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Antimicrobial properties of probiotics

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Abstract

There is an expanding request from customers for regular antimicrobial substances that can be utilised for food safeguarding and replace the synthetic food additive. The antimicrobial development of precisely critical lactic acid microorganisms as starter cultures and various probiotics microorganisms is the guideline subject of an audit. The probiotics produce metabolites, for example, natural acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, acetone, carbon dioxide, reuterin, reutericyclin, and bacteriocins, etc. The capability of utilising metabolite bacteriocin obtained from lactic acid bacteria, fundamentally utilised as bio preservatives, serves as an antimicrobial methodology for persistently expanding issues with antimicrobial obstruction. The probiotic microorganism is a useful field for the development of recombinant probiotics with antimicrobial properties. These offer the most encouraging process against the pathogen.

Key Words: ***Antimicrobial activity, bacteriocins, lactic acid bacteria, probiotics, starter cultures***

Introduction

Lactic acid bacteria (LAB) group are known to play an important role in the fermentation processes in small households, food, and dairy industry. Among different LAB, *Lactobacillus* is mainly used for the production of lactic acid, but these are also responsible for the production of organic acids, gas, flavour, aroma, and texture development in the food and dairy products. These bacteria are also known to boost therapeutic and health benefits to the consumers. The health-promoting properties of any LAB are related to its probiotic characteristics. Definition of probiotics is “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” Probiotic bacteria enters into the human gastrointestinal tract and adhere to the intestinal epithelial cells and shows various beneficial biological activities, like competitive adhesion to the epithelial layer and production of small antimicrobial molecules and peptides that suppress the harmful bacteria, stimulation of various immunomodulatory cytokines for the balance of pro-inflammatory and anti-inflammatory cytokines, production of anti-tumour compounds, bacteriocins (glycoproteins, which possesses antimicrobial and bactericidal activity) (Minj *et al.,* 2020). The expression "Probiotics" coined in the twentieth century by a Russian Noble Prize Winner and father of current immunology "Elie Metchnikoff".

- Probiotics – Probiotics are live bacteria and yeast that are beneficial in preventing health conditions.
- Bacteria are beneficial to the host organism.

Probiotics microorganisms are Gram-positive, with *Lactobacillus* and *Bifidobacterium* being the main species used as a treatment of intestinal dysfunctions (Marco *et al.,* 2006).

Antimicrobial properties of probiotics

Antimicrobial and Antagonistic are found to determine the ability of probiotics to inhibit the growth of another species (Fig. 1). Antagonism refers to an association between organisms in which one benefits at the expense of the other. The antimicrobial or antagonistic activity of probiotics is an important property that helps in the production of the antimicrobial compound, competitive removal of the pathogen, enhancement of intestinal barrier function for better health, and others. Antimicrobial is destroying or inhibiting the growth of microorganisms and especially pathogenic microorganism and antagonistic (Fijan *et al*., 2016). This antimicrobial/antagonistic ability is

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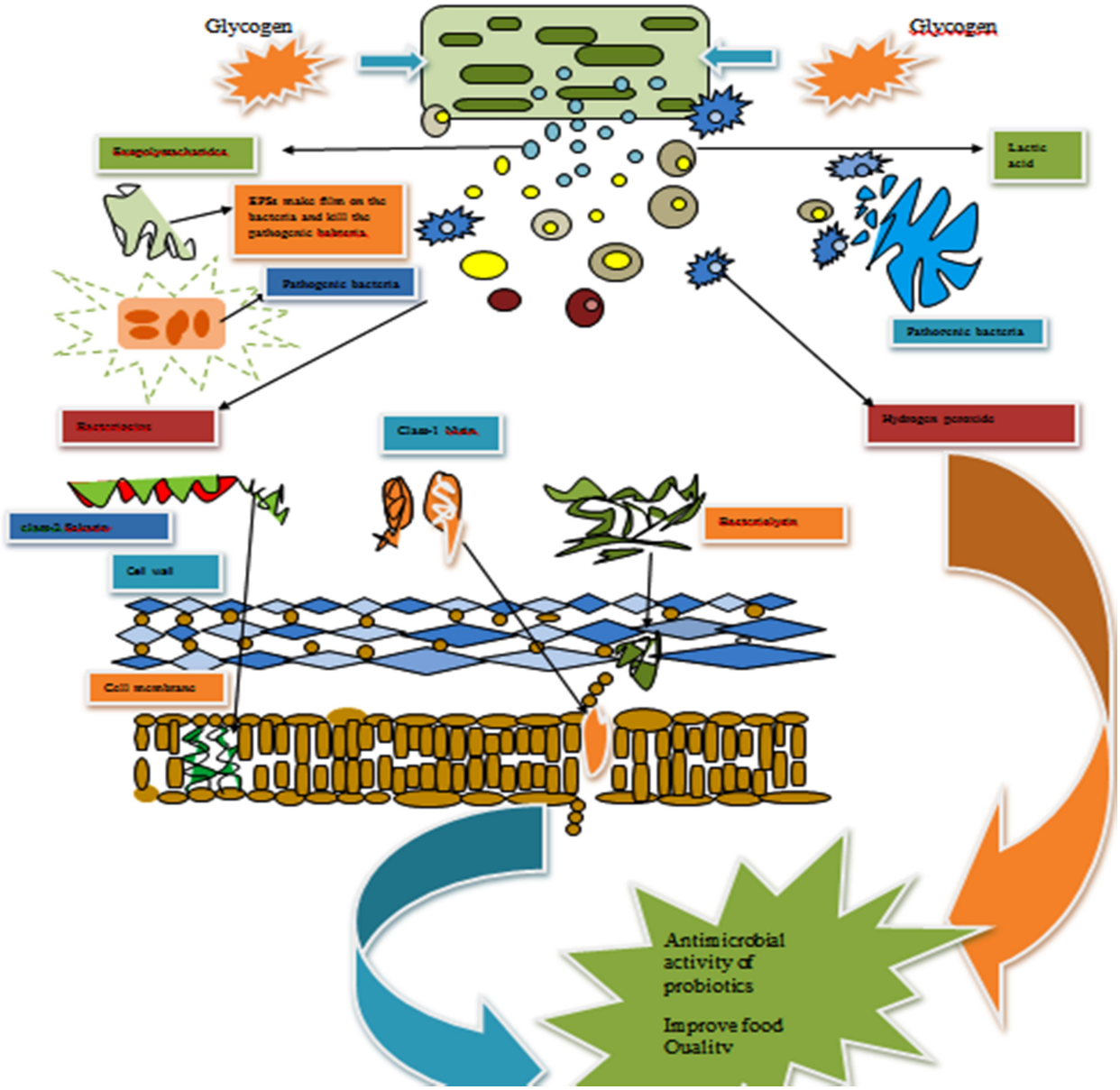

Fig. 1: Diagrammatic representation of Antimicrobial properties of probiotics

especially important for probiotics as one of the beneficial functional properties of probiotics in a broad antimicrobial activity. In a study, *Lactobacillus* and *Bifidobacterium* spp. were screened for their antagonistic activity against food-borne and human pathogens and concluded antibiotic susceptibility for the development of probiotics and food bio preservative (Georgieva *et al*., 2015). The antagonistic activity of one microorganism against another can be caused by competitive keeping out, immune modulation, stimulation of host defence system, and production of an organic acid or hydrogen peroxide that lower pH, production of antimicrobial such as bacteriocins, antioxidants, production of a signalling molecule that trigger a change in gene expression. The ability of probiotics to withstand the normal acidic condition of gastric juices and bactericidal properties of bile salts as well as the production of lactic acid that inhibits the growth of other microorganisms(Murry *et al.,* 2004). Probiotics produce a wide range of antimicrobial

metabolites, i.e., organic acids, diacetyl, acetoin, hydrogen peroxide, short-chain fatty acids, propionic acid, CO2, bacteriocins antimicrobial compound. These activities of probiotics contribute to microbiological safety by controlling the growth of other microorganisms, and inhibition of pathogenic bacteria (Hassan *et al.,* 2014).

