



**SCREENING AND CHARACTERIZATION OF BACTERIOCIN
PRODUCING HETEROLACTIC STRAINS OF BACTERIA FROM
NATIVE FOOD SOURCES**

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ABSTRACT

The use of bacteriocinogenic lactic acid bacteria (LAB) isolated directly from foods for food bio preservation is a novel approach. LABs belong to wide group of Gram-positive, non-spore forming, cocci, rods are usually non motile and are capable to ferment carbohydrates forming lactic acid as a product which is also known as probiotic bacteria. They are generally regarded as safe (GRAS) by Food and Drug Administration (FDA) and qualified presumption of safety (QPS) that has been published by European Food Safety Authority (EFSA) for their use as a starter Cultures and probiotics. In the absence of antibiotics, organisms with stable hetero resistance can maintain their resistance, whereas those with unstable hetero resistance can revert again. Bacterial hetero resistance is caused by genetically unreliable amplification of Tandem genes or a physiological mechanism. To know more about the shelf life of Heterolactic strains that are isolated and tested for Bacteriocin (Nisin A) properties. The goal is to raise awareness about the spread of antagonistic property through the food chain and the proper use of food source across the board.

Keywords: Lactic acid bacteria (LAB), Bacteriocin, Antagonistic activity, Nisin etc.

1. INTRODUCTION:

Lactic acid bacteria (LAB) are Gram positive cocci or rod shape bacteria, they play major role in fermentation process. Lactic acid is a product in the fermentation

process of glucose. The main large group of LAB are *Lactobacillus*, *Enterococcus*, *Leuconostoc*, *Pediococcus* and *Bifidobacterium*.

Most of these group of LAB are facultative anaerobe, non-spore forming, non- motile organisms. LAB are widely distributed in human intestine, Gut, Gastrointestinal tract and in some animals [1, 2]. These LABs are also considered as safe since they have probiotic components and helps to keep healthy life [3]. They are the part of healthy normal microbiota, some of them plays

important role in preventing food spoilage and controls pathogenic microorganisms [4].

LABS are divided into two main groups, namely:

- a) Homofermentative Lactic acid bacteria (LAB)
- b) Heterofermentative Lactic acid bacteria (LAB). They are classified based on their Hexose fermentation pathways. Hexose means glucose and/ or Fructose.

