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Bacteriocin production using lactic acid bacteria

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ABSTRACT

Bacteriocins, which are protenacious compounds produced by Lactic acid bacteria. In the present study the lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus pyogenes* were isolated from the curd sample. The amount of protein was also estimated and bactericidal activity was studied. The nisin extracted from the isolates can inhibit the organisms such as *Alcaligen faecalis, Bacillus subtilis, Pseudomonas aeroginosa, Serratia marcescenes* and *Staphylococcus aureus.* Compared to *Streptococcus pyogenes* the immobilized form of *Lactobacillus bulgaricus* effectively to kill the bacterial pathogens. The nisin was also employed to preserve the grapes in this study. The *Lactobacillus bulgaricus* producing nisin enhanced the self life of grapes up to 10 days, and the gapes without nisin were spoiled at 2nd day itself. In this study, it was concluded that the nisin obtained from *Lactobacillus bulgaricus* was able to inhibit the growth of some pathogens that are involved in food spoilage. So we can use nisin as a bioperservative for foods.

Key words: Bacterriocin, nisin, biopreservation, grapes.

INTRODUCTION

Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics (Wood BJB and Holzapfel, 1995). They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. In addition, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance. Whereas a food fermentation process with LAB is traditionally based on spontaneous fermentation or back slopping, industrial food fermentation is nowadays performed by the deliberate addition of LAB as starter cultures to the food matrix. This has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end products. Recently, the use of functional starter cultures, a novel generation of starter cultures that offers functionalities beyond acidification, is being explored (De Vuyst *et al.,*2004) . For instance, LAB is capable of inhibiting various microorganisms in a food environment and display crucial antimicrobial properties with respect to food preservation and safety. In addition, it has been shown that some strains of LAB possess interesting health-promoting properties; one of the characteristics of these probiotics is the potential to combat gastrointestinal pathogenic bacteria such as *Helicobacter pylori* , *Escherichia coli* , and *Salmonella* etc.,(Ashraf *et al.,* 2005 and Ma *et al.,* 2006). Nisin is a permitted food additive in more than 50 countries including in US Europe under the trade name of Nisaplin (Soomro *et al.,* 2002). In this paper we focused on nisin, a bacteriocin produced by *Lactobacillus bulgaricus* and *Streptococcus pyogenes*. We also report the effects of the components of nisin production media and their role in preservation of grapes.

MATERIAL AND METHODS

Isolation of lactic acid bacteria

The culture of *Lactobacillus bulgaricus* and *Streptococcus pyogenes* used for the present study had been isolated from curd sample using skimmed milk agar. These isolates were purified by sub culturing them on brain heart infusion agar and the isolates were identified by Gram staining and Biochemical tests like catalase, oxidase test and casein hydrolysis test (Corroler *et al.,* 1998). The motility of the organisms was observed by wet mount method.

Immobilization of lactic acid bacteria

The culture of *Lactobacillus bulgaricus* and *Streptococcus pyogenes* was grown in MRS broth to immobilize the cells. After this the cells were immobilized using 6% sodium alginate and 4% of calcium chloride solution. Then to produce the bacteriocin using both immobilized and wild type of bacterial strains.

Production of bacteriocin

The immobilized and wild type bacterial strains were grown in the medium contains fat free skimmed milk and limed milk filtrate separately. After incubation the cells were killed at 75°C for 15 minutes and pH of the medium was adjusted to 2.5, to extract the bacteriocin from the cells. The culture was centrifuged at 3000 rpm for 20 minutes and the supernatant was subjected to salt precipitation followed by dialysis for partial precipitation. After this the partially purified protein was estimated by Lowery's method (Lowery *et al.,* 1951; Yang *et al.,* 1999).

