies supplemented by the conventional molecular tools, apply key information from genome sequencing data to reveal mechanisms of the changes occurring in cells as affected by their ecological niche. Whole genome sequencing of more than 7000 bacterial genomes has been successfully completed owing to the revolutionary advances in DNA sequencing technologies (source: NCBI, GenBank), and many more are nearing completion at a fast rate. The advances in genomic analysis including the advent of functional and comparative genomics have enhanced the knowledge of microbial metabolism over the past few decades. These advances have led to detailed insights on genome structure, gene location and function, metabolic, and biological pathways, which have tremendously improved our understanding of the microbes. The attempts to understand the whole genome sequences has led to development of functional genomics and several omics platforms along the way. These omics platforms, namely transcriptomics, proteomics, metabolomics, are high-throughput technologies that aim to understand the links between the different components of a complex microbial system. Furthermore, a large amount of data is generated in analysis using these top-down, data-

driven approaches, which demands statistical analysis along with a comprehensive data analysis. These individual omics technologies have revealed immense information on microbial system and have provided deeper understanding on some of the fundamental mechanisms (Park et al., 2005). Although all the omics technologies are contributing significantly, it is also indicated that these omics platforms must be integrated together, as the complex bacterial network may not be characterized by any single omics based approach (Gygi et al., 1999).

In the past decade attempts have been made to integrate some of the omics technologies to understand the global regulatory mechanisms and the metabolic pathways and networks (Hegde et al., 2003; Mootha et al., 2003; Alter and Golub, 2004). Integrated omics approaches, though continuously offering scope of improvement, have been suggested as pivotal in understanding the microbial metabolism and functional responses to different environmental conditions.

This article presents an overview of recent progress in use of various omics approaches to investigate the stress responses in prokaryotic bacteria, with focus on probiotic bacteria. The advances in integrative omics and their potential application in this area are also discussed/presented. Introduction and application of individual omics platforms is also briefly discussed.

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GENERAL STRESS RESPONSE MECHANISM

Stress response mechanism is a complex network in the bacterial system. Some of the general stress resistance mechanisms include alterations in specific stress-sensing systems and export systems, whereas other factors contribute in a generic way by protecting the cell membrane integrity, repairing of DNA and special proteins. For instance, bacteria respond to acid stress by

up-regulation of chaperones like GroEL, GroES, DnaK, ClpE and GrpE (Frees et al., 2003), and production of novel shock proteins. Alterations in membrane fatty acids, particularly monounsaturated fatty acids, occur in bacteria as a result of high acid in the growth medium. Cyclopropane fatty acid (CFA) plays a significant role in cell responses to acid stress (Chang and Cronan, 1999; Budin-Verneuil et al., 2005). In general, release of shock proteins, alterations in membrane composition are some of the stress-induced responses in bacterial system. However, since stress tolerance is highly variable between different strains of bacteria, identification of key regulatory components involved in stress responses of probiotic bacteria is very crucial.

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OMICS APROACHES & METHODOLOGIES

Survival and adaptation mechanism of the cell is a complex system, which largely depends on the functional molecules of the cell. The presence and activity of these functional molecules are regulated by transcription, translation, various enzymatic activities, and interactions between metabolites triggered by the environmental conditions. These individual processes, each comprising of multiple components, are highly interconnected and form an extremely complex network. Although, high-throughput ‘omics’ techniques have been developed for each of these processes (transcriptomics, proteomics, metabolomics) and have been widely used (Table 1), exposure to environmental stressors affects each of these components (genes, transcript, proteins, metabolites) in a unique way. Thus, there is a need to combine these techniques to better understand the complex network of cellular responses at different molecular levels. There have been increasing applications of integrating more than one omics platforms to evaluate the stress-induced responses in bacteria (Table 2). The advent of bacterial genome sequencing in late 1990s

has opened the doors of enormous opportunities to development of advanced omics platforms. However, studying the DNA sequences alone may not be sufficient to understand the regulatory mechanisms and the functionality of these metabolites (Nierman et al., 2000). This has led to discovery of various omics techniques that have been applied individually or in combination to better understand the complex bacterial system (Figure 1). The application of individual omics approaches to understand the characteristics of probiotic microorganisms has been reviewed by Sanchez et al. (2013). They highlighted that the knowledge on probiotics will continue to evolve with the advances in omics technologies, particularly the functional gut microbiomics.

