INCORPORATING PROBIOTICS IN OUR DAILY FOOD CONSUMPTION TO COMBAT ANTIBIOTIC RESISTANCE

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE BACHELOR OF SCIENCE IN MICROBIOLOGY

Department of Mathematics and Natural Sciences

BRAC University

OCTOBER 2022

Declaration

It is hereby declared that

1. That the research work reported in this thesis title ‘INCORPORATING PROBIOTICS IN OUR DAILY FOOD CONSUMPTION TO COMBAT ANTIBIOTIC RESISTANCE ’ has been carried out under the supervision of Dr. Mahboob Hossain, Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka
2. This research work presented here is my original work while completing my degree at BRAC University.
3. The thesis has not been submitted to any other institution for any degree or diploma.
4. All the main sources of help have been acknowledged.

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Acknowledgment

First and foremost, we are very thankful to Almighty Allah for blessing us and helping us in every aspect of life. We are also thankful to Almighty Allah that we were blessed with very helpful and co-operating mentors, who helped me throughout the whole process. We are thankful to Professor Dr. A F M Yusuf Haider, (Chairperson Department of Mathematics and Natural Sciences, BRAC University) for looking after all the students and teachers in his department and always providing his helping hands whenever needed. Our most sincere acknowledgment goes to all the faculties of the MNS Department specially Akash Ahmed sir, Dr. Mahbubul Hasan Siddiqee sir, who have helped us throughout our undergraduate journey. This study would not have been possible without our supervisor, Dr. Mahboob Hossain sir, who provided us with the adequate knowledge and perspective that he possesses.He constantly guided us in the required manner with his critical feedback and pleasant regards. It was a privilege and honor for us to be able to work under his guidance.

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Table of contents

List of Figures

List of acronyms

ACRONYM	FULL FORM
1.ABR	Antibiotic Resistance
2.ARGs	Antimicrobial Resistance Gene
3.FAO	Food and Agricultural Organization
4.WHO	World Health Organization
5.IBD	Inflammatory Bowel Disease
6.IECs	Intestinal Epithelial Cells
7.LAB	Lactic Acid Bacteria
8.MUB	Mucus-Binding Protein
9.CLA	Conjugated Linoleic Acid
10.PAMPs	Pathogen Associated Molecular Patterns
11.IgA	Immuno-globiolin A
12.APC	Antigen Presenting cell
13.BOD	Biological Oxygen Demand
14.TVC.	Total Viable Count
15.TLC	Total Lactobacillus Count
16.SRBC	Sheep Red Blood Cells
17.AGD	Average Daily Gain
18.PPB	Plant Probiotic Bacteria

Abstract

An increase in antibiotic resistance throughout the globe threatens the survival of these lifesaving drugs (ABR). One of the most pressing global health issues is antibiotic resistance, which develops when bacteria or fungi acquire resistance mechanisms that make them vulnerable to treatment with the most used antimicrobials. Animals reared for human consumption are well-recognized as large reservoirs of antibiotic-resistant bacteria, and antibiotics used in food and agriculture have contributed to the present worldwide ABR issue. Exploring alternative probiotics is one of the many treatment options with promising effects. In the present study several mechanisms by which probiotics inhibit the growth of pathogenic bacteria have been described. These include probiotics' ability to alter the gut microbiota's composition, adhere to the mucosa and epithelium on favorable terms, strengthen the gut epithelial barrier, and modulate the host immune system. Some of the most widely eaten foods daily are poultry, fish, dairy, fruit, and vegetable items. In this study it was also found that many important antibiotics such as rifampin, cotrimoxazole, beta-lactams, spectinomycin, oxytetracycline, streptomycin, and many more are used during production to promote growth, maximize yield, and reduce disease risk of animals and plants; however, this practice increases the likelihood of the emergence and spread of microbial resistance in those settings and posses a threat to the safety of the foods produced as a result (due to antimicrobial residues). Estimates show that more than 60% of all antibiotics made are used in the chicken industry. To reduce the overuse of antibiotics, this review paper explores the potential function of probiotics, and how they operate to reduce infection for example modification of microbial population, competitive adhesion to the epithelial receptor, production of a specific substance, and so on, how they affect growth performance and immunological response, such as systemic immunological response, local immunological response and how they might be incorporated into the production of poultry, dairy, aquaculture sector, fruits, and vegetables.

Keywords

Antibiotic resistance. Global rise, probiotics, bacteria, antibiotics, ABR, resistance. Production, spread, poultry, aquaculture, dairy, fruit, and vegetable.

Chapter 1

Introduction

Infectious diseases that had previously killed or seriously incapacitated individuals were now considered treatable thanks to the arrival of antibiotics. (“The Discovery of Antibiotics – Part 1 – ReAct”)Their usage in animal husbandry and veterinary medicine, agriculture, and aquaculture has led to healthier and more productive farm animals, fish, vegetables, and fruits, ensuring animal and human welfare. (Economou et al., 2015).

Most developed countries consume more than 1.4 g/kg of protein per day, with animal proteins accounting for 65-70 percent of total protein intake. To meet rising demand, livestock industries of all types have used a variety of techniques to increase output (Arsène et al., 2021). As a result, overuse or misuse of antibiotics in these sectors exposes more bacterial strains to a larger range of antibiotics (Science Journal for Kids and Teens, n.d.). When bacteria become resistant to antibiotics, the original antibiotic is no longer effective in killing them. These microorganisms can multiply and spread. They have the potential to produce infections that are difficult to manage. They can even disseminate resistance to other bacteria they come into contact with. (Antibiotic Resistance | Cedars-Sinai. (n.d.). This reservoir of resistance can be passed on to people either directly or indirectly by food consumption and direct or indirect interaction. A prominent study on antimicrobial resistance has warned that if drug-resistant illnesses are not addressed, they will kill an additional 10 million people per year by 2050. (Ashiru, 2015). In the United States, more than 2.8 million antibiotic-resistant diseases occur each year. According to the CDC's 2019 Antibiotic Resistance (AR) Threats Report, more than 35,000 people die as a result. (C. 2022, July 21) The emergence and spread of antibiotic-resistant bacteria and antimicrobial resistance genes (ARGs) in the food chain have had serious health and socioeconomic consequences around the world. Foodborne illnesses that are resistant to antibiotics are one of the most serious public health concerns related to the threat of ABR in the food supply chain. Furthermore, humans may be exposed to antibiotic-resistant bacteria and ARGs indirectly through contact with or consumption of infected food products (e.g., meat, eggs, milk, and dairy products).(Founou et al., 2001)

As a result, it is critical to minimize the use of antibiotics while also looking for better alternatives. Probiotics are the subject of the most recent study among the many choices.
Microorganisms that live in symbiosis with the human host are known as probiotics. Probiotics, when consumed in sufficient amounts, may modify biological activities, resulting in health benefits. Probiotics have been found to exhibit a variety of biological features, including antibacterial action. However, few research have looked at the use of probiotics as antimicrobial therapy alternatives or as a source of novel antibiotics. (Silva et al., 2020)

Probiotics appear to be a viable option for overcoming ARGs related issues because of their ability to modulate the immune system and intestinal microflora, as well as their antagonistic role against certain pathogenic bacteria and their ability to act as a growth factor (sometimes associated with prebiotics) when used as feed additives.(Arsène et al., 2021)
According to PubMed searches, probiotic research is on the rise from 2015 to 2022. The worldwide probiotics market was estimated at USD 58.17 billion in 2021, with a compound annual growth rate (CAGR) of 7.5 percent predicted from 2021 to 2030. Probiotic usage increased exponentially in 2020, according to a survey done by Natural Marketing Institute in May 2020. According to the poll, the number of probiotic users in the United States climbed by 66%, while new users in Italy increased by 188 percent, and probiotic users in China increased by 108 percent in May 2020 compared to the previous six months. As a result, the quick increase in probiotic users in 2020 enhanced market growth substantially. (Probiotics Market Size | Industry Report, 2021 -2030, n.d.)

Several studies have also been conducted since the beginning of the twenty-first century, portraying probiotics as having the potential to play a dominant role in breeding, either as a simple supplement with good growth effects or as a viable alternative to traditional antibiotics. Adding probiotics to animal feed has been proven in several recent studies to enhance disease resistance and health by altering the gut microbiota, reducing pathogen shedding and illness symptoms, increasing gut immunity, and improving disease resistance and health. Furthermore, probiotics have an antagonistic impact on foodborne pathogens such as *Campylobacter, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella,* and *Staphylococcus aureu*s (Arsène et al., 2021)

With the increasing usage of probiotics in several areas, if probiotics can be introduced into daily food consumption, antibiotic use can be minimized, and natural immunity can be built to prevent antibiotic resistance.

