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Health Promoting and Disease Preventing Properties of Probiotics with Special Reference to Lactobacillus: A Review

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Abstract

During the last two decades, the knowledge about the proper use of probiotics has been significantly improved. By definition, probiotics are "live microorganisms which when applied in specific amounts by following a proper way can improve the host health". These beneficial bacteria follow different mechanisms to improve the host health and protect it from the harmful effects of different pathogenic microorganisms. There are different types of microorganisms that are considered as probiotics. This review specially focuses on the probiotic properties of different species of the genus of *Lactobacillus*. Here we have highlighted different aspects of *Lactobacilli* for improving animal health such as, their role in immunity, dairy industry, fermentation and their antimicrobial activity. Although, much work has been done in the field of probiotics, still a large part of it needs to be investigated. Currently research has been focused on these probiotic organisms in order to find out their beneficial aspects that are associated with food and health. Scientists are trying to prepare such foods that can provide health benefits along with overcoming nutrient deficiencies. Such kind of food known as functional food can provide better health benefits along with safe nutrient provision. However there is some uncertainty about their role regarding some diseases like their anti-carcinogenic effect, anti-cholesterolaemic effects, anti-oxidative properties, antiulcer properties, anti-inflammatory activity, prevention of genital and urinary tract infections and their potential protective effects against inflammatory bowel diseases. The present review will provide an understanding of the health promoting and disease preventing properties of probiotics in general and *Lactobacilli* in special.

Keywords: Probiotics; *Lactobacilli*; Health benefits; Early history

Introduction

Bacterial strains belonging to the genus *Lactobacillus* are mostly known to be of non-pathogenic nature that is present in most of the environments. These bacteria are involved in the processes like fermentation and preservation of food products because they are generally regarded as safe. Probiotics are defined as "living micro-organisms, which on intake in specific numbers exerts health advantages beyond innate basic immunity" [1,2].

Awareness in this area was started by Metschnikov 100 years before [3]. Conventionally, the genus *Lactobacillus* is defined by the production of lactic acid as the main end-product from carbohydrate metabolism. They are coupled with normal microbial population of gastrointestinal tract of humans and other animals. The genus *Lactobacillus* consists of a wide group of non-spore producing Gram-positive bacteria.

They are rod shaped and are generally deficient in catalase; however pseudo-catalase can be found but they are in rare cases. They are chemoorganotrophic and grow only in complex media. Fermentable carbohydrates are used as energy source.

Hexoses are broken down mainly to lactate (homofermentatives) or to lactate and supplementary products such as acetate, ethanol, CO_2, formate or succinate (heterofermentatives). *Lactobacilli* are found in foods, in sewage, on plants, as well as within the genital, intestinal and respiratory tracts of humans and other animals [4,5]. These bacteria generate a number of low molecular mass compounds together with acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide and various metabolites.

Many of those metabolites have a wide activity range against different species, and their production is mainly affected by the food medium itself. *Lactobacilli* produce a mixture of antagonistic substances that consist of end products which are resulted from metabolism, antibacterial peptides and substances similar to antibiotics which are called bacteriocins. The potential of bacteriocins produced by *Lactobacilli* to inhibit pathogenic microorganisms may be either limited that is to inhabit only those bacteria that are closely associated to them or they may inhabit a wide range of gram-positive bacteria.

The antimicrobial peptides produced by *Lactobacilli* are regarded as safe for normal preservation or biopreservation as they are considered to be digested by the enzymes present in the gastrointestinal tract [6]. Bacteriocines are protein molecules which are released extracelularly and inhibit those organisms that are closely related to the producer strain (Table 1). A number of bacteriocins have the potential to prevent the food from being spoiled by the harmful bacteria [7].

During the first half of the last century nisin was discovered which is the first bacteriocine to be used at market level for food preservation but extensive study was started during the last 20 years for searching new bacteriocine producing bacteria from different sources and right now several bacteriocines are being discovered and studied in detail [6].

