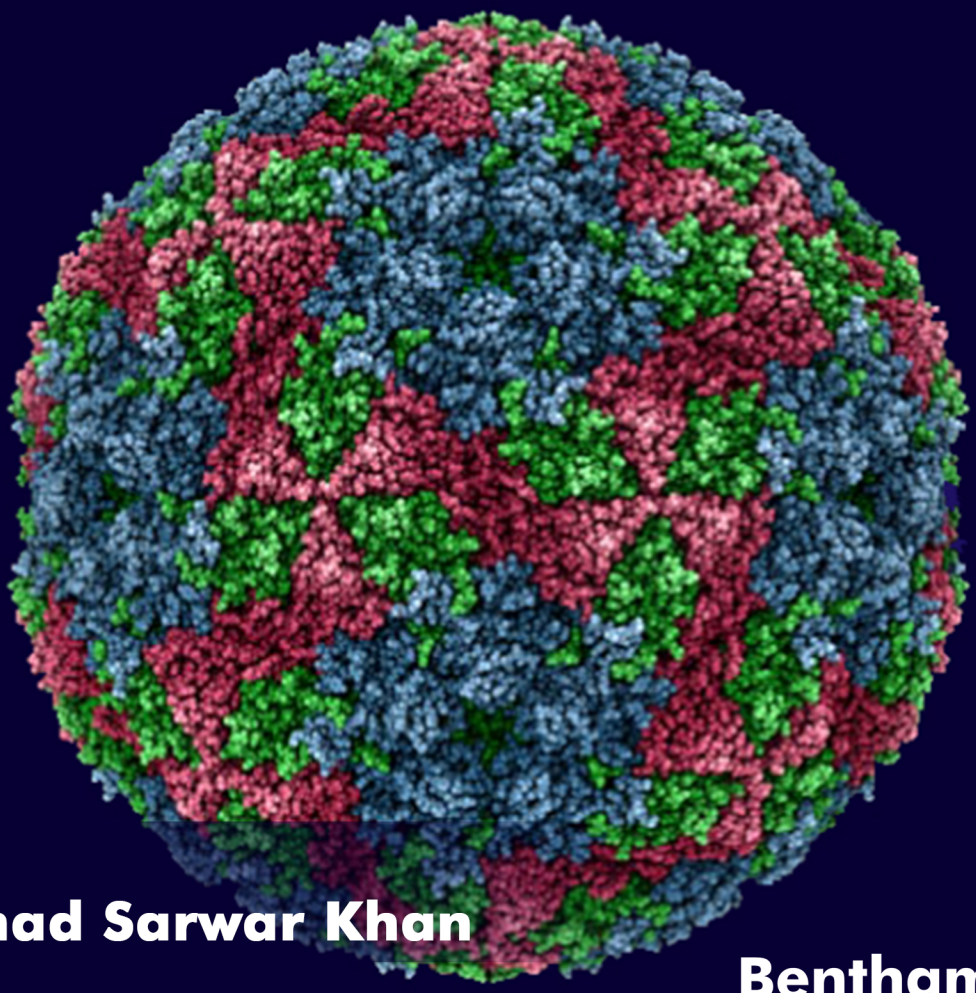
FRONTIERS IN MOLECULAR PHARMING



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Frontiers in Protein and Peptide Sciences

(Volume 2)

Frontiers in Molecular Pharming

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PREFACE

Manufacturing pharmaceuticals cost-effectively is one of the items on the wish list of biochemists and biotechnologists as drug regulatory authority in the USA has approved large-scale production and clinical trials of drugs developed through diverse production routes, including viruses, animals, and plants. Several factors are taken into account while selecting a production system of recombinant proteins since different expression systems have their own merits and demerits. The cost of expressed recombinant proteins includes production, processing, and purification costs. Normally, the production of expressed proteins costs around 70%, whereas purification costs around 30% of the total cost. Molecular pharming refers to the production of recombinant pharmaceutical proteins using plant biotechnology. This volume covers an array of topics relevant to structure, function, regulation, and mechanisms of action, biochemical significance, and usage of proteins and peptides as biomarkers, therapeutics, and vaccines for animals and human beings. Further, this book highlights the current progress from three directions, including system biology – *in silico* characterization of proteins and peptides, molecular pharming for animals, and molecular pharming for humans.

The book, Frontiers in Molecular Pharming, consists of 13 chapters subdivided into three sections. The chapters in the book are strategically organized to allow easy reading. Section I (System Biology – *in silico* Characterization of Proteins and Peptides) begins with Chapter 1 in which Dr. Rahman and his colleagues very comprehensively highlight various bioinformatics tools for predicting epitopic regions and a variety of immunological techniques to monitor the immune response generated against selected epitopic regions for the development of vaccines and diagnostics. Dr. Tahir ul Qamar and his colleagues in Chapter 2 have discussed the recent progress in the emerging field of immunoinformatics and its role in vaccine development. Dr. Ali explains the computational toolbox and its use in determining protein stability and analysis to improve thermostability in Chapter 3. In Chapter 4, Dr. Chen and her colleagues suggest how an evolving approach to Pan-proteomics is complementing our understanding of the functional complexity of emerging and highly virulent pathogens and their resistance development against drugs. Further, in Chapter 5, Drs. Haider and Niazi briefly overview the computational methods to predict the biological roles of peptides and proteins for medical or industrial applications.

Section II (Molecular Pharming for Human Beings) consists of six chapters, *i.e*., Chapters 6 through 11. In Chapter 6, Dr. Khan and his team members explain comprehensively how diverse expression systems could be used to costeffectively develop recombinant pharmaceuticals and their application to control diseases in animals and human beings. Dr. Ahmad and his team provide a snapshot of different expression systems and argue that the plant-based expression system is highly commercially feasible not only for the production of high-value targets but also to address global challenges like COVID-19 in Chapter 7. In Chapter 8, Drs. Mangena and Mkhize explain the role of antibody cross-reactivity and specificity concerning basic principles, challenges, and detection for rapid and reliable assessment in *Fusarium* pathogens. Dr. Waheed and his team in Chapter 9 and Dr. Rashid and her team in Chapter10 have discussed how the requisition of plant-based medicine is increasing day-by-day with its perspective to human diseases, and several advantages owing to United Nations' sustainable development goals (SDGs). Dr. Qasim and his colleagues in Chapter 11 explain the importance of proteins and peptides as biomarkers for the diagnosis of cardiovascular diseases to improve the risk prediction at the population level. Further, the authors explore how new technologies and innovations can be applied to advance the science of vaccine-associated biomarkers.

Section III (Molecular Pharming for Animals) consists of two chapters. In Chapter 12, Dr. Aqib and his colleagues highlight the history and recent trends in veterinary pharmaceuticals and vaccines. They further discuss the nutraceutical potential of animal products as one of the fascinating areas of research with considerable anti-microbial, anti-cancer, anti-inflammatory, anti-diabetic, and neuroprotective functions. Dr. Khan and his team in Chapter 13 highlight the importance of plant-based gene expression systems that have been exploited as bioreactors for the cost-effective production of pharmaceuticals, predominantly for the expression and accumulation of antigenic proteins, to be used as vaccines for livestock and poultry. Further, they have discussed various types of vaccines keeping in view diseases like Infectious Bursal Disease (IBD), New Castle Disease (ND), and Foot and Mouth Disease (FMD).

Molecular farming is progressively reaching the stage of being considered as an economical alternative to established systems for the production of pharmaceuticals. Thus, this volume serves as a treasured resource for students and professionals of molecular biology, biotechnology, medicinal chemistry, and organic chemistry.

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SECTION I: System Biology – *In silico* Characterization of Proteins and Peptides

CHAPTER 1

Tools for Prediction and Validation of Epitopic Regions on Protein Targets for Vaccine Development and Diagnostics

Soban Tufail[1,2], Majid Ali Shah[1,2], Maryam Zafar[1,2], Mazhar Iqbal[1,2], Amjad Ali[3], Aamir Shehzad[1,2,*] and **Moazur Rahman[1,4,*]**

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Abstract: Epitopes are parts of an antigen that are recognized by the immune system. Identification of epitopic regions on an immunogenic protein is important for several clinical and biotechnological applications. Various bioinformatics tools are currently available which can be used for the prediction of epitopic regions, and the immune response generated against selected epitopic regions can be monitored through a variety of immunological techniques. In this chapter, we provide an overview of widely used *in silico* tools for the prediction of epitopic regions, followed by biophysical methods used for their characterization. Furthermore, a brief description of important immunological approaches for measuring immune responses elicited by epitopes is also given. It is anticipated that the information provided in this chapter will help researchers in selecting appropriate tools for the prediction and validation of epitopes on a protein target for vaccine development and diagnostics.

Keywords: Diagnostics, Epitope prediction, Protein targets, Vaccine.

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Muhammad Sarwar Khan (Ed)

1. IMMUNE SYSTEM-AN OVERVIEW

A properly functioning immune system plays a key role in neutralizing 'biological threats' posed by infectious diseases and cancer. Understanding the immune system is important for devising therapeutic interventions to cure various diseases. The immune system is categorized into innate and adaptive subsystems. The innate immune system, which is non-specific, is the first line of defense against infections. On the other hand, the adaptive immune system, which is highly specific, is only found in vertebrates. Adaptive immune responses are orchestrated by lymphocytes, namely B- and T-cells, which induce humoral and cell-mediated immunity.

Importantly, specific receptors present on the surface of B- and T-cells recognize molecular components, commonly known as antigens, of pathogens. B-cell receptors, which consist of membrane-bound immunoglobulins, usually recognize parts of antigens that are solvent-exposed. Activated B-cells produce soluble immunoglobulins, also known as antibodies, which are involved in humoral adaptive immunity. The humoral immune system not only enables the recognition of antigenic determinants in pathogenic proteins but also induces the formation of memory B-cells which generate a strong antibody-mediated immune response upon re-infection. On the other hand, T-cell receptors recognize antigens by binding to antigenic peptides attached to the groove of major histocompatibility complex (MHC) molecules, also known as human leukocyte antigen (HLA) in humans, on the surface of antigen-presenting cells (APCs). In humans, the HLA system is polygenic (encoded by 21 genes on chromosome 6) and highly polymorphic. There are two distinct subtypes of T-cells, phenotypically classified as CD8+ and CD4+ T-cells, which recognize linear antigenic peptides presented by MHC molecules. Activated CD8+ T-cells, also known as cytotoxic T lymphocytes (CTLs), recognize peptides presented by MHC class I molecules (Fig. **1**). These peptides, which are typically 9 amino acids long, presented by MHC class I molecules originate from intracellular antigens degraded in the cytosol. Activated CD4+ T-cells, also known as T helper (Th) cells, recognize antigenic peptides presented by MHC class II molecules and are specific to extracellular antigens which have been endocytosed, degraded, and complexed to MHC class II molecules in endosomal compartments [1]. Typically, peptides attached to MHC class II molecules are 15 amino acids in length and protrude out of the peptide-binding groove of MHC class II molecules [2].