Chapter 2

Probiotics

The human microbiota is made up of trillions of bacteria that live in the human body. These bacteria form intricate, organ-specific, and adaptable ecosystems that have a continuous influence on the physiology of the host. Bacteria (bacteriome), archaea (archeome), fungi (mycobiome), viruses (virome), and parasites make up the microbiota and its genetic material (the microbiome) (parasitome). They influence digestion, immunological development, vitamin production, and presumably behavior and mental well-being in humans and animals when they work together. In recent years, the symbiotic link between the human host and its bacterial occupants has piqued researchers' curiosity. The gut microbiome is made up of bacteria that are mostly found in the gastrointestinal tract, and their genomes are known as the gut microbiome. The gut microbiota appears to play a crucial role in human host homeostasis, health, and illness, according to mounting data. Gut bacteria's benefits have been linked to gastrointestinal function, hunger, and immunological response. (Day et al., 2019) The name "probiotics" comes from a Greek phrase that means "for life." The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) commissioned an expert team to define probiotics as "live microorganisms." The notion of probiotics reflects the beneficial effects of these specific living organisms that when supplied in suitable proportions, impart a health benefit on the host" (Hill et al., 2014).(Spacova et al., 2001) *Lactic acid b*acteria and *bifidobacteria* are the most often utilized probiotic strains. Probiotics have been shown to offer great therapeutic promise for several disorders. (Bermudez et al.,2012)

Lilly and Stillwell used the word "probiotic" in 1965 to describe chemicals released by one organism that aid in the development of another. Antibiotics, immunosuppressive medication, and irradiation, among other treatments, may alter the makeup and affect the flora of the gastrointestinal tract. As a result, introducing beneficial bacterial species to the GI tract might be a highly appealing alternative for re-establishing microbial balance and preventing illness. (Gupta, et al., 2009) *Lactobacillus, Bifidobacterium, Escherichia, Enterococcus, Bacillus,* and

Streptococcus are some of the most often employed bacterial genera in probiotic formulations. Fungal strains from the *Saccharomyces* genus have also been employed. (Spacova et al., 2001)

2.1 The current state of probiotics:

There has been a surge in interest in the use of probiotic supplements as mediators in health and illness in recent years. This appeal is primarily motivated by mounting evidence of the involvement of the microbiota with disease pathophysiological processes in the human body. The worldwide probiotic industry is expected to be valued at USD 15 billion per year and rise at a pace of 7% each year (Van den Nieuwboer et al., 2016). There has been a surge in interest in the microbiome and probiotic research in recent years. A search for probiotics in the literature today yields over 19,000 items, including some of the most frequently read and respectable scientific journals. A MEDLINE/PubMed search for literature that includes the phrases 'probiotics' and 'health' OR 'disease' returns almost 10,000 items, indicating significant growth in research publications, with an annual increase of around thrice in the past decade and a 34-fold increase since 1998. (Day et al., 2019)

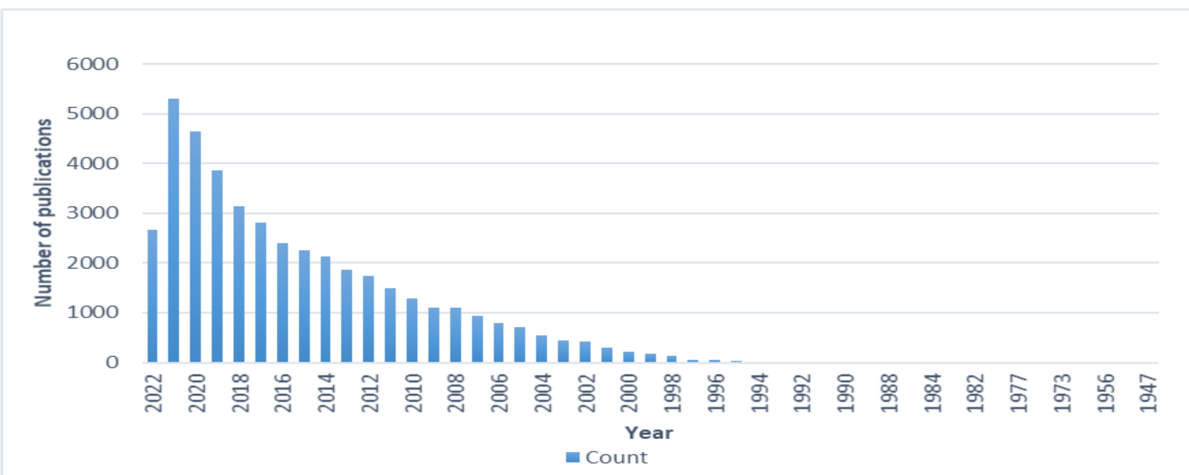

Figure 1: From 1947 through 2022, this bar graph depicts the increasing number of publications on probiotics. It can be observed that research and publishing numbers were quite low in the early 1900s, but have risen considerably in recent years. The number of articles increased rapidly from 2018 to 2020. According to Pubmed, the number of published articles in 2011 was around 1500, but in ten years the number has climbed by more than three times to 5290.

2.2 Mode of Action:

Enhancement of the epithelial barrier increased adhesion to the intestinal mucosa, and concurrent inhibition of pathogen adhesion are all major probiotic mechanisms of action, as are the competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances, and immune system modulation (fig. 1). (Brito et al., 2012)

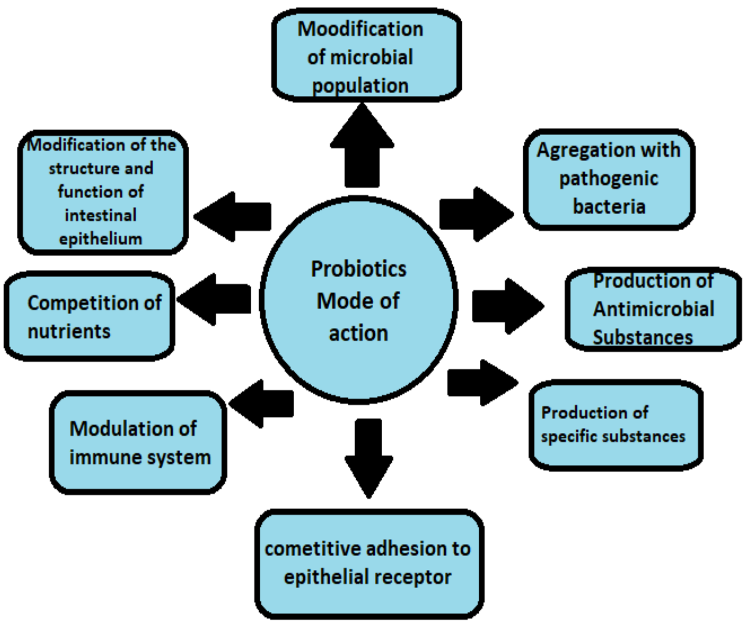

Figure 2: probiotics mode of actions graphical illustration

2.3 Enhancement of the Epithelial Barrier:

More than 500 bacteria species live in the digestive system, making it a complex habitat. Because only a single layer of epithelium separates these commensal bacteria and pathogens from the underlying immune cells, the epithelial barrier function is an important part of the arsenal of defensive mechanisms needed to avoid infection and inflammation. A thick mucous layer containing secretory IgA and antimicrobial peptides, as well as dynamic junctional complexes that govern cell permeability, make up the epithelial barrier. The epithelium has developed methods to limit bacterial growth, minimize direct contact with germs, and inhibit bacterial spread into underlying tissue to defend itself from uncontrolled inflammatory reactions. Inflammatory bowel illnesses (IBD) such as ulcerative colitis and Crohn's disease are hypothesized to emerge when this barrier is disrupted, resulting in a loss of immunological tolerance to the microbiota and inappropriate inflammatory response. The mucous layer, antimicrobial peptides, secretory IgA, and the epithelial junctional adhesion complex make up the gut barrier defenses. The consumption of probiotics, which are nonpathogenic and living bacteria, can help preserve mucosal integrity by lowering paracellular permeability, offering natural protection against pathogens, and increasing the physical impediment of the mucous layer.(Ohland et.al,.2010).

2.4 Increased Adhesion to Intestinal Mucosa:

The interaction between probiotic strains and the host depends on adhesion to the intestinal mucosa, which is regarded as a necessary condition for colonization Juntunen et al.,(2001); Beachey EH (1981); Schiffrin et al.,(1997) Probiotics' ability to adhere to the intestinal mucosa is crucial for pathogen resistance and immune system regulation Schiffrin et al.,(1997); Perdigon et al.,(2002). As a result, adherence has been a key selection factor for novel probiotic strains (Juntunen et al.,2001); (Salminen et al.,1998);(Collado et al.,2005);(Crociani et al.,1995)and has been linked to some probiotic benefits (Castagliuolo et.al2005). In their interactions with intestinal epithelial cells (IECs) and mucus, lactic acid bacteria (LABs) exhibit a variety of surface determinants. IECs release mucin, a complex glycoprotein combination that makes up the majority of mucous, preventing harmful germs from adhering (Collado et al.,2005);(González et al.,2012). The mucous gel also contains lipids, free proteins, immunoglobulins, and salts (Neutra and Forstner,1987) This particular interaction has suggested a potential relationship between the competitive exclusion of pathogens from the mucus and the surface proteins of probiotic bacteria (Ouwehand et al.,2002); (Haller et al., 2001). Numerous *Lactobacillus* proteins, as previously indicated, have been shown to facilitate mucous adhesion and bacteria exhibit surface adhesins that facilitate attachment to the mucous layer. The MUB (mucus-binding protein) generated by *Lactobacillus reuteri* is the best-researched example of bacterial adhesins that target mucus. The majority of the proteins involved in the mucous adhesion phenotype of lactobacilli are secreted and surface-associated proteins, which are either embedded in the cell wall or linked to the membrane by a lipid moiety Probiotics like *L. plantarum* have been shown to produce MUC2 and MUC3 mucins and to prevent enteropathogenic *E. coli* from adhering. These findings suggest that the intestinal epithelium increased mucus layers and glycocalyx, as well as *Lactobacillus spp* .'s of microbial binding sites, offer defense against pathogen invasion (Voltan et al.,2007);.(Kim 2010)

