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Role of Proteomics in Unraveling Bacterial Virulence in Rice

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Abstract Rice stands as a pivotal global staple, underpinning the sustenance of billions. However, its production is critically hampered by bacterial diseases, which pose substantial threats to food security. This systematic review delves into the vital role of proteomics in understanding bacterial virulence and its interactions with rice, highlighting the indispensable need for advanced proteomic techniques to address these challenges. Employing methods such as mass spectrometry and two-dimensional electrophoresis, this review consolidates key findings including the identification of specific virulence factors and detailed insights into host-pathogen dynamics. These proteomic methodologies have illuminated the pathogenic processes affecting rice, paving the way for targeted interventions. The implications of these findings are profound, offering potential strategies for the development of disease-resistant rice varieties and thus enhancing agricultural productivity. However, the field faces ongoing challenges such as the complexity of proteomic data and the need for enhanced sensitivity and specificity in detecting virulence factors. Future directions include refining proteomic techniques and integrating multi-omics approaches to foster a holistic understanding of bacterial pathogenesis in rice. This review underscores the transformative potential of proteomics in revolutionizing rice pathology for improved crop resilience and yield.

Keywords Proteomics; Bacterial virulence; Rice diseases; Host-pathogen interactions; Disease resistance

Rice is one of the most important staple crops worldwide, serving as a primary food source for more than half of the global population. Its cultivation and production are critical for food security, particularly in Asia, Africa, and Latin America (Wu et al., 2008). However, the productivity and quality of rice are severely threatened by various bacterial diseases, which can lead to significant yield losses and compromise the livelihoods of millions of farmers. Understanding and mitigating the impact of these pathogens is therefore a key challenge in agricultural science and food security efforts.

Proteomics, the large-scale study of proteins, has emerged as a powerful tool in the biological sciences. It allows for the comprehensive analysis of the proteome, which is the entire set of proteins expressed by an organism, tissue, or cell at a certain time. In the context of plant pathology, proteomics can provide insights into the complex interactions between rice plants and bacterial pathogens (Tjalsma et al., 2004). By identifying and characterizing the proteins involved in virulence, host defense, and the response to infection, researchers can uncover the molecular mechanisms that underlie disease development and progression (Hecker and Engelmann, 2000).

The data presented in this review are drawn from a range of studies that have employed various proteomic techniques to investigate bacterial pathogens. These include the discovery of virulence factors through comparative genomics, transcriptomics, and proteomics, the identification of protein biomarkers for virulent bacterial isolates, and the use of targeted proteomics for studying bacteria-host interactions (Saleh et al., 2019). The application of proteomics in clinical microbiology and the study of antimicrobial resistance further underscores the versatility of this approach in addressing complex biological questions. Finally, the review will consider how proteomics has contributed to our understanding of microbial pathogenesis and the identification of new drug targets and vaccine candidates.

This review aims to explore how proteomics can reveal the virulence mechanisms of bacterial pathogens affecting rice crops, integrate current research, demonstrate how to use proteomics technology to study rice bacterial

diseases, identify key virulence factors, and explore the relationship between pathogens and Molecular interactions between host plants. In this way we hope to highlight the important potential of proteomics in deepening our understanding of bacterial virulence and explore its applications in developing more effective disease management strategies and crop protection methods. Through a comprehensive literature analysis, this article will provide an overview of the important contributions of proteomics in the field of rice pathology and discuss its potential impact on future research directions and agricultural practices.

1 Overview of Bacterial Pathogens in Rice

1.1 Common pathogens

Rice is a staple food crop that is critical to the sustenance of a large portion of the world's population. Its production is severely hampered by various bacterial pathogens, among which *Xanthomonas oryzae* pv. oryzae and *Burkholderia glumae* are particularly notorious. *Xanthomonas oryzae* pv. oryzae is the causative agent of bacterial blight, a disease that leads to significant yield losses in rice-growing regions globally (Agrawal and Rakwal, 2006). *Burkholderia glumae* causes bacterial panicle blight, which is becoming increasingly prevalent and impactful, especially in the United States and Asian countries where high-temperature stress coincides with the flowering stage of rice. The prevalence of these pathogens underscores the need for a deeper understanding of their biology and interaction with the rice host to develop effective control strategies.

