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Inhibition of Bacterial Biofilms Formation and Stability by Modified Proteins

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ABSTRACT **: : Most antibiotics are ineffective against multidrug-resistant bacteria. Therefore, in great demand are innovative natural chemicals that can suppress and remove their biofilms. The 11S globulin isolated from lupine seeds (Lupinus termis) and its methylated derivative (M11S) inhibits the pathogenic bacteria (Gram-positive, G+) such as Listeria ivanovii LMZ11352, Listeria monocytogenes LMG10470, and Staphylococcus aureus DSM1104, and (Gram-negative, G−) including Klebsiella oxytoca LMG3055, Pseudomonas aeruginosa LMG8029, Proteus mirabilis, and Salmonella typhi LMG10395. 11S and M11S fractions triggered possible morphological alterations in both Staphylococcus aureus DSM1104 (G+) and Klebsiella oxytoca LMG3055 (G−) and were mainly characterized by cell deformation, cytoplasmic contents leaking, and destruction of the biofilm. Furthermore, the methylation of 11S generates antibacterial and antibiofilm cationic peptides against all tested G+ pathogenic bacteria.**

KEYWORDS: **11S; Antibiofilm; Lupine; Methylation; Globulin; Antibiotics**

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I. INTRODUCTION

Antimicrobial resistance and the effectiveness of antibiotics are challenges for modern medicine because of the rapidly emerging resistant bacteria. Approximately 0.7 million deaths occur annually as a result of antibacterial resistance-related problems **(O'Neill, 2016)**. Biofilms have been defined as aggregates of microorganisms in an extracellular matrix, where cells adhere to the surface, are made of extra polymeric substances (EPS), and where cells are embedded and protected **(Speranza & Corbo, 2017)**. EPS acts as a glue to keep biofilm-bacterial communities connected and protect them against the host immune system and different environmental challenges. Bacteria can embed themselves in an EPS matrix consisting of proteins, humic acids, eDNA, and polysaccharides **(Flemming et al., 2016)**. Three-dimensional structures, adhesion to one another, decreased antimicrobial resistance, adhesion to distinct interfaces, adhesion to surfaces, and host defense systems are essential survival properties of biofilms **(Shay et al., 2022)**.

The continuous increase in highly multidrug-resistant pathogenic bacteria needs new remedial options. Many years ago, scientists discussed the antibacterial activities of herbs, spices, basic proteins, and herbal extracts. Biodiversity in plants is regarded as a significant resource of biochemical substances with several medicinal uses, such as antiviral, antibacterial, anticancer, and antifungal properties (Osman et al., 2014). In many regions around the world, legumes are important in traditional diets. In particular, compared to beans and peas, lupine seeds have a higher protein level (about 34% total protein concentration) and almost no starch.

Lupine seeds have a 9:1 quantitative ratio of globulins to albumin, the two primary protein components used as a nutraceutical and functional ingredients. Globulin, β-conglutin, and a smaller protein fraction γ-conglutin their functions are not yet understood **(Abdel-Shafi et al., 2022)**. Soybean protein components may have

comparable potential uses and functions to those of other legumes due to their similarity. Antimicrobial peptides may be abundant in lupine proteins **(Osman et al., 2016)**.

Chemical alteration of proteins, which is used to obtain new cationic proteins by using esterification, is one strategy proposed to increase their antibacterial effects. Esterification, which is regarded as an essential and simple method for protein modification, inhibits free carboxylic acid functional groups, increasing the net +ve charge and basifying the altered protein **(Abdel-Shafi et al., 2019)**.Protein and peptide compounds' antibacterial and antimicrobial activities are enhanced by increasing their positive charge. By raising the net +ve charge on the changed protein structures, amidation may increase the effectiveness of bovine lactoferrin versus a broad range of G^- and G^+ bacteria **(Pan et al., 2007)**. These results originate from the antimicrobial protein's or peptide's interactions with the membrane and wall components of the bacterium **(Sitohy et al., 2013)**.

2. Biofilm formation

The biofilm, as described by **(Oliveira *et al.*, 2015)**, is an example of collaborative creativity that facilitates the collaboration of different strains and species. The quorum sensing process, whereby microorganisms use signals like AHL (acyl homoserine lactone) in G^- bacteria, was shown to be responsible for the cooperative behavior seen in the microbial community. According to the magnitude of the bacterial populations, the quorum sensing (QS) system regulates the gene expression profile of bacteria, resulting in the creation of many types of biofilm **(Płusa, 2019).** The genera1 quorum sensing technology allows bacteria to create and identify signaling substances, enabling them to synchronize their actions based on population density **(Brackman & Coenye, 2014)**.

Consequently, horizontal gene transport among microorganisms and biofilm growth are connected **(Madsen *et al.*, 2012)**. To transform from their mobile state to their immobile state, microorganisms must experience a variety of structural and physiological alterations **(Maunders & Welch, 2017)**. Consequently, these dynamical processes include (I) adhesion to the surface, (II) non-reversible adhesion, (III) microcolony or initial structural formation of biofilm, (IV) biofilm maturation which generates a tower-like or mushroom morphology, and (V) dissociation, whereby cells shed their matrix and resume their natural, mobile state (Dufour et al., 2010). Natural legume proteins and their methylation were employed as new natural agents to regulate the production of bacterial biofilm. Recent difficulties brought on by bacterial biofilm are discussed in this review.

1. Biofilm and the influence it has on antibiotic resistance

The most important health problems worldwide are bacterial antimicrobial resistance's development and spread **(Zhang *et al.*, 2018).** Besides contributing to the persistence of chronic infections, bacterial biofilm populations have been linked to the development of resistance to antimicrobial treatments **(Cepas *et al.*, 2019)**. *Klebsiella pneumoniae* is a major human pathogen that is both MDR and biofilm-forming. It is a major source of hospital infectious diseases associated with high mortality and morbidity due to a lack of effective therapeutic options **(Navon-Venezia *et al.*, 2017).** Antibiotic-resistant biofilms harboring serious adverse bacteria are a major cause of persistent infections worldwide **(Verderosa *et al.*, 2019)**. This is due to the fact that when bacteria form biofilms, they encapsulate themselves in a matrix, which might make them more difficult to kill or control using conventional methods **(Khatoon *et al.*, 2018).**

Bacteria are protected from bactericidal drugs by a matrix made of EPS **(Lebeaux *et al.*, 2014)**. Biofilm bacteria are significantly more resistant to antibiotics than planktonic bacteria by several orders of magnitude **(Rabin *et al.*, 2015)**. Biofilms are resistant to antimicrobials because of their architecture and other biological modifications, such as a sluggish development rate **(Chadha, 2014).** According to reports, the following elements contribute to the biofilm-based bacterial susceptibility: (a) the ability of a polymeric matrix to impede the spread of antibiotics, (b) antibacterial ineffectiveness due to polymer matrix interaction, (c) resistance caused by an enzyme, such as beta-lactamase, (d) alteration of metabolic processes inside the biofilm, (e) gene altering on the targeted cells or covering up the target locations, (f) antimicrobial medication flushed out with the help of efflux pumps **(Høiby *et al.*, 2010),** and (g) the existence of an outer cell membrane, as in G^- bacteria **(Singh *et al.*, 2017).**

Because of their rapid mutation rate, biofilm-forming bacteria can evolve resistance mechanisms; these bacteria's genes may then encode enzymes that inactivate antibiotics or pump them out of the cell utilizing efflux pumps **(Dzianach *et al.*, 2019)**. Biofilm-forming bacteria have many ways of evading antibiotics, including the production of metabolically dormant persister cells and the capacity to live in environments with very high

antibiotic concentrations **(Wood *et al.*, 2013)**. Due to the high concentration of microorganisms inside the matrix, close proximity between them promotes the transmission of resistance genes across microorganisms. Eventually, the whole community may be infected with the resistant gene **(Balcázar *et al.*, 2015).** Consequently, antibiotic susceptibility is mostly because of the genetic heterogeneity of bacteria in biofilm **(Płusa, 2019)**. Because of the extremely intimate vicinity of cells in this multilayered architecture, biofilm is critical for the transmission of conjugative plasmids, according to studies **(Lécuyer *et al.*, 2018).**