According to studies, the combination of probiotics and VSL3 (a medicinal food used as a probiotic) increases the production of cell surface mucins and modifies the expression of the mucin gene in a way that depends on the attachment of bacterial cells to the intestinal epithelium. Intestinal mucins are also qualitatively altered by probiotics, which prevents pathogen binding (Kim, 2010). Protease-resistant bacteria connected with the bacterial surface and engaged in the attachment of the LB and BG2FO4 *L. acidophilus* strains (Chauvière et al.,1992);(Coconnier et al.,1992);(Greene 1994). It's interesting to note that the bacterial component is also broken down into an antimicrobial peptide, which gives the host anti-pathogenic characteristics and serves as an illustration of how big surface proteins may have pleiotropic effects that are advantageous to evolution (Gopal PK 2001);(Bermudez et al., 2012)
Defensins can be released from epithelial cells by probiotic strains. These tiny peptides and proteins have antibacterial, antifungal, and antiviral properties. Additionally, these tiny proteins and peptides sustain the function of the intestinal barrier. Numerous studies have demonstrated that some probiotic Lactobacillus strains significantly increased the mRNA expression and protein synthesis of -defensin in epithelial cells, hence improving host defense against infection. (Huang et al., 2020)

2.5 Competitive Exclusion of Pathogenic Microorganisms

The phrase "competitive exclusion" was initially used by Greenberg (Greenberg, 1969) to describe the situation in which one species of bacteria fights more vigorously than another for receptor sites in the digestive tract. A variety of strategies are employed by one species of bacterium to prevent or hinder the growth of a different species, including the development of a hostile microecology, the removal of available bacterial receptor sites, the production and secretion of antimicrobial substances and selective metabolites, and the competitive depletion of vital nutrients.

Some probiotic strains have antagonistic activity against the adherence of gastrointestinal pathogens, which results in specific adhesiveness features that come from the interaction between surface proteins and mucins and may limit the colonization of pathogenic bacteria (Servin AL 2004). Numerous pathogens, including *E. coli, Salmonella, Helicobacter pylori, Listeria monocytogenes,* and *Rotavirus,* have been demonstrated to be inhibited by lactobacilli and bifidobacteria. Exclusion is the outcome of many probiotic adhesion-inhibiting processes and traits, such as the synthesis of chemicals and the activation of IECs. Based on a bacterial-to-bacterial contact driven by competition for readily available nutrients and mucosal adhesion sites, intestinal bacteria engage in competitive exclusion.

Bacteria can also alter their environment to make it unfavorable to rivals to obtain a competitive edge. One example of this kind of environmental change is the synthesis of antimicrobial compounds like lactic and acetic acid. These metabolites lower the pH of the environment, which may directly hinder the growth of hazardous organisms. Additionally, some strains of L*actobacilli* and *Bifidobacteria* share carbohydrate-binding specificities with certain enteropathogens, allowing the bacteria to compete with particular pathogens for receptor sites on host cells. Probiotic strains may generally prevent pathogenic germs from adhering to enterocyte pathogen receptors by causing steric hindrance (Coconnier et al., 1993);(Brito et al., 2012)

2.6 Production of Antimicrobial Substances

Gram-negative bacteria are effectively inhibited by organic acids, especially acetic acid, and lactic acid. They are regarded as the primary antimicrobial substances in charge of probiotics' inhibitory effectiveness against infections. The organic acid's undissociated form penetrates the bacterial cell and breaks down inside the cytoplasm. Pathogen death may result from the gradual reduction of intracellular pH. Bacteriocins and small AMPs are two examples of the antibacterial peptides that many LABs make. By creating pores or by inhibiting the creation of cell walls, bacteriocins destroy target cells. For instance, nisin inhibits the manufacture of cell walls of mostly spore-producing bacilli by creating a combination with the last cell wall precursor, lipid

II. Because of the related antibacterial action, these strains offer a competitive edge. For several Bifidobacterium strains, including *Salmonella enterica ser.* Typhimurium SL1344 and *E. coli* C1845, several antibacterial substances have been identified. The only substance known to date that is active against Gram-negative bacteria is an LMW protein known as BIF, which is produced by *B. longum* BL1928. It has been suggested that this action results in the creation of a possible LMW lipophilic compound. A wide variety of fatty acids that are good for ~~your~~ health are also produced by intestinal bacteria. Conjugated linoleic acid (CLA), a strong anti-carcinogenic compound, has been demonstrated to be produced by certain strains of intestinal *bifidobacteria* and *lactobacilli.* In mice with diet-induced obesity, *L. plantarum* has been found to have an anti-obesity effect. When compared to the bile salts produced by the host organism, deconjugated bile acids have higher antibacterial action. It is common knowledge that some probiotic strains release compounds that prevent the development of bacteria and fungi. According to several studies, *Lactobacillus* is capable of producing antifungal compounds such as benzoic acid, methylhydantoin, mevalonolactone, and short-chain fatty acids. Lactic acid, phenyllactic acid, and two cyclic dipeptides were among the four antiflogic compounds identified and chemically characterized by Dal Bello et al. in *L. plantarum* FST 1.7.(Brito et al., 2012)

2.7 immune mechanisms induced by probiotic bacteria:

It is common knowledge that probiotic microorganisms have immunomodulatory properties. These bacteria can interact with dendritic cells (DCs), lymphocytes, monocytes/macrophages, and epithelial cells. Innate and adaptive immune systems make up the immune system. B and T cells, which are specialized for certain antigens, are essential for the adaptive immune response. The innate immune system, in contrast, reacts to common elements termed pathogen-associated molecular patterns (PAMPs) that are present in the majority of infections

Moreover, A complex network of signals with numerous interactions between commensal and foreign antigens and the eukaryotic cells is necessary for the gut mucosal immune system to operate. These include mucus-producing cells like goblet cells and Paneth cells, which exude antimicrobial peptides and create cryptidins, as well as epithelial cells, macrophages, dendritic cells, and other cells from the nonspecific barriers (Rook et al., 2005).

The coordination of the defensive systems depends heavily on the mucosal epithelial cells. They release chemokines and cytokines in response to environmental cues, which attract immune cells from both innate and adaptive immune responses. When the epithelial cells are exposed to these recruited immune cells, the release of cytokines is induced. The inflammatory reaction needs to be under control, and it shouldn't be brought on by naturally occurring intestine commensal bacteria. Concerning how they first contact with the immune system, the unique properties of soluble, particulate antigens and pathogens will influence the gut immune response. Dendritic cells, specialized M cells from the Peyer's patches, and individual M cells located in the villous epithelium are at least three distinct pathways for the absorption of luminal antigens. The location and method of antigen acquisition by the immune cells from the innate response (macrophages and dendritic cells) are critical factors in defining the character of the following responses. Therefore, the immunological response elicited may be the consequence of antigen uptake by M cells from Peyer's patches or the intestinal villous, or by dendritic cells through transepithelial sampling using dendritic cells (Rescigno et al.,2001). Commensal bacteria stimulate the immune system in the gut, where antigen presentation from the luminal flora results in the production of significant amounts of local immunoglobulin A (IgA) without the activation of systemic immunity (Milling et al.,2005). The interaction of phagocytic dendritic cells with T and B cells from the Peyer's Patches with the antigen-presenting cells in isolated lymphoid follicles or the mesenteric lymph nodes is necessary for the production of local secretory IgA specific for the pathogen. Immune cell activation and the start of mucosal immune responses depend heavily on the antigen internalization route. To begin the network of immunological signals, non-pathogenic probiotic bacteria must contact the gut's epithelial cells and immune cells. The most notable effect of probiotic bacteria or fermented milk yogurt was an increase in the number of IgA-producing cells. The Peyer's patches cause the production of IgA B cells, which then move via the mesenteric lymphatic nodes, enter the blood through the thoracic duct, return to the intestinal mucosa, and repopulate distant mucosal regions like the bronchus. Intestinal T cells experience similar recirculation (Phillip et al.,1983). The IgA cycle can also be increased by some probiotic microbes, and this effect is dose-dependent. The cytokines transform growth factor (TGF-), interleukin-4 (IL-4), and IL-2, IL-6, and IL-10 function synergistically from other immune cells other than T cells and can promote the transition from IgM to IgA expression. T-independent IgA induction was also observed.