1.2 Symptoms and disease mechanisms

The symptoms of bacterial blight caused by *Xanthomonas oryzae* pv. oryzae include water-soaked lesions on the leaves, which eventually turn yellow and then brown, leading to a burnt appearance. *Burkholderia glumae* infected plants exhibit symptoms such as darkening and sterility of the rice panicles. The general mechanisms of pathogenesis involve the secretion of virulence factors that enable the bacteria to colonize the plant, evade the host immune response, and extract nutrients necessary for their proliferation. Proteomic studies have been instrumental in identifying these virulence factors and elucidating the complex interactions between the pathogens and the rice host (Wu et al., 2008; Cash, 2011; Ahmed et al., 2019).

1.3 Economic impact

The economic impact of bacterial diseases in rice is profound, with yield losses due to bacterial blight and bacterial panicle blight causing significant financial strain on farmers and the agricultural industry. These diseases not only reduce the quantity of the harvest but also affect the quality of the rice grains, thereby diminishing their market value. On a global scale, the economic repercussions extend to trade restrictions in regions where these pathogens are endemic, affecting the livelihoods of millions of rice farmers and contributing to food insecurity (Agrawal and Rakwal, 2006). The development of resistant rice varieties and effective disease management strategies is crucial to mitigate these economic losses. Proteomics research is at the forefront of these efforts, providing insights into the molecular mechanisms of pathogenesis and resistance, which are vital for the design of novel control measures (Pérez-Llarena and Bou, 2016; Khodadadi et al., 2020; Zubair et al., 2022).

2 Proteomic Techniques and Methodologies

2.1 Key techniques

Proteomic techniques have revolutionized our understanding of bacterial virulence, particularly in the context of rice pathogens. Mass spectrometry (MS) is a cornerstone of proteomic analysis, providing detailed information about the molecular weight and structure of proteins. MS-based proteomics has been extensively used to map bacterial proteomes, leading to a better understanding of the molecular mechanisms underlying bacterial infection and bacteria-host interactions, providing quantitative measurements for proteins extracted from microorganisms (Khodadadi et al., 2020). This technique, along with two-dimensional electrophoresis (2-DE), has been pivotal in identifying virulence factors and understanding the complex mechanisms of pathogenesis. This method allows for the resolution of complex protein mixtures and the identification of protein spots through subsequent mass spectrometry or microsequencing. Protein microarrays are another key technique that has been employed to study post-translationally modified proteins of bacterial pathogens, offering insights into the functional mechanisms and interactions of these proteins (Wu et al., 2008).

2.2 Sample preparation and data analysis

The process of sample collection and protein extraction is critical for proteomic studies. Proteins are typically extracted from bacteria grown under laboratory conditions or directly from the site of infection to understand the in vivo state of the pathogen (Cash, 2011). Following extraction, proteins are subjected to techniques such as MS or 2-DE for separation and identification. Data analysis in proteomics is complex and involves bioinformatics tools to interpret the large datasets generated, identifying protein biomarkers that correlate with virulence (Tjalsma et al., 2004).

2.3 Advantages over other biological approaches

Proteomic approaches offer several advantages over genomic and other molecular biology techniques (Table 1). While genomics can predict potential virulence factors, proteomics can confirm their expression and modification in response to environmental cues or during infection. Proteomics provides a dynamic view of the pathogen's biology, revealing how proteins interact and change during the host-pathogen interaction, which is crucial for understanding bacterial virulence and developing targeted therapies (Pérez-Llarena and Bou, 2016). Additionally, proteomics can uncover the secretome and surface proteins, which are often key to pathogen-host interactions and potential vaccine targets (Dwivedi et al., 2016).

3 Proteomics in Identifying Virulence Factors

3.1 Identification of pathogenic proteins

Proteomic techniques have been instrumental in identifying proteins that play a role in the virulence of bacterial pathogens. These techniques, including comparative genomics, transcriptomics, and proteomics, have been applied to a range of bacteria such as *Neisseria meningitidis*, *Yersinia pestis*, *Mycobacterium tuberculosis*, and *Staphylococcus aureus* (Figure 1) (Wu et al., 2008). Proteomics, in particular, has been successful in studying post-translationally modified proteins of bacterial pathogens, which are often critical to their virulence. The identification of these proteins provides a foundation for further investigation into their functions and mechanisms, which can be explored through phenotypic analyses such as mutagenesis and biochemical methods, as well as structural biology.

