CHARACTERISATION OF BACTERIOCIN-LIKE INHIBITORY SUBSTANCES DERIVED FROM LOCALLY ISOLATED *LACTOBACILLUS PARACASEI* FD1 AND ITS SYNERGIC IMPACT WITH ANTIBIOTICS

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by

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DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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2022

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LIST OF SYMBOLS

M	Molar concentration
g	Weight, gram
L	Volume, Litre
˚C	Temperature, Degree Celsius
m	Milli (1×10^{-3})
µ	Micro (1×10^{-6})
w	Weight
v	Volume
h	Hour
m	milli
%	Percent

LIST OF ABBREVIATIONS

L. paracaseis	*Lactobacillus paracasei*
BLIS	Bacteriocin-like inhibitory substances
Eq	Equation
LAB	Lactic acid bacteria
MIC	Minimum Inhibitory Concentrations
IC50	Half maximal inhibitory concentration
MRS	De Man, Rogosa and Sharpe
G+	Gram positive
G-	Gram-negative
FDA	Food and Drug Administration

PENCIRIAN BAHAN PERENCAT SEAKAN BAKTERIOSON DIPEROLEHI DARIPADA LACTOBACILLUS PARACASEI FD1 PENCILAN TEMPATAN DAN KESAN SINERGIKNYA DENGAN ANTIBIOTIK.

ABSTRAK

Rintangan antimikrob dalam kalangan bakteria meningkatkan kadar morbiditi dan kematian yang berkaitan dengan jangkitan bakteria, menyebabkan perbelanjaan perubatan meningkat dan penginapan hospital lebih lama. Penggunaan antibiotik yang berlebihan telah membawa kepada evolusi bakteria tahan antibiotik. Menurut Pertubuhan Kesihatan Sedunia, bakteria tahan antibiotik menyebabkan peningkatan penyakit seperti radang paru-paru, batuk kering, keracunan darah dan gonore. bacteriocin atau bahan perencat seakan bakterioson (BLIS) baru-baru ini telah menunjukkan kecekapannya dalam menyekat kesan patogenik yang berkait rapat, serta keupayaannya untuk berinteraksi secara sinergi dengan antibiotik. Matlamat penyelidikan ini adalah untuk meminimumkan penggunaan antibiotik sambil melihat kesan sinergistik antibiotik dan BLIS yang dihasilkan oleh bakteria asid laktik terpencil tempatan, *Lactobacillus paracasei* FD1, daripada durian yang ditapai. Kesan sinergistik adalah menggabungkan BLIS, BLIS MIC, dan BLIS IC50 dengan antibiotik pada kepekatan berfungsi untuk antibiotik yang menunjukkan keberkesanan sifar terhadap rintangan multidrug, manakala BLIS, BLIS MIC, dan BLIS IC50 digabungkan dengan MIC dan IC50 antibiotik yang menunjukkan keupayaan untuk menghalang rintangan multidrug. Kesannya menunjukkan peningkatan, dengan peratusan perencatan meningkat kepada julat 85.66 % hingga 100.0 %, manakala peratusan perencatan antibiotik bertambah baik kepada julat 83.7 % hingga 100.0 % untuk *Pseudomonas aeruginosa* yang tahan pelbagai ubat. Aktiviti spektrum lanjutan

beta-laktamase *Escherichia coli* bertambah baik kepada julat 93.98 % hingga 100.0 %, manakala MIC dan IC50 antibiotik dicapai dengan lebih daripada 98 aktiviti. BLIS sendiri menunjukkan perencatan yang ketara terhadap *Pseudomonas aeruginosa* tahan pelbagai ubat dan spektrum lanjutan beta-laktamase *Escherichia coli* dengan peratusan perencatan masing-masing 99.21% dan 94.35 peratus. Ini boleh disimpulkan bahawa BLIS adalah berkesan untuk digunakan untuk menghalang pertumbuhan rintangan multidrug ini serta mengurangkan penggunaan antibiotik pada masa hadapan.

CHARACTERISATION OF BACTERIOCIN-LIKE INHIBITORY SUBSTANCES DERIVED FROM LOCALLY ISOLATED *LACTOBACILLUS PARACASEI* FD1 AND ITS SYNERGIC IMPACT WITH ANTIBIOTICS.

ABSTRACT

Antimicrobial resistance among bacteria increases the rates of morbidity and death associated with bacterial infections, resulting in increased medical expenses and longer hospital stays. Overuse of antibiotics has led in the evolution of antibiotic-resistant bacteria. According to the World Health Organization, antibiotic-resistant bacteria are causing an increase in diseases such as pneumonia, tuberculosis, blood poisoning and gonorrhoea. Bacteriocin or bacteriocin-like inhibitory substances (BLIS) have recently demonstrated their efficiency in suppressing closely related pathogenic stains, as well as their capacity to interact synergistically with antibiotics. The goal of this research is to minimise antibiotic use while also looking at the synergistic impact of antibiotics and BLIS produced by locally isolated lactic acid bacteria, *Lactobacillus paracasei* FD1, from fermented durian. Synergistic effect was combining BLIS, BLIS MIC, and BLIS IC50 with antibiotics at working concentrations for antibiotics that show zero effectiveness against multidrug resistance, while BLIS, BLIS MIC, and BLIS IC50 combined with MIC and IC50 of antibiotics that show ability to inhibit multidrug resistance, The effect demonstrates an improvement, with the inhibitory percentage increasing to a range of 85.66 % to 100.0 %, while the percentage inhibition of antibiotics improves to a range of 83.7 % to 100.0 % for multidrug-resistant *Pseudomonas aeruginosa*. The activity of extended-spectrum beta-lactamases *Escherichia coli* improves to the range of 93.98 % to 100.0 %, while the MIC and IC50 of antibiotic were achieved with more than 98 activity. BLIS itself showed significant inhibition against multidrug-resistant