Probiotic bacteria may enter the gut through numerous passageways that line up with the various methods for internalizing antigens. The M cells in the Peyer's Patches, the gut epithelial cells, and the accompanying immune cells must interact with these bacteria (either as complete cells or as antigenic pieces).To up- or down-regulate the immune response upon interaction with these cells, cytokine release is stimulated.

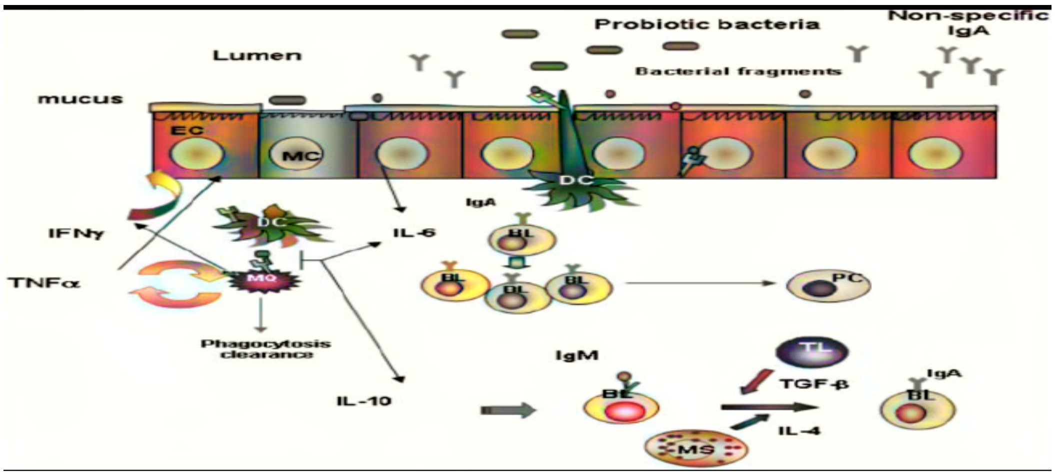

Figure 3: local immune response in the gut

FIG. 3 shows the local immune response in the gut brought on by probiotic bacteria's contact with the immunological and epithelial cells located in the lamina propria of the small intestine. It is demonstrated that the innate immune system is activated. The probiotic bacteria found in the small intestinal lumen would be internalized via many pathways: an M cell (MC) is connected to the epithelium, and epithelial cells (EC) and interdigitating dendritic cells (DC) can sample microorganisms. Probiotic bacteria or their pieces are internally absorbed after interacting with epithelial cells. Antigen-presenting cells (APC), macrophages, and/or dendritic cells connected to the gut's lamina propria are the first cells to interact with them. IL-6 is released as a result of the interaction with epithelial cells. Probiotic bacteria or their pieces are phagocytosed by macrophages and dendritic cells, which are then stimulated to release cytokines like TNF- and IFN-. These cytokines boost epithelial cell activation and start communication amongst all of the related immune cells. Additionally increased IL-4 production would occur in mast cells. To strengthen the cytokine network of signals, other cytokines are also generated, including IL-10 and IL-6. By clearing them by phagocytosis, the ingested bacteria or their particles might likewise be removed. IgA-producing cells and their transit to plasmatic cells in the lamina propria of the gut would both increase as a result of IL-6's favorable effects on the clonal growth of IgA B lymphocytes. Together with IL-4 and TGF- (not identified in our experiments), IL-6 can cause B cells to change from IgM to IgA on their surface, increasing the proportion of IgA-expressing B cells in the lamina propria of the gut. TL, T lymphocytes; BL, B lymphocytes; MS, mast cells; PC, plasma cells; EC, intestinal epithelial cells; MQ, macrophages.

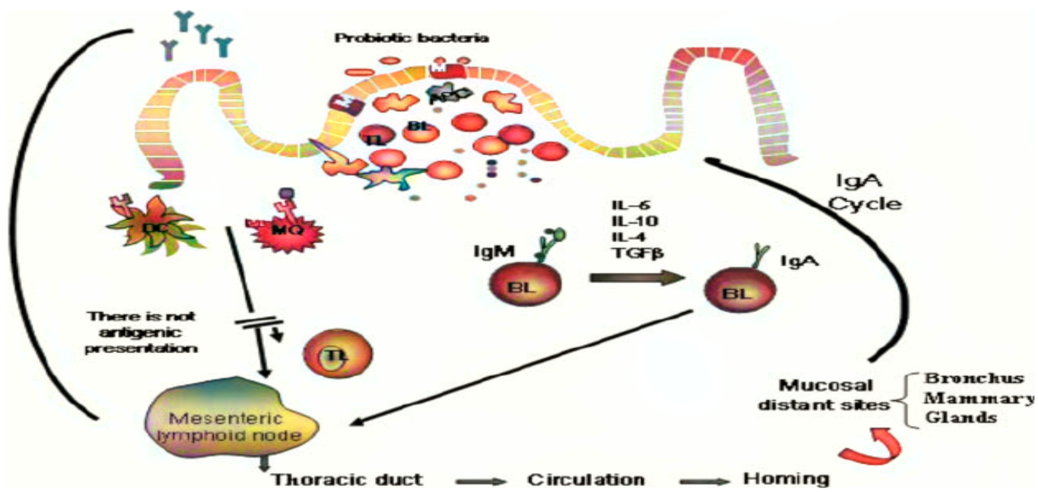

Figure 4 : systemic immunological response

FIG. 4. Systemic immunological response brought on by probiotic bacteria following contact with Peyer's patches' immune cells. Probiotic bacteria are absorbed by M cells or in a paracellular manner by follicle-associated epithelial cells of the Peyer's patches in the Peyer's patches. Following this, the bacteria or their particles engage in an interaction with macrophages and dendritic cells, which activates them and causes them to release cytokines. The bacterial activation of immune cells at this immune response induction site results in increased cytokine production and a shift from IgM to IgA B cells. Immune cells' IL-10, IL-6, IL-4, and TGF- may also encourage this T-independent transition. IgA cells are more prevalent in mucosal regions further from the colon when probiotic stimulation is present. IgA cells go from the mesenteric lymphoid node to the thoracic duct, where they enter the circulatory system and eventually reach the bronchi and mammary glands. The biological mediators of the intricate network of signals that stimulate the systemic immune response are the cytokines generated by probiotic activation

in Peyer's patches. APC, antigen-presenting cells; TL, T lymphocytes; BL, B lymphocytes; DC, dendritic cells; MQ, macrophage cells.(Galdeano et al., 2007).

Chapter 3

Incorporating probiotics in regular food

Throughout the antibiotic era, which has barely lasted for around 60 years, tons of antibiotics have been dispersed across the biosphere globally. Out of the 18,000 t of antibiotics generated annually in the United States for medical and agricultural uses, 12,600 t are used to treat animals non-therapeutically to encourage growth (SCAN 2003) 1600 t of antibiotics, or nearly 30% of the total amount of antibiotics used in agricultural animals, are utilized for growth promotion in the European Union and Switzerland (SCAN 2003). These quantities of antibiotics have put severe selection pressure on bacteria to develop resistance, and these bacteria have done so mostly through horizontal and promiscuous gene flow.(Sahu et al.,2008).

Since the use of antibiotics in food production has a direct impact on the global problem of ABR, food animals are regarded as important reservoirs of ABR. The primary ecological niches of emergence in relation to human health are community and hospital settings, with antibiotic usage serving as the primary determinant of the selection of resistant bacteria (O'Neill, 2015a; WHO, 2015a). However, the severity of the ABR worldwide threat has been exacerbated by the use of antibiotics in livestock (FAO, 2015).With the growth of food animal production and aquaculture, antibiotics are supplied not only as therapy but also as prophylactics and metaphylactic,where subtherapeutic doses are given to animals to prevent the negative effects of stress reactions that often result in infectious infections. ABR is also made worse by the widespread use of antibiotics as growth promoters for quickly expanding food animals and fish (FAO, 2015).Since antibiotics are frequently used in aquaculture, livestock production, and crop culture (Acar and Moulin, 2006;

FAO, 2015),antibiotic-resistant bacteria and antibiotic-resistant genes (ARGs) can spread easily at every stage of the food production chain and can infect humans (da Costa et al., 2013; FAO, 2015), the emergence of ABR in the food chain is regarded as a cross-sectoral problem (Chang et al., 2015; WHO, 2015a).Since many studies have found that food animals and products are colonized, infected, and contaminated by antibiotic-resistant strains like methicillin-resistant Staphylococcus aureus (MRSA), antibiotic-resistant Campylobacter spp., the emergence of ABR along the food chain is a significant global public health concern, Food animals, fish, and vegetables are regarded as significant reservoirs of antibiotic-resistant bacteria because the food production chain is an ecosystem made up of several ecological niches where numerous bacteria coexist and vast amounts of antibiotics are employed (Acar and Moulin, 2006). Additionally, contact with or ingestion of tainted food products may expose people to ARGs and microorganisms resistant to antibiotics (e.g., meat, eggs, milk, and dairy products).This more complicated and extensive indirect transmission across the food chain is a route. ARGs and large amounts of antibiotic-resistant bacteria have been found in recent reports in a variety of food products (including ready-to-eat meat, cooked meat, and bulk milk) derived from a variety of animal sources, including cattle, poultry, swine, goats, and sheep, as well as at various stages of food production (Price et al., 2012; Coetzee et al., 2016; Liu et al., 2016).