Portions of antigens that are recognized by B- and T-cells are known as epitopes. Discrete regions of antigens that are recognized by B-cell receptors or secreted antibodies and can evoke the humoral immune response are known as B-cell epitopes [3]. It has been found that B-cell epitopes predominantly consist of

solvent-exposed (hydrophilic) regions which are located on the surface of antigens [4, 5]. B-cell epitopes can be linear (continuous) or conformational (discontinuous). Linear epitopes are comprised of a contiguous stretch of amino acids of an antigen [6, 7]. Conformational epitopes consist of amino acids that are not contiguous, and residues critical for recognition by antibodies are located nearby due to the folded three-dimensional structure of a given antigen. It has been observed that the majority of conformational epitopes (more than 70%) contain 1-5 short linear segments of amino acids [5]. Moreover, most B-cell epitopes (~90%) are conformational epitopes [8, 9]. T-cell epitopes are MHC binding peptides (ligands) that elicit a T-cell immune response. Upon recognition of a T-cell epitope, T-cells produce a long-lived memory population that confers on the host the ability to respond swiftly when the same epitope is encountered again [10, 11].

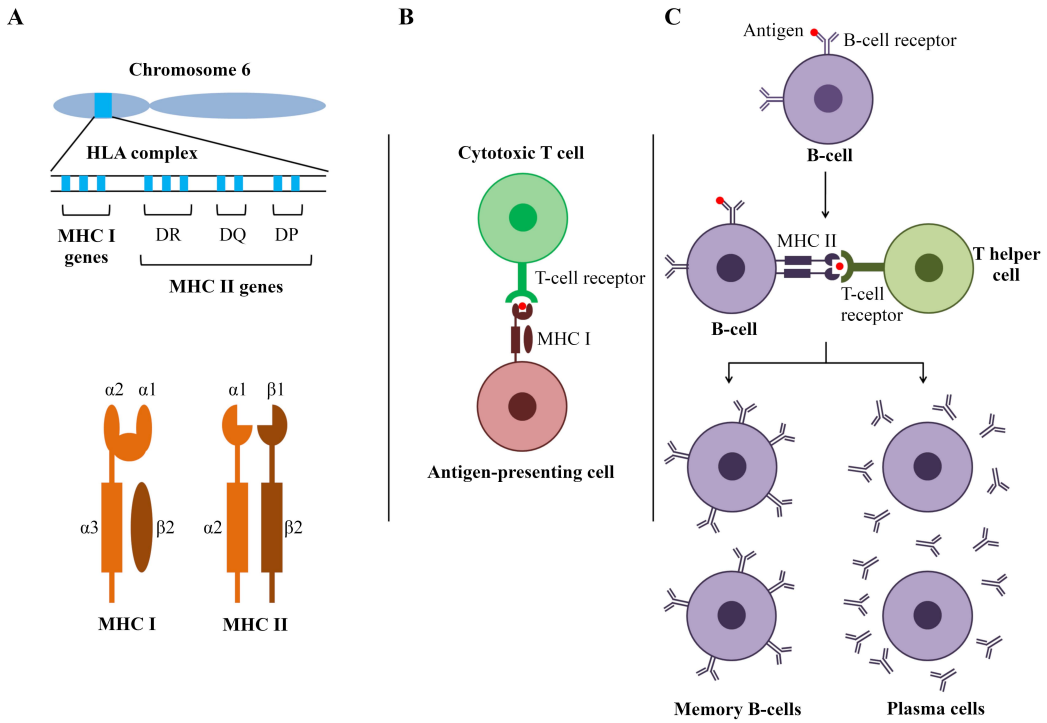

Fig. (1). Graphical illustrations of genes encoding MHC molecules and recognition of MHC-bound peptides by T cells. **(A)** The location of genes encoding MHC class I and MHC class II molecules on chromosome 6 in humans. **(B)** Recognition of peptides bound to MHC class I molecules on antigen-presenting cells by cytotoxic T cells. **(C)** The interaction of a foreign antigen with a B-cell receptor leads to the presentation of the foreign peptide to T helper cells through MHC class II molecules on the surface of B-cells. Activated B-cells proliferate and differentiate into memory B-cells and antibody-producing plasma cells.

CHAPTER 2

Immunoinformatics and its Role in Vaccine Development

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Abstract: Immunoinformatics is currently an emerging field that has accelerated immunological research to a great extent. It is playing a significant role in antigen identification, immunodiagnostic development, and vaccine design. The arrival of genome sequencing with recent advancements in immunoinformatics has provided a lot of data that can be annotated using databases and tools to reduce the cost required for antibody and vaccine development, ultimately saving time, cost, and resources. The selection and identification of immunogenic regions from the pathogen genomes by computational methods play an important role in devising new hypotheses by a comprehensive examination of immunologic data composite, which is otherwise impossible to achieve by using traditional methods alone. Presently, many epitope-based vaccines, especially multi-epitope vaccines designed employing immunoinformatics approaches, are successfully trailed and being developed against pathogens. In this chapter, we provide an outline of the recent progress in the field of vaccinology and immunoinformatics, enlisted recent tools and databases available for epitopes prediction, validation, and vaccine design, and give a brief description of the role of immunoinformatics in vaccine design against recent COVID-19.

Keywords: Computationally designed vaccines, Immunoinformatics, Reverse vaccinology, Subtractive genomics.

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1. INTRODUCTION

Vaccination is an important approach that involves the designation of vaccines and its administration in the host to protect them from various diseases [1]. The immunization journey started with the discovery of a vaccine against smallpox by Edward Jenner in 1798 [2]. Later on, many vaccines have been developed, and some are still in the process against emerging diseases. Various conventional methods being used for vaccine development generally involved inactivated or live attenuated vaccines [3], nucleic acid vaccines [4], subunit vaccines [5], and virus-like particles [6], which are discussed separately in a later section of this chapter. All these methods are generally *in vitro* and have many complications and limitations associated with them. For example, inactivated vaccines, if not properly designed, can reactivate in the host and cause diseases. Additionally, some vaccines require boosters for working effectively at later stages because they are unable to generate a robust immune response in the first attempt [2, 7, 8].

In the recent decade, whole-genome sequencing of humans and other organism resulted in a huge amount of functional, epidemiological, and clinical data [9, 10]. This assembled information available in specialist repositories and databases enables the researchers to get deep insights into the mechanisms of human diseases and to understand host immune responses. Computational immunology or immunoinformatics deals with such rapidly growing immunological data [11 - 13]. Immunoinformatics is now a crucial constituent of modern immunology research and correlates with experimental immunology and computer science. It utilizes computational resources and methods to understand, generate, process, and propagate immunological information. Immunoinformatics came into being 90 years ago with the hypothetical demonstration of malarial epidemiology [11, 12]. This emerging field includes various databases and tools that manage the observation of immunologic records produced by tentative researchers and assist in introducing and representing new therapeutic targets [14, 15]. It seems to provide the platform to develop and progress immunological research in less time. Vaccine informatics successfully utilized various bioinformatics methods and applications to accommodate different locations of the preclinical, clinical, and licensed vaccine activities. Also, the advancement in immunology and molecular biology facilitates the improvement of epitope-based vaccines, which has become a way for further research of molecular vaccines [15 - 18]. Furthermore, reverse vaccinology can expand vaccine protocols, design, and production by predicting various protein-vaccine candidates within genomes using computational approaches [19]. Immunoinformatics allow the potential B and T cell epitope prediction within selected candidate proteins, which can elicit a proper immune response against the pathogen . It has provided ease in the analysis, vaccine

design, immunization modeling, and evaluation of vaccine efficacy and safety [18, 20, 21].

In silico vaccine design, immunoproteomics, immunogenomics, and epitope prediction are some areas of Immunoinformatics. Recently, system biology methodologies have also been used to understand the various aspects and variable behavior of the complex immune system [22]. It constitutes the utilization of computational resources and methods to understand immunological evidence. It is a recent advancement in bioinformatics that uses numerous computational approaches to identify, understand, and predict a wide range of interactions among antigens and host immune system's receptors, including major histocompatibility complex (MHC) receptors also B and T cell receptors [23]. Immunoinformatics not merely aids in handling enormous data but also plays a crucial role in explaining new hypotheses associated with immune responses [24]. It is a potential and advantageous approach since conventional methods for vaccine development needs viruses to cultivate for understanding their binding patterns and to extract their antigenic proteins [25]. The term “Immunome” is used for all the data of proteins and genes in the immune system, excluding proteins and genes present in other cells except immune cells [26]. Immunoreactions that result from reactions between host cell proteins and antigens are referred to as immunome reactions, and the study of these reactions is called Immunomics [27]. Immunomics is a branch of knowledge that deals with the use of high throughput methods and techniques to determine and understand mechanisms involved in the immune system [28, 29]. In this chapter, we will briefly discuss the transformation of traditional vaccinology into modern vaccinology, recent advancements in immunoinformatics, important tools, and databases for prediction and validation of epitopes for vaccine design, and the role of immunoinformatics in COVID-19 vaccine development. It is anticipated that the information provided in this chapter will help researchers in choosing the most suitable tools and approaches for vaccine designs and immunological diagnostics development.

2. TRANSFORMATION OF VACCINOLOGY FROM CONVENTIONAL TO MODERN ERA

Previously, in the absence of effective therapies and preventive methods; infectious diseases such as measles, smallpox, diphtheria, rubella, chickenpox, and influenza were top listed child killers. Fortunately, these devastating diseases have diminished in various developed countries due to the widespread distribution and evolution of effective, safe, and affordable vaccines [30]. Vaccines can stop pathogens before they cause life-threatening damage, and millions of lives have

CHAPTER 3

Computational Toolbox for Analysis of Protein Thermostability

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Abstract: Thermostable proteins have many applications. They have utility in food and beverages, paper and pulp industries, animal feed production, laundry and detergent, and molecular biology and diagnostics. Many factors contribute to protein thermostability. These include covalent and non-covalent interactions, protein folding and conformation, and other thermodynamic factors. Although the available protein structures have been increasing over time, the increase in available protein sequences is overwhelmingly enormous. Also, structure determination can be a challenging job and many proteins are difficult to crystallize. This has resulted in a sequence-structure gap. The use of computer-assisted structure prediction has helped in filling this gap. There are many *in silico* strategies and methods available to predict and analyze protein structure and properties. These can be used to determine protein stability and are quite useful for *in silico* protein analysis to improve function and thermostability.

Keywords: *In silico*, Molecular dynamics, Mutagenesis, Protein thermostability, Rational design.