3. The influence of biofilm on the contamination of food

Globally, the contamination of food products by microbial pathogens is a serious concern for human health and causes great financial losses **(Yang *et al.*, 2017)**. Post-processing contamination by microbial biofilm, which includes both microbial deterioration and pathogens in food, reduces product quality, shortens product shelf life, and may even facilitate the transmission of pathogens **(Brooks & Flint, 2008)**. *P. aeruginosa* and *S. aureus* are among the various microorganisms that can generate biofilm on materials and apparatus **(Meesilp & Mesil, 2018)**. In the dairy sector, biofilms generated by bacteria on equipment surfaces may threaten the quality and safety of milk and other milk-based products **(Friedlander *et al.*, 2019)**. It is believed that the formation of biofilms by microorganisms like *L. monocytogenes* contributes to the persistence and resistance of the pathogen throughout the food supply chain **(Lee *et al.*, 2019).** There is a risk of pathogenic bacteria sticking around in food processing areas in the form of a biofilm on various pieces of equipment and machinery **(Chlebicz & Śliżewska, 2018).** Consequently, serious contamination issues in food, food manufacturing, and other sectors immediately harm human health and survival due to biofilm development by dangerous bacteria **(Feng *et al.*, 2015).**

Adhesion of harmful microorganisms to food surfaces may result in potential hygienic issues from a sanitary standpoint since it is resistant to hostile environments and a reservoir for contamination **(Feng *et al.*, 2015)**. In general, developing dangerous bacteria like *S. enterica* and *E. coli* O157: H7 may lead to cross-contamination of edible products and food manufacturing surfaces **(Jun *et al.*, 2010).** Biofilms not only contribute to food contamination but also decrease production and material efficiency within the food processing sector **(Achinas *et al.*, 2019).** In food manufacturing facilities, biofilms enclosed in protective EPS are difficult to eradicate **(Han *et al.*, 2017).**

4. Methods to prevent, limit, manage, and eliminate the production of biofilms on foods

These include physical and chemical techniques, sanitizers, and disinfectants, among others **(Sadekuzzaman *et al.*, 2015**). By the following processes, antimicrobial peptides suppress biofilm development and are hence efficient: (a) destruction of the membrane around bacterial cells, (b) blocking off their means of signaling or communicating with one another, (c) destroying the polymeric structure, (d) preventing a bacterial reaction by inhibiting the alarmone system, and (f) decreasing the expression of biofilm-critical genes **(Yu *et al.*, 2018).**

5. Gram-positive bacterial biofilm formation

A) *Staphylococcus aureus*

Skin infections are most often caused by *S. aureus*. It is a common component of nasal and skin flora and has a spherical shape. *S. aureus* is carried by about 20% of the population on a chronic basis. In addition, *S. aureus* may cause a wide variety of disorders, from relatively harmless skin infections like scalded skin syndrome, impetigo, carbuncles, pimples, cellulitis folliculitis, boils (furuncles), and blisters, to life-threatening illnesses like toxic shock syndrome, meningitis, pneumonia, osteomyelitis, bacteremia, endocarditis, and septicemia. Pathogens of the skin, soft tissues, respiratory tract, joints, bones, and endovascular system are common causes. It continues to be one of the top five sources of nosocomial infections, often manifesting as wound infections after surgery **(Kluytmans *et al.*, 1997; Enan *et al.*, 2020).**

S. aureus is extensively dispersed in the natural world and is carried by 25 to 33 percent of healthy persons in the anterior skin. It colonizes and infects both healthy immunocompetent individuals in the community and immunocompromised hospitalized patients. Hospital-acquired *S. aureus* is among the most frequent and major G^+ infections. It has a great ability to colonize atypical skin surfaces and open wounds, where it may stay without causing illness **(Rybak & Pharm.D, 2005).** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that triggers many complicated human diseases. MRSA is frequent in healthcare institutions, jails, and nursing residences, where people with open wounds, invasive devices, and impaired immune functions are prominent. MRSA is more resistant to infection than the normal population.

B) *Listeria ivanovii*

Infection with *L. ivanovii* may result in septicemic illness, neonatal sepsis, and even abortion. Therefore, pregnant ruminants are in the greatest danger. In Paris, France, a male in his 55th year was admitted to a hospital in January 2007, and upon evaluation, he was suspected of having listeriosis. Blood and stool samples were collected. *L. ivanovii* was determined to be an intestinal opportunistic human pathogen **(Guillet *et al*., 2010).**

C) *Listeria monocytogenes*

Listeria monocytogenes is the bacterium responsible for listeriosis, the most lethal food-borne illness in humans, with a 20 to 30 percent fatality rate, is a naturally occurring intracellular Gram-positive organism. After ingesting a large inoculum of listeria, a person may experience gastroenteritis; in immunocompromised patients, listeria may cause meningitis, septicemia, and encephalitis; and in pregnant women, the infection of the fetus's placenta can result in stillbirth, abortion, premature delivery, and neonatal illness **(Travier *et al*., 2013).**

L. monocytogenes is suited to thrive in a variety of circumstances and to colonize a variety of habitats, particularly as a biofilm. In addition, it's a kind of pathogen called a facultative intracellular pathogen since it may invade cells and spread infection throughout the body. These two complementary features of the biology of *L. monocytogenes* have been studied independently until now. Regardless of its role in illness, this is the first time a virulence factor has been linked to the survival and spread of microorganisms **(Travier *et al*., 2013).**

7. Gram-negative bacterial biofilm formation

A) *Klebsiella oxytoca*

Cultures of *K. oxytoca* may be made from various human and animal tissues, including those found in the mucous membranes, skin, intestines, and oropharynx of both healthy and diseased individuals **(Podschun & Ullmann, 1998).**Regarding humans, 8-10% of the population's feces is fertile ground for growing *K. oxytoca* **(Savino *et al*., 2009).** *K. oxytoca* is currently recognized as a clinically relevant opportunistic pathogen linked with nosocomial infections in hospitalized patients, especially infants and newborns **(Savino *et al*., 2011).**Individuals with bacteremia, septicemia, soft tissue infectious diseases, septic arthritis, urinary infections, cholecystitis, and, more recently, colicky newborns have all had *K. oxytoca* isolated from their systems. Additionally, intestinal overgrowth of *K. oxytoca* was detected in children with celiac disease **(Sánchez *et al*., 2013).**

B) *Pseudomonas aeruginosa*

P. aeruginosa is a common cause of nosocomial infections, including bloodstream infections, UTIs, and pneumonia **(Driscoll *et al*., 2007)**. *P. aeruginosa* is the major bacterium infecting individuals with cystic fibrosis, serving as the principal reason for the devastating pulmonary disease. Compared to other bacterial pathogens, infections produced by *P. aeruginosa* are prevalent and linked with significant illness and death **(Osmon *et al*., 2004)**. Opportunistic bacterium *P. aeruginosa* is responsible for various ailments in immunosuppressed individuals, including respiratory illnesses, dermatitis, bacteremia, soft tissue infections, and a variety of other systemic illnesses. In addition, around 10% of treated individuals acquire resistance to this bacterium during treatment **(Carmeli *et al*., 1999).**

Because these bacteria are pathogenic and have limited antibiotic resistance, hospital-acquired infections triggered by them are often linked with elevated disease and fatality rates **(Harris *et al*., 1999)**. Patients who have suffered burns are more vulnerable to nosocomial pathogens. Burn wounds are prone to colonization by opportunistic bacteria, such as *Pseudomonas aeruginosa* **(Lari & Alaghehbandan, 2000).**

C) *Salmonella typhi*

Typhoid (enteric fever) is a bacterial infection triggered by *Salmonella enterica* serovar Typhi. The illness is systemic and is often caught by consuming contaminated food or drink, typically from a fecal-oral source. The incidence of typhoid fever may indicate inadequate hygienic practices, both individual and ecological. The disease may be moderate or severe but is sometimes deadly **(WHO, 2008).** The illness is a significant public health concern. In Nigeria and other tropical nations where the illness is prevalent, they account for a number of instances of illness and death **(Ibekwe *et al*., 2008).** Enteric fever has persisted as a significant public health problem even with the use of antibiotics and the discovery of innovative antimicrobial drugs.