Genome sequencing is the first step to understanding the molecular responses of stressed bacteria. Owing to the revolutionary advances in high-throughput technologies, sequencing microbial genomes is now fully in reach of the scientific community as costs have fallen tremendously and faster than in some other technical fields over the past few years. With further advances in genomic analysis, functional (gen)omics have evolved over the years targeting components beyond the DNA sequence.

Transcriptomics

The next level of functional omics is the genome wide expression profiling called transcriptomics. It allows a global analysis of the transcripts, i.e. the mRNA molecules, produced in the bacteria. Genomic analysis gives static information of the bacterial species focusing on the DNA. However, the exclusivity of transcriptomic analysis is quantification and comparison of dynamic expression profile of mRNA under normal and various stress conditions. This

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information is critical to identify the genes being actively expressed or suppressed under specific environmental conditions (Ye et al., 2001; Horak and Snyder, 2002).

There are several commonly used transcriptomic techniques applied to understand the stress-induced responses in bacterial cells. The most common one is identification of mRNA differential expression under normal and stressed conditions. This approach defines the identity of the specific genes using DDGE (differential display of gene expression) or SAGE (serial display of gene expression). The second method involves application of micro-array technology, or chip-based (or slide-based) nanolitre-volume reverse transcriptase-polymerase chain reaction. These techniques provide increased sensitivity and accuracy of the levels of gene expression for several thousands of genes simultaneously (Stedtfeld et al., 2008). The third and more recent approach uses next-generation sequencing for direct cDNA sequencing converted from whole transcriptome (Frias-Lopez et al., 2008; Gilbert et al., 2008). Lastly, it is the most recent direct RNA sequencing technology that allows evaluation of the abundance of and identity of the mRNA molecules in a single process. Transcriptomics based approaches have provided insights to genome-wide stress-induced transcriptional modifications, operon structures, and comparative transcriptomic analysis. Transcriptomic techniques provide detailed analysis of induced expression of specific genes in stressed bacteria. The information from transcriptomics study also provides information for further protein expression, metabolic pathway analysis, and production of metabolites, namely proteomic and metabolomic analysis. Several studies in recent years have applied transcriptomic approaches to investigate the stress responses in prokaryotic systems. A study by Rezzonico et al. (2007) investigated global transcriptome analysis of *Bifidobacterium longum* and showed that 46% of the genes were differentially expressed upon

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exposure to heat stress. Transcriptomics have improved our understanding of stress-induced responses by revealing the changes in gene expression of known and new genes. However, like any other omics platform, an individual technique cannot provide a complete and in-depth mechanistic picture of the complex bacterial system. The major drawbacks associated with application of transcriptomics based approaches in evaluating stress responses have been reviewed in detail by Feder and Walser (2005). Some of these drawbacks are: (1) the abundance of mRNA may not be related to the activity of the corresponding protein and thus may provide limited insight into stress responses and their evolution; (2) the mRNA expression mostly does not correlate to the phenotype of the proteins transcribed during the stress response. Also, the impact of these proteins on the organism is mostly not specific to the type of stress, thereby not providing much new and specific mechanistic information (Feder and Walser, 2005); and (3) transcriptomics based approaches involve much higher cost and technical challenges as compared to non-transcriptomic microarrays to evaluate the characteristic impact of a stress factor in microbes (Letowski et al., 2003).