Lactic acid bacteria (LAB) include several groups of bacteria under the phylum firmicutes. The genus *Lactobacillus* is accepted as LAB [8].

Microorganisms considered as probiotics	
Lactobacillus species	*Bifidobacterium* species
L. acidophilus	*B. adolescentis*
L. casei	*B. animalis*
L. crispatus	*B. bifidum*
L. gallinarum[1]	*B. breve*
L. gasseri	*B. infantis*
L. johnsonii	B. lactis[2]
L. paracasei	*B. longum*
L. plantarum	
L. reuteri	
L. rhamnosus	
Other lactic acid bacteria	Non lactic acid bacteria
Enterococcus faecalis[1]	*Bacillus cereus* var. to yoi[1]
E. faecium	*Escherichia coli* strain nissle
Leuconostoc mesenteroides	*Propionibacterium freudenreichii*
Pediococcus acidilactici[3]	*Saccharomyces cerevisiae*
Sporolactobacillus inulinus[1]	*S. boulardii*
Streptococcus thermophilus[3]	

Table 1: Adopted from Maria Kechagia et al. [1]Mainly used for animals. [2]Recently reclassified as *B. animalis* and subsp. *Lactis*. [3]Little is known about probiotic properties.

Role of probiotics in immunity enhancement

Nowadays one of the major health problems is the appearance of resistant bacteria to antibiotics. In order to overcome this problem, research is mainly focused on finding such drugs that are more efficient against these resistant bacteria. In such situations, *Lactobacilli* and their products can be a better choice to be used as a substitute of antibiotics. The members of the genus *Lactobacilli* are mostly found in fermented products and commonly residing the gut of many animals including humans and presently most people including customers and researchers are attracted by these bacteria to be used as health promoters because more knowledge is available on their beneficial effects towards food, health and diet.

The mammalian immune system is a combination of different types of cells and molecules that mutually provide a defense mechanism to the body against different harmful microorganisms. Antigens are protein molecules that stimulate the immunity, are produced by most of the pathogenic microorganisms. Different parts of the body are involved in the stimulation of immunity. For example thymus and bone marrow are involved in the development of various immune cells, while lymphatic tissues of the mucosa, spleen and lymph nodes coordinate the immune reaction.

Mucosal layer is the primary target for majority of the antigens to get entry into the body where immune reaction of the mucosa acts as primary barrier for these antigens. The microbial population of the intestine has a key role in the activation of microbes that affect the animal's health both positively and negatively. Thus they are considered to be the most important agents as they are involved in the growth of postnatal immunity and oral tolerance and immunity. The microflora obtained by a child at the time and instantly after birth may be essential for the development of his immune system and they should be able to control inflammatory reactions in allergic as well as inflammatory bowel diseases. It is also possible that probiotics can obstruct such inflammatory reactions by balancing the intestinal microenvironment and the pours of the intestinal wall by enhancing the breakdown of the intestinal antigens and changing their immunogenicity. The most important future justification for mechanism of action of probiotics against pathogenic bacteria is immunomodulation and immunostimulation. Many studies have been conducted in the field of probiotics by using various organisms and models to check the immunologic importance of probiotics [9].