Antimicrobials from probiotics

Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming, catalase-negative bacteria that are without cytochrome and non-aerobic bacteria but are aero-tolerant, fastidious, acid-tolerant, and strictly fermentative bacteria. LAB is used as a starter culture for the industrial processing of fermented dairy, meat, vegetable, and cereal product. Also, the application of lactic acid bacteria and their antimicrobial metabolites in the prevention of food spoilage and the extension of the shelf life of food that is ready to eat, fresh-tasting, nutrient and vitamin-rich, minimally processed, and preserved are the major challenges for the current food industry (Galvez *et al.,* 2007). The use of bacteriocins-producing lactic acid bacteria as protective strains or bacteriocins in the form of purified or concentrated compounds as bio preservatives to control undesirable bacteria remains a primary focus of researches related to food safety and quality (Havelaar *et al.,* 2007). The antimicrobial activity of starter cultures and probiotic bacteria has been attributed to the production of metabolites (Fig. 2) such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, other low molecular mass compounds with antimicrobial activity and bacteriocins (Vanderbergh *et al.,* 1993).

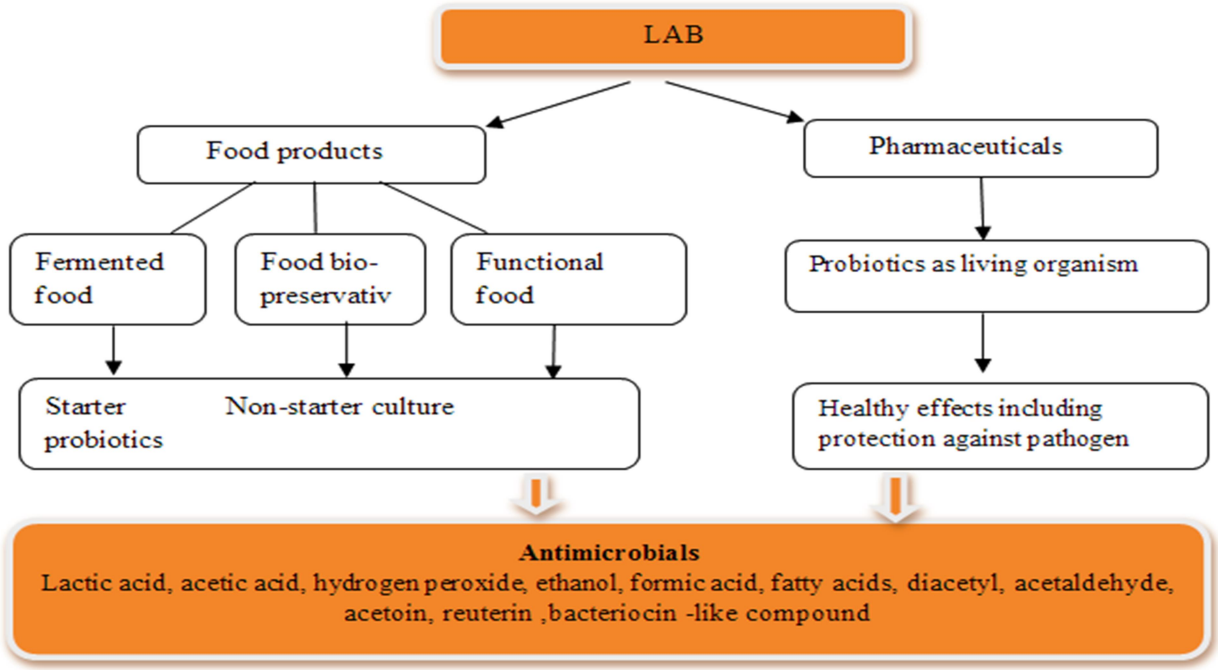

Figure 2. Sources of antimicrobials

Organic acid

As LAB is recognised as safe (GRAS), where it is safe to be consumed by a human without causing side effects, it has been widely applied in the food industry due to the production of organic acids, such as lactic acid which results in lowered pH value (Kasimin *et al.,* 2020). The most significant and best-portrayed antimicrobials natural acids delivered by LAB are acidic corrosive and lactic corrosive. The amount and type of acid produced during fermentation influence the subsequent microbial activity in the fermented material. Acetic acid, for example, is more antagonistic against yeast compared to lactic acid and some oxidative yeast can utilise organic acid as a carbon and energy source and consequently causes spoilage

through deacidification in fermented, especially plant material where they are naturally present. The inhibitory effect of organic acid is mainly caused by the un-dissociated form of the molecule, which diffuses across the cell membrane towards the more alkaline cytosol and interferes with essential metabolic functions. The toxic effect of lactic acid and acetic acid includes the reduction of intracellular pH and dissipation of the membrane potential (Daechel *et al.,* 1989).

Hydrogen peroxide

The antimicrobial property of hydrogen peroxide is attributed to its strong oxidising effect on the bacterial cell and the destruction of the basic molecular structure of cell proteins (Lindgren *et al.,* 1990). In raw milk, hydrogen peroxide produced by lactic acid bacteria can, after being catalysed by lactoperoxidase, oxidised endogenous thiocyanate. The oxidised intermediary product is toxic to different bacteria (Daeschel *et al.,*1989). Hydrogen peroxide production has been considered as the main metabolite of LAB that could protect against urogenital infections, especially in the case of bacterial vaginosis infection (Reid *et al.,*2008). The antibacterial actions of probiotic lactobacilli, mostly species/strain-specific, and they can also act against pathogenic bacteria present in the gastrointestinal tract just by releasing some substances having antimicrobial properties such as H_2O_2, lactic acid, organic acids, and bacteriocins (Jaleel and Kiliç., 2020).

Production of hydrogen peroxide by bacterial cells

Growth inhibition of one bacterial species by hydrogen peroxide generating by another species is a well-recognised mechanism of bacterial antagonism (Gotz *et al.,* 1980) and has, in some cases, been referred to as one of the possible factors responsible for the predominance of a certain species. H_2O_2 is probably generated in small amounts (Table 1) by almost all organisms growing aerobically, in aerobic culture, oxygen is used as an alternative electron acceptor and is reduced to H_2O_2 or water (Condon *et al*., 1987). The replacement of superoxide dismutase (SOD) by (Manganese) Mn^{2+} as a superoxide anion (O_2-) scavenger is a unique feature of lactic acid bacteria and the very high manganese requirement, and content of lactobacilli may reflect this function (Archibald *et al*., 1981).

