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Review Article

Proteomic Application to Study Crustacean Innate Immune Response against Pathogenes -

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ABSTRACT

Over the last decade's global aquaculture farming has shown a growth rate of a fantastic production of aquatic animal food for human consumption. Environment parameters have shown a significant impact on crustacean farm yield, which directly affects countries' economies. To obtain the world's health requirements for aquaculture products, a relentless growth in production is expected for future decades. Though a very competitive market, aquaculture is a global awareness regarding the use of scientific knowledge and emerging technologies to gain a better-farmed organism through sustainable production has improved the importance of proteomics in aquaculture biology research.

Proteomics, as a powerful comparative tool, has therefore been increasingly used over the last decade to address different questions in aquaculture regarding welfare, nutrition, health, quality, and safety. This paper will give an overview of these biological questions and the role of proteomics in their investigation, outlining the advantages, disadvantages, and future challenges. A brief description of the proteomics technical approaches will be presented. Particular focus will be on the latest trends related to the aquaculture production of fish with defined nutritional, health, or quality properties for functional foods and the integration of proteomics techniques in addressing this challenging issue.

INNATE IMMUNE SYSTEM

Innate immunity is the first line of defense against environmental changes and infectious pathogens. In multicellular organisms, innate immune system characterization revealed remarkable similarities from invertebrates to vertebrates that signify a common evolutionary ancestry [1].

Invertebrates and other arthropods share similar defense responses without the presence of immunoglobulins. Despite the absence of antigen-antibody specificity, innate system could possess good identification and respond swiftly to incapacitate and eradicate pathogens. Primarily, the exoskeleton that shield the body structure is a natural physical barrier towards any type pathogenic microorganisms [1]. The existence of cuticle serves as a lining for foregut, hindgut of a prawn and its body surface. Moreover, gills could allow any exchange due to lack of epicuticle whereas a gut present without lipid outer layer could allow the permeability [1].

Crustaceans are enclosed with an open circulatory system that transports oxygen, hormones, nutrients and cells via the hemolymph [2]. Association of humoral and cellular defense mechanisms is observed during the hemocyte flow and help to integrate with plasma cells. Humoral defense system involves in the production and release of lectins, Antimicrobial Peptides (AMP) and Prophenoloxidases (proPO) while cellular defense system includes hemocyte mediated responses for encapsulation and phagocytosis. The circulating fluids which are known as crustacean hemolymph or blood comprises of hemocytes that include fluids or plasma.

PROTEOMICS ANALYSIS

The word "proteome" was firstly proposed by Marc Wilkins as the term resulted from protein and genome word combination. It was relevant to the total proteins expressed by a genome [3]. Proteomics is a new field of research of the total proteins derived from the genome. It is oriented towards analysis of each protein's presence and its relative abundance in the cells, organisms, or tissues by measuring the expression level [3]. Consequently, it was more particularly able to describe that which proteins would be expressed at a certain time and under certain cell circumstances from the entire genome [4].

By contrast, the inherited information would be described through genome analysis (static state). It's totally different from proteome analysis that elucidates according to cells and tissues in different situations at defined times and under specific conditions (dynamic forms). This new technology's critical perspectives offer biomedical science opportunities to investigate the cells or tissues' entire protein content at different physiological states, e.g., during regulation, differentiation, or tumor development. The wide range of proteomics facilities paved the way to studying post-translational modifications, subcellular localization, protein-protein interaction, and protein function [5]. It has also been used to better understand the molecular mechanisms of disease for diagnostic purposes. Being able to do the analysis and identification of proteins produced during a particular state of disease in order to find diagnostic biomarkers is a wonderful facility in molecular science that was made possible by proteomics [6].

TWO-DIMENSIONAL GEL ELECTROPHORESIS (2-DE)

The essential protein study techniques are Two-Dimensional Gel Electrophoresis (2-DE), which is used to separate and visualize proteins; this technique is accomplished by Mass Spectrometry (MS) for protein identification. These powerful techniques have been continuously developed to deal with the challenges in the proteomics research field. The typical flow chart of proteomic analysis is illustrated in figure 1.