1. INTRODUCTION

Proteins are remarkable biomolecules. By utilizing a limited set of monomers (amino acids), the resulting variety of structure and function is extraordinary. Proteins are central to cellular processes, including catalysis, formation of cellular structures, storage, transport, communication, energy metabolism, movement, and flow of biological information.

Thermophiles are organisms that grow at temperatures exceeding 50°C. Hyperthermophiles grow at even higher temperatures. Many of them grow at temperatures close to the boiling point of water. Many industrial processes involving enzymatic reactions take place at high temperatures. So, enzymes from

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mesophiles are not suitable for these high temperature-requiring processes. In order to improve the efficiency of industrial processes, there is a continuing need for enzymes with better properties. Thermophilic enzymes meet this requirement as they are robust and can tolerate high temperatures [1]. Enzymes have applications in foods, detergents, textiles, leather, starch, and other industries. Moreover, protein engineering strategies have been useful in improving the properties of existing enzymes and tailoring them to improve process efficacy [2]. Some important applications of enzymes for industrial processes are shown in Table **1** [2, 3].

Table 1. Applications of some important enzymes.

Enzymes	Application
Amylase	Baking, paper and pulp, starch processing
Starch debranching enzymes	Starch processing, food industries
Cellulase	Plant biomass treatment, paper and pulp, biofuel production
Invertase	Food industry
Lactase	Food industry, lactose-free dairy products
Lipase and esterase	Food industry, detergents
Pectinase	Food, juice production
Peroxidase	Textile
Protease	Meat processing, detergents, leather and textile, dairy products
DNA polymerase	Polymerase chain reaction (PCR), gene cloning, diagnosis, molecular biology applications
Chitinase	Pharmaceutical products
Laccase	Bioremediation, detoxification
Amidases	Pharmaceutical products
Phytase	Feed and food industries

2. BASIS OF PROTEIN THERMOSTABILITY

During past years, much work has been done to understand protein thermostability. Many thermophilic proteins have been characterized and their structures determined. The proteins from thermophiles are not very different from their mesophilic counterparts in terms of their function. It has been reported that the thermostability of these proteins depends on various types of interactions, including hydrogen bonds, hydrophobic interaction, van der Waals interactions, and ionic interactions [4].

2.1. Electrostatic Interactions

A greater number of charged residues are found on the surface of thermostable proteins [4]. Electrostatic interactions are important for protein stability. Optimization of electrostatic interactions can result in protein stabilization while maintaining its activity [5]. Displacement of water molecules from the enzyme active site can also result in enhancing its activity. Glucanases have water molecules on their active sites, which are removed upon substrate binding. Engineering glucanases to displace water molecules during catalysis can enhance enzyme activity [6].

Electrostatic interactions of charged amino acid residues and the dielectric response of the protein are important factors in governing its thermostability. Proteins from thermophiles have been found to contain a greater number of charged residues on their surface as compared to those from mesophiles. This increasing trend of dielectric constant from mesophilic to thermophilic proteins modulates the thermal stability [7]. It has also been shown that optimizing electrostatic interactions by increasing the number of salt bridges is an important factor for high-temperature tolerance of proteins. The glutamate dehydrogenase from *Pyrococcus furiosus* has a large network of ion pairs formed by 18 charged residues [8]. Similarly, the enzyme lumazine synthase from *Aquifex* has a greater number of ions pairs as compared to corresponding mesophilic enzymes [9]. Also, the DNA polymerase from *Pyrobaculum calidifontis* (*Pca*) was shown to contain a greater number of salt bridges as compared to the ones found in *E. coli* (*Eco*) DNA polymerase [10]. *Pca* DNA polymerase contains a total of 242 charged amino acids. On the other hand, this number in *Eco* DNA polymerase is 193. A comparison of structures of these DNA polymerases is given in Fig. (**1**), demonstrating the association of electrostatic interactions with protein thermostability.

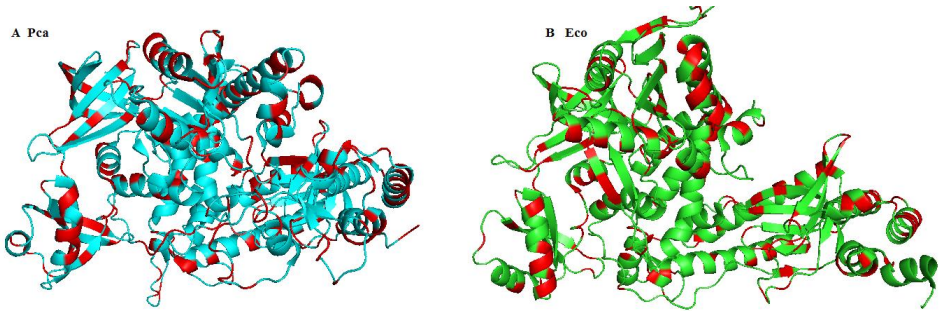

Fig. (1). Structures of **A)** *Pca* DNA polymerase – cyan (PDB ID: 5MDN) and **B)** *Eco* DNA Polymerase – green (PDB ID: 1Q8I). Charged residues are shown in red color in both structures. *Pca* DNA polymerase has a greater number of charged residues, resulting in more salt bridges, and is a thermostable enzyme. (The figure is generated in PyMol by using indicated PDB structures).

CHAPTER 4

Pan-Proteomics to Analyze the Functional Complexity of Organisms

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Abstract: Proteomics is rapidly expanding with the advent of high throughput technologies and offers a greater understanding of the complexity of life and the process of evolution. Protein profile comparison between genetically heterogeneous individuals may provide important insights into physiological diversity and function. A new term, pan-proteomics, has also been introduced that permits the qualitative and quantitative comparison of proteomes of genetically heterogeneous organisms. Here in this chapter, various aspects of pan-proteomics along with its basic methodology and applications have been discussed.

Keywords: Bioinformatics, Comparative proteomics, Proteomics, Pan-Proteomics.

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1. INTRODUCTION

Proteins are a quintessential part of all living organisms, with different functions essential for life. The word "proteome" was first coined in 1994 by Mark Wilkins. Proteomics is the large-scale protein structure and function analysis [1]. Major advances in molecular biology techniques providing new insights into the nature of genes and proteins are due to the staggering number of genomic projects performed over the past decade. However, many types of information cannot be obtained, and it is impossible to interpret the mechanism of disease and the effect of the environment solely by these results. In this context, to characterize the protein modifications, large-scale study of proteins is inevitable. Proteomics is a large-scale analysis of the whole set of proteins expressed by a cell, tissue, or organism, resulting in an information-rich landscape of expressed proteins and their modulations under a defined set of conditions. Unlike the previous studies of individual proteins or simple macromolecular complexes, proteomics offers a more comprehensive and systematic understanding of biological systems [2, 3].

Proteomics allows evaluating different cellular processes by providing a comprehensive qualitative and quantitative description of protein contents in a cell. Quantitative proteomics provided several valuable insights into proteomes, and it can be further classified in global proteome analysis and relative quantification [4]. Global proteomics strategies are often used for the absolute quantification of one or more proteins in a given sample. On the other hand, relative quantification of two samples with two different physiological states provides insights into the cellular and molecular mechanisms involved in a biological process. Quantification of mRNA content can confirm the presence of protein and its quantity in a cell [5, 6]. This concept allows the comparative analysis of protein measurements on a whole-genome level. So, the protein's detected differences can enable new insights into the processes accountable for a detected phenotype [7].

Recent technological advances in proteomics have greatly propelled our knowledge about drug development, host-pathogen interaction, and human or animal health. Proteomics offers the ability to interpret the physiological diversity and functions in a complex system. Many of the proteomes of genetically heterozygous organisms may fail to account for underlying genetic variance when compared. Therefore, recent knowledge concerning the functional heterogeneity of individuals at the protein level may be inaccurate or incomplete. To address this, there is a need to consider the impact of genetic heterogeneity on proteome comparisons more significantly. Pan-proteomics is the possible solution here that allows the qualitative and quantitative comparison between genetically heterogeneous organisms [7]. The aim of this chapter is to briefly explain the

concept of pan-proteomics and its applications in different areas of basic life sciences. In addition, proteomics strategies and bioinformatics approaches developed to increase pan-proteomic power have also been discussed.

2. CONCEPT OF PAN-PROTEOMICS

Pan-proteomics is an emerging analytical approach to compare proteome variability within genetically heterogenous organisms across species of interest [7]. Like the pan-genome, the pan-proteome also refers to the full set of non-redundant proteins thought to be expressed within members of the same species. Similarly, it can be categorized into core proteome (proteins present in all members) and variable or dispensable proteome (proteins present only in some members). The pan-proteomics complements both pan-genomics [8, 9] and pan-transcriptomics [10, 11], which helps to identify genetic variants and the presence of homologous sequences of an organism at the protein level [12, 13]. Comparative proteomic analysis of different individuals of the same species can reveal relationships and genetic variations among individuals without assessing the genomic data [14, 15].

3. APPROACHES AND SOFTWARE USED FOR PAN-PROTEOMICS

Proteomics relies on three fundamental technological cornerstones that include, Mass Spectrometry (MS) to obtain the data essential for individual protein identification; a method to separate complex proteins; and bioinformatics to analyze and assemble the MS data [16]. Just like proteomics, some steps are considered important in pan-proteomics as well, including (i) sample preparation – considering the type of protein fraction and selection of extraction buffer based on different physical-chemical properties of proteins; (ii) identification method – implementation of high-performance methodologies like MudPIT for maximum proteome coverage and dynamic range; (iii) protein sequence database – with the proteome of all species utilized in the study and curated protein sequences. So, the pan-database will contain reference proteome and unique sequences that are not present in the reference proteome [17, 18]. Once a pan-database has been constructed, sequence homology clusters can be inferred from it [7]. In this regard, different methods of homology cluster inference are available or have been reported, including: BLASTclust [19], orthoMCL [20], PipeAlign [21], OrthoFinder [22], CD-HIT [23] and OrthoVenn [24]. In addition, GROMACS [25] can be used for protein's structural clustering confirmations analysis [26]. Once inferred, clusters can be further classified into core, dispensable and specie-specific categories. Proteomic data is usually explored with the help of gene ontology (GO) and pathway analysis [7]. Several platforms are available for such

CHAPTER 5

Functional Characterization of Proteins and Peptides Using Computational Approaches

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Abstract: Bioinformatics tools have produced enormous data in different repositories over the past decade, which is of great interest today for *in-silico* analysis. Proteins contain one or more peptides that are essential molecules having biological and biomedical functions. Instead of working with sequences, proteomics, and peptidomics, researchers now concentrate more on molecules' processes and metabolic interactions in which proteins or peptides are involved. As a preliminary assessment of possible biological roles, bioinformatics methods are an essential step and greatly reduce experimental verification time and cost. This chapter offers a brief overview of computational methods for predicting the biological features of peptides and proteins. Algorithms using structural, evolutionary, or statistical patterns and strategies based on molecular docking are considered based on machine learning techniques, which are the most common today. The protein and bioactive peptide databases are reported, providing the knowledge required to develop new algorithms. The biological functions for forecasting, the features of proteins, and peptides should be considered, based on the possibility of concluding their natural role. The report includes a list of online resources that researchers can use to evaluate possible protein function and peptides.