Table 1. Substrates and reactions that might lead to the production of H_2O_2 by lactobacilli (Archibald *et al.,* 1981)

Substrates reaction	Catalyzed by	References
Pyruvate + O_2 + phosphate⇌ acetylphosphate + CO_2 + H_2O_2	Pyruvate oxidase	Gotz *et al.,*1980
Lactate + O_2 ⇌ pyruvate + H_2O_2	L-Lactate oxidase	Kandler *et al*.,1984
Saturated fatty acids + O_2 ⇌ H_2O_2	Fatty acyl-Co A	Kairuz *et al.*,1988
a-Glycerophosphate + O_2 ⇌ dihydroxyacetone phosphate + H_2O	a-Glycerophosphate oxidase	Kondon *et al*.,1987
O_2 - + 2 H+ ⇌H_2O_2	Mn2+ complexes or SOD[a]	Archibald *et al.,*1981
NADH + H+ + O_2 ⇌ NAD + H_2O_2	NADH oxidase	Kondon *et al.,*1987

Antimicrobial activity

Mechanism of H_2O_2 activation

The mechanisms of action of hydrogen peroxide were postulated by Merchand (1893)

When peroxide of hydrogen medicinal comes in contact with any open and infected surface either of the skin or the mucous membrane ozone is set free, the microbes are instantly destroyed as well as the unhealthy secretions which are caused by their action and then the diseased surface is thoroughly disinfected and made perfectly clean and healthy. The residue of this reaction is water and a small quantity of coagulated albumen (Merchand *et al.,* 1893).

The LAB metabolite hydrogen peroxide does provide antimicrobial activity and also elicit a cleaning effect and helps in the removal of debris. Hydrogen peroxide is an oxidising agent, so it would seem obvious that the main mechanism of action is due to the oxidation of various available macromolecules that make up the structure and function of microorganisms, such as protein, lipids, carbohydrates, and nucleic acids (McDonnell *et al*., 2007). Such effects would accumulate

overexposure of component-time to lead to a loss of structure, function, and therefore, the viability of microorganisms and their various components (such as toxins). It is also likely that a variety of free radicals (particularly, the hydroxyl radical •OH) and other reactive species are produced locally on the degradation of peroxide into water and oxygen during such reactions that further contribute to the overall microbial activity of hydrogen peroxide.

Reactive species may include O2 •– •OH, •OOH, •O, and •H, (Fig. 3) as well as other by-products of these species, with other components of or associated with viable microorganisms. However, despite the widespread use of hydrogen peroxide, there are remarkably few investigations into the exact mechanism(s) of action of hydrogen peroxide (Linley *et al.,* 2012).

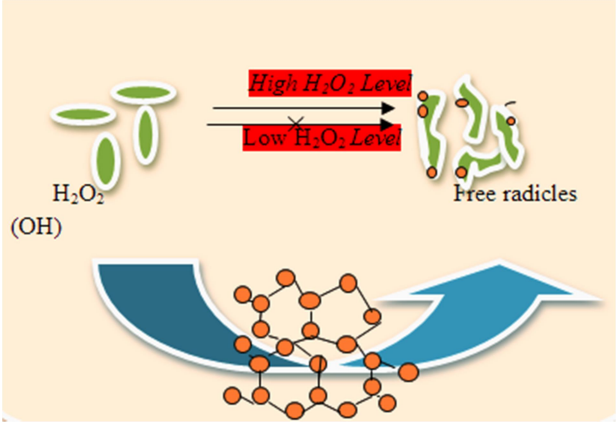

Figure 3. Reactive species (self made)

The whole cells (particularly bacteria) are directly treated, and a variety of direct and indirect treatment method affects the cell may be observed to occur that lead to cell death; "such observations

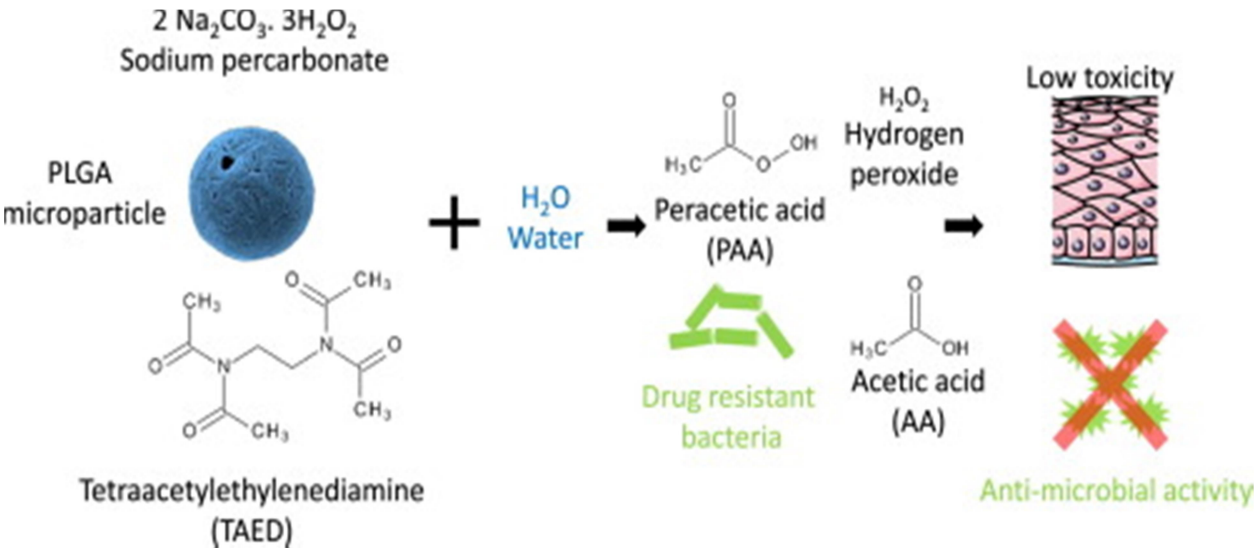

Figure 4. Action mechanism of Hydrogen Peroxide

are typical of biocide investigations but provide little detail on the true mechanisms of action (McDonnell *et al.,* 2009) (Figure 4).