D) Proteus mirabilis

P. mirabilis is a G^-, a member of the *Enterobacteriaceae* family. It causes infections of wounds, the respiratory tract, burns, the urinary system, and diabetic foot ulcers, among others. Proteus mirabilis is strongly resistant to many medications, which may result in a failure of multidrug resistance and antimicrobial therapy **(Fm *et al*., 2018).**

8. Alternative antimicrobial agents

Due to the rise in bacterial resistance to synthetic antimicrobial agents, the need for natural antimicrobial substances that are effective, non-toxic, and pose a lower environmental risk has expanded dramatically. These resistant microorganisms (like fungi and bacteria) can defend themselves against antimicrobial medications, resulting in inadequate treatment and the spread and persistence of illnesses **(Tanwar *et al.*, 2014)**. This clearly illustrates the necessity to discover new antimicrobial drugs since the growth of antibiotic resistance in bacteria is a major issue confronting civilization today.Novel chemical diversity is mostly derived from natural products. They are considered an indispensable part of the pharmacological sector of today. However, many current antibacterial and antifungal medications carry with them some unwanted toxicity, and the widespread use of these therapies has led to the rapid creation of drug-resistant strains, which is the primary cause of failure in agricultural and therapeutic applications. Thus far, many different microbial substances have been isolated, and many of them with potential medical applications require further research **(Běhal, 2001).**

8-1. Legume protein

Grain legumes are used extensively in food for animals and humans since they are a great and inexpensive source of essential elements like minerals, proteins, vitamins, lipids, and carbohydrates. In addition, among scientists involved in the development of food products, In the past few decades, there has been a rising tendency to use the functional qualities of legume seed proteins in the creation of foods that have historically relied on the functionality of animal proteins for their manufacturing and physicochemical durability. Being the greatest well-known resource of vegetable proteins, soybean protein is also frequently employed as an essential functional food ingredient. However, alternatives to soy protein in food have been explored using protein sources extracted from other grain legumes like faba beans, peas, and lupin **(Gueguen & Cerletti, 1994).**

The rising usage of plant-based proteins in food and non-food sectors has increased the industry's interest in manufacturing plant protein isolates. To lessen its dependence on the supply of protein for commercial reasons, the European Union plans to cultivate its protein sources **(Chominot, 1992)**. In general, protein isolation involves two steps: Alkaline solubilization and precipitation. Plant proteins are precipitated via acid precipitation at the isoelectric pH. In the food business, typically, protein isolates are made by alkaline extraction followed by precipitation at the isoelectric point. The low price of the chemical products and the comparative straightforwardness of the needed equipment make this method preferable to others, like the precipitation of salt-soluble proteins by dilution in water or ultrafiltration membrane separation of proteins **(Davin & Bérot, 1996).**

8-2. Natural proteins and their use as antimicrobial agents

Spices, basic proteins, herbs, and their extracts having antibacterial properties have been explored in this area for many years **(Enan *et al.*, 2020; Osman *et al.*, 2014)**. Chemical substances having antiviral, antifungal, antibacterial, and anticancer activities are abundant in plants due to their biological diversity **(Penalver *et al.*, 2005; Abdel-Shafi *et al.*, 2019; Abdel-Shafi *et al.*, 2020).**

Legumes play a significant part in the traditional diets of several areas of the globe **(Yeheyis *et al.*, 2011).**Specifically, herbaceous lupin seeds are from the *Lupinus* genus, which has more than four hundred and fifty different species. Lupin seeds have been consumed by humans and fed to animals since antiquity. Andean lupin (*Lupinus mutabilis*), blue lupin (*Lupinus angustifolius*), white lupin (*Lupinus albus*), yellow lupin (*Lupinus luteus*), and are four species of agricultural relevance **(Holden *et al.*, 2005).** Compared to other protein-rich legumes, lupin is inexpensive and can be produced in colder climates.; it is also gaining attention as a high-nutritional-value food ingredient **(Magni *et al.*, 2005).**The seeds of the lupin plant are a popular snack food, while the plant itself is used in a variety of baked products, pastries, pasta, sauces, and beverages that replace milk or soy **(Dooper *et al.*, 2006).**

Osborne classified seed storage proteins around the turn of the century into four groups depending on separation and dissolution features: albumins, globulins, glutenins, and prolamins; since then, the proteins of several species' seeds have been studied extensively **(Shewry *et al.*, 1995).** Nevertheless, it is well-known that several proteins have moderate solubility, making it difficult to differentiate them clearly **(Franco *et al.*, 1997).** Alpha-conglutin (11S and "legumin like") and beta-conglutin (7S and "vicilin like") are the two principal globulins that make up the globulin fraction of lupin protein, which together make up around 33% and 45% of its total protein composition **(Ferreira *et al.*, 1999).**

Additional minor globulins found in *L. albus* include gamma- and delta-conglutin, which together make up only 5% and 12% of the total globulin content **(Foss *et al.*, 2006; Brambilla *et al.*, 2008).** Some studies have subclassified delta-conglutin further into delta1- and delta2-conglutin **(Duranti *et al.*, 2008)**. Many strategies involving chemical changes, like esterification, have been suggested to boost proteins' antimicrobial capabilities **(Sitohy & Osman, 2010).**

8-3. Antimicrobial proteins

Plants develop a wide variety of defensive proteins to ward off the onslaught of microorganisms that cause disease. Consequently, several protein families have been discovered, extracted, and proposed as potential antimicrobial drugs due to their antifungal and antibacterial properties **(Kumarasamy *et al*., 2002)**. Antimicrobial cationic proteins or peptides (AMPs) found in plants are a large and varied group of small-molecule proteins that play an important role in the plant's intrinsic protection system by directly preventing microbial growth, reproduction, and dispersal **(Abdel-Shafi *et al*., 2020; Abdel-Shafi *et al*., 2022**). Since AMPs have a net +ve charge, they are able to attach to and pass through the phospholipid membranes (negatively charged) that are characteristic of bacteria **(Zasloff, 2002).**

The widespread and extensive usage of antibiotics gives birth to multidrug-resistant organisms **(Charpentier & Courvalin, 1999)**. Consequently, the search for novel antibacterial medications is an ongoing task. A very basic tiny protein (finotin) was isolated from *Clitoria ternatea* seeds and shown to inhibit the development of several major fungal infections in a wide and powerful manner **(Kelemu *et al*., 2004).** Antibacterial activity against seven bacteria in vitro was shown for small seed basic proteins isolated from *Robinia pseudoacacia L. Rozynskiana* (*Leguminosae*). The plant seed compounds puroindoline A and B, as well as lysozyme and the serum proteins lactoferrin, have been proven to be effective antimicrobials against the bacterium Listeria monocytogenes in experimental animal models **(Palumbo *et al*., 2010)**. However, massive molecular weight proteins, including achacin (56 kDa) and aplysianin (320 KDa), have been discovered in marine organisms and may have antibacterial characteristics. Significant pore-forming antibacterial activity was found in hydrophobic large molecular weight proteins (27 and 31 kDa) isolated from fish **(Erban *et al*., 1999).**

8-4. Mode of action of antimicrobial proteins

Both animals and plants contain cationic antimicrobial peptides, which play an important role in intrinsic immunological mechanisms against a wide variety of pathogens, from bacteria to viruses, via direct antimicrobial activity and immunoregulatory functions. They occur naturally and have shown to be effective defensive tools despite facing significant opposition throughout time **(Hancock, 2005).** However, very few resistant species strains have been discovered yet. Under certain circumstances, the outer membranes of *Morganella*, *Burkholderia*, and *Serratia* bacteria have a decreased negative charge on the surface lipopolysaccharides (LPS). In contrast, other bacteria adjust their LPS through the two-component regulators PhoPQ and PmrAB **(Devine & Hancock, 2002)**. Other organisms, such as *Porphyromonas gingivalis*, release proteases that break down peptides **(Devine & Hancock, 2002)**. The majority of resistance mechanisms have a modest (2 to 4-fold) effect on the MIC **(Hancock, 2001).** As a result, these compounds are of great interest, and novel anti-infectives based on them are now being developed. Different structural classifications (i.e., beta-sheets, loops, alpha-helixes, and extended structures) and amino acid sequences are still seen **(Hancock, 2001).** Of the approximately 700 known antimicrobial peptides, they all possess the same 3D structure. The molecules fold into amphipathic shapes, meaning they have both hydrophobic and charged sides **(Hancock, 2005)**. The majority of cationic antimicrobial peptides, due to this arrangement and the composition of bacterial membranes, function by adhering directly to the lipid bilayer.