Keywords: Bioinformatics, Biological functions, Databases, Machine learning, Molecular docking, Peptides and protein.

1. INTRODUCTION

Proteomics, including peptidomics, is one of the most rapidly developing areas of biochemistry nowadays. These science branches study the totality of proteins and protein fragments (peptides), their functions, and interactions in living organisms [1 - 3]. Mass spectrometry, various electrophoretic approaches, and liquid chro-

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matography are the primary study methods in proteomics and peptidomics [3 - 5]. To classify and further analyze peptides revealed by mass spectrometry, special algorithms are commonly used. The data obtained allow peptide abundance analysis in different samples (comparative peptidomics), determine the position of a peptide in a protein sequence, elucidate the proteases involved in peptide generation, and predict the structures and functions of the peptide [6]. One of such studies' potential objectives is to look for and evaluate bioactive peptides that, by binding to particular receptors or other targets, modulate physiological functions [7]. In higher organisms like a human, bioactive peptides can affect almost all systems. Antithrombotic peptides play a significant role in inhibiting platelet aggregation and fibrinogen binding, hypotensive peptides that inhibit angiotensin-transforming enzymes and antioxidant peptides that can scavenge free radicals (produced as by-products of cell oxidative metabolism) and inhibit lipid peroxidation are influenced by the cardiovascular system [8, 9]. Such peptides bind the cell surfaces of bacteria and thus inhibit the work of membranes or kill bacterial biofilms. In addition, certain peptides have an antiviral activity or antifungal activity [10].

Technological advances in DNA sequencing have made whole-genome sequencing a hot topic of current research. However, scientists' key challenges regarding sequence data entry are to elucidate the role of proteins and large-scale analysis of entire proteomes (the protein component of genomes) rapidly and accurately. Many local databases exist, *i.e*., UniProtKB, GenBank, RefSeq and TPA, and Swissport, PIR, PRF, and PDB that record the processed protein sequences [11]. To equate a target sequence to those of known functions, scientists typically use sequence similarity searches. Still, this approach has its drawbacks and depends on the precision of the remaining data offered to date. Protein signatures are used in alternative strategies for the classification of proteins. Some common databases that establish diagnostic protein signatures have emerged for recognized protein families or domains. Each of these databases has its methodology, signature generation parameters, and methods.

As a consequence, it also has strengths and shortcomings of its own [12]. *In-silico* draws on the techniques of bioinformatics to classify proteins and peptides. It offers references to protein and peptide databases. It works on strategies to develop algorithms to study proteins and peptides that would be of interest to specialists working in the field.

2. *IN-SILICO* ANALYSIS OF PEPTIDES

Peptides are biochemical compounds present in nature. In all living species, peptides are present naturally and play a key role in all biological activities, and are synthesized by DNA transcription, like proteins [13 - 15].

There are only 20 amino acids that can be mixed into a lot of diverse molecules. If the molecule is 2 to 50 amino acids in length, is a peptide, or is a longer chain of more than 50 amino acids, it is commonly referred to as a protein. For peptide toxicity, *in-silico* based databases and peptide sequence analysis tools have been developed., which have revolutionized peptidomics.

2.1. Classification and Databases of Peptides

Peptides are deemed to be appropriate resources in different biological fields. They can be used mainly for the rational design of molecules that are bioactive. In the manufacture of targeted drugs and diagnostics, vaccine production, or agriculture, they may be used as ligands. You can classify peptides into two broad structural classes: linear peptides and cyclic peptides. A special type of macrocyclic ring polypeptides, monocyclic peptides, exhibit advantages such as more selective binding and target receptor absorption and greater efficiency and stability compared to rectilinear forms [16]. There are many different online databases and tools available to retrieve the different types of peptides sequences. Such databases and tools are listed below in Table **1**.

SECTION II: Molecular Pharming for Human Beings

CHAPTER 6

Molecular Pharming: Research, Developments and Future Perspective

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Abstract: Plants are tamed to function as production factories of pharmaceuticals. Recently, several pharmaceuticals, including therapeutics, drugs, vaccines, vitamins, antibiotics, nutraceuticals, and diagnostic molecules, have been produced through these green factories. Compared with conventional systems, for example, bacterial, yeast, fungal, and mammalian cell cultures, plants are accepted as a cost-effective source of pharmaceuticals products. Considering plants as a versatile, cost-effective, and robust production platform, the system could be exploited in different ways like plant cell culture, transient expression and harvesting, and stable transgenics. This chapter highlights the importance and potential of molecular pharming with special emphasis on methodological aspects, proving the suitability of plants as the most appropriate biopharmaceutical production platform with recent interventions.

Keywords: Biopharmaceuticals, Cell culture, Cost-effective, Plant expression system, Recent innovations.

1. INTRODUCTION

Biopharmaceutics are biomolecules produced using biotechnological processes. These are protein or nucleic acid-based substances used for therapeutic or diagnostic purposes [1]. Pharmaceutical technology will continue to provide breakthroughs in medical research, leading to the effective treatment of noxious diseases including AIDS, cancer, asthma, Parkinson's, and Alzheimer's diseases [2]. Plant-derived pharmaceuticals have drawn special attention in this context owing to certain salient advantages of this production system as compared with others.

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The pharmaceutical market of recombinant proteins is growing steadily, with almost all major pharmaceutical firms reporting a rising share of these products. These pharmaceuticals are usually produced using mammalian or bacterial cell-based processes that are complex to operate and potentially susceptible to human pathogen contamination [3]. Current good manufacturing practice (cGMP) compliant manufacturing facilities require tremendous capital investment and include substantial financial risk. Plant biotechnology can solve some of these drawbacks, but before this emerging industry can compete successfully in the pharmaceutical market, many obstacles remain to be addressed. A variety of different technologies are now operated by plant biotechnology for recombinant protein expression. These developments constitute opportunities in the sector for current and future commercial enterprises [4].

The idea of using plants for the production of recombinant pharmaceutical proteins, known as plant molecular pharming (PMF) or pharmacy (PMP), is not new. The 1st plant-derived recombinant therapeutic protein was human growth hormone, initially developed in tobacco and sunflower in 1986 [5]. The HBs Ag (hepatitis B surface antigen) was later expressed in transgenic tobacco. Physically and antigenically, this plant-derived antigen was identical to HBs Ag obtained from recombinant yeast and human serum. HBs Ag derived from yeast is clinically used for vaccination against HBV. It has been reported that above 100 recombinant proteins were expressed and produced through a plant-based expression system [6].

The basic term 'ATMPs (advanced therapy medicinal products)' is used by the European Medicine Agency (EMA) to refer to human medicines that are based on tissue, cells, and gene engineering. Cell therapy products (CTPs) are cells/tissues containing biomedicines that have been manipulated to modify their biological properties and can be used to cure, prevent, or diagnose diseases. The common benefits of these systems are ease of manipulation, low cost, speed, and high protein yield.

The developments in functional genomics and recombinant DNA technology have been merged to provide vast opportunities for the cost-effective production of commercial-scale recombinant protein. Enzymes are now widely used in industry for bio-catalytic reactions, allowing them to be used in anything from cultivation and bioremediation to medicine and food preparation. The rise of the biopharmaceutical industry has been brought about by further exploitation and modifications in the recombinant protein structure and function for therapeutic uses [7]. Any of the therapeutic proteins, including growth hormones, pancreatic enzymes, can now be produced from alternative sources, thus available to the ultimate consumer in natural form. Further, advancements in recombinant DNA

technology have refined it by making possible the isolation and expression of protein-coding genes in the transgenic host cells. Transgenic sources of these recombinant proteins pave the way to the production of low-cost proteins with increased protection and efficiency [8].

2. HISTORY OF THE BIOPHARMACEUTICAL INDUSTRY

Proteins of mammalian origin have been produced in plants since the late 1980s. Since then, 'molecular plant farming' has widely been exploited to use plants as protein factories. Fisher introduced the concept of molecular pharming (biopharming) and highlighted that any mammalian protein, including vaccines, blood proteins, antibodies, and medicinal therapeutics, can effectively be produced in plants [9]. Over the past century, vaccine production has progressed tremendously. The burden of many life-threatening diseases has been minimized by traditional vaccines. Alternative approaches have been employed for the development of novel vaccines with the ability to effectively protect against new diseases [10, 11].

Three key groups had been striving to ascertain the notion of vaccine production in plants under the leadership of Charles Arntzen, Roy Curtsis, and Hilary Koprowski. Arntzen's research contributed first peer-reviewed publication in the area of plant molecular farming [12]. It was suggested that an edible vaccine is a fruit or vegetable with a particular antigen that can be delivered cost-effectively with increased efficacy. This proved the worth of plants as an alternative source of vaccine having the ability to compete with the existing market. Thus, using plants as edible vaccines could be an effective alternative to treat infectious diseases. The beauty of this concept was that recombinant plants could be grown near to the target population, facilitating the availability of inexpensive and effective vaccines. The reduced cost of downstream processes, transportation, and purification would result in reducing the cost as compared with traditional vaccines. Despite several advantages, there are certain limitations as well, including optimization of dosage and contamination of the food chain by producing recombinant plants [13].

Dow Agro Sciences LLC announced (on 31st January 2006) to be the global leader and obtained the World's first regulatory approval from USDA. The approved plant-based vaccine could combat the ND (Newcastle Disease). The vaccine antigen was expressed in tobacco plant cell lines through suspension culture in a traditional bioreactor system. The resulting cells were extracted and minimally processed for the final formulation of the vaccine [14, 15].

CHAPTER 7

Green Factories: Plants As A Platform For Cost-effective Production of High-value Targets

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Abstract: Transgenic plants have been developed since the early 1980s, when researchers were able to transform a piece of foreign DNA into a plant genome. Since then, the technology has expanded enormously, giving rise to many private and public ventures in the field of plant-based recombinant technology. The technology has helped in crop improvement against various biotic and abiotic stresses such as insect resistance and herbicide tolerance, as well as improving their nutritional values, for example, Golden rice. In addition to crop improvement, the technology has enabled plants to be used as green factories for the production of recombinant proteins. Several platforms are available for the heterologous expression of foreign proteins, each of which represents its own set of advantages and limitations. Plants offer many advantages for inexpensive yet large-scale production of high-value targets, making them extremely attractive for commercial applications. In this chapter, we briefly discuss the need for using plants as solar-powered cellular factories to produce recombinant proteins. We provide a snapshot of different expression systems and argue that the plant-based expression system is highly commercially feasible not only for the production of high-value targets but also to help address global challenges like Covid-19.