Diacetyl, Acetaldehyde and Acetoin Antimicrobials

Heterofermentative LAB produces active acetaldehyde compound by decarboxylation of pyruvate. These products then condense with pyruvate, forming α-acetolactate and it is converted by α-acetolactate synthases to diacetyl. The product of decarboxylation of α-acetolactate and reduction of diacetyl is acetoin (Collins *et al*., 2009).Diacetyl (2,3-butanedione) compound is best known for the buttery aroma that imparts to fermented food products, but the property of diacetyl is highly concentrated needed to provide preservation of food limit the use of diacetyl as a food preservative (Table 2). Similarly, acetaldehyde, as usual present in fermented dairy products in concentration

Table 2. Antimicrobial metabolites of lactic acid bacteria (Vanderbergh *et al.*, 1993)

Compound	Microorganisms producers	Antimicrobial spectrum
Lactic acid	All strain (LAB)	Yeasts Gram (+)ve bacteria Gram (-)ve bacteria
Acetic acid	Heterofermentative (LAB)	Yeasts Gram (+)ve bacteria Gram (-)ve bacteria
Diacetyl Acetaldehyde Acetoin	Variety of genera of (LAB) including: *Lactococcus, Leuconostoc Lactobacillus* and *Pediococcus*	Yeasts Gram (+)ve bacteria Gram (-)ve bacteria
Hydrogen peroxide	All (LAB)	Yeasts Gram (+)ve bacteria Gram (-)ve bacteria
Carbon dioxide	Hetrofermentative (LAB)	Most of the taxonomic group of microorganism
Reuterin	*Lactobacillus reuteri*	Fungi, protozoa, Gram(+)ve and Gram(-)ve bacteria
3-hydroxy fatty acids	*Lactobacillus plantarum*	Fungi
Bacteriocins	LAB	

smaller than necessary for inhibition of undesired microorganisms activity, also plays a role in controlling the growth of contaminants, together with other antimicrobial metabolites of lactic acid bacteria.

Carbon dioxide

The influence of carbon dioxide on product preservation is two folds. Namely, except for its antimicrobial activity, it creates an anaerobic environment by replacing the existent molecular oxygen. The antifungal activity of carbon dioxide is due to the inhibition of enzymatic decarboxylations and its accumulation in the membrane lipid bilayer resulting in dysfunction in permeability (Lindgren *et al.,* 1990).

Mechanism of Carbon dioxide (CO_2) action

Movement of oxygen

The activity of carbon dioxide (CO_2) was that it displaced all of the oxygen available for bacterial metabolism, thus slowing growth by a proportional amount. This possibility was discounted early in the study of this system by experiments that showed that anaerobic bacteria are also inhibited by carbon dioxide atmosphere (Frankel *et al.,* 1930). Williams (1998) confirmed these findings by replacing the bacterial growth atmosphere with 100% nitrogen. He did not observe the degree of inhibition equal to that of when carbon dioxide was present. Although reducing available oxygen may have some effect on bacterial growth, it does not appear to be the most limiting factor.

Effect on pH

Most studies on carbon dioxide (CO_2) atmosphere and bacterial growth observe that the pH of the growth medium is decreased. A brief review of the conduct of carbon dioxide (Fig. 5) in solution illustrates the chemical species present that can account for this acidification. It should first note that the solution of gaseous carbon dioxide into an aqueous mixture obeys Henry's law very closely at moderate temperature and pressure. The values for Henry's law constant for carbon dioxide were determined to be 0.797 atm/mole fraction at 10°C, 1.039 atm/mole fraction at 18°C, and 1.255 atm/mole fraction at 25°C. Adjusted the pH of bacterial growth media to standard levels (approximately pH 5.8) then grew pure cultures of *Achwmobacter,Pseudomonas,* and *Bacillus* under atmospheres of air, and others under carbon dioxide (Coyne *et al.,*1933).In all trials, the carbon dioxide treatments produced a far greater degree of inhibition, as measured by culture growth. In another investigation, (Becker *et al.,*1933) studied other acids that produced equal acidification in the cells, but found that they were not able to inhibit growth to the levels achieved through the application of carbon dioxide.

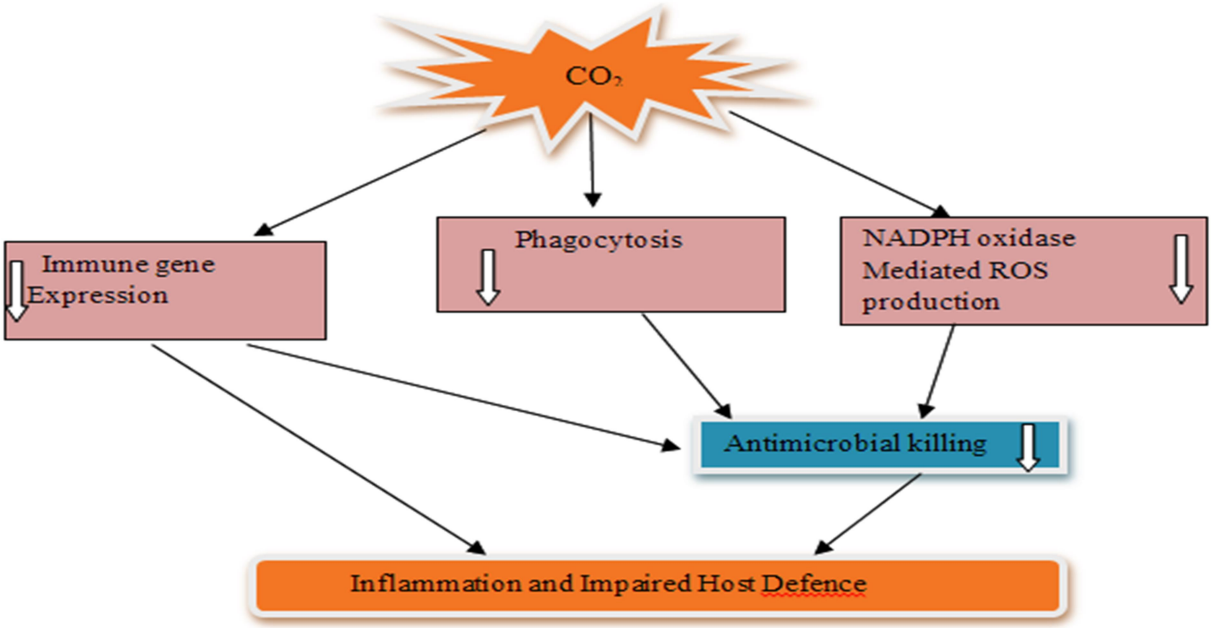

Figure 5. Antimicrobial activity of Carbon dioxide(self made)

Reutrin and Reutericyclin

Reutrin and reutericyclin are two compounds produced from the selected isolates of *Lactobacillus reuteri* which are both active towards Gram-positive bacteria. Reutericyclin is a tetrameric acid derivative and reuterin is a mixture of monomeric hydrated monomeric and cyclic dimeric forms of β-hydroxy propionaldehyde with a border spectrum of inhibitory activity, including Gram-positive bacteria, fungi, and protozoa (Leroy *et al.,* 2006).

Mechanism action of Reuterin

Strains of Lactobacillus reuteri are effective against a variety of ailments, including diarrhea and colic (Savino *et al*., 2007). One of the proposed mechanisms of action that *L. reuteri* uses to effect probiotics is the production of the antimicrobial compound 3-hydroxy propionaldehyde (3-HPA), also referred to as reuterin (Talarico *et al.,* 1988).

Reuterin is produced as an intermediate step in the conversion of glycerol to 1, 3- propanediol, a pathway proposed to regenerate NAD+ from NADH and to contribute to improved growth yield (Luthi-Peng *et al.,*2002). Reuterin is metabolised in a specialised bacterial compartment called the metabolosome**,** perhaps due to its toxicity (Sriramulu *et al.,* 2008). For unclear reasons, *L. reuteri* secretes high levels of reuterin when grows incubated in the presence of excess amounts of glycerol. Reuterin is bioactive against bacteria, viruses, and fungi (Fig. 6) (Chung *et al.,* 1989).