The proteomic analysis relies on the separation and visualization of the complex protein extracted mixtures from tissues, cells, or other biological samples in proteomic studies. Two-Dimensional Gel Electrophoresis (2-DE) technique, which was first introduced worldwide in the mid-1970s by two pioneers, O' Farrell [7,8] and Klose [9], contains two steps; the first step is the first dimension Isoelectric Focusing (IEF) that separates proteins according to their Isoelectric Points (pI) and is accomplished by second-dimension SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) that separates proteins according to their Molecular Weights (MW). Therefore, 2-DE can resolve protein Mixtures according to Two Independent Factors (MW and pI) by mixing two different steps.

To prepare the samples for 2-DE, the proteins need to be solubilized in a high concentration of urea buffer, a reducing agent (proportional to sample), and a proper no-interfering detergent. These chemicals help with sample denaturation, protein reduction, and prevention of protein aggregation or precipitation.

During the IEF stage, protein mixtures must be solubilized in a high concentrated urea solution called denaturing buffer [(8M Urea or 7M urea with 2M Thiourea)]. This buffer contains Chaotropic (CHAPS) as surfactants and (DTT) as reducing agents. A better result would obtain when this buffer does not contain non-ionic detergents.

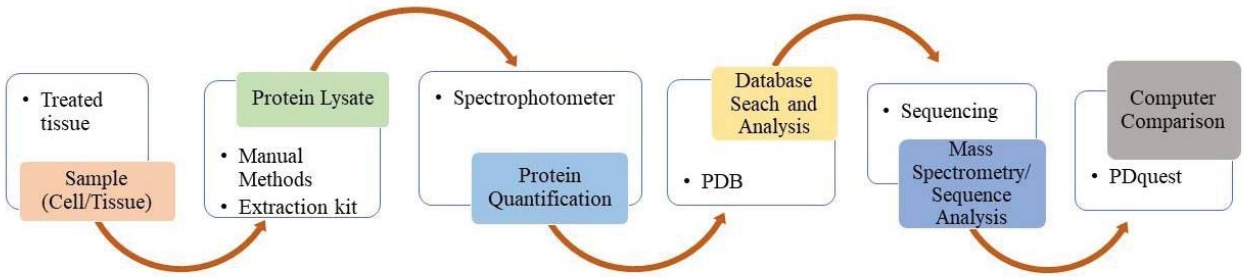

Figure 1: The flowchart of proteomic analysis is illustrated in the figure 1. The starting sample can be a treated cell or tissue. The protein lysate can be done with manual method or extraction kit. The protein quantification can be done by using spectrophotometer. Database analysis might be done through different data bases. Sequencing of the extracted proteins can be done by Mass Spectophotometery and computational analysis can be done by protein softwares such as PDquest [7]

Immobilien Drystrip with pH gradient is used to absorb and immobilize the solubilized sample on a gel strip. An electrical potential divergence is applied across two electrodes of the gel strip. Each protein will migrate under the applied electrical field until it arrives at the point equal to its pI. The protein will have no net charge at this stage and will stop migration and hence accumulate into a specific IEF band [10]. Since different types of samples have different ion content, adjusting the IEF buffer, electrical profile, and the length of the suitable dry strip gel is necessary, as well as optimizing isoelectric focusing pH gradients to each type of sample [11] to obtain high-quality data. Since any problems at this stage will influence all stages of the experiment, the IEF step is considered the most critical step of the 2-DE process.

After the first-dimension step and complete separation based on IEF, the IPG strip needs to be prepared to transfer the protein to SDS-PAGE. Providing the best transferring condition is crucial, and the IPG strip needs to be saturated with the SDS buffer using the equilibration step.

After the equilibration system, the strip is subjected to the second dimension SDS-PAGE system that separates proteins according to their Molecular Weight (MW). The SDS-PAGE gel must be prepared flat on top to have good contact between the IPG strip and the SDS-PAGE gel. The secondary separation resolution can be optimized by varying the acrylamide gel percentage according to the protein sample concentration.