Keywords: Biopharming, High-Value targets, Green factories, Plants.

1. WHY PLANT-BASED EXPRESSION SYSTEMS?

Heterologous expression of recombinant proteins for different applications has become a focus of intensive research for a while, paving the way for another revolution in the area for the development of new production technologies. The demand for cost-effective yet large-scale production of protein and secondary metabolites for various purposes, such as medical reagents, cosmetic products,

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and industrial enzymes, in terms of quantity, diversity, and, most importantly, quality has dramatically increased since the past decade [1]. The gap between demand and supply has further increased due to inefficient yet highly expensive production systems [2, 3]. Several systems, including bacteria, yeasts, animal cells, transgenic animals, plant cells, and transgenic plants, are available for the heterologous production of high-value targets [4, 5]. All available expression systems have their pros and cons in terms of cost, time, efficiency, product size, growth conditions, yield, post-translational modification, downstream processing, and regulatory approval [6]. The advantages of plant expression platforms are cited in several earlier reports [7 - 12]. Table **1** shows head-to-head comparisons of all existing platforms. Transgenic plants have become a focus of interest as new generation bioreactors mainly due to: i) reduced up-front production costs, ii) lower risk of endotoxins as well as human pathogen contamination, iii) scal-ability, iv) availability of existing infrastructure for the cultivation of transgenic plants, v) assemble complex protein with eukaryotic-like post-transcriptional modifications. However, plants lack the human-like N-glycosylation mechanism for protein processing that has been overcome by engineering tactics to ensure the authentic quality, homogeneity, and quantity [13]

Table 1. Comparison of different expression system [10].

Parameter	Bacteria	Yeast	Insect cells	Microalgae	Mammalian Cells	Transgenic Plants
Capital cost	Medium	Medium	High	Medium	Very high	Low
Operating cost	Low	Medium	High	Low	Very high	Low
Production scale	Short	Short	Medium	Short	Long	Long
Speed	Fast	Fast	Medium	Fast	Slow	Slow
Multigene engineering	Yes	No	No	Yes	No	Yes
Glycosylation	Absent	Incorrect	Yes	Yes, absent in chloroplast	Yes	Yes, absent in chloroplast
Contamination risk	High	Medium	High	Low	High	Low
Multimeric assembly	No	No	No	Yes	No	Yes
Protein folding	Low	Medium	High	High	High	High
Protein yield	High	Moderate high	Medium	High	Medium	Low-High
Scale up cost	High	High	Very high	Medium	Very high	Very low
Safety	Low	Unknown	Medium		Low	High

(Table 1) cont.....

Parameter	Bacteria	Yeast	Insect cells	Microalgae	Mammalian Cells	Transgenic Plants
Storage	Very cheap	Costly	Expensive	Low	Very expensive	Very cheap
Distribution	Easy	Feasible	Difficult	Easy	Difficult	Easy

Plant molecular farming (PMF) is termed as the technique of producing high-value proteins recombinantly in plants without disturbing their phenotype, metabolism, or performance. The proteins have been produced by this technique for more than 30 years, either in purified form, crude extract, or in planta [3, 14]. The idea of molecular farming based on the genetic transformation of plants was first proposed in the 1980s [15], which has now become a reality and is often termed as the 3rd generation of biotechnology [6]. The first examples of molecular farming using transgenic plants and plant cell suspension cultures involved the production of a human growth hormone, Nopaline synthase [16], and an antibody IgG_1 (6D4) [17]. However, the commercial application of this platform came years later when avidin was recombinantly produced in transgenic maize [18]. The breakthrough to commercial success for plant-derived biologics culminated in 2012 when the first plant-made pharmaceutical, Taliglucerase alfa, commercially known as Elelyso®, was developed by Protalix BioTherapeutics, was approved by the US Food and Drug Administration [19]. Elelyso® is a recombinant human glucocerebrocidase used for the treatment of Gaucher's disease (lysosomal storage disorder) [20].

The use of plants for the production of valuable proteins has been refined and improved over the years due to advancements in knowledge and technology. This has led to a major paradigm shift in the pharma sector, as the potential drawbacks associated with the early stages of PMF, including high expression level and efficient downstream processes, have been attained [6]. The product portfolio ranges from pharmaceutical therapeutics to non-pharmaceutical products such as antibodies, vaccine antigens, enzymes, growth factors, research or diagnostic reagents, and cosmetic ingredients. A number of 'proof-of-concept' studies have been performed to evaluate the potential of different plant species as hosts for molecular pharming [21, 22]. The host cells or the plant used for molecular farming purposes, depending upon target protein and its application, range from crop plants (rice, maize, tobacco, alfalfa, safflower, and lettuce) to pondweed, algae, microalgae, and mosses. The *Nicotiana* genus has been widely used for genetic transformation studies as it is easily genetically manipulated and has a fast growth rate. Two species, *Nicotiana benthamiana* and *Nicotiana tabacum* are considered as 'biological warehouses' for the production of many pharma or non-pharma products by the stable and transient expression [21]. Many plant-based

CHAPTER 8

Analysis of Cross-Reactivity, Specificity and the Use of Optimised ELISA for Rapid Detection of *Fusarium* Spp.

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Abstract: Many strides have been made in the development of antibody-based detection systems for rapid and sensitive analysis of *Fusarium* pathogens and their toxins. Antibody cross-reactivity, specificity, and binding affinity with antigenic molecules affect the efficacy in which these molecules serve their own functions. Researchers are, therefore, directed in investigating the principles that govern cross-reactivity, specificity, and the relationship between them, using various tools such as optimised ELISA. This is important because the ability of *Fusarium* spp. to infect and produce mycotoxins in agronomic crops passes these toxins to animals and humans after contact or ingestion. Antibodies that recognise and bind particular antigens with great affinity and specificity, especially for the effective relief of unwanted *Fusarium* pathogenic materials in humans and animals, are thus required. Furthermore, the demand for fungal contaminants free agriculture, emerging antifungal drug resistance, and the fatal health effects of fungal infections in immunocompromised humans and animals drive the need for the development of a rapid, sensitive, reliable, and accurate relief system for these pathogens. Therefore, this chapter provides a succinct review on the role of antibody cross-reactivity and specificity, with reference to basic principles, challenges, and detection for rapid and reliable assessment in *Fusarium* pathogens.

Keywords: Antibody specificity, Antigens, Cross-reactivity, ELISA, *Fusarium*, Immunoglobulins, Mycotoxins, Somatic hypermutation.

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1. INTRODUCTION

The genus *Fusarium* contains a group of filamentous fungi commonly found in soils, in which its species are linked with animal and plant pathogenesis. *Fusarium* species are toxigenic, and the mycotoxins (*i.e*., compounds with deleterious effects on susceptible host organisms) produced by these fungi are often associated with animal and human infections, including some of the diseases affecting seedlings and mature plants. According to Moretti [1], these organisms remain among some of the widely occurring plant-pathogenic species, causing diseases in several agriculturally important crops, particularly cereals, lumber, and pulses. Many of *Fusarium* spp. produce a wide range of biologically active secondary metabolites (Table **1**), with tremendous accompanying chemical diversity. Some of the bioactive metabolites serve as products of primary and secondary metabolic value, characterised by a distinct and unusual chemical structure with varying molecular weights.

Table 1. Brief outline of mycotoxins and valuable compounds produced by *Fusarium* spp., including the early and recently discovered examples of bioactive compounds.

Fungal spp.	Mycotoxins/ SMs	Brief Description	References
F. sambucinum TE-6L	Indole alkaloids	Amoenamide C and Sclereotiamide B, from angularly prenylated indole alkaloids with pyrano [2,3-g] indole moieties *Application:* Antimicrobial and insecticidal activity	Zhang *et al*. [4]
Unclassified *Fusarium* spp.	Terpenes	Orcyl aldehyde units (condensed with farnesyl side chain terminally cyclised to cyclohexanone ring in γ, ε, δ, ζ, α and β) designated LL-Z1272 *Application:* anti-*Tetrahymena pyriformis* activity	Ellestad *et al*. [13]
F. fujikuroi	Polyketides	Yellow and/ orange pigmented fusarins, encoded by a *fusA* gene *Application:* Antimicrobial activity	Diaz-Sanchez *et al*. [14]
F. graminearum	Non-ribosomal peptides	Fasaoctaxin A, product of ectopic expression of *fg3_54* gene cluster *Role:* A as a virulence factor required for cell-to-cell invasion of wheat by *F. graminearum*	Jia *et al*. [15]
F. incarnatum-equiseti	PKs + NRPs derived	Polyketides (PKs) and Nonribosomal peptides (NRPs) secondary metabolites synthesized by NRP synthetase and type-1 PK synthase *e.g*. Carotenoid, fusarubin pigment, enniatin, fusarin and zearalenone mycotoxins	Villani *et al*. [16]

(Table 1) cont.....

Fungal spp.	Mycotoxins/ SMs	Brief Description	References
F. crookwellense, *F. culmorum* and *F. graminearum*	Trichothecenes	Nivalenol (NIV), deoxynivalenol (DON) or scirpentriol (STO) chemotypes	Lauren *et al*. [17]
F. oxysporum, *F. proliferatum*, *F. moniliforme* and *F. nygami*	Fumonisins	Fumonisins A (N-acetyl analogs, FAs), B (sphingosine N-acyltransferase, ceramide synthase), C (dimethyl analogs, FCs) and P (N-3-hydroxypiridinium analogs, FPs)	Sewran *et al*. [8], Tamura *et al*. [9] and Rheeder *et al*. [10]
F. graminearum, F. culmorum and *F. crookwellense*	Zearalenone	Zearalenone (ZAN), α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL)	Tian *et al*. [11]
F. proliferatum, F. solani and *F. pseudonygamai*	Fusaproliferin	Fusaproliferin, a sesterterpene mycotoxin C_{20}-core carbon skeleton possessing an extra C_5-head/C_5-tail unit	Fotso *et al*. [18] and Liuzzi *et al*. [19]
F. concentricum	Beauvericin	Structurally related beauvericin (BEA) and enniatins B (ENN B) synthesised by multifunctional enzyme enniatin synthetase containing both peptide synthetase and S-adenosyl-L-methionine-dependent N-methyltransferase activities	Liuzzi *et al*. [19]
F. Orthoceras var. *enniatimum*	Enniatins	Structurally related beauvericin (BEA) and enniatins B (ENN B) synthesised by multifunctional enzyme enniatin synthetase containing both peptide synthetase and S-adenosyl-L-methionine-dependent N-methyltransferase activities	Liuzzi *et al*. [19]
F. proliferatum, F. phyllophilum and *F. subglutinans*	Moniliformin	Small ionic carboxylate potassium/ sodium salt (1-hydroxycyclobut-1-ene-3,4 dione) with a highly toxic one water crystallisation	Fotso *et al*. [18]
Note: SMs- Secondary metabolites Spp. – species			

Not only are mycotoxins produced by species in this genus, but some secondary metabolites potentially serve as a source of many useful novel compounds with enzymatic capability, antibacterial activity, antiviral, anti-parasitic, and growth-promoting effects, as well as other properties can be obtained [2, 3]. *Fusarium* could, furthermore, be used in industrial processing to directly or indirectly provide enzymes used to catalyse the generation of various other novel substances such as pigments, cosmetic and food compounds that may be used to substitute synthetic compounds [2, 4]. These compounds indicated in Table **1** provide the basis for developing new promising and prolific sources of bioactive secondary metabolites with prominent biotechnological applications, as well as the detection

CHAPTER 9

Plant Molecular Pharming For Human Diseases

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Abstract: Infectious diseases pose an increasing threat to global health. The world has experienced many outbreaks due to Emerging Infectious Diseases (EIDs) in the 21st century. Vaccination proves to be the most successful public health intervention to counter such outbreaks. Vaccines against many diseases are available. Most of these vaccines either consist of live or attenuated strains, thus posing health risks. There is a need for new and safe vaccines to prevent and mitigate the impact of outbreaks due to emerging and endemic infectious diseases. The requisition of plant-based medicine is increasing day by day because of their non-toxic nature with no to very few side effects and readily available at a reasonable cost. In the present chapter, we will discuss the importance of plant molecular pharming (PMP) with its perspective on human diseases. Several advantages of PMP in relation to the United Nations' sustainable development goals (SDGs) will also be deliberated.