Pseudomonas aeruginosa and extended-spectrum beta-lactamases *Escherichia coli* with inhibition percentages of 99.21% and 94.35 %, respectively. This can be concluded that the BLIS are effective to be used to inhibit the growth of these multidrug resistance as well as reduce the use of antibiotic in future.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bacteriocin is an antimicrobial peptide formed by bacteria that is synthesised by ribosomes. This peptide is made up of a short chain of amino acids that has bacteriostatic or bactericidal impact on the growth of closely associated bacteria by disrupting cell membranes with small inhibition spectrums (Ingolf F et al., 2013). The bacterium may also be identified as a non-self-propagation protein or protein-based compound that inhibits bacterial growth on a limited number of bacteria. (Kaiser et al., 1993). Several studies have shown that Gram-positive microorganisms, especially lactic acid bacteria that produce bacteriocin, are effective against a wide range of pathogens, including antibiotic-resistant strains including Staphylococcus aureus, Methicillin-resistant *Staphylococcus aureus*, *Enterococcus spp*., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (M. P. Mokoena, 2017).

Microbes' ability to manufacture bacteriocin, which destroys pathogenic bacteria, was discovered in the early 1920s by scientists. Many new papers written by scientists all over the world since then have documented on promising evaluations of bacteria that generate bacteriocin compounds. *Saccharomyces boulardii*, non-pathogenic *Escherichia coli*, and *Lactobacillus sp.* are among these bacteria (Dobson et al., 2012). Beneficial bacteria develop antibacterial peptides that can be used instead of antibiotics to treat serious

health conditions and bacterial infections. Taking antibiotics for a long time or without a prescription may cause skin allergies, nephritis, haematological problems, gastrointestinal problems, nervous system disturbances, and the development of antibiotic-resistant bacteria (Heta et al., 2018).

Antibiotic-resistant bacteria are microorganisms that are resistant to the most antimicrobial drugs, including penicillin, cephalosporins, tetracyclines, macrolides, metronidazole, clindamycin, and carbapenem, and are capable of causing severe health problems (Heta & Robo, 2018). Bacterial mutations that occur as a result of antibiotic usage cause antibiotic resistance. Antibiotic-resistant bacteria cause it to survive by reducing or removing the efficacy of antibiotics administered by doctors. Antibiotic-resistant bacteria, on the other hand, can be spread to people by a variety of means, including inadequate hygiene and a lack of infection-control procedures (Rao, 1998).

Bacteriocin is divided into four classes: lantibiotics, unmodified peptides, massive proteins, and circular peptides. Lantibiotic is a post-translationally transformed peptide containing the amino acid lanthionine or methyllanthionine, such as nisin and nukacin. Gram-positive bacteria such as *Streptococcus* and *Streptomyces* produce bacteriocin, which is used to combat other Gram-positive bacteria (Lobo-Ruiz et al., 2018). The largest class of Gram-positive bacteriocins that inhibit the growth of different bacteria is Class 2, which includes unmodified small heat-stable peptides (Carnobacteriocin X, Enterocin X, Carnocyclin A, and Aureocin). It's made by food-borne strains with the sequence -Tyr-GlyAsn-Gly-Val-Xaa-Cys, which have a wide variety

of activities against *Listeria* strains (López-Carballo et al., 2012). The third class consists of a large heat-labile protein (Lysostaphin) (>10 kDa) that destroys the cell wall and disrupts the plasma membrane, especially in *Staphylococcus aureus* (Bastos et al., 2010). Circular peptides with a peptide bond between the C- and N-termini are classified as class 4. Enterocin and Enterocina are bacteriocin. It's produced by *Enterococcus faecalis* and *Enterococcus faecium* for food preservation and inhibition of *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Escherichia coli*, *Micrococcus luteus*, *Listeria monocytogenes*, *Listeria innocua* and *Streptococcus agalactiae* (G. M. Preciado et al., 2016).

Bacteriocin's most well-known advantages are that it is low toxic; unlike antibiotics, it does not affect the host. This bacteriocin has no negative side effects while also increasing the host cell's resistance to potential pathogens (Varma et al., 2006). Furthermore, since the target cell wall lacks specific receptors, bacteriocin can bind to it anywhere on the surface. Bacteriocin has a unique mechanism in the target cell, which is linked to the process of pore formation in the outer cell membrane (Gholami et al., 2016). Bacteriocins have a wide range of uses due to their numerous benefits, the most common of which are in the food industry as a food storage agent and the pharmaceutical industry as a therapeutic agent (S. C. Yang et al., 2014).