Most antimicrobial peptides interact with membranes early in their biological processes, and this phenomenon may be explained by the Shai-Matsuzaki-Huang model. The peptides begin in solution as unstructured molecules. Upon contact with the membrane, these molecules develop a 3D architecture (e.g., beta-sheet or alpha-helix) that renders them amphiphilic in nature, having the +ve side engaging directly with the head groups of the lipid. Next, the peptide becomes embedded in the membrane's outer leaflet, contributing to its thinning. Employing XRD and AFM, new evidence for this thinning has been established **(Chen *et al*., 2003).**

After this phase, channel development is possible, but this aspect of the process is more contentious. There have been several proposed explanations for this phenomenon; they include the carpet model, the toroidal pore model, the barrel-stave model, and the micellar aggregate channel model **(Wu *et al*., 1999)**. The applicability of each model relies on the peptide **(Buffy *et al*., 2004)** and the lipids' characteristics (elasticity, phase, hydration, and length of the hydrophobic chain) **(Dave *et al*., 2005)**. In conclusion, the bacterial cells are eliminated in a variety of methods, including:

(i) Depolarization of membrane **(Westerhoff *et al*., 1989);**
(ii) Intracellular activities, such as macromolecular synthesis, are damaged **(Kragol *et al*., 2001),**

(iii) Cell wall rupture **(Bierbaum & Sahl, 1985)** and alteration of membrane bilayer lipid content **(Matsuzaki,1999)**, or

(iv) In severe instances, micelles develop, causing cell leakage **(Papo & Shai, 2005).**

While cationic peptides need rather high concentrations to be effective, their distinct mechanism of action makes them promising building blocks for developing new antimicrobial drugs **(Devine & Hancock, 2002)**. Due to the fact that these peptides' mode of action is dependent on charge-charge and hydrophobic interactions with the membrane bilayer, resistance is constrained by the fact that it would be too expensive or necessitate several mutational occurrences for a microbe to alter the structure or organization of its lipids in to reduce these interactions. Further, there is no certainty that a particular recognition site for protease cleavage exists due to the great sequence variation of these peptides. Another crucial aspect of avoiding resistance is the presence of secondary targets. Despite preferential binding to certain sites, cationic peptides may connect with some other substrates in the bacterial cell to influence processes, including macromolecular synthesis, cell wall formation or breakdown, and cell proliferation **(Hancock, 2001)**. Finally, it is important to remember that multicellular organisms often assault bacteria with several cationic peptides, hence reducing the likelihood of antibiotic resistance.

9. Chemical modifications

Chemical modification of endogenous proteins was one of the first methods used to investigate structure-function relationships. It is possible to chemically modify the primary structure of proteins to increase their functional characteristics and biological activities. This method has been used successfully to investigate various structure-function correlations. The purposeful chemical alteration of dietary proteins may affect their nutritional value, lead to the creation of potentially harmful amino acid derivatives, and contaminate the food with toxic compounds.By heating at an acidic or alkaline pH; it is possible to alter amino acid residues. Acylation, alkylation, phosphorylation, and esterification are the primary kinds of processes utilized to change the side chain of amino acids. There are three phases involved in esterification. The first stage is mixing the reactants (alcohol, protein, and acid).

The second stage is the esterification process, which typically lasts from a few hours to several days at 4°C. The last stage consists of the termination of the reaction and the collection of the resulting product **(Chobert *et al.*, 1995).**The antimicrobial and antibacterial effects of proteins and peptides are improved by providing them with a more positive charge. Negatively charged proteins have anti-HIV (human immunodeficiency virus) action in vitro**;** according to **Berkhout *et al* (2002).**

9-1. - Enhancement of antimicrobial properties of a protein by esterification reaction

Current biotechnology approaches may produce cationic proteins or peptides with antibacterial activity. Without the need for expensive and time-consuming techniques for separating the active protein component, these cationic protein combinations may be used in antimicrobial applications. Esterification is an essential technique for altering dietary proteins. Esterification with various alcohols blocks free carboxyl groups, increasing the net positive charge and making changed proteins more acidic **(Sitohy & Osman, 2010)**. The basicity of a changed protein is determined by the degree of esterification and, by extension, the initial concentration of amino acid residues that are basic.

10. Factors affecting protein esterification

Chobert, (2003) investigated this factor affecting esterification (using -lactoglobulin as a model) and identified the following parameters:

a. Influence of time-course of reaction

In an esterification procedure using β-lactoglobulin (3 percent protein concentration) dispersed in 95 percent ethanol in the presence of 0.7 N HCl, the reaction time course was monitored at 8, 16, 24, 48, 72, and 96 hours. The degree of esterification rapidly grew as the duration of the reaction progressed, as 11, 14, 23, 28, 32, and 36 percent, respectively. Even after 96 hours of reaction, it is evident that at the conditions utilized, the esterification did not achieve its maximum. In order to maximize the degree of alteration after reduced response times, settings must be dramatically altered. For instance, quantities of proteins and acids may be raised. This reaction duration is alcohol-dependent; it was found that the highest esterification with methanol occurred after 24 hours with more diluted solutions.

b. Influence of temperature

By esterifying -lactoglobulin with 99.7 percent ethanol at three different temperatures (4, 10, and 20°C), comparative studies were conducted with two percent protein and 0.7N HCl final concentration. The response was terminated after eight hours. The amount of esterification grew as the temperature rose, beginning at 2%, 4%, and 8% at 4, 10, and 20°C, respectively. After drying, however, the compounds produced at higher reaction temperatures took on a violet hue, indicating that high temperatures may trigger side reactions. **(Wilcox, 1967)** claimed that at higher temperatures, esterified products may become insoluble. In order to prevent unwanted side reactions, greater temperatures should not be used, despite the fact that they may boost reaction speeds.

c. Influence of the presence of water

During the esterification process, water is required in amounts sufficient to dissociate HCl delivering the protons necessary for the carboxyl groups activation **(Sitohy *et al*., 2000).** The water in the medium may be employed to generate a hydration layer surrounding the protein molecules, which helps arrange the hydrophobic moieties in the globulin core **(Tanford, 1980)** and makes the carboxyl groups more accessible for esterification**. (Halpin & Richardson, 1985)** implicitly evaluated the need for water for the esterification process when they esterified β-lactoglobulin using 95 percent ethanol and anhydrous methanol.

d. Influence of the type of alcohol

Among the many alcohols studied for esterification, methanol was found to be the most reactive, capable of esterifying the vast majority of -lactoglobulin's carboxyl groups for a maximum esterification extent of 97% even in the presence of minimal amounts of water (1%). Additionally, the acid's water content affects how much water is ultimately present in the reaction medium **(Chobert, 2003).**

e. Effect of protein concentration

Various settings were explored to determine the impact of protein content. Esterification was conducted for 24 hours at 4 degrees Celsius in 95 percent ethanol and 0.7N HCl. β-Lactoglobulin was added at concentrations of 2, 3, 4, and 5 percent. An increase in protein content from 2 to 3 percent increased the degree of esterification marginally. However, increasing the concentration to 4 and 5 percent had an adverse impact on the reaction, resulting in decreased esterification levels. Prior to interacting with the alcohol, protein carboxyl groups must first be activated by protons during esterification. Therefore, the most critical component for the process is not the number of proteins but the concentration of activated carboxyl groups **(Sitohy *et al*., 2000).**