Keywords: COVID-19, Human diseases, Plant molecular pharming, Sustainable development goals, World health organization.

1. INTRODUCTION

Kingdom Plantae is a huge source of biologically active molecules and compounds and it is the biggest known source of medicines since pre-historic times. According to the oldest available record (5000-3000 BCE by Sumerians on clay tablets), humans understood diseases and the use of medicines to cure their ailments [1]. Independent of each other, all big ancient civilizations such as China, Greece, and the Arab developed their medical systems, but all of them

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primarily depended on plants and plant-derived components [2]. To fight against natural selection pressure, human beings always turned to the plants, either to make them a source of food or to fight against pandemics. Along with the bigger civilizations, the smaller communities in Asia, Africa, and Latin America have a known history of reliance on traditional medicaments that are largely based on plants for prompt access to a rather safe, cost-effective, competent, and culturally acceptable solution to the primary health care [3].

Since ancient times, people around the world have relied primarily on plants to fulfill all their medicinal needs, for alleviating ailments, and discovering a cure to relieve pain and discomfort. The early man was encouraged to explore his immediate natural environment and try many plant and animal products, minerals, and a range of therapeutic agents [4]. Plants play an appealing role in the modulation of human and non-human diseases [5]. More than 75% of the medicines to control infectious diseases are of plant origin, whereas about 61% of the drugs approved by the FDA are either isolated completely from plants or are the derivative of plant-based active compounds. Not only in primitive times, but plants are also still the best source of food and medicine [6]. According to the World Health organization (WHO), about 80% of the population of the developed and developing countries believes in traditional medicines or plant-derived drugs for their primary health needs [7]. The era of pharmaceutical sciences and industries is well developed now and growing rapidly, and the market is being introduced to a variety of synthetic drugs. The modern pharmacopeia contains at least 25% of drugs that are plant derivatives or semi-synthetic, made up of prototype compounds derived from plants [8]. The requisition of plant-based medicine is increasing day by day because of the flourishing cognizance of natural products, being nontoxic, with few side effects. These are readily available at a reasonable cost and sometimes the only available source of health care available to the poor or low-income communities. Hence, plant-based medical practices hold an imperative spot in the socio-cultural and economic values of both developing and developed countries traditionally [9]. There is an undisputed belief that ‘green medicine’ is better than synthetic drugs. Since the last decade, an upsurge in the utilization of herbal medicines has been seen even in industrialized countries [10]. It is reported that over 90% of medicinal plants utilized by the pharmaceutical industries are collected in their raw form from natural sources. In about 800 species utilized by industries, less than 20 species of plants are cultivated commercially [11]. Hence, the plant collection for pharmaceutical purposes involves destructive harvesting. This massive harvesting is a big threat to the reservoirs and natural diversity of medicinal plant resources and ultimately to the economy of the country if the biodiversity is not sustainably used. The commercial cultivation of medicinal plants is more appropriate for use in the production of drugs [12].

Molecular pharming presents the best alternative to produce economic modern medicines and ensure their large-scale availability around the globe. Molecular pharming is defined as the synthesis of recombinant pharmaceutical products using plants. The field of plant molecular pharming emerged in the early 1990s as a subdivision of plant biotechnology, and the prime purpose was widely scalable production of recombinant therapeutic proteins at an affordable cost [13]. One of the main characteristics of plant molecular pharming is that it includes diverse platforms and technologies developed with recognizable and overlapping interests, linked only by the utilization of plants as bio-factory. Similarly, it includes plant cells or only the part of plants growing in small containers on the synthetic nutrient media or the plant grown on the soil at a large scale, containing a stably integrated transgene for the expression of recombinant protein [14]. Plant molecular pharming has existed since the successful transformation of the first higher plant in 1983 [15]. The expression of the first human antibodies in the plants was done by During [16] and was broaden to express secretory antibodies by Hiatt *et al*. [17]. The first protein (Avidin) expressed in plants for the commercialization purpose was done by Hood *et al*. [18], followed by Aprotinin (used as an anti-inflammatory and wound healing agent), a recombinant pharmaceutical drug [19].

For the last two decades, plant molecular pharming has been resuscitated by the commercialization of biopharmaceutical products. A significant breakthrough was achieved when the first-ever molecular pharming product enzyme taliglucerase alfa was approved for human use in 2012. This was the recombinant form of human glucocerebrosidase designed and developed by Protalix Biotherapeutics® for the treatment of Gaucher's disease, a lysosomal storage disorder [20]. Two more plant-derived pharmaceutical products approved by the European Union were insulin expressed in safflower developed by SemBioSys Genetics and HIV-neutralizing monoclonal antibody expressed in tobacco by a publicly funded consortium (Pharma-Planta; http://www.pharma-planta.net). In the production of both recombinant products, the protocols adapted were following the advanced GMP (pharmaceutical good manufacturing practice) [21].

A plant-based system has several advantages, mainly safety and high expression of recombinant proteins [22, 23]. During the Ebola outbreak in 2014, Zmapp (a plant-made monoclonal antibody cocktail) has shown the ability to fight against this disease [24]. The most promising candidates are the influenza vaccines developed by Medicago Inc. that rely on using a non-replicative vector carrying viral regulatory sequences to mediate the transient expression of Hemagglutinin (HA) in *N. benthamiana*, which has led to injectable vaccine candidates [25, 26]. Recently, functional single-chain monoclonal antibodies are also produced in plant systems, providing simpler molecules for viral neutralization for rabies [27].

CHAPTER 10

Plant Molecular Farming for Human Therapeutics: Recent Advances and Future Prospects

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Abstract: Plant molecular farming (PMF) aims to develop plants that express and accumulate proteins of our interest in considerable quantities. Transgenic plants produce edible vaccines, antibodies, therapeutic proteins for human and animal health, and other recombinant proteins required for industrial purposes. Plant systems (PS) to produce pharmaceutical products are preferred over microbial and mammalian systems as they require less input to grow and produce higher biomass. Hence, a variety of proteins are synthesized by plants that are completely free from human pathogens and mammalian toxins. Additionally, they have immunity against infectious and other life-threatening diseases such as cancer. In this review, plant-inferred therapeutic and non-therapeutic protein items that are in the position of clinical progression or commercialization are summarized. Available plant production platforms are also compared along with associated biosafety and regulatory issues. Further, plant transformation techniques are also analyzed for the development of genetically modified organisms in vaccine production. The use of PMF on a commercial scale is still a long way to go before it is achievable. New methods and techniques are needed to be developed to solve the problems of low yield, scalability, stability, and efficacy of the recombinant proteins, as well as biosafety and regulatory issues. Hence, this strategy will be the ultimate proposed solution to protect humans and animals from health threats in the future.

Keywords: Genetic transformation, Plant molecular farming, Pharmaceutical products, Plant systems, Recombinant Proteins, Transient Expression.

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1. INTRODUCTION

The production of many useful products and recombinant proteins from genetically modified plants is known as plant molecular farming. The term has many other names such as pharming, plant-made pharmaceuticals, plant bio-pharming, bio-manufacturing, bio-pharmaceuticals, plant-derived products of interest, and plants with novel traits [1]. It is a unique application of genetic engineering as it involves plants to manufacture different valuable proteins at the industrial level. Recombinant DNA technology has made it possible to isolate a gene of interest from any organism and its transformation in the plant expression system to alter the specific trait. A worldwide increase in demand for therapeutic products leads the industries and governments towards an alternative approach of pharmaceutical production, plant systems (PS). This basic research field has global worth in the future [2].

The concept of using genetically modified plants is not recent; it dates back to 1986, when tobacco was genetically modified for the first time to produce a human growth hormone [3]. Human serum albumin is reported to be the second plant-derived product obtained from genetically modified tobacco and sunflower [4]. One big breakthrough in developing the field of PMF was brought about in 2012 when the first plant-derived recombinant protein "taliglucerase alfa", a recombinant human glucocerebrosidase was synthesized and developed by Protalix Bio-therapeutics. Later on, that was approved for clinical trials for the treatment of Gaucher's disease. Afterward, many other plant-derived products, such as antibodies, vaccines, enzymes, biocatalysts, biosensors, diagnostic reagents, growth factors, and cosmetic reagents, were commercialized [5].

Several living systems like yeast, bacterial, and animal cell cultures are being used for the production of recombinant proteins. Methods of extraction and purification, along with cold storage, short shelf-lives, and transportation, make these production systems expensive [6, 7]. Plants can be used as "bioreactors" and can replace fermenters, which will reduce the upstream facility and processing of plant tissues for the oral delivery of edible vaccines. This will reduce the downstream processing [8]. The production cost of biological molecules by using plants is less as compared to other systems [9, 10]. Moreover, they also minimize the health risks with additional benefits of high stability of recombinant proteins at large-scale production [11]. The advantage of the production of therapeutically active bio-molecules in edible crops is that they are taken orally as a regular diet, without any hesitation or change in daily habits. It also offers swift scale-up and suitable storage of unprocessed plant materials. The life-threatening diseases and infections in humans and animals due to viruses, bacteria, and other pathogens are now possible to treat with edible vaccines [12, 13]. The availability of cheaper,

easy to consume, and easy to store plant products (seeds, fruits, leaves, *etc*.) that contained edible recombinant proteins, made it possible to prevent life-threatening diseases such as HIV, HBV, *etc*. [14, 15].