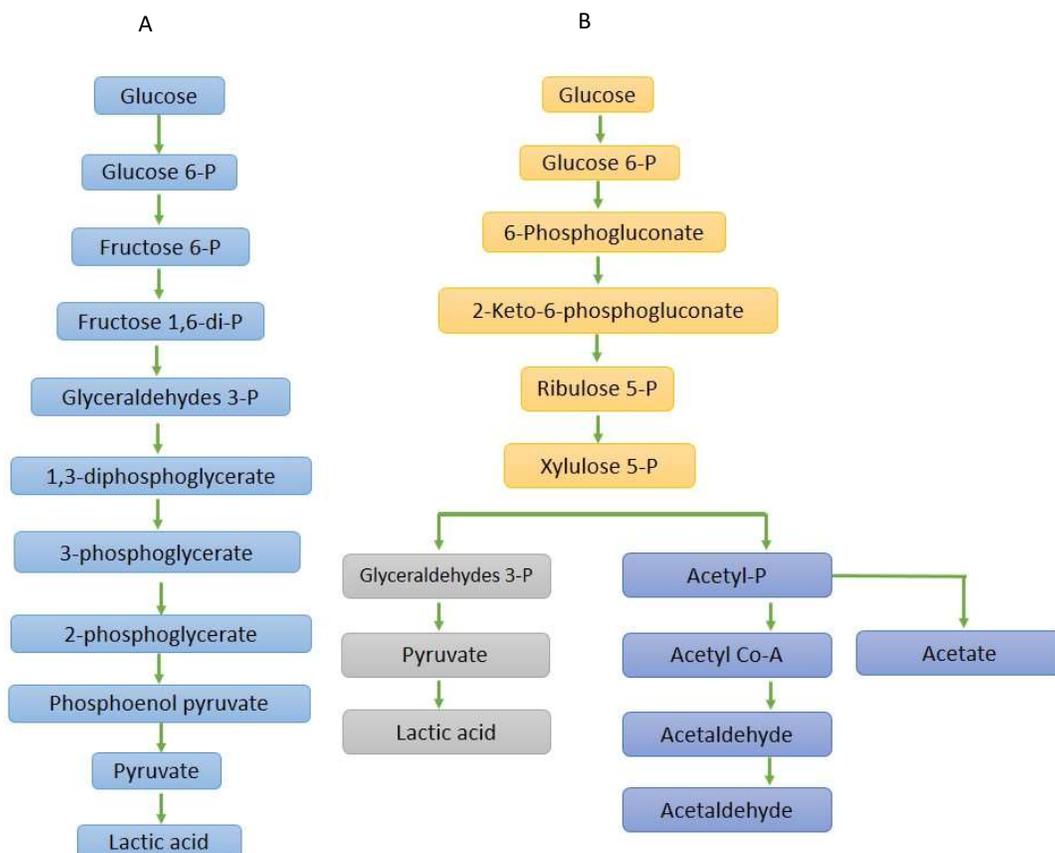


Figure 1: a) Homofermentation of Lactic Acid Bacteria, b) Heterofermentation of Lactic acid bacteria (Ravinder Kumar et al., 2015)

a) Homofermentative Lactic acid bacteria:

The Homo fermentation is the degradation of Hexose that occurs through glycolysis pathway that is carried by LAB with Lactic acid as the major product [5, 6, 7] (Figure 1, a).

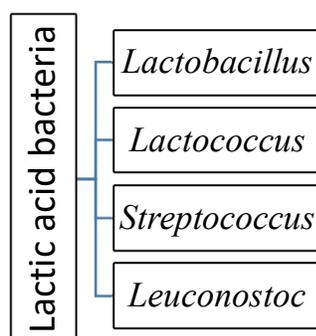
b) Heterofermentative Lactic acid bacteria: (Figure 1, b)

In Heterofermentation, LAB uses the Pentose- Phosphate pathway and gives end products such as Lactic acid as well as

Carbon dioxide, Acetic acid and/ or Ethanol [5, 6, 7]. Hetero fermentative LAB possess various benefits when compared to Homo fermentative LAB.

Heterolactic strains of Lactic acid bacteria (LAB): Heterofermentative lactic acid bacteria are an important organism which acts as a natural fermentative substance in Lactic acid fermentation [8].

3) List of Heterolactic strains of Lactic acid bacteria:



3.1) *Lactobacillus*:

The genus *Lactobacillus* is a large number among all the other species. They are thermophilic group of LAB starter cultures. There are around 50 species of *Lactobacillus* [11]. Main strains of *Lactobacillus* are:

3.1.1) *Lactobacillus delbruecki*:

It has 3 sub species, namely; a) *Lactobacillus delbruecki*, b) *Lactobacillus lactis*, c) *Lactobacillus bulgaricus* [11].

3.1.2) *Lactobacillus plantarum*:

The largest genome among all the LAB [12, 13]. Predominant in sour dough and cereals [14]. In recent studies it has been showed the antimicrobial and antifungal activities [15].

3.1.3) *Lactobacillus sanfranciscensis*:

It has 14 genomes [11]. It is mainly predominant in sour dough [14].

3.1.4) *Lactobacillus rhamnosces*:

Used mainly as a probiotic organism in food products. Also helps in cheese ripening [16, 17].

3.1.5) *Lactobacillus helveticus*:

It is quite closely related to *Lb. acidophilus*, *Lb. aerotolerance*, *Lb. amylophilus* [18].

3.1.6) *Lactobacillus fermentum*:

This type of strain of LAB mainly found in vegetables and cereal based fermented foods. Used as a probiotic [19].

3.2) *Lactococcus*:

Lactococcus are mesophilic LAB. These types of strains usually isolated from the green plants. One of the main species among *Lactococcus* are *Lactococcus lactis* used as starter culture [11].

3.3) *Streptococcus*:

Streptococcus thermophilus has been used as starter culture and it is second most important starter culture. Used in production of fermented dairy products like yoghurt, milks, feta, and Mozzarella cheese [14].

3.4) *Leuconostoc*:

Leuconostoc mesenteroides is a facultative anaerobe. It has a facultative growth factor [21]. Morphology will be varied according to growth condition [11].

4) Lactic acid bacteria as a probiotic:

Probiotics are the live supplement or organisms that can be consumed to improve the health benefits. These probiotics improve the immunity [22]. LABs are useful probiotics which has several health benefits for humans as well as animals. Role of probiotics that Prevents diarrhoea, Treats ulcers, stimulates immunity, in food preservation, Antifungal activity, Prevents infectious diseases, Production of dairy products (An interpretive review of recent nutrition research 1957).