Sitohy *et al*., (2001) reported that three milk proteins (β-lactoglobulin, α-lactoalbumin, and β-casein) were esterified to varying degrees with methanol, ethanol, and propanol, and then their solubility in the pH range of 3-10 was investigated. In the pH range of 3 to 7, their emulsifying characteristics were also evaluated by measuring the size of oil droplets using laser light scattering. In the acidic pH range, the solubility of all esterified proteins was enhanced (3-6). Increased efficacy correlates with increased esterification, grafted ester group type, and altered protein composition. In the pH range of 3–5, the emulsifying activity and stability of the esterified milk proteins were generally higher than those of similar native proteins. The protein used, the level of solubility, the level of esterification, the kind of ester group grafted, and their combination led to this improvement. At pH 5, where native proteins have weak emulsifying characteristics, methyl ester derivatives demonstrated the greatest increase in emulsifying activity.

11. Mode of action of modified proteins

CAMPs are ubiquitous in the animal and plant worlds and play a crucial role in innate immune responses, both via direct antibacterial action and immunomodulatory effects, in warding off a broad spectrum of microorganisms, from bacteria to viruses **(Lo & Lange, 2015).**

Since these peptides may attack many systems at once, resistant bacteria are harder to cultivate against them than they would be against conventional antibiotics. In 2006, it was proposed that AMPs, which may stimulate the immune system in addition to being "antimicrobial," be renamed "host-defense peptides" rather than "antimicrobial peptides," the latter designation having been given exclusively on the basis of their first identified feature **(Hancock & Sahl, 2006)**. They may be found just about everywhere, and despite widespread use, they have not lost their effectiveness as a defensive weapon.

Scientists have only discovered a few resistant species. Bacteria, including *Burkholderia*, *Morganella*, and *Serratia*, have modified their outer membrane lipopolysaccharides (LPS) to have a lower negative charge using the two-component regulators PhoPQ and PmrAB under favorable circumstances. Other organisms, such as *Porphyromonas gingivalis*, release proteases that break down peptides **(Hancock, 2005).**

12. References

Abdel-Shafi, S., Al-Mohammadi, A.-R., Almanaa, T. N., Moustafa, A. H., Saad, T. M. M., Ghonemey, A.-R., Anacarso, I., Enan, G., & El-Gazzar, N. (2020). Identification and Testing of Antidermatophytic Oxaborole-6-Benzene Sulphonamide Derivative (OXBS) from Streptomyces atrovirens KM192347 Isolated from Soil. *Antibiotics (Basel, Switzerland)*, *9*(4), 176. https://doi.org/10.3390/antibiotics9040176

Abdel-Shafi, S., Al-Mohammadi, A.-R., Osman, A., Enan, G., Abdel-Hameid, S., & Sitohy, M. (2019). Characterization and Antibacterial Activity of 7S and 11S Globulins Isolated from Cowpea Seed Protein. *Molecules (Basel, Switzerland)*, *24*(6), 1082. https://doi.org/10.3390/molecules24061082

Abdel-Shafi, S., El-Nemr, M., Enan, G., Osman, A., Sitohy, B., & Sitohy, M. (2022). Isolation and Characterization of Antibacterial Conglutinins from Lupine Seeds. *Molecules (Basel, Switzerland)*, *28*(1), 35. https://doi.org/10.3390/molecules28010035

Achinas, S., Charalampogiannis, N., & Euverink, G. J. W. (2019). A Brief Recap of Microbial Adhesion and Biofilms. *Applied Sciences*, *9*(14), 2801. https://doi.org/10.3390/app9142801

Balcázar, J. L., Subirats, J., & Borrego, C. M. (2015). The role of biofilms as environmental reservoirs of antibiotic resistance. *Frontiers in microbiology*, *6*, 1216-1216. https://doi.org/10.3389/fmicb.2015.01216

Běhal, V. (2001). Nontraditional microbial bioactive metabolites. *Folia Microbiologica*, *46*(5), 363-370. https://doi.org/10.1007/bf02814422

Berkhout, B. , Van Wamel, J.L., Beljaars, L., Meijer, D.K., Visser, S. , Floris R. (2002). Characterization of the anti-HIV effects of native lactoferrin and other milk proteins and protein-derived peptides. *Antiviral Research*, 55 , 341-355, 10.1016/s0166-3542(02)00069-4

Bierbaum, G., & Sahl, H.-G. (1985). Induction of autolysis of staphylococci by the basic peptide antibiotics Pep 5 and nisin and their influence on the activity of autolytic enzymes. *Archives of Microbiology*, *141*(3), 249-254. https://doi.org/10.1007/bf00408067

Brackman, G., & Coenye, T. (2014). Quorum Sensing Inhibitors as Anti-Biofilm Agents. *Current Pharmaceutical Design*, *21*(1), 5-11. https://doi.org/10.2174/13816128206661409051146 27

Brambilla, F., Resta, D., Isak, I., Zanotti, M., & Arnoldi, A. (2008). A label-free internal standard method for the differential analysis of bioactive lupin proteins using nano HPLC-Chip coupled with Ion Trap mass spectrometry. *PROTEOMICS*, *9*(2), 272-286. https://doi.org/10.1002/pmic.200800317

Brooks, J. D., & Flint, S. H. (2008). Biofilms in the food industry: problems and potential solutions. *International Journal of Food Science & Technology*, *43*(12), 2163-2176. https://doi.org/10.1111/j.1365-2621.2008.01839.x

Buffy, J. J., McCormick, M. J., Wi, S., Waring, A., Lehrer, R. I., & Hong, M. (2004). Solid-State NMR Investigation of the Selective Perturbation of Lipid Bilayers by the Cyclic Antimicrobial Peptide RTD-1. *Biochemistry*, *43*(30), 9800-9812. https://doi.org/10.1021/bi036243w

Carmeli, Y., Troillet, N., Eliopoulos, G. M., & Samore, M. H. (1999). Emergence of antibiotic-resistant Pseudomonas aeruginosa: comparison of risks associated with different antipseudomonal agents. *Antimicrobial agents and chemotherapy*, *43*(6), 1379-1382. https://doi.org/10.1128/AAC.43.6.1379

Cepas, V., López, Y., Muñoz, E., Rolo, D., Ardanuy, C., Martí, S., Xercavins, M., Horcajada, J. P., Bosch, J., & Soto, S. M. (2019). Relationship Between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria. *Microbial Drug Resistance*, *25*(1), 72-79. https://doi.org/10.1089/mdr.2018.0027

Chadha, T. (2014). Bacterial Biofilms: Survival Mechanisms and Antibiotic Resistance. *Journal of Bacteriology & Parasitology*, *05*(03). https://doi.org/10.4172/2155-9597.1000190

Charpentier, E., & Courvalin, P. (1999). Antibiotic resistance in Listeria spp. *Antimicrobial agents and chemotherapy*, *43*(9), 2103-2108. https://doi.org/10.1128/AAC.43.9.2103

Chen, F.-Y., Lee, M.-T., & Huang, H. W. (2003). Evidence for membrane thinning effect as the mechanism for peptide-induced pore formation. *Biophysical journal*, *84*(6), 3751-3758. https://doi.org/10.1016/S0006-3495(03)75103-0

Chlebicz, A., & Śliżewska, K. (2018). Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Food-borne Diseases: A Review. *International journal of environmental research and public health*, *15*(5), 863. https://doi.org/10.3390/ijerph15050863

Chobert, J.-M. (2003). Milk protein modification to improve functional and biological properties. In *Advances in Food and Nutrition Research* (pp. 1-71).

