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ISOLATION OF BACTERIOCIN PRODUCING LACTIC ACID BACTERIA FROM FERMENTED FOODS AND CHARACTERISATION OF BACTERIOCINS

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Abstract

This paper deals with in isolating Lactic acid bacteria from two buttermilk samples(Aavin, Hatsun), one Britannia cheese cube, two Koozh samplpes (one roadside and one home made sample). A bacteriocin screening assay has been carried out to check for bacteriocin production. A zone of inhibition against the indicator organism was obtained for each of the samples indicating presence of bacteriocin. Thus isolation of the Lactic acid bacteria from these foods and the characterization of the bacteriocins in it would have useful applications such as either inoculating these foods or non fermented foods with bacteriocin producing lactic acid bacteria or adding the bacteriocin in its purified or semi purified form or using these foods as an ingredient for the processing of some other food. One such example will be the addition of bacteriocins in Koozh to commercialize this fermented product.

Key Words: bacteriocins, lactic acid bacteria, fermented foods, natural preservatives

Introduction:

Bacteriocins (natural bio-preservatives) are proteinaceous toxins produced by antagonistic microorganisms to inhibit or destroy undesired microorganisms in foods to enhance food safety and extend shelf life. Using bio preservatives in foods compared to chemical additives ensures natural, fresher and minimally processed foods. Fermented foods like buttermilk, curd, cheese, Koozh, which are lactic acid bacteria fermented products have bacteriocins in themselves due to fermentation. Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). Bacteriocins are a heterogeneous group of anti-bacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties. Significantly however, the

inhibitory activity of these substances is confined to Gram-positive bacteria and inhibition of Gram- negatives by these Bacteriocins has not been demonstrated, an observation which can be explained by a detailed analysis and comparison of the composition of Gram-positive and Gram-negative bacterial cell walls .In both types the cytoplasmic membrane which forms the border between the cytoplasm and the external environment, is surrounded by a layer of peptidoglycan which is significantly thinner in Gram-negative bacteria than in Gram- positive bacteria. Gram-negative bacteria possess an additional layer, the so-called outer membrane which is composed of phospholipids, proteins and lipopolysaccharides (LPS), and this membrane is impermeable to most molecules. Nevertheless, the presence of Porins in this layer will allow the free diffusion of molecules with a molecular mass below 600Da. The smallest Bacteriocins produced by lactic acid bacteria are approximately 3kDa and are thus too large to reach their target, the cytoplasmic membrane. However, scientists have demonstrated that Salmonella species and other Gram-negative bacteria become sensitive to Nisin after exposure to treatments that change the permeability barrier properties of the outer membrane

Lactic acid bacteria (LAB) are among the most important groups of microorganisms used in food fermentation where they play an essential role and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable and bakery products (Noopur et al., 2010; Hassanzadazar and Ehsani, 2013). One of the most important contributions of these microorganisms is the extended shelf life of the fermented products. Growth of spoilage and pathogenic bacteria in these foods is inhibited due to competition for nutrients and the presence of starter derived inhibitors such as lactic acid, hydrogen peroxide, diacetyl and bacteriocins (Noopur et al., 2010; Noordiana et al., 2013).

Bacteriocins are extracellularly produced primary compounds of bacterial ribosomal synthesis which have a relatively narrow spectrum of bactericidal activity (Caplice and Fitzgerald 1999). They are active against other bacteria despite varying greatly in the chemical nature and mode of action. Bacteriocins have important advantage over the classical antibiotics in being easily degraded by the digestive enzymes without the risk of disruption of normal tract ecology (Caplice and Fitzgerald 1999). Bacteriocin producing LAB have the ‘generally recognized as safe’ (GRAS) status and have been shown to strengthen the barrier function of the gut microflora as well as promote the non-specific enhancement of the immune system of man and animals (Tome et al. 2008). Equally, research on the biochemical changes during the fermentation as well as the proximate composition and properties of the seeds have also received modest scientific attention (Sanni and Onilude 1997; Achi and Okereka 1999; Aremu et al. 2007 and Odibo et al. 2008).

There is presently, paucity of scientific information on the ecological contribution of the LAB and bacteriocins for the safety and biopreservation of the food condiments. Bacteriocins are antimicrobial peptides or proteins produced by strains of diverse bacterial species. The antimicrobial activity of this group of natural substances against foodborne pathogens, as well as spoilage bacteria, has raised considerable interest for their application in food preservation (Noopur et al., 2010; Gong et al., 2010; Ana, 2012). In the past years, a lot of work has aimed to detect, purify and characterize bacteriocins, as well as their application in food preservation strategies. Application of bacteriocins may help reduce the use of chemical preservatives and/or the intensity of heat and other physical treatments, satisfying the demands of consumers for foods that are fresh tasting, ready to eat, and lightly preserved. In recent years, considerable effort has been made to develop food applications for many different bacteriocins using bacteriocinogenic strains (Ana, 2012; Adenike et al., 2007). Iman et al. (2014) focused in their study on the isolation and characterization of bacteriocin producing local lactic acid bacteria isolates, beside the activity of these strains against several spoilage and pathogenic bacteria, choosing the best isolate which which has the best antibacterial activity.