Probiotics are one of the organic alternatives that must be used to prevent this antibiotic resistance. Probiotics have various qualities that make them an effective substitute for antibiotics.(Founou et al., 2001)

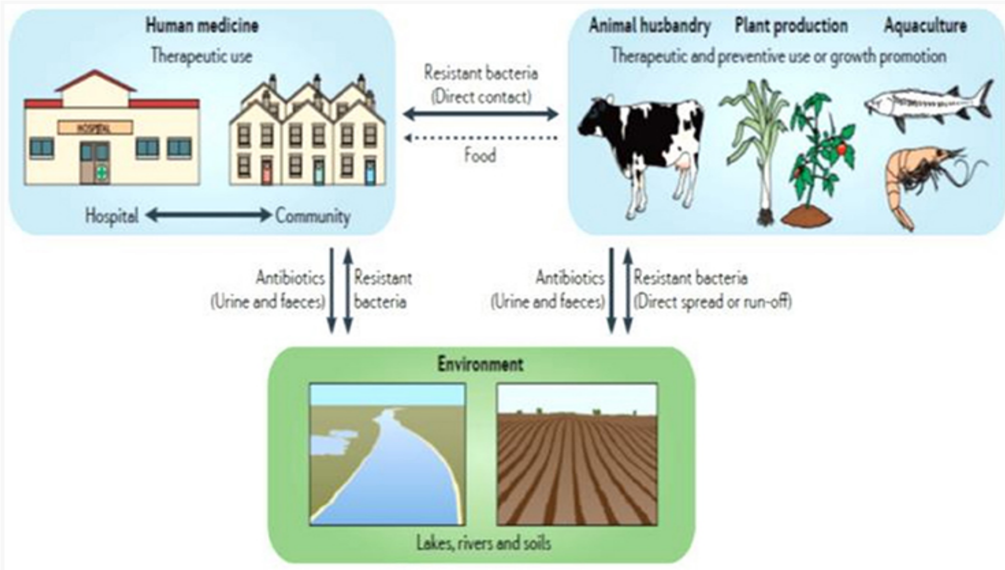

Figure 5: Antibiotic use and different transmission routes of antibiotic resistance in the food chain. Adapted by permission from MacMillan Publishers Ltd: [Nature Reviews Microbiology], (Andersson and Hughes, 2014), Copyright (2014).

Chapter 4

Incorporating probiotics in aquaculture:

Since it helps provide a significant portion of the world's need for protein-rich foods, aquaculture is a significant and quickly expanding industry. Fish are the primary ingredient in aqua products, and over 200 different fish species are raised for market value (Swapna et al.,2010).
Diseases have become a barrier in the fish farming business as a result of the industrialization and commercialization of aquaculture production (Hai 2015). Gram-negative bacteria such *Aeromonas, Flavobacterium, Pseudomonas, Vibrio,* and *Yersinia* species are the most prevalent pathogens in aquaculture that cause illness. These infections are the cause of several illnesses, including enteric red mouth disease, furunculosis, hemorrhage, septicemia, vibriosis, and others (Hamid et al., 2017; Cascales et al., 2016; Patra et al., 2016;)Chemotherapy has been used as a treatment option for prevalent illnesses in fish aquaculture. Chemotherapeutic drugs, such as antibiotics and chemicals, such as Florfenicol, Oxytetracycline, Enrofloxacin ,Chloramphenicol,Erythromycin ,Trimethoprim + Sulfamethoxazole are the standard treatment for microbial illness in aquaculture. However, frequent use of these chemotherapy drugs causes their accumulation in aquatic habitats, which has detrimental effects such as the emergence of antibiotic-resistant bacteria, accumulation of antibiotic residues in the flesh, killing off the helpful gastrointestinal microbes, and changes in the aquatic environment's microbiota (effect on non-target microbes) (Munoz-Atienzal et al., 2013; Azevedo et al. 2015). Consequently, it is now dangerous to employ antibiotics as chemotherapeutic agents in aquaculture. In addition to harming the fish's environment, eating these fish exposes the human body to the medications that eventually lead to antibiotic resistance.(Chauhan, & Singh, 2019)

Nowadays, there is a lot of worry about finding better alternatives to antibiotics that can prevent infection. Probiotics are a potential new alternative strategy for preventing fish infections because they provide fishes many ways to fight off viruses. The benefits of probiotics used in aquaculture extend beyond the gastrointestinal tract and have a significant impact on an organism's overall health. For example, probiotics promote growth, prevent disease, boost immune function, and improve water quality by altering the microbial community in water and sediments.

4.1 Benefits of probiotics in aquaculture

1. Production of inhibitory chemicals: Both gram-positive and gram-negative bacteria are inhibited by a range of chemical compounds released by probiotic bacteria. These include hydrogen peroxides, lysozymes, proteases, bacteriocins, siderophores, etc. It is well known that lactic acid bacteria (LAB) create substances such as bacteriocins that are inhibitory to other germs (Saurabh et al,.2005)

2. Competition for adhesion sites: Pathogens and probiotic microbes struggle for food and attachment sites on the gut epithelial surface, which ultimately prevents the colonization of the pathogens (Vanbelle et al., 1990) *Vibrio anguillarum* and *Aeromonas hydrophila,* two fish pathogens, have shown in vitro their ability to adhere to and proliferate in mucus from the digestive tract or the skin (Krivacek et al., 1987))

3. Competition for resources: Probiotics use nutrients that pathogenic bacteria would otherwise take. The makeup of the intestinal microbiota or the surrounding environment of aquacultured organisms can be significantly influenced by nutritional competition (Ringo et al., 1998). Therefore, it is difficult to successfully apply the theory of competition to natural conditions, and this continues to be a challenging problem for microbial ecologists.

4. Source of nutrients and enzymatic assistance to digestion: Probiotic microbes may aid aquatic animals' digestive systems, according to some studies. According to reports*, Bacteroides* and *Clostridium sp.* have helped fish maintain its nutrition by providing the host with fatty acids and vitamin*s* (Sakata T 1990) Arctic charr (Salvelinus alpinus L.) may benefit from the nutritional processes facilitated by several microbes, including *Agrobacterium sp., Pseudomonas sp., Brevibacterium sp., Microbacterium sp., and Staphylococcus sp.* By creating extracellular enzymes like lipases and proteases as well as essential growth factors, certain bacteria may also contribute to the digestion of bivalves. Similar findings have been made regarding the microbial flora of adult penaeid shrimp (*Penaeus chinensis*), which has a full complement of enzymes for compound production and digestion that the animal may ingest (Wang et al., 2000) The microbiota may act as an additional source of nutrients and microbial activity.

5. Impact on water quality: Probiotics also aid aquaculture ponds' water quality improvement. This is a result of the probiotic bacteria's capacity to take part in the ponds' organic nutrient cycle. With the notable exception of modifying the NH_3 / NO_2 / NO_3 balance, where nitrifying bacteria are used to remove toxic NH_3 (and NO_2) Fish expel nitrogen waste as NH_3 or NH_4 +, resulting in a rapid buildup of ammonia compounds that are highly toxic to fish (Hagopian et al.,1998) there are relatively few scientifically documented cases in which bacteria have helped with bio-augmentation.In comparison, nitrate is far less harmful and is tolerated in quantities of up to 4,000 mg per liter. Ammonia is converted to nitrite by several bacteria, including Nitrosomonas, and nitrite is further mineralized into nitrate by bacteria like Nitrobacter. Polymers are excreted by nitrifying bacteria enabling them to attach to surfaces and create biofilms. Skjolstrup et al. (Skjolstrup et al., 1998) showed a 50% decrease in both ammonia and nitrite in an experimental fluidized biofilter in a rainbow trout recirculating unit. Recirculating systems must use biofilters to remove ammonia. Sulfur-reducing bacteria use sulfur as a source of molecular oxygen to oxidize organic carbon. Sulfate and sulfide, which are less harmful to aquatic life, are formed when the hydrogen ion generated during the oxidation of organic carbon particles is mixed with sulfate. Carbon dioxide is used by bacteria that reduce methane as a source of molecular oxygen. Methane dissipates into the atmosphere, enhancing the water's purity.

4.2 **Methods of application of probiotics**

There are two ways that probiotics are promoted. The dried probiotics that are packaged and may be applied to water or supplied with feed must first be brewed on the farm before use. A packet of dry powder and a packet of enzyme catalyst is included in each dry probiotics package. After emptying the packets and carefully mixing, you must start the brewing process in clean, disinfected water. Typically, it is brewed with continuous aeration for 16 to 18 hours at 27 to 32 °C. Use of the final goods must happen within 72 hours. Ponds used for semi-intensive cultivation need the most aeration possible. Probiotics must be applied over two days in a row, using half of the recommended amount each time if aeration is less (Vijayakumaran, 2001)

b) Liquid forms: The majority of the time, live, ready-to-act liquid forms are used in hatcheries. These liquid forms are either mixed with farm feed or supplied straight to the tanks used for hatcheries. In indoor hatchery tanks, the liquid forms may be administered at any time of day; however, in outdoor tanks, they must be applied in the morning or the evening. Despite being less dense, liquid forms of bacteria provide favorable results faster than those in dry and spore forms (Nageswara et.al,.(2006). Probiotics have not been shown to have any negative effects, although it has been shown that their application may temporarily increase the BOD level (biological oxygen demand). For this reason, it is advised to provide subsurface aeration to hasten the establishment of probiotics organisms. During probiotics therapy, a dissolved oxygen content of at least 3 percent is advised.(Sahu et al., 2008).