Chobert, J.-M., Briand, L., Grinberg, V., & Haertlé, T. (1995). Impact of esterification on the folding and the susceptibility to peptic proteolysis of β-lactoglobulin. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, *1248*(2), 170-176. https://doi.org/10.1016/0167-4838(95)00012-j

Chominot, A. (1992). Les Proteagineux dans L'Economie Europeenne des Grandes Cultures. In Proceedings of the first European Conference on Grain Legumes. Angers, France. 529–538.

Dave, P. C., Billington, E., Pan, Y.-L., & Straus, S. K. (2005). Interaction of alamethicin with ether-linked phospholipid bilayers: oriented circular dichroism, 31P solid-state NMR, and differential scanning calorimetry studies. Biophysical journal, 89(4), 2434-2442. https://doi.org/10.1529/biophysj.105.067678

Davin, A., Berot, S., 1996. Technologie de préparation de produits enrichis en protéines végétales destinés aux consommateurs. *Protéines Végétales.* 389–390.

Devine, D., & Hancock, R. (2002). Cationic Peptides: Distribution and Mechanisms of Resistance. *Current Pharmaceutical Design*, *8*(9), 703-714. https://doi.org/10.2174/1381612023395501

Dooper, M. M. B. W., Holden, L., Fæste, C. K., Thompson, K. M., & Egaas, E. (2006). Monoclonal Antibodies against the Candidate Lupin Allergens α-Conglutin and β-Conglutin. *International Archives of Allergy and Immunology*, *143*(1), 49-58. https://doi.org/10.1159/000098224

Driscoll, J. A., Brody, S. L., & Kollef, M. H. (2007). The Epidemiology, Pathogenesis and Treatment of Pseudomonas aeruginosa Infections. *Drugs*, *67*(3), 351-368. https://doi.org/10.2165/00003495-200767030-00003

Dufour, D., Leung, V., & Lévesque, C. M. (2010). Bacterial biofilm: structure, function, and antimicrobial resistance. *Endodontic Topics*, *22*(1), 2-16. https://doi.org/10.1111/j.1601-1546.2012.00277.x

Duranti, M., Consonni, A., Magni, C., Sessa, F., & Scarafoni, A. (2008). The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends in Food Science & Technology*, *19*(12), 624-633. https://doi.org/10.1016/j.tifs.2008.07.002

Dzianach, P. A., Dykes, G. A., Strachan, N. J. C., Forbes, K. J., & Pérez-Reche, F. J. (2019). Challenges of biofilm control and utilization: lessons from mathematical modelling. *Journal of the Royal Society, Interface*, *16*(155), 20190042-20190042. https://doi.org/10.1098/rsif.2019.0042

Enan, G., Al-Mohammadi, A.-R., Mahgoub, S., Abdel-Shafi, S., Askar, E., Ghaly, M. F., Taha, M. A., & El-Gazzar, N. (2020). Inhibition of Staphylococcus aureus LC554891 by Moringa oleifera Seed Extract either Singly or in Combination with Antibiotics. *Molecules (Basel, Switzerland)*, *25*(19), 4583. https://doi.org/10.3390/molecules25194583

Ebran, N.; Juliena, S.; Orangeb, N.; Saglioc, P.; Lemaître a, C. and Molle, G. **(1999):** Poreforming properties and antimicrobial activity of proteins extracted from epiderma mucus of fish. Comprative Biochemistry and Physiology. 122: 181-189.

Feng, G., Cheng, Y., Wang, S.-Y., Borca-Tasciuc, D. A., Worobo, R. W., & Moraru, C. I. (2015). Bacterial attachment and biofilm formation on surfaces are reduced by small-diameter nanoscale pores: how small

is small enough? *NPJ biofilms and microbiomes*, *1*, 15022-15022. https://doi.org/10.1038/npjbiofilms.2015.22

Ferreira, R. B., Franco, E., & Teixeira, A. R. (1999). Calcium- and Magnesium-Dependent Aggregation of Legume Seed Storage Proteins. *Journal of Agricultural and Food Chemistry*, *47*(8), 3009-3015. https://doi.org/10.1021/jf981151c

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology*, *14*(9), 563-575. https://doi.org/10.1038/nrmicro.2016.94

Fm, S., Se, G., & Ha, A. (2018). Antimicrobial resistance of clinical Proteus mirabilis isolated from different sources. *Zagazig Journal of Pharmaceutical Sciences*, *27*(1), 57-63. https://doi.org/10.21608/zjps.2018.38156

Foss, N., Duranti, M., Magni, C., & Frøkiær, H. (2006). Assessment of Lupin Allergenicity in the Cholera Toxin Model: Induction of IgE Response Depends on the Intrinsic Properties of the Conglutins and Matrix Effects. *International Archives of Allergy and Immunology*, *141*(2), 141-150. https://doi.org/10.1159/000094716

Franco, E., Ferreira, R. B., & Teixeira, A. R. (1997). Utilization of an Improved Methodology To Isolate Lupinus albus Conglutins in the Study of Their Sedimentation Coefficients. *Journal of Agricultural and Food Chemistry*, *45*(10), 3908-3913. https://doi.org/10.1021/jf9702813

Friedlander, A., Nir, S., Reches, M., & Shemesh, M. (2019). Preventing Biofilm Formation by Dairy-Associated Bacteria Using Peptide-Coated Surfaces. *Frontiers in microbiology*, *10*, 1405-1405. https://doi.org/10.3389/fmicb.2019.01405

Gueguen, J., & Cerletti, P. (1994). Proteins of some legume seeds: soybean, pea, fababean and lupin. In *New and Developing Sources of Food Proteins* (pp. 145-193): Springer US.

Guillet, C., Join-Lambert, O., Le Monnier, A., Leclercq, A., Mechaï, F., Mamzer-Bruneel, M. F., Bielecka, M. K., Scortti, M., Disson, O., Berche, P., Vazquez-Boland, J., Lortholary, O., & Lecuit, M. (2010). Human listeriosis caused by Listeria ivanovii. *Emerging infectious diseases*, *16*(1), 136-138. https://doi.org/10.3201/eid1601.091155

Halpin, M. I., & Richardson, T. (1985). Selected Functionality Changes of β-Lactoglobulin upon Esterification of Side-Chain Carboxyl Groups. *Journal of Dairy Science*, *68*(12), 3189-3198. https://doi.org/10.3168/jds.s0022-0302(85)81226-1

Han, Q., Song, X., Zhang, Z., Fu, J., Wang, X., Malakar, P. K., Liu, H., Pan, Y., & Zhao, Y. (2017). Removal of Food-borne Pathogen Biofilms by Acidic Electrolyzed Water. *Frontiers in microbiology*, *8*, 988-988. https://doi.org/10.3389/fmicb.2017.00988

Hancock, R. E. W. (2001). Cationic peptides: effectors in innate immunity and novel antimicrobials. *The Lancet Infectious Diseases*, *1*(3), 156-164. https://doi.org/10.1016/s1473-3099(01)00092-5

Hancock, R. E. W. (2005). Mechanisms of action of newer antibiotics for Gram-positive pathogens. *The Lancet Infectious Diseases*, *5*(4), 209-218. https://doi.org/10.1016/s1473-3099(05)70051-7

Hancock, R. E. W., & Sahl, H.-G. (2006). Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology*, *24*(12), 1551-1557. https://doi.org/10.1038/nbt1267

Harris, A., Torres-Viera, C., Venkataraman, L., DeGirolami, P., Samore, M., & Carmeli, Y. (1999). Epidemiology and Clinical Outcomes of Patients with Multiresistant Pseudomonas aeruginosa. *Clinical Infectious Diseases*, *28*(5), 1128-1133. https://doi.org/10.1086/514760

Høiby, N., Bjarnsholt, T., Givskov, M., Molin, S., & Ciofu, O. (2010). Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents*, *35*(4), 322-332. https://doi.org/10.1016/j.ijantimicag.2009.12.011

Holden, L., Christiane K. Fæste, a., & Egaas, E. (2005). Quantitative Sandwich ELISA for the Determination of Lupine (Lupinus spp.) in Foods. *Journal of Agricultural and Food Chemistry*, *53*(15), 5866-5871. https://doi.org/10.1021/jf050631i

Ibekwe, A., Okonko, I., Onunkwo, A., Donbraye, E., Babalola, E., & Onoja, B. (2008). Baseline Salmonella agglutinin titres in apparently healthy freshmen in Awka, South Eastern, Nigeria. *Scientific Research and Essays*, *3*(9), 425-430.

