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Biosurfactant production by *Enterococcus* spp and their promising applications in therapeutic and environmental managements

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Abstract

Enterococci, primarily *E. faecalis* and *E. faecium*, can produce inhibitory chemicals including H_2O_2 , bacteriocins and lactic acid which can stop food pathogens and spoilage bacteria from growing. Fats and phosphates, lipid fats, lipoproteins, glycolipids, polymeric biological agents, and unique biological factors are some of the numerous types of biosurfactants. Biosurfactants can be used to improve oily extraction, ingredients of herbicide and pesticide, detergents, health care and cosmetics, charcoal, textiles, ceramic processing, pulp and paper and food industries, as well as uranium processing and mechanical dewatering from peat. They can also be utilized in a range of industries, such as pharmaceuticals, beverages, preservatives, and environmental remediation procedures like biodegradation, soil washing, and soil flushing.

Key words: *Probiotics, Enterococci, biosurfactant*

Enterococci

Enterococci are a type of commensalism that is positive to Gram stain, lacked the ability to spore formation , negative to catalase and oxidase tests, and grows facultatively aerobically. For many years, these bacteria were thought to be safe to humans. In addition, *Enterococcus* species are widely employed as probiotics and as starter cultures in the food sectoras showed by Anagnostopoulos and colleagues (2018).

Enterococcus is a lactic acid bacteria (LAB) genus with 37 species categorized depending on the phylogenetic analysis utilizing sequencing of 16S rRNA as well as hybridization with DNA-DNA. *Enterococcus* is the third level of size inside lactic acid bacteria genera after *Lactobacillus* and *Streptococcus* (Franz *et al*.,2011). *E. thailandicus*, *E. ureasiticus*, *E. pallens*, *E. caccae, E. cammelliae*, *E. lactis*, and other new species have recently been found (Henning *et al.*,2015).

Distribution and pathogenecity

The existence of *Enterococci* in every environment, including water, sewage, soil, and even plants, characterizes the genus *Enterococci*'s habitat. (Braek *et al.*, 2017). Currently, *E. faecalis*, *E. faecium*, *E. durans*, and *E. hirae* are the most common *Enterococcus* species in the digestive system then *E. faecium*, *E. faecium*, *E. durans, and E. hirae* which came after them(Hanchi *et al.*,2018). In numerous meals, as in the case of meat, vegetablesas well as dairy derivatives, *enterococci* are the most common lactic acid bacteria (LAB). A few strains have a remarkable track record of safe use as probiotics, with these organisms balancing the gut's microbial balance and thereby boosting intestinal health. Furthermore, their ability to lysis of proteins and lipids as well as their ability to utilise citrate and pyruvate as carbon sources and create bacteriocins (known as enterocins when produced by *enterococci*).

The positive characteristics of lactic acid producing bacteria sparked a lot of curiosity, and many of distinct species that produced as probiotics that enhanced human health. Human and animal gastrointestinal tracts are home to *Enterococcus faecium* and *Enterococcus faecalis*. Due to their helpful health enhancements in the host, such as reduction the growth of tumor cell lines, enhancement the immune system activity for intestinal (Olvera-Garcan *et al*., 2018), reduction of diarrhoea besides the treatment of hypercholesterolemia. *Enterococci* can be found in various dairy derivatives included cheeses and raw milk) (Elmoslih *et al.*,2017), fermented vegetables like olives, fermented sorghum (Tamang *et al.*,2016) besides the foods with high protein level like meats, fish, and sea foods (Tamang *et a.*,2016). (Vinderola *et al.*,2017).

Enterococci specis, primarily *Enterococcus faecalis* and *Enterococcus faecium*, produce compounds with inhibitory activity like lactic acid, hydrogen peroxide, and bacteriocins, which can stop food pathogens and spoilage microorganisms from growing (Raafat *et al.*, 2016).

The majority of infections caused by *Enterococcus* species considered as opportunistic infections and they revealed higher resistance to b-lactams, aminoglycosides, and vancomycin, so that their treatment becoming increasingly difficult to treat particullatily *Enterococcus faecalis* infections as presented by Cattaneo *et al.*(2010). Virulence genes, like resistance genes, are typically carried on plasmeds so that they can spread rapidly inside *Enterococcus* genus (Leclercq *et al.* 2013).

Bacteriocins

Bacteriocin is a protein that kills bacteria while also having a tiny inhibitory impact that is limited to the bacteria that produce it or similar species, and the encoded genes must be plasmid-based. It is not complete, particularly for Gram positive bacteria, which may have a bactericidal impact (Harder,1997) that interacts with and eliminate the development of another bacteria, which closely related with the same source(Hanchi *et al.*, 2018).