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Proteomics

Proteins being the vital macromolecules play a significant role in metabolic and cell signaling pathways. From building the cellular structure to bearing multiple functionalities, understanding the role of proteins and to identify and quantify proteins is very crucial. High-throughput proteomic approaches target at qualitative and quantitative analysis of all cellular proteins induced by exposure to various environmental stresses. The term proteomics is analogous to genomics and transcriptomics and was designated as a tool for understanding the structure and

function of proteins (Wilkins et al., 1996; James, 1997). The two technologies adopted in the proteomics approach are: a) two-dimensional gel electrophoresis, where proteins are separated based on their mass and charge and then identified using several ways; and b) liquid chromatography (LC) based separation (high performance LC or micro-capillary LC), or size exclusion chromatography coupled with tandem mass spectrometry (Baggerman et al., 2005; Nie et al., 2008).

Although there was tremendous potential in proteomic technologies and its applications, yet identification of less than fifty percent of the bacterial proteins only could be achieved. Therefore, to target more accurate and quantitative proteomic analysis advanced techniques such as isotope labelling-based isotope-coded affinity tags (ICAT), isobaric tag for relative and absolute quantification (iTRAQ) (Yan et al., 2008) or label-free comparative quantitative proteomics need to be employed (Haqqani et al., 2008) were developed. Proteomics based technologies continue to have improved and increased applications in microbial systems. Studies have been conducted in employing proteomics as a tool to understand the stress-related responses in probiotics *Bifidobacterium longum* (Xiao et al., 2011) and *Lactobacillus rhamnosus* (Koponen et al., 2012). There are continued attempts to improve and enhance the degree of information revealed by proteomics and with the application combined proteomic techniques, the future looks promising. However, there are also some drawbacks of proteomics-based technologies. Limitations of proteomic based approaches have been reviewed in detail by Garbis et al. (2005). Proteomics technologies lack the capacity to detect low abundance, hydrophobic, and basic proteins. Further, two-dimensional gel electrophoresis does not include the intrinsic bacterial membrane proteins that play a significant role in stress responses. For instance,

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adaptation to salt stress involves ABC transporter systems which regulate the uptake of compatible solutes (involving OpuAB, OpuD, OpuE proteins), or the uptake of potassium (involving KtrB and KtrD proteins). These proteins are intrinsic membrane proteins which play a significant role in osmo-adaptation but are not evaluated by gel-based proteomic technologies (Hoper et al., 2006). These drawbacks of proteomics based approaches drive the advancements in technologies and enable in achieving a deeper understanding of the biological systems.

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Metabolomics

Metabolomics, or the omics of cellular metabolites, targets the complex metabolic pathways, which are highly interlinked and thus requires a holistic approach for accurate analysis. Metabolite analysis appears to be more challenging due to the diverse and dynamic nature of the metabolites, and the number of metabolites present in a single microbial system. Dunn and Ellis (2005) estimated the metabolome to be over 7-9 magnitudes of concentrations, i.e. pmol-nmol, indicating that a metabolomic based approach should have the potential to detect compounds that span a diverse chemical spectrum and a large dynamic concentration range.

Metabolomic based approaches include multiple aspects such as targeted and non-targeted, quantitative and qualitative etc. Some of these are metabolite fingerprinting, targeted metabolomic analysis, and metabolite profiling. While metabolic fingerprinting targets numerous compounds, it does not differentiate or quantify the individual compounds. Targeted metabolomic analysis is limited to specific known metabolites, which may only represent a small percentage of the whole bacterial metabolome (Halket et al., 2005). Metabolite profiling, the most pivotal in metabolomic analysis, is based on detection of a known or unknown metabolites

belonging to a particular metabolic pathway (Fiehn, 2001; Dunn and Ellis, 2005). A major challenge in metabolomic studies lies in extent of coverage of cellular metabolites; however, metabolomics has still been of increasing interest in an attempt to complete more components in the complex cellular network.