Several factors that are responsible for the inhibition of harmful bacteria from multiplying on and attaching to the intestinal epithelium include bacteriocins and organic acids (antimicrobial agents) production and secretion [Yusuf, 2013], adherence via competition for the binding sites and steric hindrance [Yusuf, 2013]. pH reduction as a result of lactic acid production from sugar fermentation process is one of the major factor for the prevention of the proliferation of this undesirable microorganisms [Yusuf, 2013]. Lactic Acid Bacteria (LAB) reduce the pH in food to such an extent that it become unfavourable for the growth of other microorganisms including pathogenic microbes common to humans which helps to lengthen the shelf-life of the food. The production of lactic acid from the fermentation processes of LAB lead to the resultant pH reduction resulting in the liposolubility of organic acids thereby enhancing the ease with which the LAB penetrate the cell membrane and gain entrance into the pathogen cytoplasm. Other contributing factors include the LAB competing for required nutrients. Fig. 1 shows typical mode of action bacteriocins [Yusuf, 2013; Breukink, 2006].

The bacteriocins produced by LAB offer several desirable properties that make them suitable for food preservation: (i) are generally recognized as safe substances, (ii) are not active and nontoxic on eukaryotic cells, (iii) are readily inactivated by the action of digestive proteases, with slight impact on the microorganism inhabiting the gut, (iv) they

can tolerate a wide range of pH and temperature, (v) they have a fairly broad antimicrobial spectrum, against many food-borne pathogen of food origin and food spoiling bacteria, (vi) they exhibit bactericidal action on the target host, typically acting on the bacterial cytoplasmic membrane: no cross resistance with antibiotics, and (vii) their genetic determinants are usually plasmid-encoded, facilitating genetic manipulation.

From wide literature review, it is observed that lactic acid bacteria appear to be a major producer of bioactive peptides known to be inhibitory to other closely related microbes. Though, they are known to have some detrimental properties, the advantages over weight these disadvantages. Most of all they are known to impact little or no side effects on the hosts. Amongst all bacteria, LAB happens to be the highest producer of bacteriocins and enterococci spp leads them all despite its ambivalent nature. Lactic acid bacteria and its bacteriocins have also found diverse usage in human, livestock and poultry.