Bacteriocins are classified mostly by species, such as Pediocin, which is produced by the bacteria *Pediococcus acidilactici* (Papagianni and Anastasiadou, 2009). Furthermore, the differentiation of bacteriocin is based on the type of bacteria generated, such as colicin, which is created by E. coli (Tishvarian,1996), plantaricin, which is produced by *L. plantarum* (Todorov,2012), and epidermicin, which is produced by *S. epidermidis* (Halliwell *et al*,. 2017).

Optimization of bacteriocins production

Bacteriocin can be manufactured in enormous quantities in the laboratory under ideal dietary and physiological conditions. Bacteriocin production, on the other hand, is highly dependent on what is available to the producing organism in terms of nutrients, incubation temperature, and primary pH. (Furtado *et al*., 2009).

Carbon is an important source of microbial cell growth because it is used in cell building and reproduction as well as providing energy. Several studies have found that glucose is the optimal carbon source for the synthesis of bacteriocins. Ogunbanwo *et al.,* (2003) used 0.25 percent glucose to produce bacteriocin from *Enterococcus* sp F1 in this context. Furthermore the glucose and fructose as a carbon sources led to over production of biomass and bacteriocin production as mentioned by Drosinos and his team in(2005), respectively . However, contrary to the previously stated cases of glucose being the best carbon source for bacteriocin production, *Enterococcus* sp. showed an antagonistic relationship with rising glucose concentration.

In the fermentation and bacteriocin manufacturing processes, nitrogen supplies are also crucial. They are used to make amino acids, which are the basic building blocks of proteins (Ogunbanwo *et al*.,2003). Many studies, on the other hand, have found that a pH of 6 to 7.5 is ideal for *S. epidermidis* bacteria growth and bacteriocin production. According to Sahl *et al.* (1981), the maximum bacteriocin production from *S. epidermidis* occurred at pH 7.3, while the highest growth occurred at pH 6 and 7.

Bacteriocin Classification

The first form of bacteriocin is already known for its restricted ability to influence, whereas the second type of bacteriocin has wide spectrum of inhibition toward another organisms (Jiménez *et al*.,1993). The bacteriocins that secreted by positively Gram stain microorganisms can affect on other sorts of bacterial isolates (Mehta *et al*.,1983). There are some differences between the bacteriocins that belong to positive and negative bacteria sice the positive organisms have a wider spectrum of efficacy than those produced by negative bacteria (Savadogo *et al*., 2006).

Bacteriocins that belong to *Enterococci* classified to two categories(Nes et al., 1996). The class II bacteriocins composed cationic, hydrophobic, and heat-stablthermal stable e peptides. The subclass IIa has potent anti-Listeria action. The bacteriocin isolated from *E. faecium* displayed thermal stability and represion action forward *L. monocytogenes*, as well as other subclass IIa features.

Bacteriocins properties

Enterococcus sp bacteriocins which affected on intestinal and food-borne pathogenic organisms, so that they used in vegetables and fermented dairy products as biopreservatives. little molecular weight, thermal stability, and peptide chain that loses lanthionine . The class II of bacteriocins comprise enterocins A, B, P and L 50. The pediocin-like peptides in the class II a subgroup, in particular, have hydrophilic N-area that covered of YGNGVXC motif.

Mechanism of action in bacteriocin

Bacteriocins from positively bacteria (including enterocins) are initially active forward another bacteria that are closely related, though some, such as nisin, have shown activity against Gram-negative bacteria (Ennahar *et al.*, 2000). Bacteriocins have been shown to be membrane active peptides that cause membrane pores to form, destroying the integrity of the cytoplasmic membrane (Moll *et al.*, 1999).

The positively bacterial specis contain a lot of concentration of anionic lipids in their membranes, as is well known. The mechanism of action in bacteriocin is derived from its ability to bind with the membrane of bacteria. The attraction power between the membrane lipids with the negative charge and bacteriocin residue which has positive charge, which is restricted in the C- or N-terminal area. This is due to bacteriocin penetration into the lipid bilayer of the bacterial membrane and formation the pores during the bacterial cell membrane, as shown in fig.1 and this state lead to spill minerals, ions and metabolites critical ions and that required remaining the life of cells, resulting in bacterial disease (Ruiz-Larrea *et al.,* 2007).