Metabolomics is a newer omics platform as compared to the three discussed above, and as a result, the advancements in the methods and applications are still also being improved continuously. The metabolomic approaches are based on mass spectrometry, nuclear magnetic resonance spectroscopy, and vibrational spectroscopy (Dunn et al., 2005). Metabolomics, involving the most diverse of all components from other omic approaches, require techniques to be able to detect and identify hundreds of distinct chemical compounds in different forms and largely varying amounts. Several studies have shown that various cell functions are mediated by and acted upon at the metabolome and metabolic network level (Raamsdonk et al., 2001; Mandal et al., 2003). Furthermore, some environmental stressors may not induce changes in the transcriptome and proteome, but they do have significant impact on the individual metabolites and the whole bacterial metabolome (Wang et al., 2006). Moreover, it has been reported that bacterial metabolomic analysis reveals more specific responses (to the kind of stress factor) as compared to transcriptomic analysis, which reveals more generic stress responses (Jozefczuk et al., 2010).

The application of metabolomics to evaluate stress responses has been gaining momentum over the past few years. One of the most common bacterial stress responses is synthesis of compatible solutes. Metabolomic analysis has the potential to provide time-dependent release

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(based on different growth phases) of these compounds, such as proline, glycine, trehalose, choline etc., which may contribute to development of improved strains (with enhanced stress resistance) for industrial application. For instance, trehalose, released as a response to stress also confers additional resistance to bacteria during harsh conditions such as freeze-drying (Lippert and Galinski, 1992). Furthermore, in a study evaluating metabolomic changes in *Streptomyces coelicolor* under salt stress revealed synthesis and accumulation of several solutes (Kol et al., 2010). The authors proposed that owing to high accumulation of ectoine (produced under salt stress) in the mutant strain of *S. coelicolor* could potentially make it suitable as a producer of ectoine in (large-scale) bacterial milking (Sauer and Galinski, 1998).

Several studies have been conducted which have used metabolomics with another omics platform to investigate the stress-induced responses in cellular metabolites (Ghobakhlou et al., 2013; Zhu et al., 2015). Limited studies have been conducted on metabolomic analysis alone of stress-induced responses of bacteria (Kol et al., 2010; Ye et al., 2012; Zhu et al., 2015), and there is no published study yet on metabolomics of stress responses in probiotic bacteria. Metabolomics are relatively new as compared to transcriptomic and proteomic platforms, and thus has untapped potential in stand-alone and integrated approaches, particularly in probiotic bacterial systems.

Interactomics

The cellular components form a complex network and several elements (DNA-protein and protein-protein) interact with each other. Several proteins, mostly from the same pathway, form complex aggregates. Interactomic analysis is targeted on such the role of such interactions and

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their contribution to the cellular metabolism alterations (Singh and Nagaraj, 2006). These interactomes are analyzed either by using computational approaches for determination of protein interactions or by proteomic based approaches to separate the proteins and identify using mass spectrometry based methods. The interaction between DNA and protein may regulate the gene expression and thereby altering the regulatory and metabolic pathways. The common approach to study this interactome is large-scale genetic interaction screening by phenotype analysis of gene deletion mutants. Interactomic based studies have revealed the presence of new reactions and novel pathways that involve not only functionally uncharacterized genes, but also well-characterized genes. Together, they have improved our understanding of gene function and network connections in bacteria. Interactomics based analysis of stress-induced responses has the potential to address some of the drawbacks of genomics, transcriptomics, and proteomics approaches mentioned above. The DNA-protein interactome data define the fundamental genetic regulatory network in the bacterial cell. Knowing the structure of this network is crucial to understand the modifications in the transcriptional state of the cell in response to environmental stressors (Joyce and Palsson, 2006). More in-depth research has been focused on understanding the protein-protein interactions networks for bacteria such as *E. coli* (Butland et al., 2005) and *H. pylori* (Rain et al., 2001). Establishing these comprehensive protein-protein interactome networks (Droit et al., 2005), then drive the analysis of interaction patterns paving ways for several biological interventions. For instance, additional efforts are now being made to develop high throughput small-molecule screens in order to identify the molecules interacting and interfering the protein-protein interactions (Roehrl et al., 2004).