Chapter 5

Incorporating probiotics in Poultry:

The poultry industry has expanded into a major economic driver in many countries. Products from chickens and turkeys are among the most eaten worldwide, yet the widespread use of antibiotics in poultry production in many countries poses risks to human health (through antimicrobial residues) and the development and dissemination of microbial resistance in poultry ecosystems. (Agyare et al., 2019).Meat production in the chicken industry is boosted by the use of antibiotics because of their positive effects on feed conversion, growth rate, and disease prevention.(Mehdi et al., (2018).

Chickens are the most commonly grown animal, with an annual production of almost 90 billion tons of chicken meat (FAO Publications Catalog 2017). Poultry is often reared with a broad variety of antimicrobials in the majority of countries. The use of several of these antimicrobials is considered vital in human medicine for example rifampin, cotrimoxazole, gentamicin, vancomycin, and chloramphenicol (World Health Organization; 2010:1-43. 14 World Health Statistics, 2017). This critical usage of antimicrobials in animal production is likely to promote the development of AR in both commensal and pathogenic bacteria. There would be treatment failures, financial losses, and the risk of the sickness spreading to humans. There are concerns for human health owing to the presence of antimicrobial residues in animal products such as meat (Mirlohi et al., 2013); (Darwish et al., 2013), eggs, and other foods.

When an antibiotic is used, the strains of bacteria that can be killed by the drug are often wiped out, leaving only those that are resistant to the drug. The genes that make them resistant can then be passed on to other bacteria, both horizontally and vertically, as these resistant bacteria grow and take over (Laxminarayan, 2013). People who eat or handle infected meat have a chance of getting resistant germs from poultry products (van, den Bogaard, et al., 2000). Once these pathogens are in the body, they may take up residence in the intestines and share or pass on their resistance genes to the native intestinal flora. This could make it harder to treat infections caused by these organisms in the future. Estimates show that more than 60% of all antibiotics made are used in the chicken industry.(Agyare et al., 2019).

Disease problems and damage to the environment are common in large-scale chicken farms where chickens are kept in stressful conditions. These problems cause huge economic losses. Disease prevention and control measures have led to a big rise in the use of veterinary pharmaceuticals over the past several decades. Pathogenic bacteria have a long history of becoming resistant to antibiotics. Because of this, antimicrobial agents have been called into question as a preventive tool.

Because of this, both consumers and producers are looking for alternatives because antibiotics might not be used to help chickens grow and because people are worried about the bad effects of using them as medicines. Probiotics are being looked at to fill this gap.

For a healthy gut, one needs to eat a diet that is well-balanced and full of nutrients and energy. Nutritionists and veterinarians have recently put a lot of effort into helping chickens grow by making sure they get the right nutrients and using probiotics.

In broiler nutrition, probiotic species from the families *Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida, and Saccharomyces* have a positive effect on broiler performance, modulation of intestinal microflora and pathogen inhibition, intestinal histological changes, immunomodulation, specific haemato biochemical parameters, improving the sensory qualities of dressed broiler meat, and Probiotics keep normal intestinal microflora in chickens through competitive exclusion and antagonistic interactions. They also change the metabolism by increasing the activity of digestive enzymes and decreasing the activity of bacterial enzymes and the production of ammonia. improving feed intake, digestion, and stimulation of the immune system. Probiotics keep normal intestinal microflora in chickens through competitive exclusion and antagonistic interactions. They also change the metabolism by increasing the activity of digestive enzymes and decreasing the activity of bacterial enzymes and the production of ammonia. improving feed intake, digestion, and stimulation of the immune system.

Probiotic is an umbrella term for products that encourage microorganisms that can change the gut environment in a way that is good for health and makes feed more efficient (Dierck, 1989). There may be yeast cells, bacterial cultures, or both in these products. Some of the ways probiotics improve feed conversion efficiency are by changing the intestinal flora, encouraging the growth of nonpathogenic facultative anaerobic and gram-positive bacteria that make lactic acid and hydrogen peroxide, stopping the growth of intestinal pathogens, and making digestion and nutrient use better (Yeo, 1997). So, using probiotics has three main effects: it makes plants grow faster, it makes people live longer, and it makes feeding more efficient. In a previous study, Tortuguero and Fernandez (Tortuero F, Fernandez E1995) found that adding probiotics to feed increased the efficiency of feed conversion.

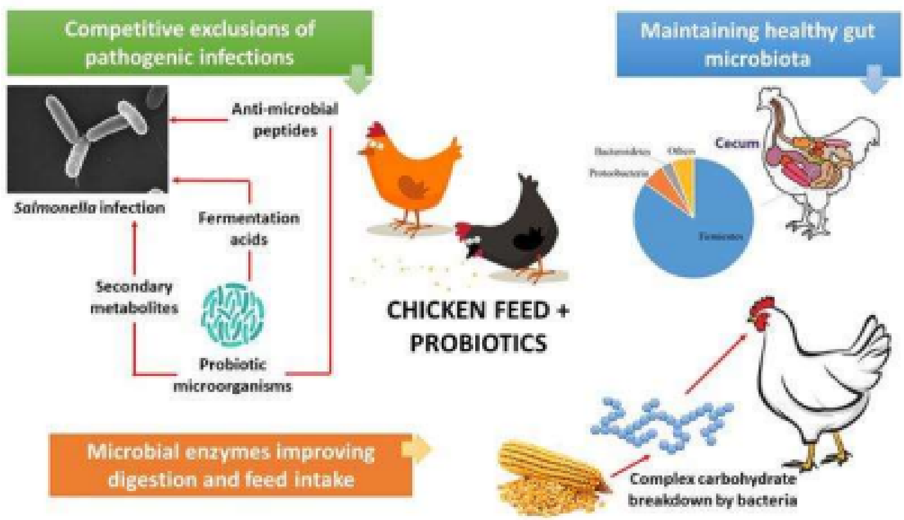

Figure 6: The mode of probiotic actions in poultry (Suresh et al. 2020)

5.1 Evaluating Probiotic Effects on the Intestinal Microbiota and Intestinal Morphology

Kabir et al. (Kabir et al.,2005) . measured the total viable count (TVC) and total *lactobacillus* count (TLC) of the crop and cecum samples of the probiotics and conventionally fed groups at the 2nd, 4th, and 6th week of age to see how well probiotics clear up bacterial infections and control intestinal flora. In the end, they found competition. The results of their study also showed that some harmful bacteria were stopped by probiotic organisms because they took up space on the gut wall. They also found that broilers given probiotics had more dramatic changes in the histology of their intestines than controls, such as active cell division and bigger nuclei.

Watkins and Kratzer (Watkins et al.,1983). found that chicks who were given *Lactobacillus strains* had fewer coliforms in their feces than the control group. Francis et al. found that adding a *Lactobacillus* product at a rate of 75 mg/kg of feed made a big difference in the amount of coliform in the ceca and small intestine of turkeys. (Fuller, 1977) found that *Escherichia coli* in the colon and small intestine might be lessened by host-specific *Lactobacillus* strains. Kizerwetter-Swida and Binek (Kaiserwetter-Swida et ai.,2009) found that *Salmonella enteritidis* and *Clostridium perfringens* were less common in the group of hens given *Lactobacillus*. This was true for the *L. salivarius* 3d strain. Watkins and others found that when *L. acidophilus* was given to gnotobiotic chicks, pathogenic *E. coli* was pushed out of the GI tract. This was published in the journal Int. J. Mol. Sci. 2009, 10:3538. (Yaman et al.,20060; (Mountzouris et al.,2007), and (Higgins et al.,2007) recently found that probiotic species from the families *Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida,* and *Saccharomyces* may be able to change intestinal microflora and stop pathogen growth.