1.2 Problem Statement

The issue has sparked several concerns and debates about the rise of antibiotic-resistant bacteria and the use of successful antibiotics. Antibiotic misuse or overuse, as well as poor infection control in humans and animals, have recently accelerated the development of antibiotic-resistant pathogens. Antibiotic-resistant pathogens have been the most significant threat to food security and global health in decades. Antibiotic-resistant diseases and infections cause a slew of issues and are difficult to treat because common antibiotics are ineffective against many antibiotic-resistant pathogens. To combat this serious problem, a growing number of highly dangerous drugs and chemical antibiotics are being developed. It's also likely that modern antibiotics will have side effects as a result of this. As a result of the emergence of this severe drug resistance epidemic, people are beginning to think about the advantages of bacteriocin. However, worldwide use of bacteriocins in medical and health care tends to be slower. High purity without antimicrobial peptides is required to meet the FDA Medication Guide requirements and the requirements specified by the Minister of Health, particularly in Halal issues.

1.3 Research Objective

The objectives of this project are:

I. To investigate the antimicrobial effects of Bacteriocin-Like Inhibitory Substances (BLIS) from locally isolated *Lactobacillus paracasei* FD1 against Multidrug Resistant *Pseudomonas aeruginosa* and Extended Spectrum Beta-Lactamase producing *Escherichia coli*.

II. To characterize the highly effective Bacteriocin-Like Inhibitory Substances (BLIS) from selected lactic acid bacteria against Multidrug Resistant *Pseudomonas aeruginosa* and Extended Spectrum Beta-Lactamase producing *Escherichia coli*.

III. To determine the synergistic effect of 13 antibiotics and Bacteriocin-Like Inhibitory Substances (BLIS) against Multidrug-Resistant *Pseudomonas aeruginosa* and Extended Spectrum Beta-Lactamase *Escherichia coli*.

CHAPTER 2

LITERATURE REVIEW

2.1 Lactic acid bacteria

Lactic acid bacteria is a very beneficial microorganism that primarily exhibits probiotic effects in humans and animals by enhancing their immune systems, avoiding certain infections, and improving digestion (Fuller, 1989). Lactic acid bacteria are gram-positive facultative anaerobic microorganisms with a high tolerance for low pH (van Geel-Schutten et al., 1998). They are able to live in our bodies because of their ability to thrive in low pH environments. Lactic acid bacteria can be isolated from saliva, the intestine, and the vaginal canal in humans, and they can also be present in almost all fermented foods and beverages (Mduduzi Paul Mokoena, 2017). Lactic acid bacteria consume carbohydrates in the medium and create lactic acid as a byproduct of the fermentation method. *Lactobacillus, Pediococcus, Leuconostoc, Streptococcus,* and *Lactococcus* are some of the genera of lactic acid-producing bacteria. (Du et al., 2011).

2.1.1 Lactobacillus paracasei

Lactobacillus paracasei is known as part of the *Lactobacillus casei* group due to a high degree of gene sequencing similarity when the 16S rRNA gene sequence is used to classify it (M.Gobbetti et al., 2014). It is a gram-positive, heterofermentative facultative found in a variety of fermentation products. It can be contained in humans in places like the

intestine, the mouth, and fermented foods. *L. paracasei* has been shown in studies to have very promising properties in the treatment of gastrointestinal diseases (Lombardo, 2008). *Lactobacillus paracasei* is said to live up to three months longer than other bacteria species. (Lavermicocca et al., 2005).

Lactobacillus paracasei has been shown in a variety of studies to be proteolytic, capable of releasing small peptides and amino acids (X-prolyl dipeptidyl-aminopeptidase) with a molecular weight of 145 kDa (Ong et al., 2006). *Lactobacillus paracasei*, *Lactobacillus plantarum*, and other lactobacilli are used in the production of cheese. The use of both types of strains could increase the cheese's flavour intensity and provide technical benefits such as longer storage times (J. Castro et al., 2015). The bacteria's survival would be improved if they were encapsulated in alginate microcapsules. The initial mean counts for free and encapsulated bacteria were 3.29 107 and 2.55 108 cfu/g, respectively. During the cheese-making process, alginate-microencapsulation of a probiotic *L. paracasei ssp. paracasei* strain resulted in a high survival rate (FATİH Ortakci et al., 2012). *Lactobacillus paracasei* has another benefit in that it has a significant antagonistic effect. Both Gram positive and Gram-negative pathogen strains are significantly inhibited by the antagonistic effect of *Lactobacillus paracasei* IMC 502 and *Lactobacillus rhamnosus* IMC 501. *L. paracasei* IMC 502 showed the greatest growth inhibitory activity against *Bacillus cereus*, with an inhibition zone of 24.0 mm.

