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ARTICLE ID: 20

Application of Bacteriocins for Biopreservation of Traditional Dairy Foods

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INTRODUCTION

The food industry is under continues consumer pressure to produce fresh, unprocessed (or minimally processed) natural, healthy and safe food. Bio preservation is the best preservation method than chemical preservation (i.e. use of nitrates & propionates chemicals for preservation) & physical preservation (i.e. sterilization, pasteurization etc.). Bio preservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life. They reduces the amounts of chemical preservatives as well as the intensity of heat treatments, both of which can otherwise negatively affect the food quality. In bio preservation, most microorganisms are capable of producing a large variety of molecules. Beneficial bacteria or the fermentation products produced by these bacteria are used in bio preservation to control spoilage and render pathogens inactive in food. These molecules may be inhibitory either to the producing cell or to other bacteria and include Organic acids, toxins, antibiotics and bacteriocins etc. Lactic acid bacteria (LAB) play an essential role in the majority of food fermentation. Growth of spoilage & pathogenic bacteria in these foods is inhibited due to competition for inhibitors such as lactic acid, hydrogen peroxide and bacteriocins.

Bacteriocins:

Bacteriocins are ribosomally synthesized polypeptides possessing bactericidal activity that are rapidly digested by proteases in human digestive tract. Antimicrobial peptides (AMPs) or protein produced by bacteria are categorized as a bacteriocins. Bacteriocins differ from most therapeutic antibiotics in being proteinaceous and generally possess a

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narrow specificity of action. (Tagg et al., 1976) Bacteriocins are bactericidal antibiotic like substance. In nature it is protein produced by many bacteria. It reduces the growth of bacterial strains that are similar or closely associated.

Classification of Bacteriocins of LAB

Main category	Characteristics	Subcategory	Examples
Class I	**Lantibiotics** **Smaller molecular weight of (<5 kDa)**	**Type A** **Type B**	Nisin Mersacidin
Class II	Nonmodified heat-stable bacteriocins containing **peptides with molecular masses of 10 kDa**	**Subclass IIa** **Subclass IIb**	Pediocin AcH Enterocin FH99 Pediocin 34 Lactacin F and Lactococcin G
Class III	**Protein bacteriocins** **with molecular masses** **of 30 kDa**		Helveticin J Lactacins A and B
Class IV	**Bacteriocins that form large complexes with other macromolecules**		Leuconosin S Lactococcin 27

Factors promoting use of bacteriocins as bio preservatives

- Do not alter acceptance quality of food and are safe for human consumption.
- Effective under wide pH & temperature range
- Activity is not lost in the presence of food additives and effective in dairy Foods during storage
- Effective in low concentrations advent of novel bacteriocins with broad spectrum of activity from food grade LAB

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- Consumer resistance to traditional chemical preservatives and concern over the safety of existing food preservatives such as sulfites and nitrites
- Safe and efficacious use of nisin for > 40 years in several countries

The important bacteriocins are:-

1) Nisin

Nisin is produced by certain strains of lactic acid bacterium – *Lactococcus lactis* that functions by interacting with the phospholipids in the cytoplasmic membrane of bacteria, thus disrupting membrane function. Nisin is used in over 50 countries, has food & drug administration approval & Nisaplin is sold as a natural food protectant. Nisin has been shown to be effective in a number of food systems, inhibiting the growth of a wide range of gram positive bateria, including many important food borne pathogens such as *Listeria monocytogenes* (Tagg et al., *1976*). It is used predominantly in canned foods, dairy products & is especially effective when utilized in the production of processed cheese & cheese spreads where it protects against heat-resistant spore forming organisms such as those belonging to the genera *Bacillus* and *Clostridium.*

The level of nisin used depends on:

- Food composition
- Likely spores
- Required shelf life
- Temperatures likely to be encountered during storage

2) **Pediocin**

Pediocin is produced by *Pediococcus acidilactici.* Generally Recognized As Safe (GRAS) organism and used in fermented sausage production. Most pediocins are thermostable proteins & function under a wide range of pH (Rodriguez *et al.*, 2002) Pediocin has been proven to be effective against both spoilage & pathogenic organisms, including *Lactobacillus monocytogenes, Enterococcus faecalis and Staphylococcus aureus*. (Bhunia *et al.*, 1988). A natural antimicrobial called Inovapure is said to be effective against a wide range of food spoilage organisms and can be successfully used to extend the shelf life of various food products, including raw & processed meats, cheese, and other dairy products.