Integrated transcriptomics (translatome) and proteomics

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Integrated transcriptomic and proteomic analysis have been the most common integrated approaches, probably due to the obvious central dogma of molecular genetics. However, not all of the studies have found a strong correlation between protein and mRNA levels (Gygi et al., 1999; Nie et al., 2007). These variations may be attributed to several post-translational and post-transcriptional changes. This combination of omics approaches (transcriptomics and proteomics) being the most common has also been applied in studies involving probiotic bacteria mainly to investigate the bile salt resistance mechanisms. One such study targeting the *Lactobacillus casei* BL23 revealed (Alcantara and Zuniga, 2012) revealed mechanistic insights to bile stress adaptation. The studies using combined transcriptomic and proteomic technologies aim at complementing the findings from each omics platform, or to unravel novel information that cannot be achieved by individual omics platform.

Integrated transcriptomics and metabolomics

Integrating transcriptomics and metabolomics approaches provides a link between the information carrying components and the functional components in a microorganism. Several studies have integrated transcriptomics and metabolomics approaches to better understand the microbial system as affected by environmental stresses. In one such study (Jozefczuk et al., 2010), metabolite composition analysis was incorporated with gene expression study in *Escherichia coli* subjected to four different stressors (cold, heat, oxidative stress and lactose diauxie). The alterations in metabolites showed higher specificity in early adaptation phase as compared to stationary phase, and were found to be more specific as compared to the transcriptional alterations. Nevertheless, the alterations in both elements (transcripts and

metabolites) followed similar dynamics in the central energy related pathways. Comprehensive statistical analysis including co-clustering and canonical correlation analysis of data from metabolomic and transcriptomic technologies revealed several significant condition-dependent associations between metabolites and transcripts. Their findings restated the power of integrated omics based analysis in microbial system.

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Integrated proteomics and metabolomics

Proteomic and metabolomics technologies target the fundamental structural and functional cellular components, thereby making it critical to understand the unqiue and dynamic cellular responses under normal and stressed conditions (Hecker et al., 2010; Kriegeskorte et al., 2011). Proteomic and metabolomic have been less commonly applied as compared to transcriptomics and proteomics, which could be attributed to the recent advances in metabolomics. To the best of our knowledge, there is no published literature on the application of these two omic approaches in evaluating stress responses in probiotic bacteria. However, the very recent studies by Liebeke et al. (2011); Alreshidi et al. (2015) in *Staphylococcus aureus* provide a good example of the integrated application of proteomics and metabolomic in stress related studies. As reported by Liebeke et al. (2011), individual genomic, proteomic, and transcriptomic data analysis of *S. sureus* had enabled them to reconstruct metabolic networks, however, the detailed information on metabolites was still lacking. Thus, they conducted a parallel metabolomic and proteomic analysis on stress responses to evaluate the correlation between proteomic changes with the metabolic activity. This would then enable an integrated analysis of the bacterial physiology, with detailed information on which pathway metabolites are most affected by environmental

stress. They investigated the integrated time resolved changes in the proteins and the enzymes of the bacteria during different growth phases. This was crucial because of the dynamic changes in the proteins from exponential to stationary phase, and increased metabolites in stationary phase of the bacterial growth, and could be accomplished conveniently by integrating metabolomic and proteomic study. Furthermore, through their study, Alreshidi et al. (2015) proposed that the activation of phenotypic shift as an adaptive measure of *S. aureus* (to cold stress) was triggered by the significant alterations in both, proteome and metabolome the bacteria.