Lactobacillus strains have a high antagonistic activity against all pathogens, ranging from 75 to 100 percent antagonistic effectiveness. However, even this combination inhibit the clinical isolates of *E. coli* DSM 1103 and *Pseudomonas aeruginosa*, it did not give to a high inhibition (Coman et al.).

Lactobacillus paracasei subsp. paracasei D6, D14, and N14 were found to have the most inhibition in another study, with two of them being able to inhibit *Candida albicans*. N14, D14, and D6 strains of *Lactobacillus paracasei subsp. paracasei* showed antibacterial, anticandidal, and both antibacterial and anticandidal activity, respectively. N14 and D6 both inhibited 11 of 15 and 10 of 15 bacterial pathogens isolated from HIV-seropositive patients' mouths, respectively while D14 inhibited 11 of 22 candidal pathogens isolated from HIV-seronegative patients' mouths and 12 of 18 pathogens isolated from HIV-positive patients' mouths (Sookkhee et al., 2001). *Lactobacillus paracasei* FJ861111.1 was studied for its probiotic properties in vitro and anticolonization potential in treated mice with harmful microbes. *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were among the pathogens that the test successfully inhibited. *L. paracasei* FJ861111.1 can survive in acidic conditions (pH 2.5) with a 0.3 percent bile salt concentration. The in vivo findings also revealed that yoghurt containing *L. paracasei* increased the population of total bacteria and *Lactobacillus* in mice's faeces, at the same time it inhibit *C. albicans* colonisation of the mice's intestines after infection (Deng et al., 2015).

According to a scientific paper published in Taiwan, the native lactic acid bacterium isolated from newborn infant faeces has been described as *Lactobacillus paracasei subsp. paracasei* NTU 101. This strain demonstrated strong low-pH survival, resistance to high bile concentrations, and the ability to minimise cholesterol levels in vitro (Pan et al., 2002). Many benefits can be obtained from fermented products containing this strain, including blood cholesterol and blood pressure control, gastric mucosal lesion prevention, immunomodulation and allergy relief, anti-osteoporosis, and fat tissue inhibition. Feeding fermented milk resulted in slightly lower serum cholesterol levels. With a reduction of 32.9 percent, *L. paracasei subsp. paracasei* NTU 101 ranks second behind *L. acidophilus*. (Chiu et al., 2006). The same strain was used in another research where according to the findings, *L. paracasei subsp. paracasei NTU 101* could be a promising candidate for probiotic use in disease prevention. In BALB/c mice infected with *Escherichia coli* O157:H7, *Lactobacillus paracasei subsp. paracasei* NTU 101 was found to have immunomodulatory activity. During the *E. coli* challenge, mice were given *L. paracasei* NTU 101 for 7 days. When compared to untreated mice, feeding *Lactobacillus paracasei* for 7 days resulted in increased weight gain and lower cumulative morbidity rates and antibody development in post- and pre-treated mice (Tsai et al., 2010).

2.2 Synergic effect of bacteriocin and antibiotic

Antibiotic resistance has been demonstrated in a significant number of pathogenic bacteria. Seeking new antimicrobial compounds to mitigate these issues has become a priority as a result of resistance. While bacteriocin has a strong antimicrobial effect against a number of pathogens that are closely related, a combination of bacteriocin and antibiotics has a more promising bactericidal effect. The implications of this synergy have been researched, and the results have recently improved. *Enterococcus faecalis* was not clearly destroyed by penicillin or chloramphenicol alone but was seriously disrupted by either antibiotic in conjunction with nisin. Furthermore, researchers discovered that when chloramphenicol, penicillin and ciprofloxacin were given together with nisin, they had greater antibiofilm effects than when these antibiotics were given alone. Another antibiotics such as streptomycin, roxithromycin, cephazolin, and cefuroxin initially had no effect on the test strain *E. faecal* however these antibiotics showed much better antibiotic activity when they were added with 200 U/mL (Tong et al., 2013). The combination of penicillin or chloramphenicol and nisin was thought to have a synergistic effect against the three test *E. faecalis* strains. The cumulative fractional inhibitory concentrations of nisin and penicillin for the synergistic effect were 0.31, 0.375, and 0.28, respectively. The cumulative fractional inhibitory concentration FICs of nisin and chloramphenicol, on the other hand, were 0.155, 0.155, and 0.15, respectively. Checkerboard assays revealed synergistic interactions between nisin and penicillin or chloramphenicol against three *E. faecalis* strains.

Furthermore, transmission electron microscopy showed that these combinations were extremely successful at killing *E. faecalis* cells. (Tong, Zhang, et al., 2014).

Another research looked at the possible synergistic effects of triclosan or chlorhexidine combined with the bacteriocin PsVP-10 produced by *Pseudomonas sp*. R-10 against *Streptococcus mutans* and *Streptococcus sobrinus* strains (Kleinberg, 2002; Marsh, 2003). The synergistic activity of bacteriocin PsVP-10 + chlorhexidine and bacteriocin PsVP-10 + triclosan was studied using fractionary inhibitory concentrations. When bacteriocin and chlorhexidine were combined, a synergistic effect on the both species tested was identified, with all 240 strains that developed a biofilm and strains that did not develop a biofilm being suppressed by the synergic effect. When the strain is partly synergistic, there is no inhibition. The results demonstrate the effectiveness of chlorhexidine and the bacteriocin PsVP-10 could minimize the amount of pathogenic microorganisms in vitro (Lobos et al., 2009).