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Difference between Bacteriocin and Antibiotics

	Bacteriocin	Antibiotics
1.	Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth bacterial strain.	An antibiotics is an agent that either kills or inhibits the growth of microorganisms.
2.	Bacteriocins are kill the bacteria of same or closely related species.	Antibiotics are harmful to the growth of variety of microorganisms.
3.	Bacteriocins are the primary metabolites.	Antibiotics are the secondary metabolites.
4.	Bacteriocins producing strain usually show immunity.	Antibiotics do not show immunity.

Isolation of Bacteriocin

Fermented milk was directly submitted to isolation of lactic acid bacterial strains, while cheese is serially diluted in saline solution and then plated on MRS agar. The plates were incubated aerobically at 37°C for 48 h and then several colonies were picked randomly for identification of lactic acid bacteria. The isolated colonies were further sub cultured and transferred to MRS slants incubated at 37°C for 48 h and preserved at 4°C for further work. Optimum temperature for growth of *L. lactis: Lactococcus lactis* culture was inoculated in 100 ml MRS broth at 100 rpm in a shaking incubator at different temperatures ranging from 30, 35, 37 and 40°C. Samples were takenevery hour for the first 12 h and then at 14, 16, 20 and 24 h for enumeration of LAB in MRS agar and incubated at 37°Cfor 24-36 h.

Effect of Bacteriocin in Dairy Product

Product	Nisin Conc.	Observation
Milk	100 mg/ml	Shelf life extension 2-12 days at 25°C
Cheese	500 IU/gm	Inhibition the growth of nonstarter LAB for one month
Yoghurt	25 IU/gm	Enhance shelf life of 10 days at 7°C
Dahi	1000 IU/gm	Effective in controlling lipolysis in dahi during storage
Kheer	200 IU/gm	Enhancing shelf life for six month

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Role of bacteriocins in food preservation

- There is a continuous raise of awareness in the public regarding the amount of chemical intake by their body as food preservative.
- In the view of the above problem, bio-preservatives are in very high commercial demand at present, in the protective cultures of their metabolites i.e. enzymes and bacteriocins.
- As there is an increase in demand for natural, minimal processed, microbiologically safe products, bacteriocins provide the consumer with high health benefit.
- The bacteriocins from food grade Lactic acid bacteria (LAB) qualify as an ideal food bio preservative primarily because;
    1. It is proven non-toxic to humans
    2. Does not alter the nutritional properties of the food product
    3. Effect at low concentration
    4. Active under refrigerated storage
    5. It is degraded immediately during digestion
- There are at least 3 ways, in which bacteriocins can be incorporated into a food to improve its safety i.e.,
    1. Using a purified bacteriocin preparation as an ingredient in food,
    2. By incorporating an ingrent previously fermented with a bacteriocin-producing strain, or
    3. By using a bacteriocin-producing culture to replace all part of starter culture in fermented foods to produce the bacteriocin *in situ.*

Advantages:

- Safer preservatives than chemical.
- More accurate efficacy.
- Good acceptance from consumer.
- To overcome various drawbacks such as toxicity of chemicals and nutritional alteration of food.

CONCLUSION

Nisin is only bacteriocin biopreservative that has received acceptance in countries worldwide. It would be naive to believe that bacteriocins represent the ultimate solution to

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food safety problems. The effectiveness of bacteriocins as a incorporated & a consumer desire for minimally processed food. Continued research on bacteriocins will undoubtedly lead to our increased understanding & with the emergence of new bacteriocins, new potential bio-preservatives.

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