5.2 Evaluating Probiotic Effects on Immune Response

When Kabir et al. (Kabir et al., 2004). looked at how probiotics affect the immune responses of broilers, they found that the experimental birds made a lot more antibodies than the control chickens (P 0.01). They also found that the difference in spleen and bursa weight between broilers that were fed probiotics and those that weren't could be explained by the different amounts of antibody production in response to SRBC (sheep red blood cells, or SRBC, which are frequently used by scientists to study humoral immune responses in poultry). They also thought that the probiotic had a positive effect on some of the early immune responses against *E. acervulina*, as shown by early production of IFN- and IL-2, which led to better local immune defenses against coccidiosis. Brisbin and the others (Brisbin, 2008)

When probiotics are taken, they change the gut microbiota, which in turn affects how the immune system works (McCracken et al.,1999). The immunomodulating effects of probiotics seem to be caused by unknown mechanisms. Researchers have found that probiotics cause different types of immune system cells to make cytokines, which in turn help to start and control the immune response

5.3 Evaluating Probiotic Effects on Meat Quality

When Kabir (Kabir SML, n.d) and Kabir et al. looked at the effects of probiotics on the sensory qualities and microbiological quality of dressed broiler meat, they found that adding probiotics to broiler rations improved the quality of the meat both before and after freezing. According to Mahajan et al. (Mahajan et al., 2000) the probiotic (Lacto-Sacc) fed group's scores for how the meatballs looked, how they felt, how juicy they were, and how well they were accepted overall were significantly ($p < 0.001$) higher, but their scores for how they tasted were lower. In the same way, Mahajan et al. found that beef from the International Journal of Molecular Sciences 2009, 10 3539 was contaminated with a virus.

When compared to meat from chickens that weren't given probiotics (Lacto-Sacc), the total viable count was lower in animals that were given probiotics. But Loddi et al. found that neither probiotics nor antibiotics changed the way breast and leg meats tasted, smelled, felt, and looked, But Zhang et al. (Zhang, 2005) used 240-day-old male broilers to study the effects of *Saccharomyces cerevisiae (SC)* cell components on the quality of meat. They found that whole yeast (WY) or *Saccharomyces cerevisiae* extract might make the meat softer (YE)(Lutful Kabir, 2009).

Chapter 6

Incorporating probiotics in Dairy:

Dairy products have been enjoyed for almost 6,000 years, and now you can find them in almost every country. Dairy product preparation, fermentation, and consumption have seen remarkable levels of innovation during the last few millennia. There will be an increase from the expected $871 billion in 2021 to $1,128 billion in 2026 in the value of the global dairy market. As the demand for dairy products rises, producers face more pressure to keep up with supply. Antibiotics are being used more often because people are afraid to become sick (Shahbandeh, 2022).

Fluid milk is the most widely eaten kind of dairy in the developing world. Liquid milk is in higher demand in cities, while fermented milk is more popular in rural regions, although processed goods are on the rise in many nations. Six billion people throughout the globe, the vast majority of whom are residents of developing nations,(Dairy Production and Products: Products, n.d) eat milk and milk products regularly. Antibiotic such as beta-lactams, spectinomycin, florfenicol, and tetracyclines residue in milk is used for several things by producers, including:

Therapeutic Antibiotic Uses: Using antibiotics inappropriately to treat infectious disorders such as clinical mastitis and viral infections is a major contributor to the prevalence of ARs in milk.

Antibiotic usage as prevention: Antibiotics are sometimes used in dry cow treatment and post-surgical risk management, both of which contribute to antibiotic resistance (AR) in milk.

Inadequate adherence to labeled procedures may result in antibiotic residues in milk.

Inappropriate use of an antibiotic that has only been licensed for human consumption, use in species for which it has not been approved, use in a setting in which it has not been approved, or use over the recommended dose are all examples of extra-label use.

Antibiotics may be retained in tissues for longer and at greater levels in ill animals because their normal metabolic process of antibiotics is hindered.

6.1 Results of ARs on public health and the dairy industry:

One major problem is the development of antibiotic-resistant bacteria owing to the presence of even trace amounts of antibiotics in milk and other dairy products. Resistant microorganisms can spread between people either via personal contact or by the spread of resistance genes in the environment.

The normal microbial flora of the gut colonizes and lives in harmony with a wide variety of different bacteria to keep harmful ones from causing illness. It has been hypothesized that antimicrobial resistance (AR) in milk caused by the use of broad-spectrum antibiotics can kill a wide range of microflora in the intestine, including the non-pathogenic organisms, which can make the disease-causing microorganisms more prominent and disrupt the normal intestinal environment.

Seventhly, the impact on the dairy industry: Even at trace levels, the presence of ARs in milk is a cause for alarm. Cheese and yogurt fermentation may be stunted by antibiotic residues, which can also contaminate the finished product.

To keep the calves' productivity up and the scours down, antibiotic treatment has been used. However, probiotic additives have been developed as an alternative to improve animal health and productivity in light of rising safety concerns regarding the risks of antibiotic resistance brought on by the release of antibiotics into the environment and the persistence of chemical residues in animal products. (Uyeno et al., 2015). (By 2020, the estimated value of the worldwide market for probiotics in animal feed was $4.4 billion. Assuming a CAGR (compound annual growth rate) of 8.8% from 2021-2026, the market is expected to reach $7.3 billion (USD) by that year. As more people learn about the advantages of probiotics in animal feed, they are more likely to buy high-end goods that include probiotics in animal feed. This should keep demand high. (Probiotics in Animal Feed Market by Livestock, Source, Form, and Region | MarketsandMarkets, 2022)

Therefore, it is time to hunt for superior alternatives to minimize the usage of antibiotics in this business, and one of the most notable options is probiotics.

Because fermented dairy products have been proven to be the most effective delivery mechanism for live probiotics to date, the dairy sector is responsible for the production of the majority of the probiotic foods that are now available to consumers. There is evidence to suggest that dietary matrices have a key part in the probiotics' positive impacts on the host's health. (Espirito et al., 2011); (Shori et al.,2014) The majority of the time, probiotics are delivered to the gut through fermented meals, notably dairy products. Because fermentation is a very low-cost method of preserving food, increasing the food's nutritional worth, and enhancing the sensory characteristics of the meal, it makes a significant contribution to the human diet in many nations. However, the growing demand for novel probiotic products has pushed the development of additional matrices that may carry probiotics. Some examples of these matrices are ice cream, newborn milk powder, and fruit juice. (Shori et al., 2018)

Lactobacillus, Bifidobacterium, Bacillus, Saccharomyces, and *Enterococcus* are only a few of the many lactic-acid bacteria strains employed as probiotics in functional foods and animal feed. Probiotic species such as *Lactobacillus* and *Bifidobacterium* have been demonstrated to prevent gastrointestinal infections. Single or many strains of various microorganisms, such as bacteria and yeast, make up these helpful microbes. There is a synergistic adhesion effect between the various strains in multi-strain probiotics, which may amplify the positive benefits of the probiotics (Timmerman et al., 2004). Common uses for probiotics include protecting against viral infections and reducing gastrointestinal symptoms like diarrhea and bloating (Liong, 2007). Multiple studies have shown positive effects from giving ruminants probiotics orally. Probiotics boost the host's resistance to illness, encourage growth and development in animals, and maintain a healthy balance of gut bacteria (Xu H et al.,2007). Probiotics have been shown in recent research to have a number of positive effects on the health, growth, and productivity of ruminants when used as a feed supplement. It has been proven that the use of probiotics may lessen the environmental damage caused by the production of ruminants, particularly the release of methane.

6.2 Probiotics' Impact on Weight Gain and Strength Gain

Dry or live probiotics are natural feed additions that have been demonstrated to increase animal performance and welfare by influencing the gut microbiome, which plays a crucial role in maintaining host homeostasis (O'Hara et al.,2007) When given to animals as a single strain or as part of a combination of strains, probiotics improve their growth rate and production output. There is evidence that probiotics given orally to sheep, goats, and cattle enhance feed intake, daily weight growth, and total weight gain. Studies have demonstrated that administration with probiotics including beneficial microorganisms like *Lactobacillus* and *Bifidobacteria* enhances their development. This is especially important since the population of beneficial microbes is low in newborn calves. Live yeast probiotics enhanced feed intake, feed efficiency, ADG, and total weight growth in dairy cows. Milk production and quality were both improved by probiotics (Stein et al.,2006) ;(Poppy et al., 2012).

6.3 The Milk Probiotic Effects

Supplementing a bovine diet with probiotics improves milk output, milk quality, and functional components such as protein and fat content. Probiotic dairy products are safe for widespread use in scientific studies. Milk production and milk protein levels were shown to be higher in cows given the probiotic strains *Aspergillus oryzae* and *Saccharomyces cerevisiae*, according to research by (Yu et al.,1997) Probiotics boosted dry matter intake in dairy cows, as well as improved feed utilization, milk output, and milk component profiles, according to studies by (Stein et al., 2006).,and (Stella et al., 2007). Somatic cell count was decreased, udder inflammation was decreased, and milk production was increased after probiotic treatment, as reported by(Xu et al.,2017)Milk output, milk fat, protein, and lactose yield, and milk somatic cell count were all observed to rise after probiotic treatment to dairy cows by (Sun et al.,2011) and (Lehloenya et al.,2008) The following effects of probiotics on the quantity of cellulolytic and fiber-degrading bacteria, as well as changes in the volatile fatty acid in the rumen, are responsible for the beneficial effects of probiotics on milk production and milk quality attributes.