The figure from Pivard et al. 2023, offers a detailed analysis of the proteomic variations in Staphylococcus aureus linked to genetic differences among strains. The use of principal component analysis (PCA) clearly differentiates the strains into distinct clusters based on two genetic markers: agr specificity groups and clonal complexes. Panel A showcases the distribution of various agr types, while Panel C does the same for clonal complexes, each represented by different colors, helping visualize the genetic diversity and its correlation with virulence profiles. The eigenvalue plot in Panel B quantitatively supports the PCA by showing the percentage of variance each principal component accounts for, with a significant drop after the first two dimensions. This study effectively highlights the complex relationship between genetic background and virulence in *S. aureus*, potentially guiding targeted therapeutic approaches.

3.2 Function of virulence proteins

The functions of virulence proteins identified through proteomic analyses are diverse, but they often include toxins or enzymes that compromise plant health. The secretome, which encompasses proteins secreted by bacteria, plays a crucial role in bacterial survival and pathogenicity. For instance, the gram-positive bacterium Bacillus subtilis secretes approximately 90 extracellular proteins, many of which are involved in virulence. These proteins can include nonenzymatic toxins and enzymes that directly affect the host. Proteomics has revealed that the actual composition of the extracellular proteome can differ significantly from genome-based predictions, highlighting the importance of direct proteomic analysis in understanding bacterial virulence (Tjalsma et al., 2004; Bonar et al., 2015).

3.3 Comparative proteomics

Comparative proteomics involves analyzing the protein profiles of pathogenic versus non-pathogenic strains or the changes in protein expression before and after infection onset. This approach has been used to identify protein biomarkers for virulent bacterial isolates and to correlate these biomarkers with the outcome of bacterial infections

(Bhavsar et al., 2010). By comparing the proteomes of bacteria grown under laboratory conditions to those during in vivo infection, researchers can gain significant insights into bacterial pathogenesis. Proteomic technologies have been used to identify and characterize bacterial genes expressed specifically in vivo, which is crucial for understanding the molecular boundaries of microbial pathogenesis and for the development of targeted therapies and vaccines (Cash, 2011).

In the context of rice bacterial pathogens, proteomics has been applied to clarify the interaction between rice and microbes, including bacteria. This research is essential for plant pathology and has led to discussions on the proteomic analysis of interactions between rice and various microbes, such as fungi, bacteria, and viruses. The identification of specific proteomic signatures in these interactions can help in understanding the complex dynamics between rice and bacterial pathogens.

Table 1 Different quantitative proteomic approaches with the associated advantages and disadvantages (Source: Pérez-Llarena and Bou, 2016)

Proteomic tools	Advantage	Disadvantage
GEL-BASED METHODS		
2DE	(1) Simple (2) Robust (3) Suitabie for MS analysis	(1) Involves large amount of sample (2) Low throughput (3) Poor recovery ol hydrophobic proteins (4) High inter-gel vaiabilitl
2-DIGE	(1) Multiplexing (2) Better quantitation (3) Minimal gel to gell variation	(1) Expensive Cy dyes (2) Poor recovery of hydrophobic proteins (3) Difiulty in separating low molecular weigh compounds
GEL-FREE METHODS		
SILAC	(1) High throughput (2) Robust (3) Sensitive and simple	(1) Only suitable for tissue culture models (2) Costly reagents (3) Not ppliable to tissue sample
ICAT	(1) Selectvely isolates peptide (2) Compatible with any amount o protein (3) Complexity of the peptide mixture is reduced	(1) Cannot identify proteins with less than eight cysteines (2) Large ICAT label (>500 Da) (3) Post-translational modificationo information is frequently lost
iTRAQ	(1) Applicable to versatle samples (2) Multiplexing (3) Better quantitation	(1) Involves high amount o sample (2) Incomplete labeling (3) Expensive reagents
ICPL	(1) High-throughput quantitative proteome profing on a global scale (2) Able to detect post-translational modifications and protein isoforms (3) Applicable to protein samples such as tissue extracts or body fluids	(1) lsotopic effect of deuterated tags interferes with retention time of the peptides labeled during LC
Label-free	(1) Involves less amount of sample (2) Higher proteome coverage (3) Awoids labeling	(1) High throughput instrumentation (2) Not suitable for low abundant proteins (3) Incomplete digestion may introduce eror (4) Multiplexed analysis not possible in one experiment
SRM	(1) Highly sensitive, quantilatively accurate and highly reproducible (2) Protein detection is relativly rapid and straightforward (3) Enables detection of non abundant (> 10 ng/m) proteins (4) Quantification of post -ranslationai modfications	(1) Limiled broad scale appcalion because of dfiully in generating high-quality SRM assay (2) Sensitivty is not comparable to immunological assays (3) Detection and quantification of non abundant proteins (i., ~10 ng/ml or less)