Use of probiotics in fermentation

Conventionally, cultures of probiotics are related with fermented products and cultured milk. Evidences show that these probiotics have optimistic effect on human health [10,11]. Many Lactobacillus strains have been studied for their antagonistic activity against various pathogenic bacteria such as *E. coli*, *Clostridium difficile*, *Helicobacter pylori* and *Campylobactor jejuni*. All the strains of *Lactobacilli* of human origin have the ability to inhibit the growth of pathogenic bacteria of gastrointestinal tract of humans. Moreover, bacteriocines also have anticholestrole and antitumor like properties. Through various chemical reactions bacteriocins can reduce the nitrates, enhance the immune system and vitamin B absorption. The transity *Lactobacilli* of the gastrointestinal tract are able to transport enzymes and other chemicals inside the intestine that almost help to normalize the microflora of the intestine [12]. The rising population of the world needs to use the present livestock resources in order to fulfill the need for animal protein requirements. The people are using beneficial microorganisms from the past hundred years when they were using fermented milk. The word probiotic was used for the first time by Lilly and Stillwell in 1965 for this kind of microorganisms and according to them probiotics are substances secreted by one microorganism that stimulate the growth of others [13]. According to Fuller, probiotics are live microbial feed supplement that beneficially affects the host animal by improving the balance of the intestinal microflora [14]. As the antibiotic growth promoters used for promoting animal growth and prevention of diseases have harmful effects on eggs and meet, therefore the concept of probiotics to be used as alternative to antibiotics in poultry industry has become very much significant in the last few years. *Lactobacilli*, *Bafidobacteria* and *Streptococci* are lactic acid producing bacteria that are mostly used in the preparation of probiotics [15]. Due to its good probiotic potential, the genus Lactobacillus has been regarded as a better probiotic bacteria and it has significant importance [16, 17]. *Lactobacilli* are thought to be the most important lactic acid bacteria. In chickens they are mostly found in the areas of ileum and crop [18]. The aim of the present study is to isolate and characterize the genus *Lactobacillus* from chickens and to check their probiotic potential for their use in poultry industry.

Mechanism of action of probiotics

There are multiple ways through which probiotics improve the host health. Probiotics are able to avoid diarrhea and irritation either individually or in combination with the host immune system. These

probiotic bacteria attach to the mucosal wall of the gut and therefore prevent the pathogenic microorganisms to attach to the mucosal wall. Bacteriocines produced by *Lactobacilli* working like antibiotics against many harmful microorganisms and may reduce the formation of pro-inflammatory cytokines like IL-12, IFN-γ and TNF-α. Probiotic *Lactobacilli* are also involved in the stimulation of IgA formation [19]. *Lactobacilli* inhibit the growth of pathogenic bacteria by producing lactic acids and acetic acids. It is believed that probiotic *lactobacilli* compete with other pathogenic microorganisms either by modifying the toxic substances secreted by the pathogenic organisms or by competing for the available nutrients in the gut. According to Saavedra et al, the characteristics of a probiotic to be efficient are resistance to bile and gastric acids, potential to prevent the attachment, settlement and replication of pathogenic microorganisms in the gastrointestinal tract and they should also be resistant to being digested by the pancreatic enzymes [20]. There are several members of the family *Lactobacilli* that are regarded as probiotics like *Lactobacillus Rhamnosus* GG, *Lactobacillus* acidophilus and *Bifidobacteria*. A lot of bacteria can be considered as probiotics but everyone has a different mechanism of action in various situations of diseases, taking into consideration that some of the diseases can be better treated with mixture of bacteria and that there is a concern about the dosage and the element of bacteria i-e non-viable vs. viable. Treatment with the help of probiotic bacteria may be safe but not risk free because they can be potentially harmful. In a recent study it has been reported that 3 patients with fungaemia who were found with probiotic origin through DNA fingerprinting. Studies on the infections caused by probiotics underline the truth that those patients were generally immunosupressed with several ways of entrance such as urinary catheter and venous [21].

Probiotics benefiting the host health

It is generally believed that species of *Lactobacilli* that are identified as probiotics, can promote the health through different mechanisms when they are given to rats either orally or through some other ways. They can prevent intestinal infections, control the level of cholesterol in the serum, enhance the immune system in humans and rates and may enhance the growth in pigs and chickens. There are different methods through which probiotics can improve the host health and enhance the gut wall such as: adherence to the binding sites to prevent the pathogenic microbes to attach to the gut wall, may produce substance to inhibit the pathogens and restructuring of the microflora of gastrointestinal tract and metabolism [22]. The effectiveness of *Lactobacilli* in rabbits is uncertain because they are not the normal residents of the digestive tract of such species and also their poor adherence to the epithelial cells. Different studies on pet rabbits have shown that the species of *Lactobacilli* can be used effectively for the treatment of enteritis caused by antibiotics. These studies have recommended two manners in which they act. Firstly the *Lactobacilli* can inhibit the growth of pathogenic *E. coli* therefore it will be better in situations when the infection is caused by *E. coli*. The next assumption is also about the rabbits which say that, as *Lactobacilli* are commonly found in the gut, therefore they may be eliminated by the improper use of antibiotics. Third condition is that, as probiotics are living organisms, feeding of these probiotics to animals at commercial level should be successful, applied immediately after birth and they must be able to minimize the uncontrolled factors the quality of water and proportioner/medicator role and stability. Such drawbacks can be pointed out and they can be reduced if we apply the probiotics through spray method as they can be seen in the poultry industry [23,24].