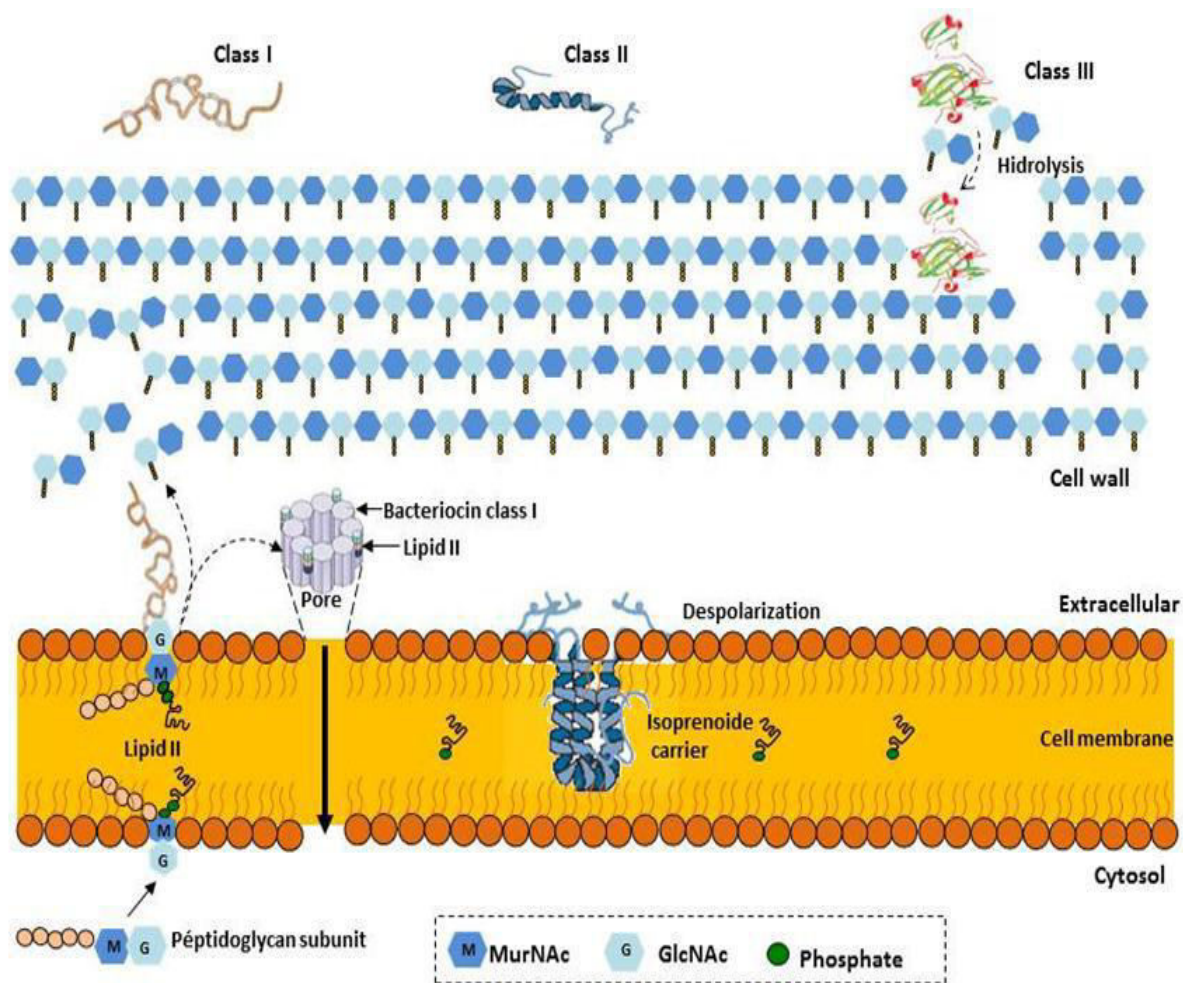


Fig. 1: Lactic acid bacteria bacteriocin mode of action. [Yusuf, 2013; Breukink, 2006].

The primary objective of this include (i) Isolation and partial characterization of Lactic acid bacteria from some fermented food samples and (ii) Bacteriocin screening

Materials and Media:

The following materials and media were used for this study.

- Two buttermilk samples (Aavin, Hatsun), one Britannia cheese cube, two Koozh samples (one roadside and one home made sample).
- Autoclaved Petri plates, test tubes, sterile pipette-man tips 30 Peptone water
- Elliker's Broth
- MRS agar
- Soft agar (1% nutrient agar)
- *Micrococcus luteus* (indicator organism)

Isolation and Partial Characterisation of Lactic Acid Bacteria

A small portion of the food sample was aseptically inoculated in 2.5ml of Elliker's broth in a test tube.

It was then incubated in a shaker bath at 37°C at 110 rpm for 24hrs.

- Serial 10 fold dilutions (100-10⁵) were plated on MRS agar medium (selective medium for LAB).
- Plates were incubated at 37°C for 24hrs.
- Selected colonies with different morphologies were purified on MRS agar.
- Gram positive, Catalase negative and Oxidase negative non-motile cells were presumptively identified as LAB.
- The bacteria were inoculated in 3ml Elliker's Broth.
- The test tubes were incubated at 37°C for 24hrs.

Bacteriocin Screening Assay

Horizontal Agar diffiision assay (Tramer and Fowler, 1964) using *Micrococcus luteus* as indicator organism:

- Approx 10⁵ cells of the indicator strain (*Micrococcus luteus*) were inoculated into the appropriate soft agar medium (1% nutrient agar) and poured into empty plates.
- The plates were pre-incubated for 1 hr. at 4°C for proper setting.

- Wells of 8mm diameter were cut and ISOul of the culture supernatant fluid of the potential Bacteriocin producing strain placed into each well.
- The plates were then incubated at 30°C for 24-36 hrs.
- At the end of the incubation period the diameter of the inhibition zones were measured.
- The supernatant fluids were obtained by centrifuging the cultures at 10,000 rpm for 15 mins.

The following is the result that was obtained from the study.

RESULT:	
FOOD SAMPLE	DIAMETER OF ZONE OF INHIBITION mm
AAVIN BUTTERMILK	21.6
HATSUN BUTTERMILK	20.9
BRITTANIA CHEESE CUBE	22
Raggi	22.1
KOOZH HOME	22.3

Discussion:

The zone of inhibition produced against the indicator organism confirms the isolation of the Bacteriocin producing lactic acid bacteria. Characterization of the Bacteriocins is being carried out.

Summary and Conclusion:

The biopreservation of foods using bacteriocinogenic lactic acid bacteria (LAB) isolated directly from foods is an innovative approach. The primary objective of the present investigation is to (i) Isolation and partial characterization of Lactic acid bacteria from some fermented food samples and (ii) Bacteriocin screening. Characterization of the Bacteriocins is being carried out. Further investigations into the mechanisms of inhibition and determination of the optimal growth conditions for LAB to produce bacteriocin-like substances (BLS) are necessary. Moreover, more studies are required to envisage the effectiveness of LAB and BLS on other food products.

Once the characterization of the Bacteriocins is over, it is possible to find useful applications such as either inoculating these fermented foods or nonfermented foods with bacteriocin producing lactic acid bacteria or adding the Bacteriocin in its purified or semi purified form or using these fermented food as an ingredient for the processing of some food.

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