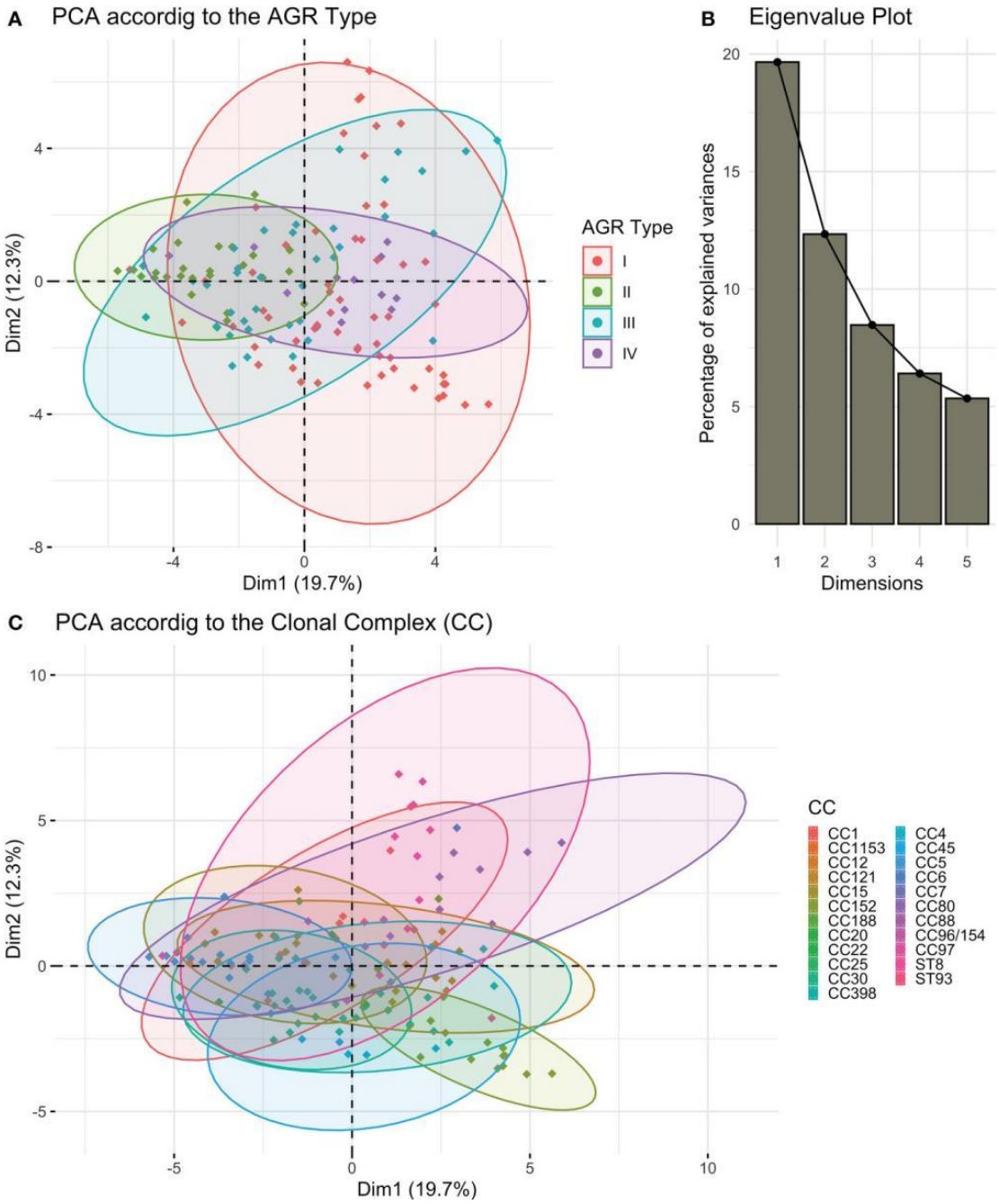

Figure 1 *S. aureus* proteome according to the strains' genetic backgrounds (Photo credit: Pivard et al., 2023)

Image caption: (A) Variations in the virulomes according to the AGR type are visualized in the PCA analysis on the first and second dimensions. (B) The eigenvalue plot for the first five dimensions of the PCA is represented using a histogram. (C) Variations in the virulomes according to the clonal complex (CC) are visualized in the PCA analysis on the first and second dimensions. The colored ellipses represent different AGR types and CCs, explaining the variations in virulence gene expression under different genetic backgrounds (Adapted from Pivard et al., 2023)

4 Host-Pathogen Interaction Studies

4.1 Host response profiling

Proteomics has significantly contributed to our understanding of host response during bacterial infections. In the context of rice, proteomic analysis has been instrumental in profiling the changes in protein expression upon infection by various pathogens, including bacteria. Studies have shown that upon infection, rice plants undergo a complex reprogramming of their proteome, which includes the upregulation and downregulation of numerous proteins involved in defense and stress responses. These proteomic changes are crucial for the plant's survival and adaptation to the pathogenic attack. The use of advanced proteomic techniques, such as mass spectrometry, has

allowed for the identification and quantification of these protein changes (Figure 2), providing a comprehensive view of the host response to bacterial infections.