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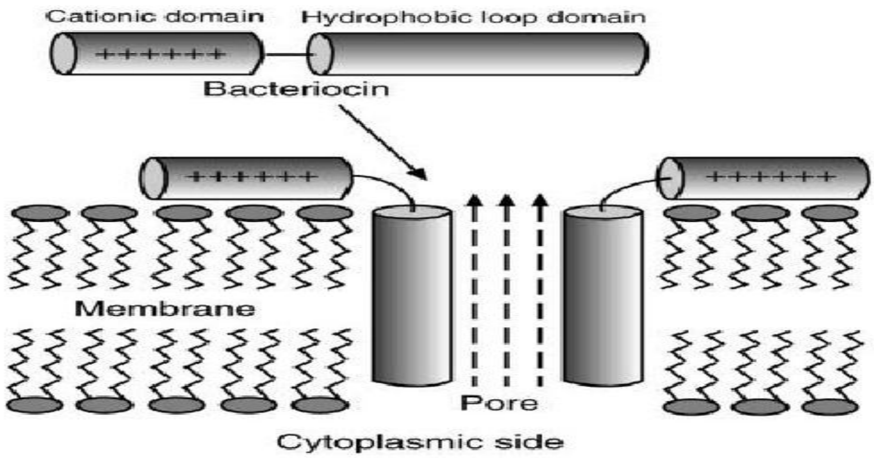

Figure (1): Formation of pore in the bacterial surface by bacteriocin (Oscáriz *et al.*, 2001).

Bacteriocin function

Bacteriocins can have a number of distinct effects. Bacteriocins can help introduce a product that inhibit the another pathogenic bacteria and compatative organisms, change the content of microbiota and affect the immune system of the host as demonstrated by Dobson and his colleagues (2012).

In animal models like rodents, pigs, and birds, probiotic bacteriocins can reduce pathogen quantity or change the nature of the target microbiota. In animal trials, Bernbom *et al.* (2006) found that purified nisin, nisin + *Lactococcus lactis* strain CHCC5826, and non-nisin-producing *Lactococcus lactis* strain CHCH2862 all had the ability to influence the configuration of the intestine microbiota. Another attempt was to use bacteriocin-producing S. epidermidis as vital cells to treat a variety of infections, including acne (Yang *et al.*, 2019) and sinusitis, as well as to eradicate MRSA and inhibit the ability of other pathogens to form biofilms (Halliwell *et al.*, 2017;).

The peptides in bacteriocins can allow for producer to enter and/or dominate an already crowded niche (Dobson *et al.,* 2012). They can act as antimicrobials or kill peptides, inhibit rival strains or pathogens, or as signals for peptides that either signal other bacteria in communities of microorganisms or cells signals in the immuno system of the host through quorum sensing (Riley and Wertz., 2002).

Probiotics considered a benefit living organisms that provided the enhancement to the host health when taken in probates amounts,. They are thought to help maintain or improve the beneficial-to-harmful component ratio in the human microbiota (Pineiro and Stanton, 2007, Muslim *et al.*, 2021). Probiotic activity has been reported in a variety of ways, the most common of which is their ability to reinforce the intestinal wall, adjust host immunity, and produce antimicrobials such as bacteriocins (Cotter *et al*., 2005).

Applications of bacteriocin

1- Medical importance

The resistant of bacteria to widely used antibacterial agents has become a growing source of worry. New antibacterial molecules that are not intimidated by current resistance mechanisms are urgently needed to prevent future widespread outbreaks of infectious diseases (Velásquez *et al.,* 2011, Muslim *et al.,* 2017). The use of bacteriocins in various technological applications is largely dependent on their effects. A clear learning for the significance of this action is required to develop novel strategies (Balciunas *et al.*, 2013). The fast rise and spread of multi-resistant infections has underlined the importance of research investigations targeted at finding new ways to combat infection.

Bacteriocins with significant antibacterial activity might thus be used as promising natural antimicrobials in a variety of industrial and food-related applications (Balciunas *et al.*, 2013). Bacteriocins offer a lot of potential in human health applications when compared to current antibiotics. Bacteriocins have several advantages, including minimal toxicity, a high target specific action mechanism, the presence of several forms in nature, and potency at nanomolar doses. Antibiotics are employed in the treatment of high blood pressure, inflammation, and allergies, as well as the treatment of skin infections, herpes, and dental infections. The glycolipids that excreted extracellularly can cause cell differentiation more than proliferation in the cell lines of promyelocytic leukemia (Riley and Wertz, 2002).

2- Industrial application

The benefit of bacteriocin in the maintenance different products is one of the most extensively used bacteriocin applications for various reasons. The first is the lethal action of a number of varieties of bacteriocins in preventing food damage. The second reason is the bacteriocin's heat tolerance, and the third is the protein nature of the bacteriocin; this exposes them to the enzymes that analyze protein in the digestive tract, so they don't cause any side effects like antibiotic-induced allergies. Biosurfactants were used asalternative for chemically manufactured surfactants in the cosmetic industry because of their emulsification ability besides to bind, spread and wet capacities that affect on the viscosity and consistency of product. Also they used as insect repellents, antacids, bath products toothpaste, contact lens solutions, infant goods, lipsticks and teeth cleaners(Ruiz-Larrea, 2010).

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