INTEGRATING OMICS: CHALLENGES & FUTURE PROSPECTS

The increasing interest in the application of omics technologies for studying microbial systems in general have led to significant progress in this field. However, there are still several challenges that restrict its wider application, including but not limited to variability in experimental platform in terms of precision, accuracy, linearity of responses, and several other sources of error. These challenges make it difficult to compare the findings between experiments within the same omics platform, and more so between different omics platforms (Searls, 2005). Within one omics platform, different technologies may still reveal (totally or partialy) relating views of the required component. In this case, challenges are faced in comparing the data obtained from multiple experimental platforms as they have a weak or no correlation (Klipp et al., 2005). It has been suggested that these variations may be due to noise in the readings, attributed to long protocols, or due to much more complex non-linear bacterial system. These non-linear correlations demand more advanced methods of detection and large data sets to be able to confirm the findings between different platforms.

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After knowing the important stumbling blocks one has to be aware of when combining genome-wide datasets, some of the available tools for such analyses have been previously discussed (Zhang et al., 2010). It is important to note that any application of these tools has to build upon a sound understanding of how datasets have to be prepared for an integrated analysis, considering different biases and methods for their normalization as well as multiplicity. System-wide experiments require sound experimental designs such that data from disparate 'omics'-classes become usable in integrated analyses. Without such a design, the strict requirements for dataset combination frequently result in a very sparse intersection of testable hypotheses. In this respect, standardization remains the most important task for future system-wide approaches.

CONCLUSION

Integrating the information from gene expression to proteins to metabolites and metabolic fluxes, and interactions between all these components will lead to both, fundamental understanding and revolutionary advances. The lack of resources and financial constraints made it challenging to integrate omics and most studies just focused on one of the omics platform. However, there has been an increasing application of integrated omics technologies over the past few years. Although the application of high-throughput individual omics approaches have provided some insights to the complex microbial metabolism, it has also indicated the missing links, thereby highlighting the importance of integrating omics technologies. The findings from studies based on integrated omics approaches strongly indicate its potential in unraveling the stress-response mechanisms in probiotic bacteria as well. There has been a great impetus to such integrated omics studies; nevertheless, there is still scope for improvement in terms of accuracy,

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sensitivity, and specificity of these technologies. Overcoming the current challenges, and integrating the individual omics approaches along with interactomics analysis that will eventually expose more fundamental insights into cellular stress responses.

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Table 1: Summary of stress response studies in bacteria using individual omics platforms

Species	**Goals of the study**	**Omics platforms applied**	**Reference**
Bacillus subtilis	General stress response	Transcriptomics	Petersohn et al. (2001)
Bacillus subtilis	Cold stress induced responses	Proteomics	Budde et al. (2006)
Bifidobacterium animalis	Bile stress response	Proteomics	Sanchez et al. (2007b)*
Bifidobacterium longum	Low-pH and acid stress response	Proteomics	Sanchez et al. (2007a)*
Bifidobacterium longum	Heat shock response	Transcriptomics	Rezzonico et al. (2007)*
Bifidobacterium longum	Oxygen stress response	Proteomics	Xiao et al. (2011)*
Desulfovibrio vulgaris	NaCl induced stress response	Genetics	Mukhopadhyay et al. (2006)

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Escherichia coli	Heat stress	Metabolomics	(Ye et al., 2012)
Lactobacillus casei	Bile salt stress response	Proteomics	Wu et al. (2010)*
Lactobacillus casei	Low acid stress	Proteomics	Wu et al. (2011)
Lactobacillus plantarun	Bile stress response	Proteomics	Hamon et al. (2011)*
Lactobacillus reuteri	Transient acid stress response	Proteomics	Lee and Pi (2010)*
Lactobacillus reuteri	Bile stress response	Proteomics	Whitehead et al. (2008)*
Lactobacillus sanfranciscensis	High pressure stress response	Proteomics	Hormann et al. (2006)*
Lactobacillus rhamnosus GG	Acid stress response	Transcriptomics	Koponen et al. (2012)*
Propionibacterium freudenreichii	Bile salt stress response	Proteomics	Leverrier et al. (2003)
Propionibacterium	Acid and bile salt	Proteomics	Leverrier et al. (2004)