Jun, W., Kim, M. S., Cho, B.-K., Millner, P. D., Chao, K., & Chan, D. E. (2010). Microbial biofilm detection on food contact surfaces by macro-scale fluorescence imaging. *Journal of Food Engineering*, *99*(3), 314-322. https://doi.org/10.1016/j.jfoodeng.2010.03.005

Kelemu, S., Cardona, C., & Segura, G. (2004). Antimicrobial and insecticidal protein isolated from seeds of Clitoria ternatea, a tropical forage legume. *Plant Physiology and Biochemistry*, *42*(11), 867-873. https://doi.org/10.1016/j.plaphy.2004.10.013

Khatoon, Z., McTiernan, C. D., Suuronen, E. J., Mah, T.-F., & Alarcon, E. I. (2018). Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon*, *4*(12), e01067. https://doi.org/10.1016/j.heliyon.2018.e01067

Kluytmans, J., van Belkum, A., & Verbrugh, H. (1997). Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. *Clinical microbiology reviews*, *10*(3), 505-520. https://doi.org/10.1128/CMR.10.3.505

Kragol, G., Lovas, S., Varadi, G., Condie, B. A., Hoffmann, R., & Otvos, L. (2001). The Antibacterial Peptide Pyrrhocoricin Inhibits the ATPase Actions of DnaK and Prevents Chaperone-Assisted Protein Folding. *Biochemistry*, *40*(10), 3016-3026. https://doi.org/10.1021/bi002656a

Kumarasamy, Y., Fergusson, M. E., Nahar, L., & Sarker, S. D. (2002). Bioactivity of Moschamindole from Centaurea moschata. *Pharmaceutical Biology*, *40*(4), 307-310. https://doi.org/10.1076/phbi.40.4.307.8467

Lari, A. R., & Alaghehbandan, R. (2000). Nosocomial infections in an Iranian burn care center. *Burns*, *26*(8), 737-740. https://doi.org/10.1016/s0305-4179(00)00048-6

Lebeaux, D., Ghigo, J.-M., & LUCET, J.-C. (2014). Physiopathologie et prévention des infections liées aux dispositifs médicaux implantés. *La Revue du praticien*, *64*(5), 620-625.

Lécuyer, F., Bourassa, J.-S., Gélinas, M., Charron-Lamoureux, V., Burrus, V., & Beauregard, P. B. (2018). Biofilm Formation Drives Transfer of the Conjugative Element ICEBs1 in Bacillus subtilis. *mSphere*, *3*(5), e00473-00418. https://doi.org/10.1128/mSphere.00473-18

Lee, B.-H., Cole, S., Badel-Berchoux, S., Guillier, L., Felix, B., Krezdorn, N., Hébraud, M., Bernardi, T., Sultan, I., & Piveteau, P. (2019). Biofilm Formation of Listeria monocytogenes Strains Under Food Processing Environments and Pan-Genome-Wide Association Study. *Frontiers in microbiology*, *10*, 2698-2698. https://doi.org/10.3389/fmicb.2019.02698

Lo, J. C. Y., & Lange, D. (2015). Current and potential applications of host-defense peptides and proteins in urology. *BioMed research international*, *2015*, 189016-189016. https://doi.org/10.1155/2015/189016

Madsen, J. S., Burmølle, M., Hansen, L. H., & Sørensen, S. J. (2012). The interconnection between biofilm formation and horizontal gene transfer. *FEMS Immunology & Medical Microbiology*, *65*(2), 183-195. https://doi.org/10.1111/j.1574-695x.2012.00960.x

Magni, C., Herndl, A., Sironi, E., Scarafoni, A., Ballabio, C., Restani, P., Bernardini, R., Novembre, E., Vierucci, A., & Duranti, M. (2005). One- and Two-Dimensional Electrophoretic Identification of IgE-Binding

Polypeptides of Lupinus albus and Other Legume Seeds. *Journal of Agricultural and Food Chemistry*, *53*(11), 4567-4571. https://doi.org/10.1021/jf0500785

Matsuzaki, K. (1999). Why and how are peptide–lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1462*(1-2), 1-10. https://doi.org/10.1016/s0005-2736(99)00197-2

Maunders, E., & Welch, M. (2017). Matrix exopolysaccharides; the sticky side of biofilm formation. *FEMS Microbiology Letters*, *364*(13). https://doi.org/10.1093/femsle/fnx120

Meesilp, N., & Mesil, N. (2018). Effect of microbial sanitizers for reducing biofilm formation of Staphylococcus aureus and Pseudomonas aeruginosa on stainless steel by cultivation with UHT milk. *Food science and biotechnology*, *28*(1), 289-296. https://doi.org/10.1007/s10068-018-0448-4

Navon-Venezia, S., Kondratyeva, K., & Carattoli, A. (2017). Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiology Reviews*, *41*(3), 252-275. https://doi.org/10.1093/femsre/fux013

Oliveira, N. M., Martinez-Garcia, E., Xavier, J., Durham, W. M., Kolter, R., Kim, W., & Foster, K. R. (2015). Biofilm Formation As a Response to Ecological Competition. *PLoS biology*, *13*(7), e1002191-e1002191. https://doi.org/10.1371/journal.pbio.1002191

O'Neill J. (2016).Tackling drug-resistant infections globally: Final report and recommendations. London: HM Government and Welcome Trust. Review on Antimicrobial Resistance, chaired by Jim O'Neill. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf .

Osman, A., El-Didamony, G., Sitohy, M., Khalifa, M., & Enan, G. (2016). Soybean glycinin basic subunit inhibits methicillin resistant-vancomycin intermediate Staphylococcus aureus (MRSA-VISA) in vitro. *International Journal of Applied Research in Natural Products*, *9*(2), 17-26.

Osman, A., Mahgoub, S., & Sitohy, M. (2014). Hindering milk quality storage deterioration by mild thermization combined with methylated chickpea protein. *International Food Research Journal*, *21*(2), 693-701.

Osmon, S., Ward, S., Fraser, V. J., & Kollef, M. H. (2004). Hospital Mortality for Patients With Bacteremia Due to Staphylococcus aureus or Pseudomonas aeruginosa. *Chest*, *125*(2), 607-616. https://doi.org/10.1378/chest.125.2.607

Palumbo, D., Iannaccone, M., Porta, A., & Capparelli, R. (2010). Experimental antibacterial therapy with puroindolines, lactoferrin and lysozyme in Listeria monocytogenes-infected mice. *Microbes and Infection*, *12*(7), 538-545. https://doi.org/10.1016/j.micinf.2010.03.010

Pan, Y., Shiell, B., Wan, J., Coventry, M. J., Roginski, H., Lee, A., & Michalski, W. P. (2007). The molecular characterisation and antimicrobial activity of amidated bovine lactoferrin. *International Dairy Journal*, *17*(6), 606-616. https://doi.org/10.1016/j.idairyj.2006.08.005

Papo, N., & Shai, Y. (2005). Host defense peptides as new weapons in cancer treatment. *CMLS Cellular and Molecular Life Sciences*, *62*(7-8), 784-790. https://doi.org/10.1007/s00018-005-4560-2

Penalver, P., Huerta, B., Borge, C., Astorga, R., Romero, R., & Perea, A. (2005). Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. *APMIS*, *113*(1), 1-6. https://doi.org/10.1111/j.1600-0463.2005.apm1130101.x

Płusa, T. (2019). Znaczeniebiofilmu w kontekscienarastaniaopornoscibakteriinaantybiotyki the importance of biofilm in the context of increasing bacterial resistance to antibiotics. *Pol MerkurLekarski*, *47*(281), 197-202.