The beneficial effects of probiotics on human health got the attention of the people after 1908 when Metchnikoff proposed that people should use milk fermented with *Lactobacillus* for prolonged life. It is just a recent concept that the association between intestinal micro flora and their beneficial effects are still to be explored. Recently it is accepted that the optimal balance of the micro flora in the human digestive tract depends upon the quality of food and health. The primary microorganisms that play a key role for this balance are members of the genus *Lactobacillus* and *Bifidobacterium*. Factors such as diet and stress that exert harmful effects on the relation between intestinal micro flora may leave harmful effects on health. There are many proofs revealing the fact that probiotic *Lactobacilli* can facilitate to maintain a favourable microbial balance which may leave beneficial effects on the host. During the last few years the use of probiotics has been increased and they added into the food as nutritional adjuncts. Bio-yogurt, which is one of the most important fermented foods, is used for the deliverance of probiotic Lactobacillus acidophilus and *Bifidobacterium bifidum*. Adequate amount of probiotic bacterial cells like 'therapeutic minimum' should be used repeatedly by the consumers to get health benefits. *Lactobacillus acidophilus* is a non-pathogenic probiotic bacterium and normally found in the intestine, is extensively used in fermentation industry and clinical studies because it has the potential to inhibit the growth of pathogenic bacteria and yeast found in small intestine. This bacterium also produces the enzyme lactase which is necessary for milk digestion [25]. Milk products fermented with *Lactobacillus acidophilus* may positively affect the human health. In order to determine its particular function in balancing the microbial environment of the intestine, the characteristics of *Lactobacillus acidophilus* have been studied both for humans and other animals. *L. acidophilus* is known to be the dominant probiotic of the intestine in healthy people [26].

Use of probiotics in dairy industry

The strains of *L. acidophilus* have widely been used as starter culture in dairy due to their therapeutic potential for microbial equilibrium of the intestine, in food fermentation and used as a probiotic in food supplements. In recent years studies have been focused on the role of bacteriocins and bacteriocin-like substances produced by *Lactobacilli* as they have inhibitory potential against pathogenic microorganisms and can be used to improve the safety of food products. The most widely used probiotic bacteria are the species of *L. acidophilus* belonging to the family *Lactobacilli*. Production of bacteriocins is referred to as an important characteristic of probiotic bacteria [27].

Species of probiotic *Lactobacilli* may help to improve the potential of the host to resist the colonization which in turn protects the gastrointestinal tract from pathogenic microorganisms. The strain *L. acidophilus* isolated from dairy products shows antimicrobial activity against Salmonella and *Escherichia coli* therefore it may have probiotic potential [28].

Early history of *Lactobacilli* to be used as probiotic organisms

The concept of the use of microorganisms for the treatment of specific medical problems goes back to the work of Doderlein who in 1892 used *Lactobacilli* for the treatment of vaginal infections [29]. In the year 1907, the use of specific group of microorganisms for promoting human health was proposed for the general public by Elie Metchnikoff who was awarded with Nobel Prize. In a book named The Prolongation of life, Metchnikoff proposed that microbes in the colon