Antibacterial activity of bacteriocin

The partially purified both wild and immobilized protein was subjected to the bactericidal activity against common pathogens involved in food spoilage using agar disc diffusion method (Bruno, 1993). The organisms used in this study was *Alcalingen faecalis, Bacillus subtilis, Pseudomonas aeroginosa, Serratia marcescens,* and *Staphylococcus aureus.*

Biopreservation of grapes using bacteriocin

The grapes were taken aseptically in polythene bag separately and both wild and immobilized *Lactobacillus bulgaricus* and *Streptococcus pyogenes* nisin 5 mg/L was added to the grapes. Then the grapes were stored for 10 days with control that is without the addition of nisin and stored in refrigerator grapes also. All these samples were incubated at 25ºC and examined at appropriate intervals for bacterial load.

RESULTS AND DISCUSSION

In the present study *Lactobacillus bulgaricus* and *Streptococcus pyogenes* was isolated from curd sample. The cells were Gram positive rod and cocci and arranged in pairs. They were non facultative anaerobic and non motile organisms. They formed small round, convex, transparent and pale white colonies. The cells were immobilized and then killed to extract the bacterriocin from the cells. The extracted bacteriocin was partially purified and then estimated by Lowery's methods. Compared to *Streptococus pyogenes* the immobilized from of *Lactobacillus bulgaricus* contain high protein content (410 mug/ml).

In this study, bactericidal activity of nisin was determined against some bacterial pathogens and result was showed in Table 2. Compared to

Table 1: Activity of nisin against bacterial pathogens

S.No	Bacteria	Inhibition of zone (mm)	Result
1	*Alcaligen faecalis*	12	Intermediate
2	*Bacillus subtilis*	25	Sensitive
3	*Pseudomonas aeroginosa*	Pigment lysis	Resistant
4	*Serratia marcescens*	18	Sensitive
5	*Staphylococcus aureus*	23	Sensitive

wild type strains the both immobilized type of bacterial cells producing bacteriocin effectively to kill the bacterial food borne pathogens. The immobilized bacteriocin of *Lactobacillus bulgaricus* effectively to kill the *Alcaligen faecalis* (12 mm) and *Staphylococcus aureus* (23 mm) respectively. In case of Gram positive bacteria, it forms pores on the cytoplasm membrane through that pores the cell contents are released out which results in cell death. In Gram negative bacteria, the bacteriocin showed only intermediate activity, because the gram negative bacteria need some sub lethal stresses to destabilize the membrane vesicles.

Biopreservative activity of bacteriocin

Nisin Z, carnocin U149 and crude bavaricin A have potential in extension of the shelf – life of brined shrimp, salmon and other sea foods like fish and rainbow trout (Chen and Hoover, 2003). He proved that these products were showed good result when inoculated with nisin producing Lactococci (Michael, 1996). Nisin at a level of 5 mg/L makes a significant increase in refrigerated shelf – life of grapes from between 5 to 10 days. In our study nisin used at level of 100 mu g in 20 ml to the grapes enhances the shelf life of grapes up to 10 days but the grapes without nisin were spoiled at

Table 2: Changes occur during preservation of grapes without nisin

Days	Aerobic CFU/ml	Anaerobic CFU/ml	pH	Appearance	Odour
1	17×10^2	4.5×10^2	7.6	Good	Nil
4	4.4×10^2	5.8×10^2	7.6	Good	Nil
7	5×10^2	3.5×10^2	7	Loss of colour	Slight
10	7.8×10^2	4.8×10^2	6.95	Complete loss of colour	Strong

Table 3: Changes occur during preservation of grapes with nisin

Days	Aerobic CFU/ml	Anaerobic CFU/ml	pH	Appearance	Odour
1	2	2	7.9	Good	Nil
4	6	4	7.7	Good	Nil
7	2.1×10^1	8	7.6	Good	Nil
10	3.1×10^1	13	7.4	Good	Nil

5th day itself. Spoilage of control was associated with strong odor, loss of color and a small drop in Ph. It was noted that the control spoiled mainly due to the growth of *Pseudomonas aeroginosa* and *Bacillus cereus* (Table 2)

CONCLUSIONS

From the present work it is concluded that the nisin can be used as a bioperservative for several food materials. It will be marketed as a safe and natural preservative instead of chemical food additives.

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