The combination of first-dimension IEF and second dimension SDS-PAGE techniques in 2-DE allows efficient separation of protein mixtures into individual proteins. Nowadays, highly technical improvements are available to achieve a high quality of reproducible 2- DE gels to prepare experimental gels for profile comparison. By comparing the 2-DE gels, protein spots cannot be visualized by the eye. Therefore accurate spot excision is not possible.

In order to have an accurate comparison between experimental 2-DE gels, it is necessary to visualize proteins, so staining or labeling methods are applied (chemical or radioactive) to protein detection. Several methods for protein detection, quantification, and visualization on 2-DE gel have been developed; each method has its advantages and technical problems [12]. The two main conventional and classical staining methods are Silver and Coomassie Brilliant Blue. In Coomassie Brilliant Blue staining, CBB G-250 is used for protein detection in conventional SDS-PAGE. It is generally used in the experiments that about 30-50 ng protein sensitivity detection is required [13]. Silver staining is more sensitive and compatible than CBB G-250, making it a better choice for further mass spectrometry analysis [14]. Difficulty in spot matching and quantification is the main problem with these two conventional methods, so high sensitivity fluorescent staining has been developed as a choice of staining, but fluorescent staining also has some difficulties such as; it is more expensive than conventional CBB and silver staining and requires specific gel documentation instrumentation [14]. To reduce staining expenses, some Commercial dyes are available that include 5-hexadecanoylamino-fluorescein and SyproRuby™, deep Purple™ [15].

After staining the image, scanning showed protein spots in 2-DE gels that revealed different thousands of spots that show complex protein mixtures. This image also can determine protein spots concentration and expression level. The resulting "protein profile maps image" can be compared between experimental and control samples to identify differentially expressed proteins between two samples using image processing software. Some proteins would be chosen according to the project purpose.

The next step is to identify the excision cut protein spot from SDS-PAGE gel through in-gel digestion and mass spectrometry techniques. Recently, Mass Spectrometry (MS) has been introduced as a capable tool that facilitates sensitive protein identification and proteomic analysis [16]. In this method, proteolytically digested proteins are used to achieve higher protein identification analysis accuracy [17]. Proteins proteolytic digestion is often carried out in the gel plaque so, it is called 'in-gel digestion.' Proteolysis is achieved using available enzymes such as trypsin, proteinase K, and pepsin. Also, some chemical digestion reagents are available for protein digestion, such as Cyanogenbromide (CNBr).

The identification procedure begins with cutting out the protein spot from the 2- DE gel. The staining chemical would be removed by using a suitable de-staining method, shrivel up the gel pieces, and apply protease treatment [17]. Among the proteolytic enzyme, trypsin is the most common one in-gel digestion. Because of its unique characteristic, it can hydrolyze peptide bonds on arginine residues and the C-terminal side of lysine while other enzymes such as proteinase K, pepsin, and even CNBr do not have that much accuracy. Also, the use of CNBr causes high yields of large peptide fragments, which cause cleaves proteins at methionine residue; these

chemical phenomena make it useless for peptide sequencing by Mass spectrometry.

The obtained eluted peptides from the gel are subjected to MS analysis. The basic modules of mass spectrometers are composed of three important parts; Ion production, resolving the ion, ion detection. Ion would be produced from the samples through an ionization source. Determining ions is based on their mass-over-charge (m/z) ratio by the mass analyzer, and ion detection is based on the ion passing from the analyzer to the ion-sensitive detector.

Peptides and proteins ionization is a crucial step in MS analysis. Therefore, two different techniques have been developed. One is Electrospray Ionization (ESI) [18]. The other is Matrix-Assisted Laser Desorption/Ionization (MALDI) [19].