were responsible for poor health in adults and the use of sour milk or yogurt will neutralize these pathogenic bacteria (Table 2). He stated that the strain *Bulgaricus bacillus* which was later identified as *Lactobacillus bulgaricus* has the ability to confer better health and to survive for long time in humans [30]. In 1911 the bacillus of long life was discovered by Douglas which supports the theory of human's long life as well as the use of fermented milks. Alfred Nissle in 1911 reported a strain of *Escherichia coli* and he used this strain to treat the acute diseases of intestine such as shigellosis and salmonellosis in which most of the cases were successful. Nowadays this strain has been given the name *E. coli* Nissle which is still used as probiotic but this is a non-lactic acid producing probiotic [31]. Retteger in 1935, at Yale University stated that *Lactobacillus acidophilus* is a suitable choice to be used in clinical tests for humans [32].

This was followed by another study which was proven to be successful for chronic constipation. The use of particular strains of bacteria for the treatment of human diseases was started in 20th century however the name "probiotic" was not used in this regard till 1974. In 1974 Parker defined probiotics as "Organisms and substances that contribute to intestinal microbial balance". An EEC knowledgeable committee defined the probiotics as "Living microorganisms that when ingested in sufficient amount confer health benefits beyond inherent general nutrition" [33].

Summary of the quality of evidence for the use of probiotics in different diseases		
Type of disease	Comments	Quality of evidence
Pouchitis	Efficacy clearly shown in adult studies with VSL no. 3	I
Pediatric Crohn disease	No clear efficacy (mostly Lactobacillus GG data)	I
Ulcerative colitis	Efficacy suggested (equivalent to ASA preparations)	I
Irritable bowel syndrome	Efficacy possible	I
AAD	Efficacy clearly shown but not all probiotics are effective (mainly S. boulardii and Lactobacillus GG)	I
C.difficile diarrhea	Efficacy clearly shown but mainly in severe recurrent disease using S. boulardii and Lactobacillus GG	I
Mild to moderate acute diarrhea	clearly shown; treatment shortens duration of illness by 1 day (mostly *Lactobacilli*, 10 billion per dose or more) Prevention, modest effect with some conflicting reports	I
Necrotizing enterocolitis	Efficacy possible	I
Hepatic encephalopathy	Efficacy possible; small studies favoring efficacy in adults; large studies as well as pediatric studies are necessary	I
H. pylori eradication	No efficacy supported	I
Allergy	Clearly shown in preventing atopic dermatitis	I
Cancer therapy and prevention	Efficacy possible; inconsistent clinical data	II
Urogenital disorders		
Respiratory tract infections		

Table 2: Adopted from Lippincott Williams and Wilkins.

Historical background of *Lactobacilli*

The process of fermentation with the help of lactic acid production is a new concept. The process of fermentation has been used for different purposes such as to improve the storage qualities and nutrition value of fresh food products such as milk, cereal, vegetables and fish meat. Lactic acid bacteria are the organisms that are used for such kind of fermentation and they also play a key role in the preservation of foods. In advanced countries these bacteria are primarily used for the fermentation of dairy products such as yogurt, buttermilk and cheese. The use of starter cultures in dairy has turned into business nowadays. The grouped name "Lactic acid bacteria" was applied for the bacteria causing the coagulation and fermentation of milks and include those bacteria that turn lactose into lactic acids. Orla-Jensen in 1919 applied the family name Lactobacteriaceae for a group of bacteria that produce lactic acids alone or in combination with acetic acids, carbon dioxide and alcohols [34]. Nowadays the family Lactobacteriaceae and lactic acid bacteria are regarded as synonyms [35].

According to the Russian scientist Metchnikoff, it is believed that *Lactobacilli* are beneficial for the health of humans. Today various foods have been promoted which beneficially affect the human health like pharmaceuticals and these beneficial effects are based on the properties of certain strains of *Lactobacilli*. However majority of those strains have not been fully characterized therefore their beneficial effects can not completely be verified. Moreover, the specific strains used during the studies are not properly identified. Therefore most of the data about the beneficial effects of *Lactobacilli* is unreliable.