The aldehyde group of reuterin is highly reactive and thus reuterin can form additional compounds in aqueous solution (Vollenweider and Lacroix, 2004). Reuterin can dimerise, forming HPA dimer, or can be hydrated to form HPA hydrate. Reuterin can also be dehydrated into the toxic compound acrolein. Because reuterin can be converted into these various compounds, the mechanism by which reuterin exerts its antimicrobial effects has been difficult to determine.

Two main hypotheses have been proposed:

- First, the aldehyde group of reuterin is proposed to be highly reactive with thiol groups and primary amines, and therefore reuterin could inactivate proteins and small molecules containing these groups (Vollenweider and Lacroix, 2004). Broad-spectrum of pathogenic microorganisms such as bacteria, fungi, and viruses would be affected by reutrin.
- On the other hand, "the dimeric form of reuterin, HPA dimer, which is structurally similar to a ribose sugar, could specifically block the enzyme ribonucleotide reductase by acting as a competitive inhibitor. This enzyme is required for the generation of deoxynucleotides, which are required for DNA synthetase. An earlier report has suggested that

reuterin could indeed inhibit ribonucleotide reductase and possibly exert its broad-spectrum effects through this inhibition (Talarico and Dobrogosz, 1989)."It is impossible to determine which mode of action is correct of reuterin from earlier experiments because the active site of this enzyme contains a thiol group. Oxidative stress can occur by modifying thiol groups inside the cells as the aldehyde form of reuterin is the bioactive agent. Direct contact of reuterin with other bacteria can lead to stimulating the production or secretion of reuterin solely by the enzyme glycerol dehydratase.

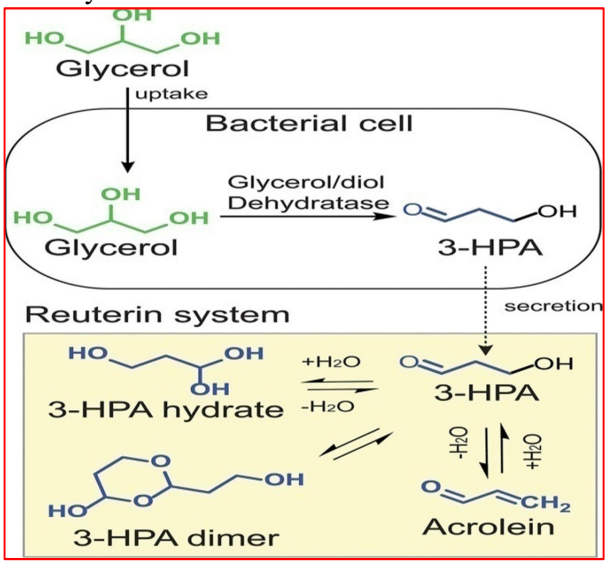

Figure 6. 3-HPA (reuterin) can adopt different forms. 3-HPA can react with water to form HPA hydrate or interact with itself to form HPA dimer. 3-HPA can also undergo dehydration to form acrolein (Vollenweider *et al.*, 2004).

Reurterincyclin

Reuterincyclin is a naturally occurring metabolite, low molecular weight tetrameric acid antibiotic produced by sourdough isolates of *Lactobacillus reuteri* (Holtzel *et al.*, 2000). "The production of reuterincyclin in sourdough is thought to prevent stable colonisation of competing for Gram-positive bacterial, Reutrincyclin works against Gram-positive microorganism, including pathogen-associated with a tropical infection such as methicillin-resistant *Staphylococcus aureus* (MRSA), S*treptococcus sp.* and *Clostridium diffuicle* (Hurdle *et al.*,2011).

Bacteriocins of Lactic Acid Bacteria

LAB produced bacteriocins, antibacterial proteinaceous substances with bactericidal activity against related species (narrow spectrum), or across genera (broad spectrum of activity) (Rogelj *et al*., 1994). Bacteriocin biosynthesis is a desirable characteristic for strain selection as it serves as an important mechanism of pathogen exclusion in fermented food as well as in the gastrointestinal environment. Bacteriocins are ribosomally synthesised peptides or protein with antimicrobial activity produced by many Gram-positive or Gram-negative bacteria; however, those produced by food grade LAB have received considerable attention due to their potential application in the food industry as natural preservatives (bio preservatives).

The bacteriocins are classified into 3 or 4 classes(Table 3):

Class:1 (a) Lantibiotics or small, heat-stable, lanthionine-containing, single- and two-peptide bacteriocins

(b) Biologically inactive propeptides are subjected to extensive post-transitional modification

Class: II. Small, heat- stable, non-lanthionine-containing bacteriocin

Class (IIa) including pediocins like or *Listeria*-active bacteriocin

Class (IIb) two peptide bacteriocin

Class (IIc) circular bacteriocin

Class: III bacteriolysins or large, heat-stable, lytic proteins, often murein hydrolases (Cotter *et al.,* 2005).

Mode of action

Bacteriocin that is produced by LAB can be of broad-spectrum, but in general, the activity is directed against low G+C Gram-positive species (Cotter *et al.,* 2005). The antibacterial spectrum includes spoilage organisms and food-borne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus.* Wide ranges of a mode of action have been described for bacteriocins, such as enzyme activity modulation, inhibition of outgrowth of spore formation of pores in the cell membrane. Most bacteriocins interact with an anionic lipid that is abundantly present in the membranes and consequently initiate the formation of pores in the membranes of susceptible cells (Chen, 2003). However, generalised membrane disruption models

Table 3. Classification, major characteristics, and some examples of bacteriocin (V*uyst et al.,* 2007)

Classification	Major characteristics	Examples
Class I		
Lantibiotics/lanthionine-containing bacteriocins subdivided into: Type A lantibiotics Type B lantibiotics	Small(<5 k Da) membrane-active unusual amino acids -elongated peptides with a net positive charge -small globular peptide with negative or no net charge	Type A Nisin, lactocin S, lacticin 481 Type B. Mersacidin
Class II		
Non-lanthionine contains Bacteriocin subdivided into: Subclass IIa Subclass IIb Subclass IIc	Heterogeneous class(<10 kDa) Post-translation unmodified non-lantibiotics IIa: pediocin-like IIb: two peptide IIc with a wide range of effects on membrane permeability and cell wall formation	IIa: pediocin PA1, sakacin A, sakacin P, leucocin A., curvacin A IIb: lactococcin G, lactococcin M, lactacin F,plantaricin A IIc: acidocin B, enterocin P, enterocin B reuterin 6
Class III		
Bacteriolysins	Large(>30 kDa)heat-labile antimicrobial complex proteins with domain type structure that function through the lyses of sensitive and cell by catalyzing cell wall hydrolysis	Lysostaphin,enterolysin A,helveticin J, helveticin V-1829
Class IV		
	Complex bacteriocin carrying lipid or carbohydrate moieties	Plantaricin S, leuconosin S,lactocin 27, pediocin SJ1

cannot adequately describe the mode of action of bacteriocins. Rather, a specific target seems to be involved in pore formation and other activities (fig 7). For the Nisin and epidermis family of lantibiotics, the membrane-bound cell wall precursor lipid II has been identified as a target (Hechar*d et al.,* 2002). Most of the class II bacteriocin dissipates the proton motive force (PMF) of the target cell via pore formation (Law *et al.,* 1995). The subclass IIa bacteriocin activity depends on a mannose permease of the phosphotransferase (PTS) specific target. The subclass IIb bacteriocin (two-component) also induce dissipation of the PMF by forming cation or anion-specific pores; a specific target has not yet been identified. Finally, subclass IIc comprises miscellaneous peptide with various modes of action such as membrane permeabilisation, specific inhibition of spectrum formation, and pheromone activity (Cotter *et al.,* 2005).