Presently, PMF is becoming a very profitable industry with the synthesis of several new recombinant proteins, and many biotechnological companies and governments of different countries are adopting it. Profit associated with PMF can be envisaged by comparing the development of Bt-cotton with that of Bt-corn. An international PMF society has also been formed, and it will become a foundation to support the production of recombinant proteins from PS [16]. In upcoming years, the main focus of scientists will be the development of pharmaceutical products from PS against complex diseases like metabolic disorders, infectious and neurodegenerative diseases and cancer, *etc*. [17].

The current study summarizes the applications and benefits of PMF associated with health issues in humans and animals. It also summarizes available PS, their benefits and limitations, and the solutions to solve these limitations in the future. It also covers health and environmental concerns associated with PMF and different approaches to minimize these limitations so that PMF products may not be rejected by Genetically Modified Organisms' legislation bodies and the general public. Furthermore, it also covers available methods of genetic transformation to develop transgenic plants that synthesize the required proteins on a small as well as on a commercial scale because benefits associated with PMF will only be realized when production is taken up at a commercial scale.

2. SIGNIFICANCE OF PLANT MOLECULAR FARMING (PMF) APPLICATIONS

Several reports have shown benefits associated with PMF in comparison with other production systems [18]. The pattern of protein synthesis in plants is not very much different from the mammalian pattern but plant systems are very cost-effective. Plants also have protein disulfide isomerases and certain chaperons that ensure proper folding and assembly of native as well as non-native proteins and this capability makes them superior over bacterial production platforms [19]. Types of post-transcriptional modifications specifically N- and O- type glycosylation offered by different plant species make PS the most suitable and cost-effective platforms for the synthesis of human-like proteins as well as bio-betters. Such modifications play their role in efficiency, storage as well as downstream processing [20]. Production cost is also reduced to a considerable extent because downstream processing is not required in some cases like edible vaccines and it is also free from human pathogens. Many protein products are

CHAPTER 11

Proteins and Peptides as Biomarkers for Diagnosis of Cardiovascular Diseases

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Abstract: With the increase in the prevalence of cardiovascular diseases internationally, particularly cardiac failure (CF) and atherosclerosis, the investigation for new biological markers remains one of the main priorities. In contrast to complicated diagnostic methods that might not be appropriate to be employed on a larger population, biological markers are effective for the screening of the population. Owing to their non-invasive detection with typically high accuracy and sensitivity, circulating biomarkers have become increasingly significant for routine medical practice. Cardiac troponins and natriuretic peptides (NPs), specifically brain NP (BNP), mid-regional pro arterial NP, and N-terminal (NT) pro BNP, are validated blood biological markers in the diagnosis of CF and prediction of CF-associated outcomes. Inflammatory proteins like C-reactive protein can also have increased importance in anti-inflammatory treatment guidance. Moreover, next-generation biological markers like galectin-3, growth/differentiation factor 15, diverse miRNAs, and soluble suppression of tumorigenicity-2 might have additional value in the analysis of ventricular remodeling and differentiation of CF subtypes. In this chapter, we will first discuss the biological markers as per the major categories of cardiovascular disease, *i.e*., myocardial stress, inflammation, plaque instability, myocardial injury, systemic stress, calcium homeostasis, and platelet activation. Lastly, we will describe the multi-marker methods, including various combinations of novel and established biomarkers that may improve the risk prediction of CF at the population level.

Keywords: Acute cardiac infractions, Cardiovascular diseases (CVD), Cardiac failure (CF), Congenital heart diseases (CHD), Natriuretic peptides (NPs).

1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death internationally, in

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both developing as well as industrial countries, and involves arteriosclerotic (for example, peripheral arterial disease (PAD), cerebrovascular disease, coronary heart disease (CHD)), and nonarteriooosclerotic disease (venous thromboembolism, congenital heart defect (CHD) and valvular disease). The incidence of CVD rises with age, and numerous risk factors, like obesity, alcohol abuse, physical inactivity, and tobacco use, are involved in the progression and development of CVD [1]. Moreover, an additional increase by almost 50% is expected till 2030, thus rising social and economic challenges with cost explosion in the coming decades [2]. The lifetime risk for cardiac failure (CF) is still 20-45 percent and highly age-dependent [3]. The rates of CF hospitalization declined significantly over the years; however, the one-year mortality rate due to CF has not yet substantially improved [4].

One key issue in the development of preventive approaches is that modern risk assessment tools have reduced predictive capacity and undergo miscalibration when employed on various population groups. Biomarkers have been increasingly attracting the scientist, as they enable the non-invasive identification of high-risk patients. The development of unique and highly prognostic CVD biomarkers possesses the potential of improving risk stratification and facilitate the targeted prevention approaches in the pre-clinical phase when treatment would most probably be effective. The use of markers in a clinical setting is also recommended in modern CF guidelines, which indicates their growing importance in the field of CF. Furthermore, circulating markers can provide valuable information regarding causal pathways involved in disease and possess the potential of pathway-targeted treatments and personalized treatment approaches.

This chapter aims to describe both the novel as well as established biological markers in CVD risk assessment. Biological markers will be characterized based on various pathophysiologic processes they indicated and placed within the framework of unmet needs in the area of CVD risk estimation and prevention.

2. BIOMARKERS FOR CVD

A biological marker can perform various functions when utilized within a clinical setting. It is described as a “characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” and performs a variety of functions according to different phases in the evolution of disease (Fig. **1**) [5]. Therefore, biomarkers are considered as an indicator of a disease state, disease rate, or disease trait [6]. A biological marker for cardiovascular disease is not restricted to a particular molecule, for example, RNA, metabolite, or protein, measured within a biospecimen like tissue or bodily fluids (cerebrospinal fluids,

urine, plasma), but also a determination of various physical parameters, for example blood pressure, echocardiogram or electrocardiogram.

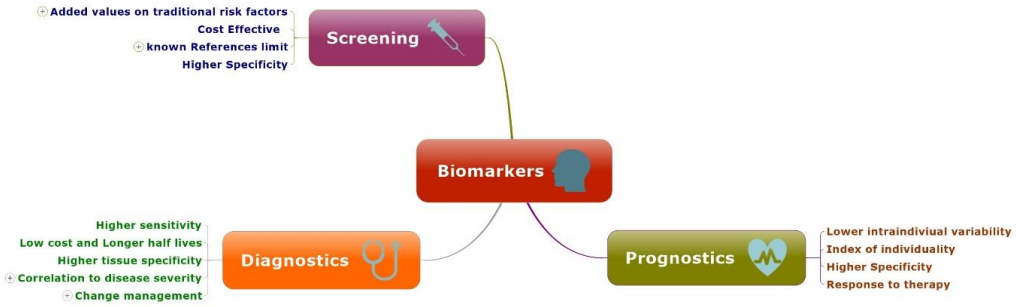

Fig (1). Characteristics and use of Biomarkers.

Preferably, a CVD biomarker should improve the capability of the clinician to treat the affected patient in the best possible way. Though numerous studies have suggested many protein markers for cardiovascular diseases, only a few of them have been employed successfully within cardiology practice – most of them are used for diagnostic purpose (Table **1**).

Table 1. Commonly used diagnostic biomarkers for CVDs.

Biomarkers	Pathology	References
Mammary derived growth inhibitor	Critical illness	[114]
	Myocardial infarction	[115]
Cardiac troponins	Coronary heart disease	[116]
	Myocardial infarction	[29]
Copeptin	Critical illness	[117]
	Myocardial infarction	[118]
Atrial natriuretic peptide	Ischemic stroke	[119]
Brain natriuretic peptide	Heart failure	[120]
Myosin-binding protein-C	Myocardial infarction	[121]

Biomarkers for cardiovascular disease are usually categorized according to various pathological processes they indicate. As many biological markers reflect numerous pathophysiological processes involving extra-cardiac pathology (for example, kidney failure), this method can result in oversimplification. Yet, to be in accordance with the current research, the cardiac biological markers in this chapter are categorized based on the major classes of CVDs, *i.e.*, myocardial stress, myocardial injury, inflammation, plaque instability, calcium homeostasis,

SECTION III: Molecular Pharming for Animals

CHAPTER 12

Veterinary Nutraceutics, Pharmaceutics and Vaccine

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Abstract: Animals have been utilized extensively as part and parcel of pharmaceutical and vaccine development. Many studies from animal models for human diseases have re-affirmed inventions in the field of medicine. Animal nutraceuticals of biological origin have marked exceptional promise by enhancing the production and performance of commercial animals. Transgenic animals have helped transform laboratory-scale developments into clinical applications. The nutraceutical potential of animal products is a fascinating area of research with considerable anti-microbial, anti-cancer, anti-inflammatory, anti-diabetic and neuroprotective functions. Vaccines in veterinary sciences have been revolutionized based on the efficacy demonstrated by animal models. Vaccines are being routinely used against bacteria, viruses and some parasites at commercial levels. Third-generation vaccines that were thought to be very expensive

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in the last century are now being commercially produced and marketed worldwide for animal health. Most recently, many avenues have opened that encourage the use of biologically derived pharmaceuticals and vaccine products. This chapter deals with a very comprehensive contrast of history and recent trends in veterinary pharmaceuticals and vaccines. It concludes that more research focus is required to come up with more efficient treatment and prophylactic approaches amidst mutating pathogens of concern.

Keywords: Animal peptides and proteins, Nutraceutics, Passive immunization, Pharmaceutics, Vaccines.

1. INTRODUCTION

Over the 300 years of the history of biomedical and pharmaceutical work, animals are of prime importance in regard to testing models as well as the source of pharmaceutical products [1]. It is an essential part of toxicological studies to utilize animal paradigms to explore the pharmaceutical pros and cons of the underlined products [2]. Animal carcass and its by-products have been used as a potent medicinally important biochemical for a long time. These biochemicals are highly labile to proteolytic enzymes, microbial as well as mal-handling degradation, which renders their quality and quantity. Market analyses showed that animal health pharmaceutics has emerged as a progressive and proactive industry in world-leading forums [3]. Animal pharmaceutics has a collection of nutrition, reproduction, and production-related entities; entitled in Chinese, herbal and modern medications [4]. After the down shock from Transmissible Bovine Spongiform Encephalopathy (TBSE), quality maintenance has become an optimistic point of concern in the animal pharmaceuticals and veterinary products market. Provision and critical analysis of the anatomical, biological, and geographical justification of animal products and by-products are of prime importance in the drug development industry. Reports from the Swiss Market showed 438 out of 655 pharmaceutical products of animal origin undergone the strict screening of TBSE [5]. Another study revealed that 530 out of 535 laboratory synthesized pharmaceutical products in the market are positive to at least one carcinogenic testing assay. Moreover, about half of the pharmaceutical ingredients used in Germany are reported to be of environmental relevance and are potent contaminants. This indicates the safety ranges of artificial products and empowers the concept of utilizing natural resources in this regard [6].