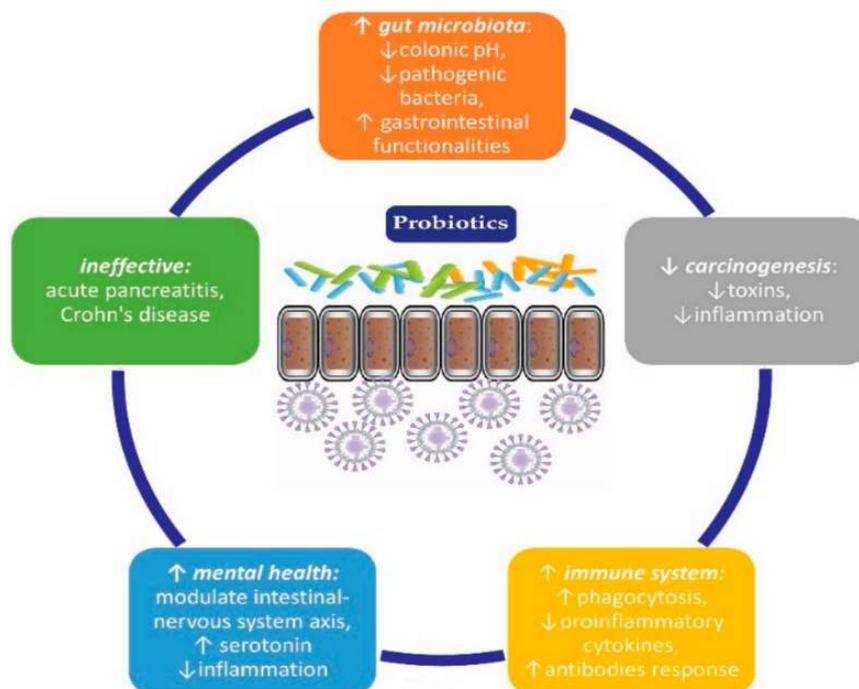


Figure 2: The most important beneficial properties of probiotics in promoting human health (Kechagia, M, et al., 2013)

5) The antimicrobial substances:

The substance viz, Acetic acid, Carbon dioxide, Hydrogen peroxide, Diacetyl, Bacteriocins, Bacteriocins like inhibitory substances, Acetoin, Ethanol, Ammonia etc., [24, 25] that tend to produce antagonistic activity against other pathogens. This antimicrobial activity of LAB is the result of competition with pathogenic organisms. Among the above substances bacteriocins tends to get more attractions and has enormous antimicrobial activity produced in it. Bacteriocin is one of the major substances among all.

6) Bacteriocin:

Bacteriocins are antimicrobial peptides of proteins. They have antimicrobial activity against the closely related microorganisms. They represent has bio preservatives [24-27]. Bacteriocins are produced by both Gram- positive and/ Or Gram-negative bacteria [28]. In the current years, evaluation of bacteriocin producing LAB are considered as safe because of their GRAS status. And these types of LAB can be used as a food additive to preserve food and as probiotics [29].

Table 1: Most important bacteriocins produced by LAB [31-33, 36, 37]

Bacteriocin	Bacteriocin producing strain
Lactacin F	<i>L. johnsonii</i> spp
Lactocin 705	<i>L. casei</i> spp
Lactococcin MN	<i>Lactococcus lactis</i> var <i>cremoris</i>
Nisin	<i>Lactococcus lactis</i> spp
Leucocin H	<i>Leuconostoc</i> spp

6.1) Classification of Lactic acid bacteria (LAB) bacteriocins:

Most of the LAB bacteriocins are smaller in size (<10 kDa). They are membrane permeabilizing peptides and heat- stable. It is divided into 3 major classes [30, 31]. Most of the bacteriocins exhibit small amount of adsorption specificity. LAB bacteriocins shows high antibacterial activity at low Ph [32].

Class 1: The Lantibiotics:

They are class of peptide substances which contains characteristics of polycyclic thioethers amino acids, lanthionine or

methyllanthionine and unsaturated amino acids. Class 1 is further is divided into two types: Type A has elongated, screw shaped, positively charged flexible molecules. Nisin and Lactacin 3147 are the major types of this class 1. Molecular mass of this is 2-4 kDa. Type B Lantibiotics are globular in structure and interfere with cellular enzymatic reaction. Molecular mass of this type varies between 2-3 kDa [33, 34]

Class 2: The non- Lantibiotics:

The class of non- Lantibiotics are also small (<10 kDa). Divided into 2 subclasses. Subclass 2. A) pediocin like or listeria

active bacteriocins [35]. Subclass 2. B) It requires two peptides to work simultaneously to achieve an antimicrobial activity. Lactacin F and Lactococcin G are the major types [36].

Class 3: Bacteriocins:

This class has high molecular weight compared to other classes (>30 kDa) [37-40].