For MALDI, peptides or proteins are mixed with a matrix, deposited on a special plate, and irradiated with a pulsed laser. The mixture is heated and expands, then the matrix absorbs energy, and the result is the ionization of the analytes and transferring into the gas phase. For ESI, a high voltage current is applied between a slim duct tube that delivers the inlet and the mass spectrometer's analytic stream to construct the charged droplets. The charged droplets are finally released from the tip of the capillary. The formation of analytic ions in the gas phase occurs by rapid solvent evaporation and subsequent droplet fission.

Two techniques are developed for protein identification. One is Peptide Mass Fingerprinting (PMF). The peptide masses obtained from mass spectrometry or the tandem masses from intact proteins peptide fragmentation are then compared against theoretical tryptic peptide masses of each protein sequence in a protein database, and the most matched sequence would confirm the protein result. PMF studies by MALDI-TOF MS has been used widely in proteomics. The other is the Time of Flight (TOF) mass analyzer, which measures the m/z ratio of a peptide ion by the time it took to progress through a vacuum and field-free tube to a detector. It allows for inexpensive and rapid high-throughput identification. The m/z ratio of the peptide ion progresses through a vacuum tube to a detector measured by the Time of Flight (TOF) mass analyzer. However, there are several limitations [20] for this technique.

The limitation by comparing experimental PMF data with the databases is that there is insufficient protein sequence. Sometimes splice edited transcripts, variants, unaccounted isoforms, or other differences cause incorrect identification.

Another critical limitation for MALDI-TOF is that it cannot correctly identify a protein in a complex experimental sample that contains more than one protein. Whenever MALDI-TOF is failed to identify a protein, the Tandem MS/MS instrument can overcome the problem by selecting a precursor parent ion or a particular ion from the mixture of ions and fragment it within a collision module or fragmentation, and therefore identify the resulting ion fragments.

In addition, the peptide MS/MS spectrum contains information that can explain the peptide sequence (Patterson and RH, 2003). Also, using MS/MS data in database search gives higher accuracy and confidence in comparison with PMF data. Additionally, MS/MS data can search against coding sequence databases or Expressed Sequence Tag (EST) databases.

The extracted MS/MS or MS (PMF) data can be used as the input data for Computer software analysis and Database searching for matching input data to a protein sequence in databases; later on, the software matches the input data to a particular protein sequence and reports the most matched queries as output results. It also can determine the protein modification, which is not possible to identify from the nucleotide sequence.

Some software is well-known for analyzing MS/MS or MS (PMF) data such as MASCOT, PHENYX, SEQUEST, Profound, and Protein Prospector [21]. They have been developed using advanced MS technology. Currently, more and more internet websites are offering advice on these techniques. They also provide open access to various software that allows searching of the databases such as MASCOT and Protein Profound.

PROTEOMICS APPLICATION TO STUDY CRUSTACEAN IMMUNE RESPONSE

Knowledge of the interactions between the environment, pathogens, and the immune system is critical to understanding the disease's pathogenesis. These interactions may result in immune responses against the invader or cause changes in the gene expression levels of favor virus replication in the host genes. Studies on prawn's antiviral response show that prawns' responses to viral infections is not at the translational level but is highly observed at the transcriptional level.

Therefore, proteomic analysis has been done on prawn's differential organs such as lymphoid organs, stomach, and hemocytes to identify differentially expressed proteins in the immune response to viral and bacterial pathogens. Some studies have previously been done on the transcription level using molecular methods such as expressed RT-PCR; Sequence Tags (ESTs), microarray chips, differential hybridization, and suppression subtractive hybridization. These studies focused on the most critical immune organs (hemocytes and lymphoid organs). The results of these studies have provided good insights into biological defense mechanisms. The latest studies on prawn immune response have indicated that proteomic-based techniques are useful for studying immune response and prawn's immune-related proteins identification.

So far, Two-Dimensional Gel Electrophoresis (2-DE) was used in studies related to protein expression upon Vibrio harveyi infection in Penaeus monodon hemocytes [22], White Spot Syndrome Virus (WSSV) Litopenaeus vannamei stomachs [23], in hemocytes of Penaeus vannamei during Taura Syndrome Virus (TSV) infection [24] , and also studies involving functional lumphooid organ of Chinese shrimp Fenneropenaeus chinensis (*F.chinensis*) [25].