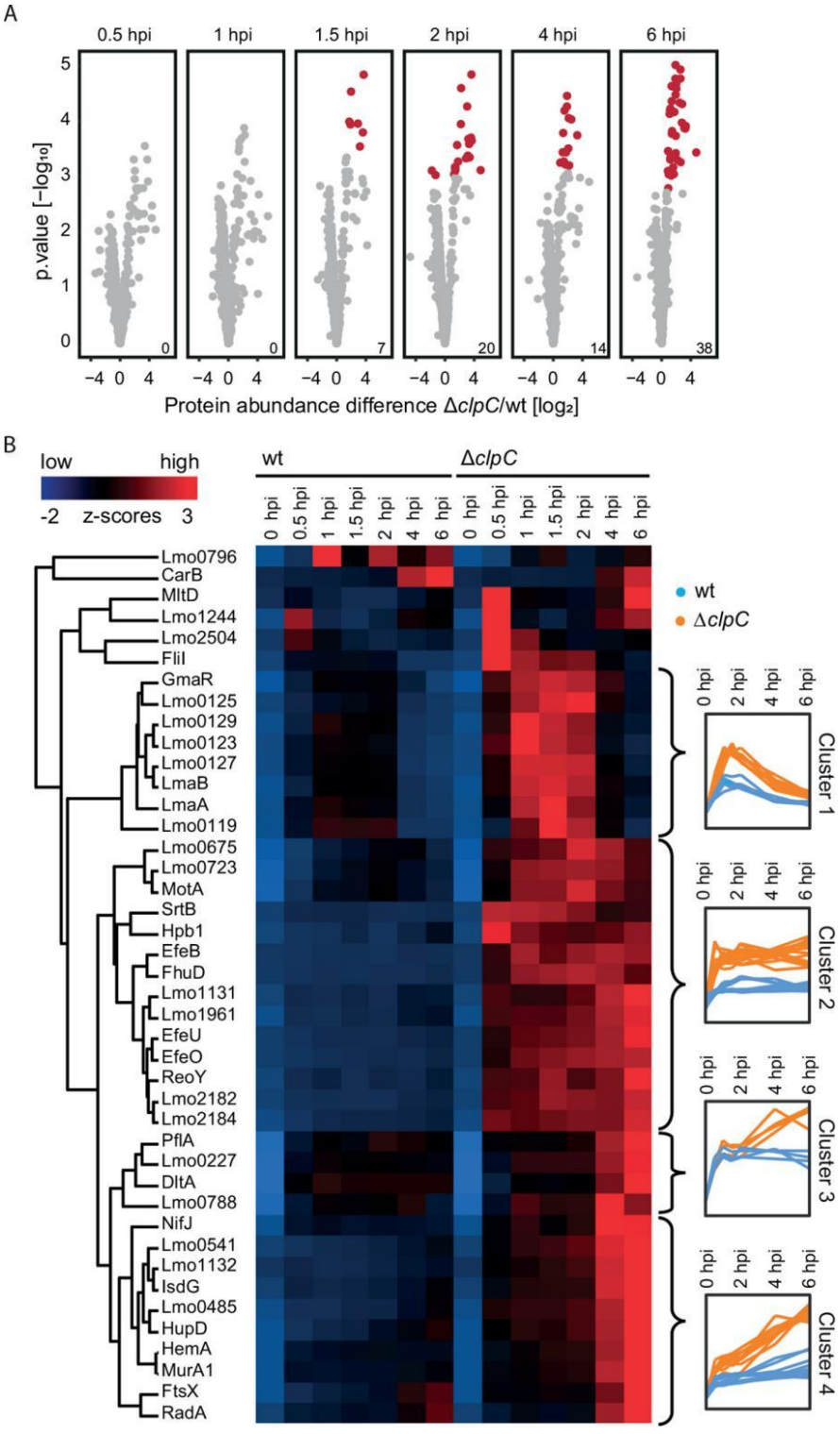

Figure 2 Abundance ratios of new proteins between the mutant and the wt strain and the statistical significance level (Photo credit: Birk et al., 2021)

Image caption: Panel (A) presents volcano plots indicating the protein abundance differences between the two strains from 0.5 to 6 hours post-infection, with red dots representing proteins that are significantly different (Padj≤0.05). The numbers in the bottom right corner denote the number of significantly different proteins at each time point. Panel (B) shows a heatmap illustrating the hierarchical clustering based on Euclidean distance of Z-scored abundances of de novo synthesized proteins upon infection. The profile plots on the right display the Z-scored protein levels for the corresponding clusters (Adapted from Birk et al., 2021)

Figure 2 from Birk et al. 2021, provides a detailed comparative proteomic analysis between a mutant strain (Δ clpC) and wild-type (wt) of a bacterium across several time points post-infection. Panel A uses volcano plots to effectively illustrate the differences in protein abundance between the strains, highlighting significantly different proteins with red dots. This visualization allows for easy identification of proteins with notable abundance changes, as well as the statistical significance of these changes at different time points. Panel B features a heatmap coupled with hierarchical clustering, showcasing the patterns of protein expression over time. The heatmap utilizes Z-scores to standardize protein abundance levels, making patterns easier to recognize and compare. The accompanying line plots provide a focused look at the temporal expression trends within identified clusters, revealing how protein expression dynamics differ between the mutant and wild-type strains.

4.2 Molecular interaction mapping

Proteomic studies have significantly advanced our understanding of the molecular interactions between bacterial and plant proteins, particularly in the context of bacterial virulence in rice. Proteomic studies have been employed to map these molecular interactions, revealing how bacterial proteins interact with host proteins to promote virulence or trigger defense responses. Proteomic approaches also involve the use of two-dimensional electrophoresis (2D-PAGE) combined with mass spectrometry to identify and quantify proteins. This method has been used to create comprehensive protein indices for various bacteria, such as Bacillus subtilis, which can be applied to study the regulation, structure, and function of bacterial regulons, including those encoding virulence factors. Techniques such as targeted proteomics and mass spectrometry-based approaches have been used to identify and characterize these interactions. These studies have provided insights into the molecular cross-talk between rice and bacterial pathogens, highlighting potential targets for disease control and contributing to our understanding of the complex dynamics of host-pathogen interactions (Saleh et al., 2019).