Uses of bacteriocins in combination with other antimicrobial factors

The antimicrobial spectra and activity of bacteriocins can be extended through the synergy between different antimicrobial factor such as inorganic salts (especially sodium chloride), organic acid and their salts, chelating agent (such as EDTA), essential oil and their active component, phenolic compounds, as well as other natural antimicrobials. Application of bacteriocins together with different physicochemical treatments, like heat treatment, modified atmosphere packaging, high hydrostatic pressure, pulsed electric field, pulsed magnetic field, and gamma irradiation, has received great attention in recent years (Deegan *et al.,* 2006). The effectiveness of bacteriocins, in combination with hurdle technology, will depend upon the type of food and its microflora. Thus with acidification of the food, acid-tolerant bacteria may be selected, while heat treatment may favour bacterial

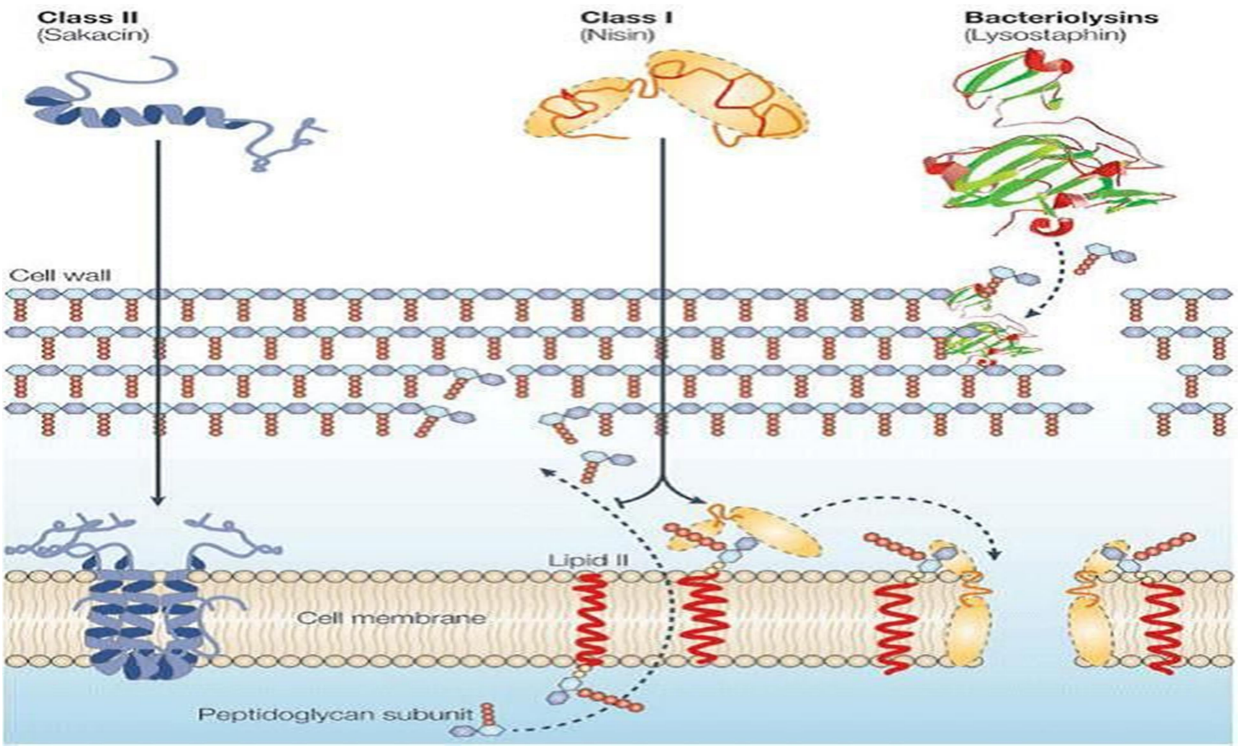

Figure 7. Mechanism action of Bacteriocin (Juodeikiene *et al.*, 2011).

endospores, but in combination with bacteriocins higher sanitisation may be achieved after optimisation of doses and condition. Furthermore, the Gram-negative bacteria could become sensitive to bacteriocins activity upon exposure to hurdles such as a chelating agent that destabilises the bacterial outer membrane (Fang *et al.,* 2003).

Application of Nisin

Nisin is the only bacteriocin licensed as food preservatives (E234). Commercial production of nisin by *Lactococcus lactis* ssp. lactis began in England in 1953, and international acceptance of nisin was given in 1969 by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) (FAO/WHO *et al*., 2002). Nisin is widely used in food products including cheese, salads, canned soups, ice for storing fish, baby foods, milkshakes, and baked goods. Nisin is a small peptide of 34 amino acids.

Pulsed electric field (PEF) has been evaluated in combination with other thermal and non-thermal techniques and resulted in a synergistic reduction of the microbial population (Buckow *et al.,* 2014). The effectiveness of nisin and PEF showed promising resulted in inactivating *Bacillus cereus* (Lopez *et al.,* 2008).

Nanotechnology and Food Integration

When nisin is utilised in food, it is affected by numerous components present in food. The binding/interaction of nisin with the food matrix component can reduce the efficacy of nisin and hence decrease food stability (Bernela *et al.,*2014). Nisin can be adversely affected by several food components, such as glutathione, protease, sodium metabisulphite, and titanium dioxide (Quintavalla and *V*icini, 2002). Furthermore, the use of nisin in its free form loses effectiveness due to its degradation by enzymes. Jung *et al.* (1992) observed a substantial loss of nisin efficacy in milk due to its interactions with milk components. A positive charge present on the bacterial cell wall also reduces nisin effectiveness by preventing the interaction of nisin and bacterial surface molecules due to electrostatic repulsion (Taylor *et al.,* 2007). The controlled release and delivery of nisin can be extensively improved through the use of nano-particulate systems (Quintavalla and Vicini, 2002). Finally, to overcome the hindrances associated with nisin as a food preservative, the use of nanotechnology for the synthesis of nisin-loaded/coated nanoparticles has been introduced.