The proteomic approach is a useful method to study prawn immunity, which involves the proteome events associated with IHHNV infection in M.rosenbergii. In a prawn immune response study, the comparative a gel-based proteomics approach was used to identify differentially expressed proteins in the hemocytes of M.rosenbergii during IHHNV. Identified proteins paved the way toward improving the understanding of the cellular pathways necessary for resistance against IHHNV infection. Proteomic analysis of prawn hemocytes was used to elucidate the M.rosenbergii immune responses at the translational level upon IHHNV infection [26].

PROTEOMIC STUDY IN VIRAL AND BACTERIAL DISEASES

Viral and bacterial diseases can cause mass mortalities in a commercial crustacean farms. In this regards, proteomic studies has been done to get more insights about antiviral and antibacterial responses [27].

Proteomic studies have improved our understanding of the microbial world. The most recent advances in this field have helped us to explore aspects beyond genomics. For example, by studying proteins and their regulation, researchers now understand how some pathogenic bacteria have adapted to the lethal actions of antibiotics. Proteomics has also advanced our knowledge of mechanisms of bacterial virulence and some important aspects of how bacteria interact with human cells and, thus, of the pathogenesis of infectious diseases [28].

Examination of protein profiles by proteomic analysis has become an essential tool for studying the basic mechanisms of bacterial resistance and virulence. This has led to a better understanding of the biology of pathogens that cannot be investigated by reductionist or even genomic studies [29].

The potential application of proteomics to bacterial pathogen research is huge. The basic knowledge concerning resistance and virulence obtained recently through proteomic technology should be useful for developing new diagnostic and therapeutic applications for the treatment of infectious diseases [30].

Hemocyte protein profiling study from vibrio. Harveyi challenged shrimp , Penaeus monodon suggests the usefulness of a proteomic approach to the study of shrimp immunity and revealed hemocyte proteins whose expression were up regulated upon V. harveyi infection such as hemocyanin, arginine kinase and down regulated such as alpha-2-macroglobulin, calmodulin and 14-3-3 protein epsilon. The information is useful for understanding the immune system of shrimp against pathogenic bacteria [31].

Hemocyte protein profiling study from Infectious hypodermal and hematopoietic necrosis virus, Macroracium rosenbergii suggests the usefulness of a proteomic approach to the study of shrimp immunity and revealed hemocyte proteins whose expression were up regulated upon IHHNV infection such as hemocyanin, arginine kinase and down regulated such as up/down-regulation of Prophenoloxidas and hemocyanin isoforms. The information is useful for understanding the immune system of shrimp against pathogenic virus [32].

CONCLUSION

Proteome modification in different conditions may occur in many different ways that are not predictable from genomic analysis. A better understanding of this changes may have a sustainable impact in aquaculture industry. Currently, some studies has been conducted comparing the protein profiling of the infected animal's versus healthy animal and confirmed that the changes that occurred in the proteome content of the infected animals are spectacular. Proteomic techniques will pave the way for new biomarker discovery and develop new ways to manage the diseases. Proteomic analysis of biological fluids by 2DE mass spectrometry is the initial step of the proteomic analysis and knowledge development.

Although significant progress has been made in developing proteomics technologies still the lack of knowledge on crustacean proteome profiling limits the coverage of proteome power to control the outbreak caused by disease and environmental changes in crustacean.

AUTHOR CONTRIBUTIONS

TA and SM, conceived the study. SB and RYO were team supervisor. TA, SM drafted, and revised the manuscript. The first and second authors have the same credit for this manuscript.