Podschun, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical microbiology reviews*, *11*(4), 589-603. https://doi.org/10.1128/CMR.11.4.589

Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y., Bonsu, E., & Sintim, H. O. (2015). Biofilm formation mechanisms and targets for developing antibiofilm agents. *Future Medicinal Chemistry*, *7*(4), 493-512. https://doi.org/10.4155/fmc.15.6

Rybak, M. J., & Pharm.D, K. L. L. (2005). Community-Associated Methicillin-ResistantStaphylococcus aureus: A Review. *Pharmacotherapy*, *25*(1), 74-85. https://doi.org/10.1592/phco.25.1.74.55620

Sadekuzzaman, M., Yang, S., Mizan, M. F. R., & Ha, S. D. (2015). Current and Recent Advanced Strategies for Combating Biofilms. *Comprehensive Reviews in Food Science and Food Safety*, *14*(4), 491-509. https://doi.org/10.1111/1541-4337.12144

Sánchez, E., Donat, E., Ribes-Koninckx, C., Fernández-Murga, M. L., & Sanz, Y. (2013). Duodenal-mucosal bacteria associated with celiac disease in children. *Applied and environmental microbiology*, *79*(18), 5472-5479. https://doi.org/10.1128/AEM.00869-13

Savino, F., Cordisco, L., Tarasco, V., Calabrese, R., Palumeri, E., & Matteuzzi, D. (2009). Molecular identification of coliform bacteria from colicky breastfed infants. *Acta Paediatrica*, *98*(10), 1582-1588. https://doi.org/10.1111/j.1651-2227.2009.01419.x

Savino, F., Cordisco, L., Tarasco, V., Locatelli, E., Di Gioia, D., Oggero, R., & Matteuzzi, D. (2011). Antagonistic effect of Lactobacillus strains against gas-producing coliforms isolated from colicky infants. *BMC microbiology*, *11*, 157-157. https://doi.org/10.1186/1471-2180-11-157

Shay, R., Wiegand, A. A., & Trail, F. (2022). Biofilm Formation and Structure in the Filamentous Fungus Fusarium graminearum, a Plant Pathogen. *Microbiology spectrum*, *10*(4), e0017122-e0017122. https://doi.org/10.1128/spectrum.00171-22

Shewry, P. R., Napier, J. A., & Tatham, A. S. (1995). Seed storage proteins: structures and biosynthesis. *The Plant cell*, *7*(7), 945-956. https://doi.org/10.1105/tpc.7.7.945

Singh, S., Singh, S. K., Chowdhury, I., & Singh, R. (2017). Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. *The open microbiology journal*, *11*, 53-62. https://doi.org/10.2174/1874285801711010053

Sitohy, M., Chobert, J.-M., & Haertle, T. (2000). STUDY OF FACTORS INFLUENCING PROTEIN ESTERIFICATION USING ?-LACTOGLOBULIN AS A MODEL. *Journal of Food Biochemistry*, *24*(5), 381-398. https://doi.org/10.1111/j.1745-4514.2000.tb00708.x

Sitohy, M., Chobert, J. M., & Haertlé, T. (2001). Improvement of solubility and of emulsifying properties of milk proteins at acid pHs by esterification. *Nahrung/Food*, *45*(2), 87-93. https://doi.org/10.1002/1521-3803(20010401)45:2<87::aid-food87>3.0.co;2-z

Sitohy, M., Mahgoub, S., Osman, A., El-Masry, R., & Al-Gaby, A. (2013). Extent and Mode of Action of Cationic Legume Proteins against Listeria monocytogenes and Salmonella Enteritidis. *Probiotics and Antimicrobial Proteins*, *5*(3), 195-205. https://doi.org/10.1007/s12602-013-9134-2

Sitohy, M., & Osman, A. (2010). Antimicrobial activity of native and esterified legume proteins against Gram-negative and Gram-positive bacteria. *Food Chemistry*, *120*(1), 66-73. https://doi.org/10.1016/j.foodchem.2009.09.071

Speranza, B., & Corbo, M. R. (2017). The Impact of Biofilms on Food Spoilage. In *The Microbiological Quality of Food* (pp. 259-282).

Tanford, C. (1980). *The hydrophobic effect: formation of micelles and biological membranes 2d ed*. J. Wiley.

Tanwar, J., Das, S., Fatima, Z., & Hameed, S. (2014). Multidrug resistance: an emerging crisis. *Interdisciplinary perspectives on infectious diseases*, *2014*, 541340-541340. https://doi.org/10.1155/2014/541340

Travier, L., Guadagnini, S., Gouin, E., Dufour, A., Chenal-Francisque, V., Cossart, P., Olivo-Marin, J.-C., Ghigo, J.-M., Disson, O., & Lecuit, M. (2013). ActA promotes Listeria monocytogenes aggregation, intestinal colonization and carriage. *PLoS pathogens*, *9*(1), e1003131-e1003131. https://doi.org/10.1371/journal.ppat.1003131

Verderosa, A. D., Totsika, M., & Fairfull-Smith, K. E. (2019). Bacterial Biofilm Eradication Agents: A Current Review. *Frontiers in chemistry*, *7*, 824-824. https://doi.org/10.3389/fchem.2019.00824

Westerhoff, H. V., Hendler, R. W., Zasloff, M., & Juretić, D. (1989). Interactions between a new class of eukaryotic antimicrobial agents and isolated rat liver mitochondria. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *975*(3), 361-369. https://doi.org/10.1016/s0005-2728(89)80344-5

Wilcox, P. E. (1967). [74] Esterification. In *Methods in Enzymology* (pp. 605-617): Elsevier.

Wood, T. K., Knabel, S. J., & Kwan, B. W. (2013). Bacterial Persister Cell Formation and Dormancy. *Applied and Environmental Microbiology*, *79*(23), 7116-7121. https://doi.org/10.1128/aem.02636-13

World Health Organization (WHO)**,(2013):** Global Strategy for Containment of Antimicrobial Resistance. Geneva: World Health Organization, 2001. Available at **HYPERLINK"http://whqlibdoc.who.int/hq2001/WHO_CDS_DR_2001.2.pdf.Ibekwe"**

Wu, M., Maier, E., Benz, R., & Hancock, R. E. W. (1999). Mechanism of Interaction of Different Classes of Cationic Antimicrobial Peptides with Planar Bilayers and with the Cytoplasmic Membrane of Escherichia coli. *Biochemistry*, *38*(22), 7235-7242. https://doi.org/10.1021/bi9826299

Yang, S.-C., Lin, C.-H., Aljuffali, I. A., & Fang, J.-Y. (2017). Current pathogenic Escherichia coli food-borne outbreak cases and therapy development. *Archives of Microbiology*, *199*(6), 811-825. https://doi.org/10.1007/s00203-017-1393-y

Yeheyis, L., Kijora, C., Wink, M., & Peters, K. J. (2011). Effect of a Traditional Processing Method on the Chemical Composition of Local White Lupin (Lupinus albus L.) Seed in North-Western Ethiopia. *Zeitschrift für Naturforschung C*, *66*(7-8), 403-408. https://doi.org/10.1515/znc-2011-7-812

Yu, S., Zhu, X., Zhou, J., & Cai, Z. (2018). Biofilm inhibition and pathogenicity attenuation in bacteria by Proteus mirabilis. *Royal Society open science*, *5*(4), 170702-170702. https://doi.org/10.1098/rsos.170702

Zasloff, M. (2002). Antimicrobial Peptides in Health and Disease. *New England Journal of Medicine*, *347*(15), 1199-1200. https://doi.org/10.1056/nejme020106

Zhang, J., Li, W., Chen, J., Qi, W., Wang, F., & Zhou, Y. (2018). Impact of biofilm formation and detachment on the transmission of bacterial antibiotic resistance in drinking water distribution systems. *Chemosphere*, *203*, 368-380. https://doi.org/10.1016/j.chemosphere.2018.03.143