Lactic acid bacteria are a group of Gram-positive bacteria followed by a collection of physiological, metabolic and morphological

properties. They are non-sporulating, fermenting carbohydrates and producing lactic acids, non-aerobic, acid tolerant and catalase negative. These bacteria are typically non-motile and also not capable of scaling back nitrite. They are divided into four sub-genera including *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconstoc*. Current taxonomic modification recommends that the group of lactic acid bacteria include the genera *Lactobacillus*, *Carnobacterium*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *aerococcus*, *Pediococcus*, *Vagococcus*, *Leuconostoc* and *Tetragenococcus*. Initially, *Bifidobacteria* were placed into the genus *Lactobacillus* and thus the strain was named as *Lactobacillus bifidus*. However the arrangement of lactic acid bacteria into absolutely different genera is primarily based on the properties used by Oral-Jensen in 1919, but the uncertainty was still present when the monograph of Oral-Jensen was appeared [6]. This work has had a huge impact over the systematics of lactic acid bacteria, and however, it was modified up to some extent, but is still applicable and consequently the basis of classification still remains unchanged. The taxonomy of lactic acid bacteria is principally based on mode of glucose fermentation, morphology, pattern of lactic acid production, growth at various temperatures, growth at high salt concentration and tolerance to acidic and basic pH. Some other characteristics may also be used for the purpose of classification such as motility and composition of fatty acids for some recently reported members of lactic acid bacteria. Originally the term lactic acid bacteria were applied for "Milk souring organisms". Significant advancement was observed in the field of classification of these bacteria as the similarity was found between the milk souring organisms and lactic acid bacteria of other origins [36]. Lactic acid producing bacteria are usually found in nutrient rich environments like food products such as vegetables, milk and meat while some of their members may also be found in mouth, vagina of mammals and intestine. The genera that most likely posses the characteristics of lactic acid bacteria are: *Lactobacilli*, *Streptococci*, *Leuconostoc*, *Aerococci* and *Pediococci*. Three of them including *Lactobacilli*, *Leuconostoc* and *Pediococci* are still in their original position while some bacteria that were formerly the members of *Lactobacilli* are nowadays included in a separate genus which is named as *Carnobacterium* [35].

Properties necessary for a probiotic

For an efficient probiotic strain it is necessary to have some properties which are believed to be the primary selection criteria and without those properties a strain cannot be considered a beneficial probiotic. Some of the characteristics are discussed in detail but it is not necessary for a probiotic to meet all these requirements.

Tolerance to low pH and bile salts: The bacterial strains that are used as potential probiotics are mixed with food materials and taken through the mouth and therefore they have to cross the lower part of the intestine. For this situation the bacteria must be able to resist the enzymes of the oral cavity such as lysozyme. After passing through the oral cavity, they have to pass through the stomach and join the upper part of the intestine where bile is present. During this situation the bacteria must have the potential to tolerate the process of digestion. It has been concluded that food materials take a time of three hours when it passes through the stomach, therefore the organisms should be able to tolerate this harsh situation in the stomach where the pH ranges from 1.5 to 3.0 and the upper part of the intestine where bile is present [37,38]. In order to prove themselves to be effective potential probiotics, the strains should be able to keep themselves alive in the lower part of the intestine. The first and most important condition for a probiotic strain to be efficient is its survival in the presence of low pH and high bile concentration. Liver produces bile acids from cholesterol and transfer them to the gall bladder and from there these bile acids are poured into the Duodenum area in combined form at a concentration of 500-700 ml/day. Due to the activity of microbes, these bile acids undergo various chemical changes. These bile acids both singly and in combination exert antimicrobial activity against the species of E. coli and Salmonella. In combined form these bile acids are more efficient against most Gram positive bacteria [38,39].

The strain *Lactobacillus acidophilus* is used as probiotic bacteria most widely in the products such as capsules and dairy industry. During a study, species of *L. acidophilus* were isolated that were resistant to acids and bile. These probiotic bacteria were of different origins and some of the strains showed tolerance when they were subjected to pH 3.5 for duration of 90 minutes and temperature was 37°C. They were also able grow in the presence of 0.2% bile salt and pH 3 [37].