Bacteriocins developed by *Lactobacillus plantarum* LS6 and *Pediococcus pentosaceus* LU11 from fermented products in India were tested for synergistic potential with antimicrobial drugs against clinical β-lactamases producing pathogens, and they inhibited growth significantly more than antibiotics alone. Bacteriocins were found to have synergistic activity when combined with antibiotics such as cefotaxime, polymyxin B, imipenem, and tigecycline (Lee et al., 2005; Upadhyay et al., 2015). The efficacy of polymyxin B and imipenem in combination with LU11 and LS6 extracts significantly enhanced activity, with the highest average inhibition zones for polymyxin B

and LU11 and LS6 extracts being 20.3 mm and imipenem with LU11 and LS6 31.6 mm and 31.3 mm, respectively, against multidrug resistant *E. coli* and *K. pneumoniae*. The extract from isolate LU11 has the maximum mean inhibition zone of 32.3mm against MDR strains of *E. coli* IB9 (Biswas et al., 2017).

Another synergic effect research was conducted on the action of colistin alone or in conjunction with two bacteriocins, nisin A and pediocin PA-1/AcH, against *Salmonella choleraesuis* ATCC 14028, *Pseudomonas aeruginos*a ATCC 27853, *Yersinia enterocolitica* ATCC 9610, and *Escherichia coli* O157:H7. When nisin A with concentration of 1.70 g/mL or pediocin PA-1/AcH with concentration of 1.56 g/mL were added with colistin, the inhibitory concentrations for *E. coli* O157:H7 were 0.01 and 0.03 g/mL, respectively. Colistin inhibited all of the target strains, with MICs varying from 0.12 to 1.21 g/mL. When colistin was combined with nisin A or pediocin PA-1 / AcH, the MIC decreased, suggesting a synergistic effect and a higher likelihood of inhibiting *E. coli* O157:H7 enterohemorrhagic bacteria. *Escherichia coli* O157: H7 was inhibited at very low concentrations of about 0.01 and 1.56 g/mL when colistin was combined with nisin A or pediocin PA-1 / AcH (Naghmouchi et al., 2013).

2.3 Characteristic and Utilization of antibiotic.

Antibiotics are secondary metabolites produced by bacteria that have a wide bioactivity range and are either cytotoxic or cytostatic (I. M. Gould et al., 2013). It functions by killing or inhibiting bacterial cell, protein, DNA, and

RNA synthesis, as well as other specific actions. (Levy et al., 2004). In short, peptidoglycan protects bacterial structures, but during duplication, weak structures with holes emerge. When a bacterial cell divides, antibiotics such as penicillin block the peptidoglycan binding protein, preventing the closure of holes in the cell wall, allowing high amounts of surrounding fluid to penetrate the cell and cause it to burst (Canzani et al., 2017). Penicillin resistance became a major clinical issue in just a few years (Spellberg et al., 2014).

The use of inadequate antibiotic treatment or low adherence to treatment and cure is a major contributing factor to antibiotic resistance (Laxminarayan et al., 2006). About 34 million antibiotic prescriptions for acute respiratory infections are excessive per year, according to the Center for Disease Control and Prevention (Fleming-Dutra et al., 2016). Antibiotic medications were also found to be inappropriate in 31% of patients in a study conducted in 2008-2009. According to other reports, outpatients do not need more than half of the antibiotics prescribed for acute respiratory tract infections. (Fleming-Dutra et al., 2016; Gonzales et al., 2001; Kronman et al., 2014). These make up to over 30% to 50% of antibiotic abuse is excessive in human.

As a result of the rising demand for livestock. Antibiotics are fed to animals in the form of food to aid development, prevent infection, and increase yields and product quality (McEwen et al., 2002; Michael et al., 2014). Antibiotic overuse in animals increases the risk of bacteria developing resistance to antibiotics (Van Boeckel et al. 2015). It is estimated that the antibiotic used is 45 mg/kg in cattle, 148 mg/kg for chickens, and 172 mg/kg in pigs (Van Boeckel

et al. 2015). Antibiotic therapy is administered to 16 percent of all dairy cows in the United States each year (USDA, 2007). Every year, the amount of antibiotics used is expected to increase. Antibiotics such as tetracyclines, virginiamycin, streptomycin, and bacitracin are widely used in animal feed (Gadde et al., 2018; Granados-Chinchilla et al., 2017; Rusoff et al., 1957). Tetracycline and penicillin are widely used by farmers to improve poultry immunity and egg production (Puvača et al., 2020). The sewage and water from the animal farms were immediately dumped into the river or surrounding farmland. Farmers are forced to use antibiotic-polluted water to irrigate their crops as a result of this. Researchers also discovered that lettuce, corn, and potatoes also uptake sulfamethazine from livestock that is fertilised with livestock, with sulfamethazine concentrations in plants varying from 0.1 to 1.2 mg/kg dry weight and the remaining 70% in the soil (Dolliver et al., 2007). Another trial found that corn, green onions and cabbages were grown in a fertilizer that absorbed an antibiotic called chlortetracycline with a concentration of 0.002–0.017 mg/kg fresh weight (Kumar et al., 2005).