Pharmaceutical proteins of animal origin have been used since the 1920s, when insulin was extracted from the pig pancreas for human use. Later in the 1980s, this job was done using biotechnologically prepared recombinant microbes. In 2006, the pharmacologically active protein “antithrombin III” was approved as a medicinal product by European Medicinal Evaluation agencies. Other

pharmaceutically important proteins include human growth factors, hormones, coagulation factors, monoclonal antibodies, interferons, enzymes, and collagen [7].

2. VETERINARY ORIGIN NUTRACEUTICS

Milk peptides are proven excellent dietary supplements as well as potent pharmaceutics. According to numerous studies, milk contains several biologically active peptides that can enhance antibacterial activity. These peptides have a variety of physiological functions, including metabolic, immunomodulatory, antibacterial, thrombolytic, and prebiotic/probiotic functions [8]. Alpha1-casei--derived peptides are the first antibacterial peptides to be effective against a wide variety of gram-positive bacteria, including *Staphylococcus aureus*. The N-terminal α1-casein peptide can be used *in vitro* to counteract lactobacilli and gram-positive bacteria. It also provides effective protection against *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Listeria monocytogenes* [9]. Therefore, according to the literature, these biologically active peptides change their biological function due to their ability to bind specific receptors to target cells and induce various biological reactions in the host organism, as shown in Table **1**. Bovine casein can produce peptides such as SKVLPVPQK (β-CN; f168-176), YQKFPQY (αs2-CN; f89-95), LPYPYY (κ-CN; f56-61), LPQNIPPL (β-CN; f70). I will. -77), FLPYPYY (κ-CN; f55-61), and two novel angiotensin-converting enzyme (ACE) inhibitor peptides [10]. Compared to milk casein, milk casein contains more α-S2 and β casein than κ180 casein. Alpha S2-casein consists of two main components and several secondary components with varying degrees of post-translational phosphorylation. Kappa-casein can inhibit toxin invasion and pathogenic adhesion to the cell wall and protect cells from infections mediated by *Porphyromonas gingivalis*, *Salmonella, Rhodococcus*, *Streptococcus mutans*. It is hydrolyzed by mineral binding peptide 1 and binds zinc and iron ions to form a soluble dialyzable complex. This product is best at removing 2,2-diphenyl-1-pyridhydrazino (DPPH), which strengthens the immune system [11].

Table 1. Therapeutic usage of milk constituents [11 - 19].

Sr. No.	Milk Derived Bio-Active Constituents	Action Spectrum
1.	Opioid peptides	Agonist of opioid receptors
2.	Angiotensin inhibitory peptides	Antihypertensive effect
3.	Antimicrobial peptides	Bactericidal
4.	Immunogenic casein peptides	Lymphocytic and macrophage proliferation
5.	Antithrombotic peptides	Inhibit fibrin binding and platelets aggregation

CHAPTER 13

Plant Molecular Pharming For Livestock And Poultry

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Abstract: Plants are exploited as bioreactors for the cost-effective production of pharmaceuticals, predominantly for the expression and accumulation of antigenic proteins, to be used as vaccines for livestock and poultry. Due to the high body mass of large animals and large population of poultry and other birds, a larger quantity of vaccines is needed continuously. It increases the production costs of vaccines for these animals. Under high biomass production ability, plants represent promising biofactory with added advantages of pathogen-free production of desired proteins in bulk quantities. Hence, plant-based transient, as well as stable expression systems, have been exceedingly applied to express immunogenic proteins. We have been using various plants like soybean and *Trifolium* to produce edible vaccines for poultry and livestock, respectively. Here we have reviewed various types of vaccines with a special focus on their plant-based expression with examples of Infectious Bursal Disease (IBD), New Castle Disease (ND), and Foot and Mouth Disease (FMD).

Keywords: Biopharming, Molecular pharming, IBD, ND, FMD, Vaccine, Edible vaccine, DNA vaccine, Subunit vaccine.

1. INTRODUCTION

Plants have been used for medicinal purposes since ancient times. More than 120 plant-derived drugs/pharmaceuticals have been approved for commercial utilization. Plant-derived pharmaceuticals include pain killers, wound healers, diagnostic reagents, antibiotics, therapeutic proteins, antimalarial and anticancer drugs [1]. These exogenous proteins are mostly produced as monoclonal antibodies, drugs, and vaccines not only to treat diseases but also to provide

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additive nutrients. Plant molecular farming gained more importance after the production of insulin using a bacterial expression system in 1982 [2]. Since then, numerous medicinal products have been produced by using different expression systems, *i.e*., mammalian cell lines, insect cell lines, and yeast cell lines. Compared to the conventionally produced pharmaceuticals, recombinant therapeutic proteins can be complex with an intricate mode of action. Owing to the involvement of complex biochemical pathways, their chemical synthesis is quite difficult and has numerous limitations so they must be produced in a living system to exploit the host cellular machinery for protein synthesis [3]. Each of the production systems has certain advantages and pitfalls regarding production cost, risks of contamination, post-translational modifications, downstream processing cost, regulatory and approval issues, *etc*.

Most of the commercial pharmaceuticals are produced through mammalian cell lines, yet the plant expression system has its significance [4]. Pioneering research on plant expression systems (plant-based pharmaceuticals) has proved its competitiveness for commercial applications. Compared to other production platforms, plants have several distinctive attributes to produce a wide variety of valuable pharmaceuticals. Various industrial enzymes, vaccine candidates, and monoclonal antibodies have been successfully expressed in plants because they are easy to scale up and do not require capital investment for infrastructure. They can be cultivated in a greenhouse or open fields, and as a result, the cost of production is very low. They are also free from human and animal pathogens; therefore, they are biosafe with limited chances of contamination [5]. Furthermore, plants can produce recombinant proteins with proper post-translational modifications. Hence, effective recombinant therapeutic proteins can be produced at the commercial level in a short period [6 - 8].

Elelyso® was the first recombinant therapeutic protein that was commercially produced in plants in 2012 and was approved for enzyme replacement therapy. Numerous plant species have been explored to produce various proteins of pharmaceutical importance. The most notable are: production of diagnostic reagent (avidin) in maize, commodity chemicals in rice, veterinary medicine in strawberry, and nutraceuticals in barley [9]. A large number of whole plant-produced pharmaceuticals are in pipeline for commercialization, *i.e*., influenza vaccine is in 3rd phase of the trial. A large number of proteins including pharmaceuticals have been attempted to be produced through plant expression systems. This chapter highlights the use of plants as a valuable source for the production of biopharmaceuticals with special emphasis on the proteins of worth for livestock and poultry. Further, comparisons among different expression systems and transmission of diseases from animals to humans have also been discussed.

2. GLOBAL IMPORTANCE OF LIVESTOCK AND POULTRY SECTORS

Animal-derived food demand has drastically increased owing to the rapidly increasing population, increased income, and urbanization. The livestock species play a critical role not only to fulfill protein needs but also in the well-being of poor farmers, all over the world [10]. Besides meat and milk, several other products (animal fat, skin, hides, and horns) are used for domestic consumption as well as for the manufacture of various industrial products. Total world meat production has increased to 342.42 million tonnes where major contributors are poultry, pork, cow, buffalo, sheep, goat, camel, horse, and ducks. In 2018, approximately 302 million cattle, 479 million goats, 574 million sheep, 456 million turkeys, 1.5 billion pigs, and 69 billion chickens were killed or slaughtered to obtain meat. Likewise, milk production has increased to 800 million tonnes, but still, there is a continuous increase in the demand for animal protein and milk [11].

Compared with other sources of animal proteins, poultry meat has shown the fastest trend of growth during the last decades. During the last 50 years, the growth rate of poultry meat was 5%, that of beef was 1.5%, for pork it was 3.1%, and for small ruminants, it was 1.7% [12]. This growth trend is even higher in developing countries, particularly South East and East Asia (7.4%). The top-most producer of poultry meat is United States (20 million tons), followed by China (18 million tons), European Union (EU), and Brazil (13 million tons per annum). More than 23 billion poultry birds are present on the planet whereas efforts are in progress for its improvement through breeding and advanced molecular research. Poultry meat and eggs are the most common animal food, consumed all over the world. It is categorized as the most efficient sub-sector regarding protein provision and use of natural resources. Increased per capita consumption is a clear-cut indication of the importance of poultry. Egg consumption has increased from 4.55 kg to 8.92 kg whereas meat consumption has increased from 2.88 kg to 14.13 kg. Though the poultry sector has contributed a lot to ensure food security by providing proteins, energy, and essential nutrients to humans yet further efforts are direly needed to fulfill the ever-increasing demand for eggs and meat [13]. Further, it has widespread implications in the manufacture of non-food products which need to be explored and worked out. Thus, producing more from less without affecting the environment is the key to meet the sharply increasing demands of animal protein and its by-products.

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Muhammad Sarwar Khan

Muhammad Sarwar Khan has an established academic career in Plant Molecular Biology, Biopharming, and Education. He started his career as a graduate student researching nutritious plants' biotechnology and afterwards, he earned his Ph.D. in Plant Molecular Biology specializing in Chloroplast Biotechnology from the University of Cambridge, UK. Dr. Khan was awarded a prestigious postdoctoral fellowship by The Rockefeller Foundation for researching at the Waksman Institute of Microbiology, Rutgers, the State University of New Jersey. His findings—research of first-of-its-kind-was published in Nature Biotechnology. Dr. Khan was appointed as National Coordinator to intermediate level students for participation in the International Biology Olympiads. He served as the founding Group Leader of Chloroplast Biotechnology and Biopharming Group, then as founding head of the Biotech Interdisciplinary Division (BID) at NIBGE, and is currently serving as Professor and Director of the Center of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan. He has served as Director General of the Social Safety Net Program of the Government of Pakistan.

Dr. Khan has supervised more than 100 Ph.D. scholars, MPhil students, and researchers who are now serving at national and international levels in various research institutes and universities. He has vastly published in high-impact journals and is the author of several book chapters and books. His current research interests include the development of plant-based cost-effective therapeutics and edible vaccines for animals. Dr. Khan has received prestigious awards, including the President's Medal for Technology, a Gold Medal in Agriculture from the Pakistan Academy of Sciences, a Performance Gold Medal by PAEC, the Biotechnologist Award by the National Commission of Biotechnology, and the Best University Teacher Award by the Higher Education Commission of Pakistan. He is also a fellow of the Cambridge Commonwealth Society, the Cambridge Philosophical Society, and a member of the International Association for Plant Biotechnology. Currently, he is serving as Editor/Guest Editor on several international research journals and books, being published by publishers like *Springer, Taylors* & *Francis, Bentham,* and *IntechOpen*.