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freudenreichii	stress response		
Streptomyces coelicolor	Salt stress	Metabolomics	(Kol et al., 2010)
Synechocystis sp. PCC 6803	Ethanol tolerance	Metabolomics	(Zhu et al., 2015)

*Studies on probiotic bacteria

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Table 2: Summary of integrative omics studies for evaluating stress responses in bacteria

Species	Goals of the study	Omics platforms applied	Reference
Bacillus subtilis	Oxidative stress response	Proteomics and transcriptomics	Mostertz et al. (2004)
Desulfovibrio vulgaris	Low-oxygen induced stress response	Proteomics and transcriptomics	Mukhopadhyay et al. (2007)
Escherichia coli	Cold, heat oxidative and lactose stress responses	Metabolomics and transcriptomics	Jozefczuk et al. (2010)
Lactobacillus casei	Bile salt stress response	Proteomics and transcriptomics	Alcantara and Zuniga (2012)*
Lactobacillus reuteri	Bile salt stress response	Proteomics and transcriptomics	Lee et al. (2008)*
Lactobacillus rhamnosus GG	Bile salt stress response	Proteomics and transcriptomics	Koskenniemi et al. (2011)*
Pseudomonas pseudoalcaligenes	Response to metal exposure	Metabolomics and transcriptomics	Tremaroli et al. (2009)

Rhodobacter sphaeroides	Oxygen exposure stress response	Proteomics and transcriptomics	Berghoff et al. (2013)
Shewanella oneidensis	Actue chromatic stress response	Proteomics and transcriptomics	Brown et al. (2006)
Staphylococcus aureus	Glucose starvation response	Metabolomics and proteomics	Liebeke et al. (2011)
Staphylococcus aureus	Prolonged cold stress response	Metabolomics and proteomics	Alreshidi et al. (2015)
Synechocystis sp. PCC 6803	Heat shock response	Proteomics and transcriptomics	Suzuki et al. (2006)
Thermobifida fusca	Osmotic exposure response	Proteomics and transcriptomics	(Chen and Wilson, 2007)

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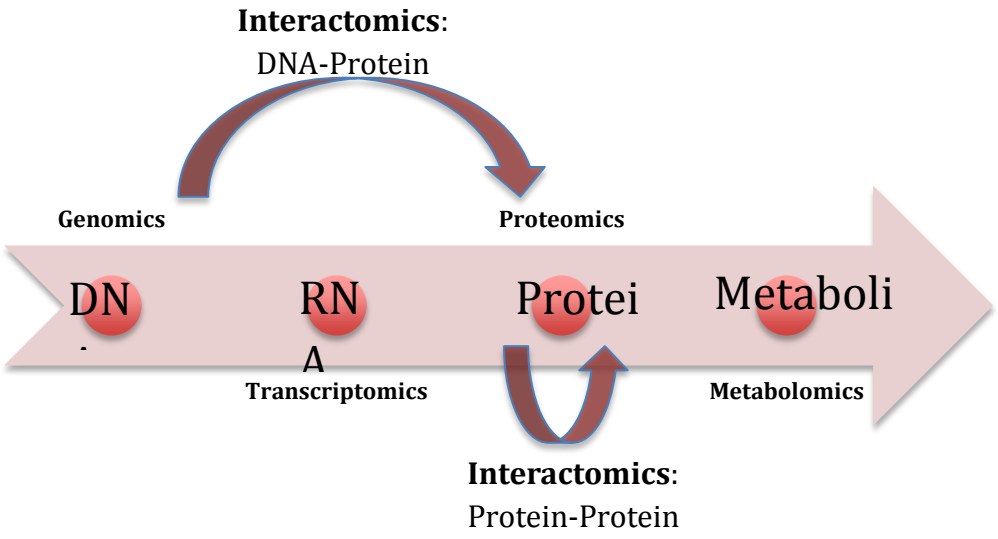

Figure 1: Towards integrated omics: diagrammatic representation of various omics technologies available for bacterial system

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