4.3 Insights into host defense mechanisms

Proteomic data have shed light on the defense mechanisms employed by rice plants against bacterial attacks. The primary immune response in rice, known as pathogen-associated molecular pattern (PAMP)-triggered immunity, is designed to recognize common features of microbial pathogens and initiate defense responses. The identification of proteins involved in the plant's immune response, such as pathogenesis-related proteins, has been facilitated by proteomic analyses. These proteins are part of the plant's innate immune system and play a critical role in recognizing and responding to pathogenic bacteria (Birk et al., 2021). Proteomics has revealed the role of post-translational modifications in the regulation of defense proteins, further enhancing our understanding of the host defense system.

5 Future Perspectives and Challenges

5.1 Emerging technologies

The field of proteomics is rapidly evolving, with new technologies and methodologies on the horizon that promise to significantly enhance our understanding of bacterial virulence, particularly in rice pathogens. Recent advancements in mass spectrometry-based proteomics, such as quantitative proteomics using selected or parallel reaction monitoring, have already improved the sensitivity and specificity of proteome studies of pathogenic bacteria (Saleh et al., 2019). These targeted proteomics approaches are instrumental in identifying biomarkers, characterizing bacterial virulence, and understanding antimicrobial resistance. Furthermore, the integration of proteomics with other 'omics' technologies, such as genomics and transcriptomics, is paving the way for a more comprehensive understanding of bacterial pathogens. The application of these emerging technologies is expected to lead to the discovery of novel virulence factors and the elucidation of complex host-pathogen interactions, which are crucial for developing new therapeutic strategies and vaccines.

5.2 Challenges in proteomic research

Despite the progress made in proteomic research, several challenges remain. One of the primary limitations is the detection sensitivity, which is critical for identifying low-abundance proteins that may play key roles in bacterial virulence (Khodadadi et al., 2020). Additionally, quantitative accuracy in proteomics is essential for comparing protein expression levels under different conditions and for validating potential virulence factors. Another

significant challenge is the complexity of data interpretation, as the vast amount of data generated by proteomic studies requires sophisticated bioinformatics tools for meaningful analysis. The complexity of bacterial proteomes, including post-translational modifications and the dynamic nature of protein expression, further complicates the interpretation of proteomic data (Bonar et al., 2015) Addressing these challenges will require continued technological innovation and the development of more advanced analytical tools.

5.3 Applications and implications

The implications of proteomic research in understanding bacterial virulence are vast. By identifying and characterizing virulence factors, proteomics can contribute to the development of disease-resistant rice varieties. For instance, the identification of secreted proteins involved in bacterial pathogenesis can inform breeding programs to select rice strains with enhancedresistance to these factors. Additionally, proteomic studies can aid in the development of targeted treatment strategies, such as antimicrobial therapies that specifically inhibit virulence factors without affecting the host or beneficial microbes. The knowledge gained from proteomic analyses of bacterial pathogens can also lead to the development of rapid diagnostic tools, which are crucial for the timely management of rice bacterial diseases (Pivard et al., 2023). Ultimately, the integration of proteomic data with other biological insights will be instrumental in devising comprehensive strategies to combat bacterial infections in rice, thereby ensuring food security and agricultural sustainability.

6 Concluding Remarks

This review systematically highlights the important role of proteomics in understanding bacterial virulence, especially rice pathogens. Complementing genomic data, proteomics provides insights into an organism's proteins and biological processes. It enables the identification of mechanisms behind bacterial virulence, antimicrobial resistance and host-pathogen interactions. Technologies such as MALDI-TOF and targeted proteomics are key to identifying virulence factors and biomarkers of pathogenic bacteria. Research has also expanded into the secretome and surfaceome to explore potential vaccine candidates and understand immune responses to bacterial infections.

Proteomics has proven valuable in studying interactions between rice and various microorganisms, elucidating the complex dynamics of plant-pathogen relationships. This study also highlights the importance of conducting in vivo studies to more accurately characterize bacterial pathogenesis. However, it also points the way for future research. More comprehensive in vivo proteomic analyzes are urgently needed to better understand what happens during host infection. Advances in more sensitive and specific quantitative proteomics techniques could improve the identification and understanding of virulence factors. Combining proteomics with other omics approaches, such as genomics and transcriptomics, can provide a more complete view of bacterial virulence mechanisms. Studying the rice microbiome and its interactions with pathogens through proteomic studies may lead to new disease management strategies.