REFERENCES

1. Pillai D, Johnson SK, Beuno SLS. Freshwater Prawns-Biology and Farming. Health Management. Oxford: Blackwell Publishing Ltd. 2010;(1).
2. Vazquez L, Alpuche J, Maldonado G, Agundis C, Pereyra-Morales A, Zenteno E. Review: Immunity mechanisms in crustaceans. Innate Immun. 2009 Jun;15(3):179-88. doi: 10.1177/1753425909102876. PMID: 19474211.
3. Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphery-Smith I, Hochstrasser DF, Williams KL. Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnol Genet Eng Rev. 1996;13:19-50. doi: 10.1080/02648725.1996.10647923. PMID: 8948108.
4. Nägele E, Vollmer M, Hörth P, Vad C. 2D-LC/MS techniques for the identification of proteins in highly complex mixtures. Expert Rev Proteomics. 2004 Jun;1(1):37-46. doi: 10.1586/14789450.1.1.37. PMID: 15966797.
5. Panicker RC, Chattopadhaya S, Yao SQ. Advanced analytical tools in proteomics. 2006;556(1):69-79. https://tinyurl.com/ybbbc9nb
6. Macarthur DJ, Jacques NA. Proteome analysis of oral pathogens. J Dent Res. 2003 Nov;82(11):870-6. doi: 10.1177/154405910308201105. PMID: 14578497.
7. Walker, John M. The Protein Protocols Handbook. University of Hertfordshire, Hatfield, UK. 2002; https://tinyurl.com/y9wvcndx
8. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. J Biol Chem. 1975 May 25;250(10):4007-21. PMID: 236308; PMCID: PMC2874754.
9. Klose J. Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. A novel approach to testing for induced point mutations in mammals. Humangenetik. 1975;26(3):231-43. doi: 10.1007/BF00281458. PMID: 1093965.
10. Garfin D, Two-dimensional gel electrophoresis: An overview. Trends in Analytical Chemistry 2005;22(5):263-272.
11. Görg A, Obermaier C, Boguth G, Harder A, Scheibe B, Wildgruber R, Weiss W. The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis. 2000 Apr;21(6):1037-53. doi: 10.1002/(SICI)1522-2683(20000401)21:6<1037::AID-ELPS1037>3.0.CO;2-V. PMID: 10786879.
12. Switzer RC 3rd, Merril CR, Shifrin S. A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. Anal Biochem. 1979 Sep 15;98(1):231-7. doi: 10.1016/0003-2697(79)90732-2. PMID: 94518.
13. Candiano G, Bruschi M, Musante L, Santucci L, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L, Righetti PG. Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis. 2004 May;25(9):1327-33. doi: 10.1002/elps.200305844. PMID: 15174055.
14. Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem. 1996 Mar 1;68(5):850-8. doi: 10.1021/ac950914h. PMID: 8779443..
15. Steinberg TH, Chernokalskaya E, Berggren K, Lopez MF, Diwu Z, Haugland RP, Patton WF. Ultrasensitive fluorescence protein detection in isoelectric focusing gels using a ruthenium metal chelate stain. Electrophoresis. 2000 Feb;21(3):486-96. doi: 10.1002/(SICI)1522-2683(20000201)21:3<486::AID-ELPS486>3.0.CO;2-Q. PMID: 10726748.
16. Yates JR 3rd. Mass spectrometry and the age of the proteome. J Mass Spectrom. 1998 Jan;33(1):1-19. doi: 10.1002/(SICI)1096-9888(199801)33:1<1::AID-JMS624>3.0.CO;2-9. PMID: 9449829.
17. Rosenfeld J, Capdevielle J, Guillemot JC, Ferrara P. In-gel digestion of proteins for internal sequence analysis after one- or two-dimensional gel electrophoresis. Anal Biochem. 1992 May 15;203(1):173-9. doi: 10.1016/0003-2697(92)90061-b. PMID: 1524213..

18. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. Science. 1989 Oct 6;246(4926):64-71. doi: 10.1126/science.2675315. PMID: 2675315..

19. Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Analytical Chemistry. 1988 Oct;60(20):2299-2301. DOI: 10.1021/ac00171a028.

20. McDonald W, Yates J. Proteomic tools for cell biology. Traffic. 2000;1(10): 747-754. https://tinyurl.com/yavn3xlw

21. Palagi PM, Hernandez P, Walther D, Appel RD. Proteome informatics I: bioinformatics tools for processing experimental data. Proteomics. 2006 Oct;6(20):5435-44. doi: 10.1002/pmic.200600273. PMID: 16991191.