Antimicrobial activity: The antimicrobial activity against the pathogenic microorganisms is one of the primary characteristics for the selection of potential probiotic organisms. The primary target of the antimicrobial activity is the elimination of pathogenic and unwanted microorganisms [40]. The inhibitory potential of *Lactobacilli* is the result of production of compounds including lactic acids, propionic acids, H_2O_2, CO_2, bacteriocins and other antimicrobial compounds [40,41]. Currently few species of *Lactobacilli* have been investigated for the production of inhibitory substances. *Lactobacillus reuterii* is an example of such species which normally occurs in humans and other animals, produces reuterin which is an antimicrobial peptide. Similarly *Lactococcus lactis* produces a bacteriocine called nisin A and *Lactobacillus plantarum* produces a bacteriocine called plantericine S while *Lactobacillus acidophilus* produces acidophilucine A [41]. The production of the antimicrobial peptides largely depends upon the environment of the producer organism such as nutrients availability, pH, temperature and time. *L. lactis* subspecies lactis produces a bacteriocine called nisin which is mostly used for the preparation of food materials [40].

In an experiment, the antibacterial activity of *Lactobacilli* of human origin was evaluated against different pathogenic bacterial strains including *Pseudomonas*, *S. aureus*, *Streptococci*, *E. coli*, *Enterococci*, *Bifidobacteria* and *Clostridium*. It was found that the strain *L. salivarus* has considerable inhibitory potential against both Gram positive and gram negative bacteria like *Bacillus subtilus*, *Bifidobacterium bifidium*, *Enterococcus faecalis* and *Streptococci* etc [39].

During a study, four strains of *Lactobacilli* were isolated from pig feces and their probiotic potential was evaluated. In the experiment, the indicator strains used were *S. typhi*, *E. coli* and *S. aureus*. It was observed that the cell free supernatant of these probiotic strains inhibited the growth of all the indicator strains used in the study [42]. In a similar study four strains of *Lactobacilli* (one strain of *Lactobacillus salivarus*, two strains of *Lactobacillus gasseri* and one strain of *Lactobacillus fermentum*) were isolated from human milk and their antibacterial activity was evaluated. Maximum inhibitory activity was observed for all the probiotic strains against *S. aureus*, *E. coli*, *Listeria monocytogenes* and *Clostridium tyrobutyricum* which usually cause spoilage [43].

Safety requirements of probiotics: Nowadays, it has been proved that the probiotic bacteria that are routinely used for probiotic purposes do not have any harmful aspects. Bacterial strains to be used for probiotic purposes should have the following properties.

The isolation source for the strains used for humans should be of human origin.

The gastrointestinal tract from which the probiotic strains to be isolated should be healthy.

They should not be pathogenic.

These bacteria should not cause any disease related to the gastrointestinal tract.

They must not have any mechanism of deconjugation with bile salts.

They must not possess any infectious genes that are responsible for resistance to antibiotics [44].

Conclusion and Future Recommendations

From the past forty years, people are using antibiotics to enhance the growth and as preventive measures in poultry to control diseases and consequently, to improve their growth results, enhanced feed competence and shield them from the harmful effects of pathogenic microbes. The use of antibiotics was banned by the European Union in 2006 as they had concerns about the appearance of extensive antibiotics resistance in human pathogenic microbes. Thus, people around the world are looking for alternatives to antibiotics in order to sustain well-organized health facilities. Probiotics can be used as alternatives to antibiotics to limit the chances of infections caused by most of the pathogenic strains of bacteria. Many probiotics are available at commercial level that can be used in poultry production.

Consequently further studies should be conducted to use these probiotic bacteria in a reliable way. It will be advantageous to test the following characteristics;

1. To study their mucosal adhesion.

2. To study their clinical features for the health of human beings.

3. To study their Technical features like strain stability, viability in products and bacteriophage resistance.

4. Their antibiotics resistance pattern should also be studied.

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