6.4 Immunity and probiotics

The capacity of probiotic bacteria to alter the immune system and increase both innate and adaptive immune responses has been linked to their favorite health benefits. Multiple in vivo and in vitro investigations have shown that probiotics improve gut health by boosting the immune system's natural defenses. A variety of probiotic bacteria, such as *Lactobacillus casei* and *Shirota, Streptococcus thermophilus, Lactobacillus fermentum,* and yeast, have been shown to stimulate the immune system in tests (Matsuzaki, 2000)

6.5 Bovine probiotics

Dairy and beef cattle of all ages and stages of development have benefited greatly from the use of probiotics in modern cattle farming. Directly fed microbial or probiotic bacteria like *Lactobacillus* and *Bifidobacteria* have been shown to improve the health of dairy cows, beef cattle, neonatal calves, and periparturient cows in several ways, including their growth, production performance (milk production, milk functional components, and milk composition), and immune response (Worku et al.,2016). Improved immunological response in stressed calves was another positive outcome of probiotic administration. Probiotics also showed promise in reducing ruminal acidosis in feedlot cattle and dairy cows. The use of probiotics enhanced the ADG, feed efficiency, and feed intake of dairy cows. Milk production and quality were also improved by probiotics (Stein et al.,2006) and Poppy et al., 2012].(Adjei-Fremah et al., 2018)

Chapter 7

Probiotics in fruits and vegetables :

Since the 1950s, antibiotics have been used to mitigate the effects of microorganisms on high-value crops such as fruits, vegetables, and flowers.(McManus et al., 2002). Antibiotics were first developed to combat the bacterial illnesses that plagued crops and fruits, namely fire blight. Sadly, streptomycin-resistant plant infections have impeded plant disease management in the same way as antibiotic-resistant bacterial strains have hampered the efficacy of similar medications in human medicine.

Because of their great nutritional content, fruits and vegetables are recommended by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) as part of a healthy diet (WHO2013 and Darmon N et al.,2005). Many fresh leafy and non-leafy vegetables, root vegetables, sprouts, and fruits pose a risk of exposing people to foodborne bacterial pathogens, including antibiotic-resistant bacteria (ARB). Over the last several decades, there has been a rise in reports of foodborne illness outbreaks attributed to exposure to antimicrobial-resistant bacteria along the food chain (Rahman et al., 2021). Animal feces and manure in the soil, water, or fertilizer may taint fruits and plants. (C. 2021, October 26).

New technical advances have become necessary to lessen the usage of ecologically hazardous chemical fertilizers and pesticides as interest in sustainable agriculture have grown in recent years. Many researchers have looked at the use of plant probiotics as a source of alternative soil fertilization due to their positive impacts on nutrient delivery, reduced need for field maintenance, and lack of negative side effects (Bhardwaj et al., 2014)

Many biogeochemical processes are mediated by the microflora of soil, with certain species influencing organic matter and soil pollutant biodegradation (rhizoremediation), and abiotic stress tolerance.(de Souza et al.,(2017).

Many diverse processes in nature are referred to as ecosystem services, and the soil plays a crucial role in establishing links between the air, water, rocks, and creatures. Because it contains antibiotic resistance determinants from every known species of plant, fungal, soil bacterial, small animal, and protist, it may be thought of as a sizable reservoir (Manyi-Loh et al., 2018).

(More than 175 million tons of chemical pesticides and fertilizers are applied to soils annually to increase agricultural yields, as reported by (Shahzad et al.,2013) Agriculture now relies heavily on this commodity to meet the needs of a hungry globe. Chemical fertilizers are widely used, however, they are very pricey and detrimental to both human and environmental health. For plants, the group of beneficial bacteria (probiotics) known as plant probiotic bacteria (PPB) was defined by (Haas 2003) based on their efficiency in niche colonization, ability to induce systemic resistance in their host, improvement in plant nutritional content, and increase in crop quality (Menéndez et at., 2017). How bacterial plant probiotics may boost vitamin C and disease-preventing plant components, and vice versa. Humans rely heavily on horticultural crops for a wide range of vitamins and minerals. (Ramsay et al., 2017), found a correlation between eating a variety of horticulture fruits and having a higher quality diet overall. Vitamins have an important role in human health because they correct nutritional imbalances that may lead to deficiency symptoms. Researchers are working on a variety of strategies to boost the vitamin density of horticulture crops. According to (Bona et al.,2017) inoculating tomato plants with *Pseudomonas sp.* 19Fv1T increases crop output and raises vitamin C content in tomatoes compared to the control treatment. When two different strains of *Bacillus amyloliquefaciens* were introduced to the tomato fruits, Gül et al. [94] found that the fruit's vitamin content increased to a maximum (FZB2 and FZB42). Tomato yields and vitamin C fruit content were shown to be boosted by a combination of *Bacillus amyloliquefaciens, Bacillus megaterium,* and *vermicompost*, as demonstrated by (Shen et al.,2016). Tomatoes are widely grown for their fruit, but they are also prized for the antioxidant compounds they contain. After inoculation with *Bacillus licheniformis,* (Ochoa-Velasco et al.,2016) reported a significant increase in vitamin C and total phenols levels at lower nitrogen dosages. The basil plant (Ocimum basilicum L.) is a fascinating culinary and medicinal herb. The addition of plant probiotic bacteria has boosted its antioxidant power. Antioxidant levels were maximum when a combination of *Pseudomonas putida, Azotobacter chroococcum,* and *Azospirillum lipoferum* bacteria was injected into the system. Basil's antioxidant activity increased both under water stress and after being inoculated with *Pseudomonas sp Bacillus lentus* and *Azospirillum brasilense .(Jiménez-Gómez et al., 2019).*

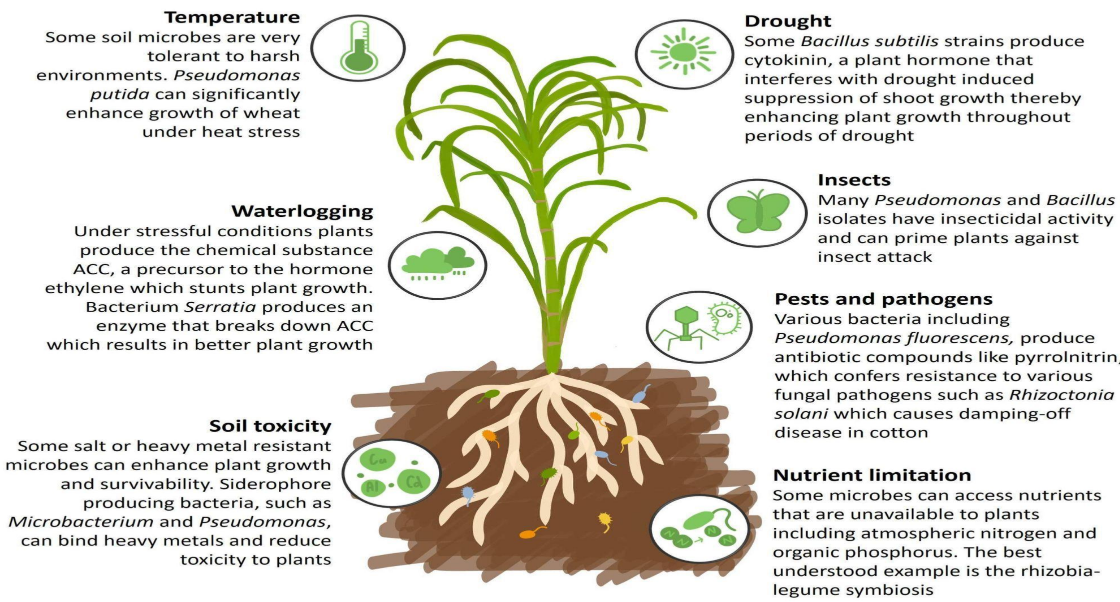

Figure 7: Confirmed benefits of crop probiotics on plants .(Credit: Mrs Shelby Berg)

Crop probiotics are beneficial microorganisms administered to crops to aid in their recovery from environmental challenges such as drought, heat, pests, toxic soil, and nutrient deficiency.

Some strains of *Bacillus subtilis* produce cytokinin, a beneficial plant hormone that lowers the impact of drought-induced restriction of growth, while *Pseudomonas putida* promotes wheat growth under heat stress, as Mrs. Berg points out. (*Promoting Plant Probiotics*, n.d.)

Conclusion:

Antibiotic-resistant bacteria (ABR) in human consumption and agricultural production have had far-reaching effects on human health and economic development across the globe. ABR can be transmitted to humans directly or indirectly through food consumption and direct or indirect interaction. With no action taken, drug-resistant diseases would claim the lives of an extra 10 million people every year by 2050.

Probiotics are microorganisms that have learned to live with humans and may even affect biological processes for the better. Probiotics have not been extensively studied as alternatives to conventional antimicrobial therapy or as a source of new antibiotics. Due to their capacity to alter intestinal microbiota and the immune system as well as their antagonistic effects on some pathogenic bacteria, probiotics seem to be a promising solution for resolving ARG-related problems.

Recent research has shown that supplementing animal feed with probiotics enhances disease resistance and health by altering the gastrointestinal tract's microflora. Probiotics may prevent the growth of harmful bacteria in the digestive tract, including those that cause food poisoning. These bacteria include *Campylobacter, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella*, and *Staphylococcus aureus.* According to the WHO, preventing antibiotic resistance may be achieved by the regular intake of probiotics, which will lead to reduced antibiotic usage and the development of natural immunity.

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