2.4 Development and transmission of antibiotic-resistant

The development of antibiotic resistance is confirmed when bacterial mutations occur. It is likely that these mutations will minimise or even eliminate the ability of the antibiotics to treat the infection. In other words, this promotes the bacterial proliferation, which causes more damage to both to humans and animals (Ventola, 2015). When humans take antibiotics to kill the bacteria that causes disease, they kill all bacteria, including those that are beneficial, with the

exception of those that are resistant to antibiotics (Fair et al., 2014). This gives antibiotic resistance more room to develop and become dominant in the gut. Worse, antibiotic-resistant bacteria may pass on their drug-resistance to other bacteria (Munita & Arias, 2016). Transmission from animal to human. High levels of antibiotic resistance were discovered in the intestines of livestock and farmers 35 years ago, marking the beginning of the transition of resistant bacteria from livestock (Bartlett et al., 2013). Antibiotic-resistant can survive in animal meat if not well cooked, contaminated fertilizer or water containing animal faeces used in food agriculture (Overdevest et al., 2011; Voets et al., 2013). All these foods are eaten by humans and allow them to stay in the human gut and cause infections (Marshall et al., 2011). Antibiotic resistance can easily spread from person to person, from a contaminated drinking water source to a hospital with inadequate infection control. In the hospital, healthcare professionals are still worried with patient-to-patient transmission. *Methicillin-resistant Staphylococcus aureus* (MRSA) caused 9,670 deaths in 2012, according to the Centers for Disease Control and Prevention (CDC, 2012; Laxminarayan et al., 2006). Many people assume that the main way MRSA spreads from patient to patient is by health professionals' contaminated hands. This aspect has been proven by studies that indicate that health workers are responsible for approximately 17% of MRSA transmission to patients (McBryde et al., 2004). As a result, hand washing procedures to disinfect their hands are needed at all times to reduce the spread of this disease.

2.5 Mechanism of action of antimicrobial resistance (AMR)

Antibiotic resistance, also known as antimicrobial resistance (AMR), has had a negative impact on crops, humans, and animals over the years. Antimicrobial resistance can build defensive mechanisms to counteract antibiotics and survive. Antibiotic resistance is currently being combated by modifying existing antibiotics to destroy emerging resistance pathogens (Davies et al., 2010). Antibiotic-resistant bacteria have many successful protection mechanisms, according to the Centers for Disease Control and Prevention. These bacteria either create new cell processes to avoid using antibiotic targets, modify or kill them with enzymes, inhibit or alter cell permeability to antibiotics, eliminate antibiotics by pumping, or alter antibiotic targets to render them inactive and unable to work (CDC, 2020; Munita, Arias, et al., 2016).

2.6 Bacteriocin: An alternative to antibiotic

Over the last few decades, bacteriocin has steadily gained attention in several fields, including food preservation, cancer pathogenic and therapeutic care, and human health maintenance (S. C. Yang et al., 2014). Bacteriocin serves as a defence mechanism, allowing its producers to thrive in their natural environments. Antimicrobial proteins (AMPs) or antibacterial peptides synthesised by ribosomes are known to undergo post-translational modifications that can kill or inhibit the growth of bacteria that are closely related (Ingolf F et al., 2013; Ventura et al., 2015) and some bacteriocins even show a broad-spectrum activity (Chi et al., 2018). In addition, it will not cause

any damage to the bacteria itself by certain immunity proteins (S. C. Yang et al., 2014).

Many bacteriocins produced by Gram-positive bacteria are formed by less than 60 amino acids (G. M. Preciado et al., 2016), especially bacteria that produce lactic acid. Nisin, for example, is formed by *Lactococcus lactis*, a polycyclic antimicrobial peptide that is commonly used as a food preservative due to its thermal stability and low pH tolerance (Cleveland et al., 2001a; Kitagawa et al., 2019). As they go through bioengineering, bacteriocin can improve their activity or specificity to target pathogenic strains due to their relatively simple biosynthetic mechanisms (Perez et al., 2014b).

Table 2.1: Comparison between bacteriocin and antibiotic.

	Bacteriocin	**Antibiotic**
Biosynthesis	Primary metabolite	Secondary metabolite
Size	15-80 kDa	0.3-0.5 kDa
Activity spectrum	Narrow to closely related species	Narrow to a broad spectrum
Active pH range	Wide range	Small range
Thermal stability	High	Low
Mode of action	Pore-forming	Cell membrane and intracellular
Colour, taste and odour	No	Yes
Adverse effect	No	Yes
Application	Food and clinical	Clinical
Causing resistance	No	Antibiotic-resistant
Toxicity	Low	Low to high

2.7 Bacteriocin producing bacteria

The majority of lactic acid bacteria (Michael et al. 2014) contain a wide range of bacteriocins. Bacteriocin is an inhibitory proteinaceous molecule or bactericidal antibiotic-like substance developed as a primary metabolite during the primary phase of development (Gaspar et al., 2018). It is produced by non-pathogenic bacteria that are mostly lactic acid bacteria to destroy or inhibit other pathogenic bacteria in the host (Parada et al., 2007). Bacteriocin itself is

characterized by biochemistry and genetics, and structure, biosynthesis, and mechanism of action and many other aspects.