Nanoemulsion

Nanoemulsions are nanoscale (200-600 nm in diameter) oil-in-water or water-in-oil dispersion composed of a combination of water, surfactants, oil (soybean oil), and co-solvent (ethanol) (Imran *et al.,* 2015). Nanoemulsion is not only kinetically stable but also long-term physically stable; a unique characteristic sometimes referred to as approaching thermodynamic stability (Jaiswal *et al.,* 2015). Nanoemulsions possess stability against flocculation, creaming, sedimentation, and coalescence. Generally, Nanoemulsions are non-toxic, non-irritant when formulated with oil, and approved as GRAS for human consumption by the FDA (Acevedo-Fani *et al*.,2015). Nanoemulsion (fig. 8) has been receiving more attention in the food industry, due to the potential application in clear beverages and fortified drinks (Zhang *et al.,* 2015).

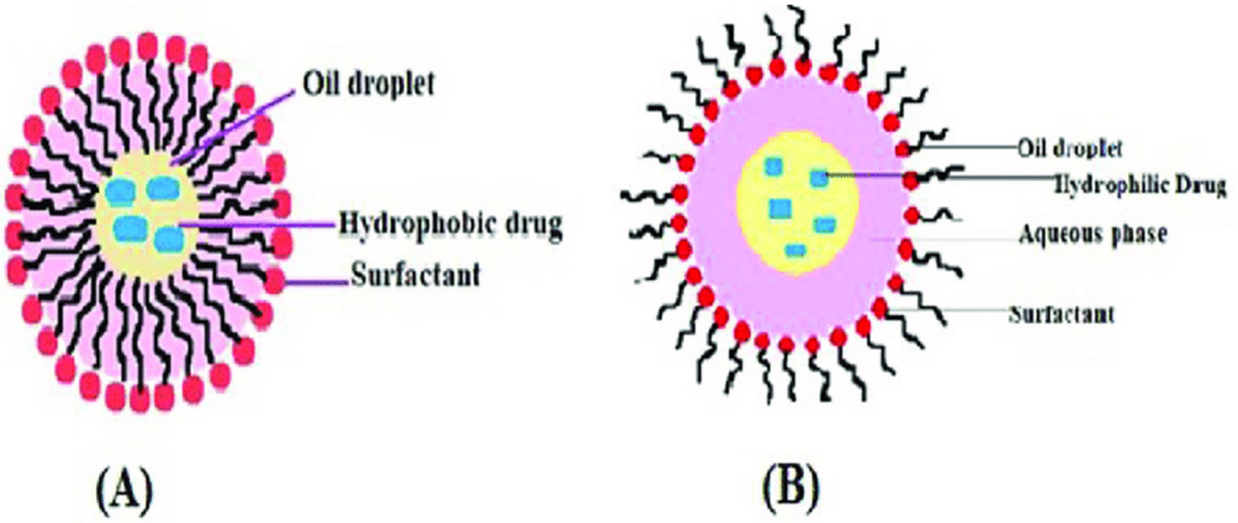

Figure 8. Nanoemulsion (Pande *et al*., 2018).

Antimicrobial property of EPSs

The probiotic effects ascribed to fermented dairy products arise not only from whole microorganisms and wall components but also from metabolites such as peptides and extracellular polysaccharides (EPS) produced during fermentation. EPSs produced by lactic acid bacteria (LAB) have gained increasing attention due to their potential health benefits. LAB are food-grade organisms that are generally recognised- as safe (GRAS) and can produce EPSs that are potentially useful as additives to improve the texture and viscosity of naturally fermented milk products and to prevent syneresis. It has also been suggested that some EPSs produced by LAB may confer health benefits to the consumer. The formation of EPSs occurs in two forms depending on location: as a capsule (capsular polysaccharides) where the polymer is closely associated with the bacterial surface; and as slime polysaccharide loosely associated with the bacterial surface. Such a distinction may be difficult since some strains release capsular polysaccharide material at the periphery. For bacteria, EPSs are thought to play a role in protection against desiccation, toxic compounds, bacteriophages, osmotic stress, and to permit adhesion to solid surfaces and biofilm formation (De Vuyst and Degeest, 1999). Antimicrobial components produced by lactic acid bacteria and bifidobacteria include organic acids, hydrogen peroxide, carbon dioxide, diacetyl, bacteriocins, and low molecular weight antimicrobial substances (Ouwehand and Vesterlund, 2004), but few studies have investigated their polysaccharide metabolites, especially exopolysaccharide (fig. 9). Interleukin-10 (IL-10), a 17- to 20-kDa glycoprotein (Moore *et al.,* 2001), is a potent inhibitor of the production of the pro-inflammatory cytokines such as interferon-γ (INF-γ), tumour necrosis factor-α (TNF-α), IL-1, IL-2, IL-6, IL-8, IL-12, and IL-18, all of which are associated with cell-mediated immunity and inflammatory responses. On the other hand, IL-10

is involved in the promotion of humoral immunity (Ghildyal *et al.,* 1992). IL-10 stimulates the secretion of IgG, IgM, and Ig A by B cells (Rousset *et al*., 1992), but suppresses antigen-specific IgE secretion involving allergy. IL-10 enhances B cell survival in culture and mast cell development survival (Ghildyal *et al*., 1992). Mice carrying a null mutation of the IL-10 gene (IL-10−/−) show increased spontaneous development of inflammatory bowel disease and extreme susceptibility to infection-induced immune pathology (Steidler *et al.,* 2000). In contrast to IL-10, tumour necrosis factor-α (TNF-α) is a pro-inflammatory interleukin frim inflammatory cells.