7) Nisin:

It is one of the most abundant Bacteriocin that is present in the LAB, especially in *Lactococcus* and *Streptococcus* species [38, 39]. Nisin was first identified in fermented milk in the year 1928 [40, 41]. Nisin plays a significant role in the food industry, it can be used as a bio preservative to store different types of food and food products [39]. In the year 1988 Nisin was approved as safe by Food and drug administration (FDA), United States (US) [42]. Nisin A is one of the originally

described Variant of Nisin. This Nisin A is produced by LAB i.e., *Lactococcus lactis* [43]. Nisin belongs to Class- 1, Lantibiotics [44]. Nisin has several beneficial properties in the field of biomedical applications and treating bacterial infections and diseases such as cancer and oral disease etc.

Nisin Solubility, stability and biological activity depend on the factors such as pH, temperature, and nature of the substrate. The solubility and stability of Nisin increases in high acidity. But in neutral or in alkaline conditions, Nisin will be insoluble [45]. Nisin has antimicrobial effect on Gram positive Bacteria and spore germination but they are inactive against Gram negative Bacteria, fungi, or viruses [46]. Nisin has shown high antibacterial activity against pathogenic bacteria such as, *Listeria monocytogenes*, *Staphylococcus aureus*, *Micrococcus luteus*, *Micrococcus flavus* [37, 47, 48].

Table 2: Types of Nisin and their target pathogens

Type of Nisin	Origin	Target pathogens
Nisin A	<i>Lactococcus lactis</i> strains	<i>Micrococcus flavus</i> DSM1719, <i>Streptococcus thermophilus</i> Rs, <i>Clostridium trybutaricum</i> Bz 15, <i>Bacillus cereus</i> P7 & C5, <i>Listeria monocytogenes</i> 13.
Nisin Z	<i>Lactococcus lactis</i> NIZO	<i>Micrococcus luteus</i> IFO12708, <i>Bacillus circulans</i> JCM2504
Nisin F	<i>Lactococcus lactis</i> subsp. <i>lactis</i> F10	Clinical <i>Staphylococcus aureus</i> (hVISA and MRSA)
Nisin Q	<i>Lactococcus lactis</i> 61- 14	<i>Enterococcus faecalis</i> , <i>Lactobacillus plantarum</i>
Nisin U	<i>Streptococcus uberis</i>	
Nisin P	<i>Streptococcus galloyticus</i> subsp. <i>pasteurianus</i>	<i>Streptococcus agalactiae</i> DPC7040 (Human faecal isolate)

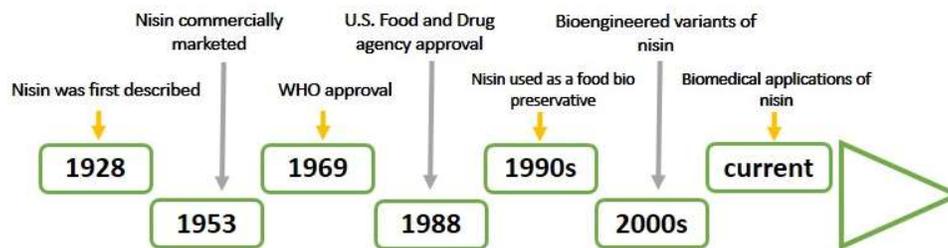


Figure 3: Development of Nisin over the years Jae M shin *et al.*, 2015

CONCLUSION:

Recent studies have shown that LAB, being a probiotic bacterium, it has several importance in the field of food microbiology and to enhance the immunity in human beings as well as other living beings like cattle, poultry, pigs, and aquatic organisms etc.,. These Heterolactic strains of LAB also act as an alternative antibiotic, since they contain antimicrobial substance called Nisin, these Nisin are naturally present in traditional foods like, Appam, Dosa, Pickle, Idli, etc., and in some vegetables like Tomato, capsicum, chilly etc. If the practice of consuming these traditional foods, their nutritional value gets increases and helps to fight against other pathogenic microorganisms and controls from infection and/ or diseases. Nisin can also be used for biomedical purposes and as a bio preservative. These nisin are also commercially available. The food sources which contain high amount of nisin is consider as a nutritional food, since it gives protects from getting affected from

foodborne pathogens. To keep the shelf life of food and food products many scientist and researchers came up with an idea with LAB, and considered them as a safe organisms. LAB and the bacteriocins that are produced are emerged as viable sources in industrial field. Furthermore, more research is needed to determine the efficacy of LAB on various food products.

Conflict of Interest

The authors declare no conflict of interest.

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