Lactic acid bacteria are a major gram-positive non-sporulation-type microorganism group. They're also acid-tolerant, odourless, mesophilic, rod-shaped (bacilli) or sphere-shaped (cocci), and facultative anaerobic bacteria (Ali, 2010; Bintsis, 2018). Lactic acid bacteria can live in the intestine, gall bladder, and pancreas and attach to the intestinal epithelium, modifying and improving intestinal microflora (Bezkorovainy, 2001; Ohland et al., 2010; F. Ortakci et al., 2012). These microorganisms are characterised by their ability to transform glucose, the most basic type of carbohydrate, into lactic acid as a major metabolic end product throughout the fermentation phase (Vinderola et al., 2017).

The method can be divided into two classes based on the biochemical properties of lactic acid bacteria fermentation: homolactic and heterolactic (Kandler, 1983). Homolactic fermentation converts one molecule of glucose into two molecules of lactic acid (Romero-Garcia et al., 2009) while during heterolactic fermentation, lactic acid bacteria use the phosphoketolase pathway to generate carbon dioxide and ethanol as additional metabolites (Kandler, 1983). The lactic acid bacteria that normally produce only lactic acid as the final product are *Pediococcus sp.*, *Lactococcus sp.*, *Streptococcus sp.* and some *lactobacilli sp.* while bacteria that produce other addition metabolites of lactic acid are *Carnobacterium sp.*, *Enterococcus sp.*, *Leuconostoc spp.* and some *Lactobacilli sp.* (Bintsis, 2018; Elshaghabee et al., 2016; Hammes et al., 2006;

Hladíková et al., 2012; Teuber, 2008). These helpful lactic acid bacteria are also known as probiotics and can be present in a number of fermented foods. If eaten in adequate amounts, fermented foods have many health benefits for both humans and animals. Therefore, it adheres to the World Health Organization's (WHO) concept of probiotics as "live microorganisms that confer a health benefit on the host when administered in sufficient quantities." Probiotics are still used in many industries today to improve certain biological functions in human and animal health (H. Kumar et al., 2016; Lorenzo et al., 2012).

2.8 First identified bacteriocin and its mode of action

Belgian scientist André Gratia discovered the first known plasmid-encoded bacteriocin almost simultaneously with Alexander Fleming's discovery of the antibiotic (S. C. Yang et al., 2014). A colicin was produced by coliform bacteria; *Escherichia coli* under stress conditions showed and gave early results on antimicrobial properties (Sharma et al., 2013). At low concentrations in the nano-to-micro molar range, it passes through by creating pores in the target bacteria's inner membrane (Cascales et al., 2007).

Bacteriocin's antimicrobial properties inhibit cell wall synthesis and reduce internal cell components such as DNA and RNA of non-host pathogenic strains by a very high proteolytic enzyme degradability due to their proteinaceous properties, and it has demonstrated relatively no toxicity to eukaryotic cells (Jin et al., 2018; Perez et al., 2014b). Bacteriocin is a fast-acting antimicrobial that kills and prevents pathogenic bacteria. This is because, since

their discovery, there has been no study on the growth of bacteriocin-resistant bacteria. Bacteriocin is also said to be present in the human body for a limited period of time, which means the target strain has a lower risk of interacting with the reduced antibiotic fragment, which is the starting point for the development of most antibiotic resistance (Perez et al., 2014b).

However, due to the various difficulties encountered in the development of bacteriocin and the low consistency in regulating microbial growth as a result of a lack of study and understanding of their biology, bacteriocin production and use has declined since then. Since antibiotics have shown considerable success in treating bacterial infections, this has resulted in more work being put into discovering the chemical synthesis of broad-spectrum antibiotics until now (Williams et al., 2013).

2.9 Classification of Bacteriocins

After the controversy over the new developments related to the structure of bacteriocins and their mode of action, researchers agreed with Heng and Tagg in 2006 that bacteriocins can be distinguished and divided into four distinct groups (Heng et al., 2006). Small post-translationally modified peptides with a size less than 5 kDa that contain 19-39 amino acid moieties, as well as rare modified amino acids such as -methyl lanthionine, lanthionine, and a few dehydrated amino acids, are known as polycyclic antibacterial peptides or Lantibiotics. It is heat stable due to the internal rings created by the covalent bonds of certain amino acids by the unusual modified amino acids (Guder et