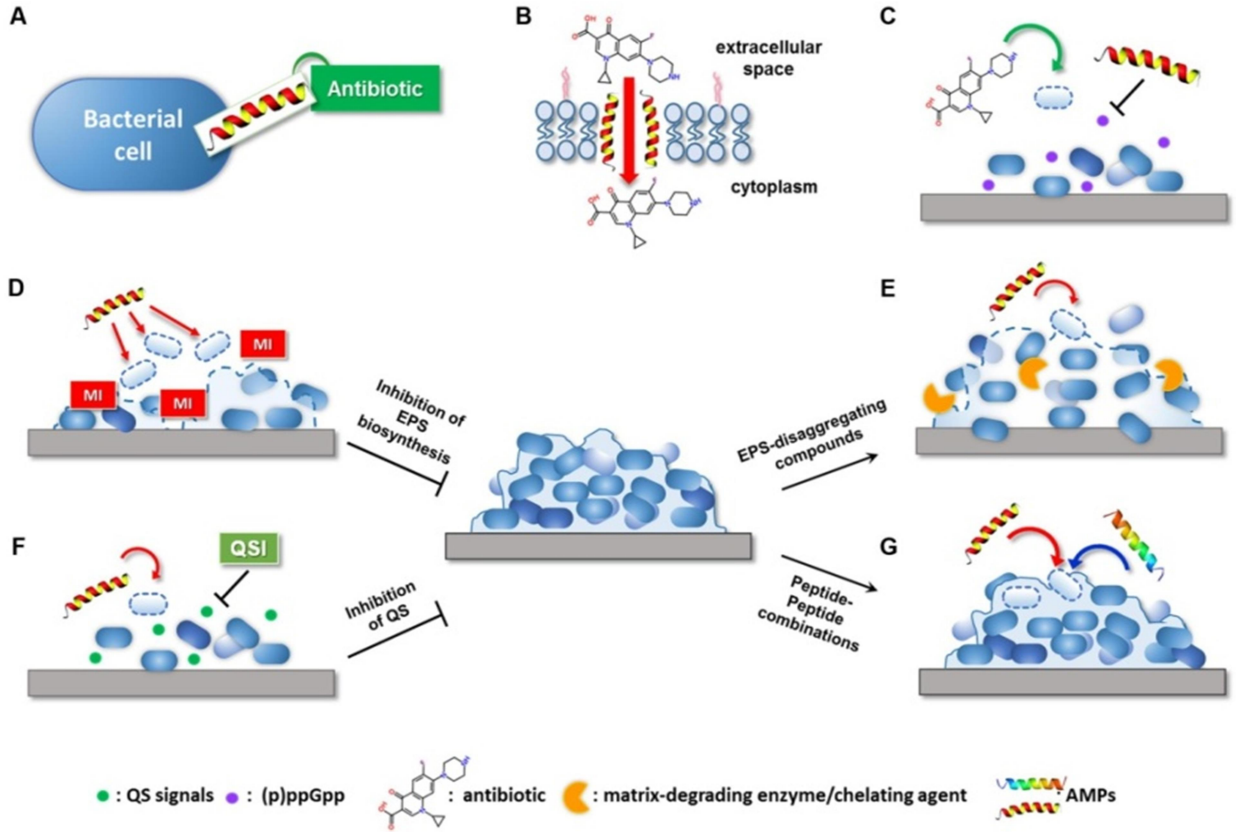

Figure 9. Mechanism action of exopolysaccharides (Grassi *et al*., 2017).

Diacyetylated microbial biofilm exopolysaccharides

Cationic exopolysaccharides can also be mediated resistant and to antimicrobial agents through repulsion or sequestrants of these molecules. "PNAG (Poly-N- Acetyl glucosamine) exo poly saccharide production increases the biofilm resistance of A*ggregati bacter actinomycete comitans* to the cationic detergent cetylpyridinium chlorides and protects *S.epidermidis* against microbial action of glycopeptides antibiotics (fig. 10), such as vancomycin (Farber *et al*., 1990). The production of GAGs by fumigants limits intracellular penetration of the hydrophobic antifungal posaconazole and reduces its activity (Snarr *et al*., 2017). The ability of Pel to enhance antimicrobial resistance may be strain or condition-dependent, however, because other studies have reported that disruption of the Pel A deacetylase failed to alter susceptibility to tobramycin or ciprofloxacin (Baker *et al*., 2016).

Conclusion

Antimicrobial from natural sources gives a guarantee of microbial safety and increase the shelf life of foodstuffs. Normal additives are undependable, however, comprise a variable choice to take care of the issue of microbial opposition, meeting the necessity of sound nourishments simultaneously. The fuse of a characteristic additive

to meat, organic products, and vegetables, just as edible packaging leads to shelf life extension. The regular antimicrobial can inactivate microorganisms and chemicals without weakening organoleptic or dietary properties. Nevertheless, it has been accounted for that in complex food networks; dynamic bio mixes can tie to the hydrophobic moieties of protein or lipids. This can limit the accessibility of common antimicrobials.

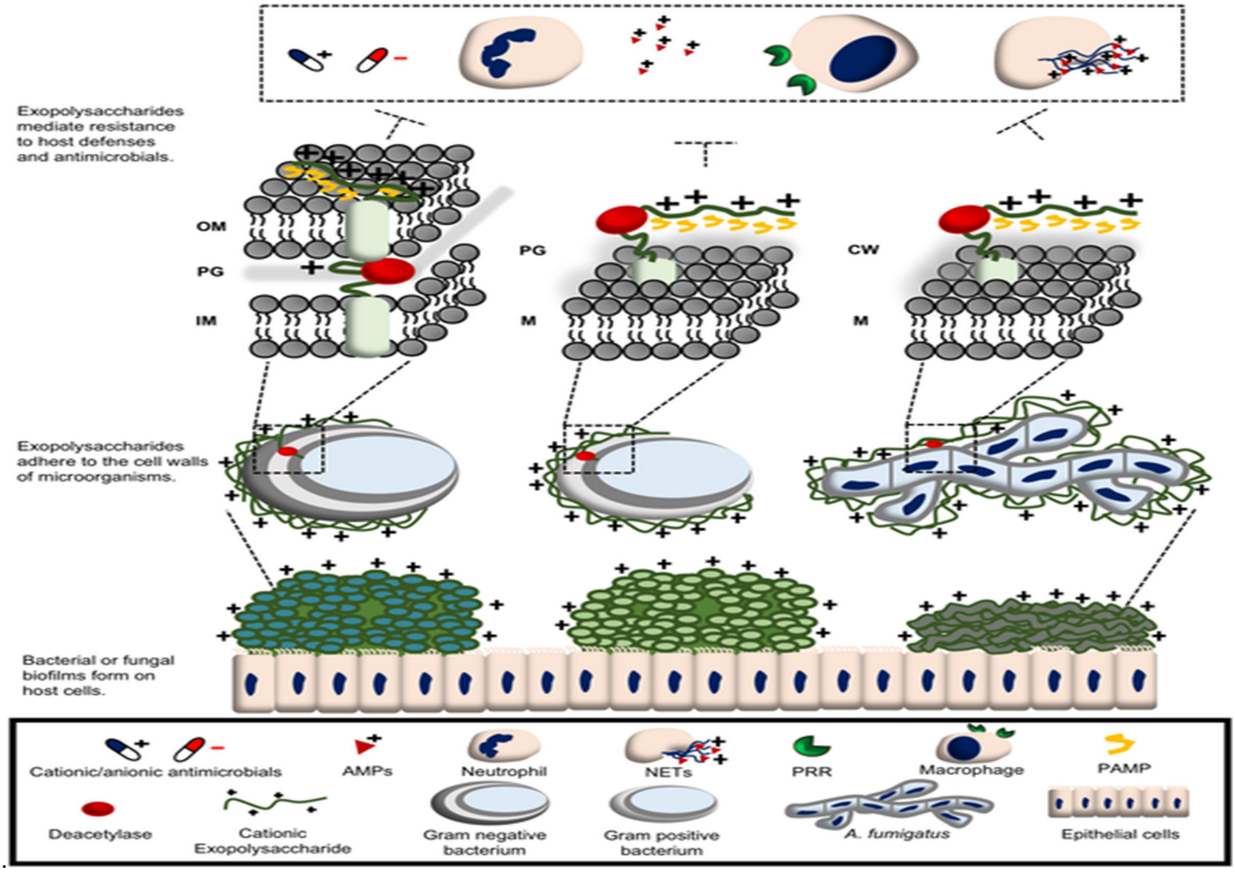

Figure 10. Mechanism of Diacetylated microbial biofilm exopolysaccharides (Ostapska *et al*., 2018)

Besides, a reduction of the antimicrobial movement can be a consequence of handling. Nanoparticle joining has been affirmed as a viable and safe antimicrobial conveyance framework. With an expanding interest for a characteristic item concerning food safety, buyer acceptance, and food conservation, there is more scope for biopreservation.

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