22. Somboonwiwat K, Chaikeeratisak V, Wang HC, Fang Lo C, Tassanakajon A. Proteomic analysis of differentially expressed proteins in Penaeus monodon hemocytes after Vibrio harveyi infection. Proteome Sci. 2010 Jul 13;8:39. doi: 10.1186/1477-5956-8-39. PMID: 20626881; PMCID: PMC2915975.

23. Wang YC, Chang PS, Chen HY. Tissue expressions of nine genes important to immune defence of the Pacific white shrimp Litopenaeus vannamei. Fish & Shellfish Immunology. 2007;23:1161-1177.

24. Chongsatja PO, Bourchookarn A, Lo CF, Thongboonkerd V, Krittanai C. Proteomic analysis of differentially expressed proteins in Penaeus vannamei hemocytes upon Taura syndrome virus infection. Proteomics. 2007 Oct;7(19):3592-601. doi: 10.1002/pmic.200700281. PMID: 17722205.

25. Zhang J, Li F, Jiang H, Yu Y, Liu C, Li S, Wang B, Xiang J. Proteomic analysis of differentially expressed proteins in lymphoid organ of Fenneropenaeus chinensis response to Vibrio anguillarum stimulation. Fish Shellfish Immunol. 2010 Aug;29(2):186-94. doi: 10.1016/j.fsi.2010.03.011. Epub 2010 Apr 13. PMID: 20394824.

26. Alinejad T, Bin KQ, Vejayan J, Othman RY, Bhassu S. Proteomic analysis of differentially expressed protein in hemocytes of wild giant freshwater prawn Macrobrachium rosenbergii infected with infectious hypodermal and hematopoietic necrosis virus (IHHNV). Meta Gene. 2015 Jun 6;5:55-67. doi: 10.1016/j.mgene.2015.05.004. PMID: 26106581; PMCID: PMC4473098.

27. Carrera M, Piñeiro C, Martinez I. Proteomic Strategies to Evaluate the Impact of Farming Conditions on Food Quality and Safety in Aquaculture Products. Foods. 2020 Aug 4;9(8):1050. doi: 10.3390/foods9081050. PMID: 32759674; PMCID: PMC7466198.

28. Pérez-Llarena FJ, Bou G. Proteomics As a Tool for Studying Bacterial Virulence and Antimicrobial Resistance. Front Microbiol. 2016 Mar 31;7:410. doi: 10.3389/fmicb.2016.00410. PMID: 27065974; PMCID: PMC4814472.

29. Van Oudenhove L, Devreese B. A review on recent developments in mass spectrometry instrumentation and quantitative tools advancing bacterial proteomics. Appl Microbiol Biotechnol. 2013 Jun;97(11):4749-62. doi: 10.1007/s00253-013-4897-7. Epub 2013 Apr 27. PMID: 23624659.

30. Otto A, Becher D, Schmidt FJP. Quantitative proteomics in the field of microbiology. 2014;14(4-5):547-565.

31. Somboonwiwat K, Chaikeeratisak V, Wang HC, Fang Lo C, Tassanakajon A. Proteomic analysis of differentially expressed proteins in Penaeus monodon hemocytes after Vibrio harveyi infection. Proteome Sci. 2010 Jul 13;8:39. doi: 10.1186/1477-5956-8-39. PMID: 20626881; PMCID: PMC2915975.

32. Alinejad T, Bin KQ, Vejayan J, Othman RY, Bhassu S. Proteomic analysis of differentially expressed protein in hemocytes of wild giant freshwater prawn Macrobrachium rosenbergii infected with infectious hypodermal and hematopoietic necrosis virus (IHHNV). Meta Gene. 2015 Jun 6;5:55-67. doi: 10.1016/j.mgene.2015.05.004. PMID: 26106581; PMCID: PMC4473098.