al., 2000; Heng & Tagg, 2006; Kraaij et al., 1999; McAuliffe et al., 2001; Poltronieri, 2017). These peptides have a rigid structure and protease resistance (Poltronieri, 2017). Lantibiotics are usually produced to kill other types of gram-positive bacteria (Lagos, 2013). Lantibiotic interferes with or disrupts cell wall activity by binding nisin and epidermin to the amphathatic peptidoglycan, while duramycin disrupts many physiological functions (Islam et al., 2012). Furthermore, bacteriocins of class I can be classified into three subclasses: Lantibiotics subclass Ia, subclass Ib and subclass Ic. The distinction between these three forms of lantibiotics is that subclass Ia is linear, cationic, and kills by rapidly forming pores (L. Smith et al., 2008). Recently, a novel class I bacteriocin, Subtilin L-Q11, was discovered with promising applications in the food industry and agriculture. *Bacillus subtilis* L-Q11 isolated from the orchard. Subtilin L-Q11 inhibits the growth of many human pathogens and foodborne bacteria, most notably *Staphylococcus aureus* (Qin et al., 2019).

Subclass Ib is a non-cationic, globe-shaped enzyme inhibitor that prevents the biosynthesis of peptidoglycan. Mersacidin, an antimicrobial peptide developed by *Bacillus sp.* stretch HIL Y-85.54728, is the smallest known lantibiotic with a molecular weight of 1825 Da (Brötz et al., 1998). Macacidin works by inhibiting the transglycosylation reaction of cell wall biosynthesis (Brötz et al., 1998). Mersacidin has been shown to inhibit Gram-positive bacterial growth in many trials, including the *methicillin-resistant Staphylococcus aureus strain* (MRSA) (Chatterjee et al., 1992; Hoffmann et al., 2002; Kruszewska et al., 2004).

Subclass Ic is a multicomponent lantibiotic having two peptides known as non-active lantibiotic (Brötz et al., 2000; Dufour et al., 2007; Veskovic-Moracanin et al., 2014). *Lactobacillus plantarum* MBSa4 strains isolated from fermented and dried meat in cured Italian sausages in Brazil are one example. The molecular mass of this substance is 2.3 kDa. Plantaricin MBSa4 is a two-peptide with excellent thermal stability and low pH resistance. Plantaricin MBSa4 was found to be active against all strains of *Listeria monocytogenes* tested as well as some strains of *Staphylococcus aureus* and *Enterococcus faecium* in a follow-up analysis. However, it did not show inhibitory activity against *Escherichia coli, Bacillus cereus, Enterobacter aerogenes, Salmonella Typhimurium, Pediococcus pentosaceus*, and almost all *Lactobacillus* strains (Barbosa et al., 2016).

Class II bacteriocin is non-lantibiotic, with a molecular mass of less than 10 kDa produced by bacteriocin producing bacteria (Parada et al., 2007; Perez et al., 2014a). This bacteriocin has antibacterial properties against a variety of bacteria (S.-C. Yang et al., 2014). It is a stable heat that retains more than 60% of its original activity at 60 ° C for 30 minutes (Elayaraja et al., 2014). The unmodified peptide, also known as Class II bacteriocin, is the main class of gram-positive bacteria. This bacteriocin is mainly cationic and does not undergo posttranslational modifications (Poltronieri, 2017). Subclass IIa, subclass IIb, subclass IIc, and subclass IId bacteriocins can be found in class II.

Subclass IIa is a cationic pediocin PA-1-like bacteriocin that belongs to a small heterogeneous class of peptides with several glycine peptides (L. Cintas

et al., 2001; Cotter et al., 2005). The bacteriocin is heat resistant and does not contain lanthionine or hydrophobic peptides. Bacteriocins of subclass IIa are all related to lactic acid bacteria found in our food (Mduduzi Paul Mokoena, 2017). Almost all subclasses IIa bacteriocins come from a range of sources, including fermented foods and natural sources like dairy, vegetables, fruits, and meat (Ennahar et al., 1999). At the same time, it's important to realise that it's anti-listeria. These bacteriocins, like the majority of bacteriocins, destroy pathogens by permeating the cell membrane and causing harm. The degree of membrane damage varies depending on the bacteriocin (Martinez-Cuesta et al., 2006). The novel Avicin A was discovered in an *Enterococcus avium* strain isolated from a sample of healthy baby faeces, and garvicin ML was discovered in a *Lactococcus garvieae* strain isolated from mallard ducks. (G. Preciado et al., 2016).

Two cationic peptides make up Subclass IIb. To be triggered, two complementary peptides must function together (Oppegård et al., 2007). It acts as a pore-forming toxin, passing through the cell membrane and interfering with the movement of ions (Balla et al., 2000; Ojcius et al., 1991). Bacteriocin of subclass IIb is made up of two distinct peptides that are encoded by two unrelated genes that are similar to each other (Alvarez-Sieiro et al., 2016; Nes et al., 2000). Antimicrobial activity of the peptides is minimal or non-existent. However, when the two peptides are combined in a one-to-one molar ratio, they have excellent antibacterial activity. Only the immunity protein is needed to protect the producer from bacteriocin destruction (Nes et al., 2013; Nissen-Meyer et al., 2010; Nissen-Meyer et al., 2009; Oppegård et al., 2007).