Integrating proteomics into rice bacterial disease research is expected to have a significant impact. It could lead to the discovery of new virulence factors, aid in the development of new antimicrobial drugs, and aid in the design of effective vaccines. Harnessing proteomics in this area will transform our understanding of bacterial diseases of rice, contribute to global food security, and support people who rely on rice as their staple food. Continued advancements in proteomic technologies and their application in the study of bacterial pathogens will undoubtedly provide important insights into disease mechanisms and resistance, thereby fostering innovative solutions against infections in rice and other crops.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Agrawal G., and Rakwal R., 2006, Rice proteomics: a cornerstone for cereal food crop proteomes, Mass spectrometry reviews, 25(1): 1-53.
https://doi.org/10.1002/mas.20056

Ahmed F., Kumar G., Soliman F., Adly M., Soliman H., El-Matbouli M., and Saleh M., 2019, Proteomics for understanding pathogenesis, immune modulation and host pathogen interactions in aquaculture, Comparative biochemistry and physiology, Part D, Genomics and proteomics, 32: 100625.
https://doi.org/10.1016/j.cbd.2019.100625

Birk M., Ahmed-Begrich R., Tran S., Elsholz A., Frese C., and Charpentier E., 2021, Time-resolved proteome analysis of listeria monocytogenes during infection reveals the role of the aaa+ chaperone clpc for host cell adaptation, mSystems, 6.
https://doi.org/10.1128/mSystems.00215-21

Bonar E., Wojcik I., and Władyka B., 2015, Proteomics in studies of Staphylococcus aureus virulence.. Acta biochimica Polonica, 62(3): 367-81.
https://doi.org/10.18388/abp.2015_1083

Cash P., 2011, Investigating pathogen biology at the level of the proteome, PROTEOMICS, 11.
https://doi.org/10.1002/pmic.201100029

Dwivedi P., Alam S., and Tomar R., 2016, Secretome, surfome and immunome: emerging approaches for the discovery of new vaccine candidates against bacterial infections, World Journal of Microbiology and Biotechnology, 32: 1-9.
https://doi.org/10.1007/s11274-016-2107-3

Hecker M., and Engelmann S., 2000, Proteomics, DNA arrays and the analysis of still unknown regulons and unknown proteins of Bacillus subtilis and pathogenic gram-positive bacteria, International journal of medical microbiology : IJMM, 290(2): 123-34.
https://doi.org/10.1016/S1438-4221(00)80080-6

Khodadadi E., Zeinalzadeh E., Taghizadeh S., Mehramouz B., Kamounah F., Khodadadi E., Ganbarov K., Yousefi B., Bastami M., and Kafil H., 2020, Proteomic applications in antimicrobial resistance and clinical microbiology studies, Infection and Drug Resistance, 13: 1785-1806.
https://doi.org/10.2147/IDR.S238446

Pérez-Llarena F., and Bou G., 2016, Proteomics as a tool for studying bacterial virulence and antimicrobial resistance, Frontiers in Microbiology, 7.
https://doi.org/10.3389/fmicb.2016.00410

Saleh S., Staes A., Deborggraeve S., and Gevaert K., 2019, Targeted proteomics for studying pathogenic bacteria, PROTEOMICS, 19.
https://doi.org/10.1002/pmic.201800435

Tjalsma H., Antelmann H., Jongbloed J., Braun P., Darmon E., Dorenbos R., Dubois J., Westers H., Zanen G., Quax W., Kuipers O., Bron S., Hecker M., and Dijl J., 2004, Proteomics of protein secretion by bacillus subtilis: separating the “secrets” of the secretome, Microbiology and Molecular Biology Reviews, 68: 207-233.
https://doi.org/10.1128/MMBR.68.2.207-233.2004

Wu H., Wang A., and Jennings M., 2008, Discovery of virulence factors of pathogenic bacteria., Current opinion in chemical biology, 12(1): 93-101.
https://doi.org/10.1016/j.cbpa.2008.01.023

Zubair M., Wang J., Yu Y., Faisal M., Qi M., Shah A., Feng Z., Shao G., Wang Y., and Xiong Q., 2022, Proteomics approaches: A review regarding an importance of proteome analyses in understanding the pathogens and diseases, Frontiers in Veterinary Science, 9.
https://doi.org/10.3389/fvets.2022.1079359