A Study of the Characteristics of Lactobacillus Plantarum Isolated from Sausage in Iran

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Abstract

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Introduction: Lactic acid bacteria are widely used for the fermentation and preservation of dairy and meat products and to improve their aroma and texture. The aim of this study was to screen Lactobacillus plantarum isolated from sausage for detection of plasmids, protein bands and phages, to find possible linkage of bacteriocin production to genetic location.

Material and Methods: Two Lactobacillus plantarum with antibacterial activity were isolated from sausage. Bacterial plasmids were isolated by alkali lysis and electrophoresis through agarose gel. Proteins were precipitated from cell-free supernatants by ammonium sulphate and analysed by SDS-PAGE. For detection of phages, mitomycin C of final concentration of 2.5 μg/ml was used and phages were detected by transmission electron microscopy.

Results: One plasmid of about 4.5 kbp was detected in one Lactobacillus plantarum strain. Two bands of proteins were found on SDS-PAGE. The molecular weight of protein bands of Lacto. plantarum without plasmid was higher than the protein bands of Lacto. plantarum with plasmid. A phage was detected on the cell wall of one strain of Lacto. Plantarum; no plasmid was detected in this Lacto. plantarum. It appears that antibacterial activity is located in the phage of this strain.

Conclusion: The high molecular weight of proteins with a wide spectrum effect on bacteria may indicated chromosome-coded bacteriocin. The role of phages in lactobacilli could be a factor which inhibit meat product starter cultures or attributed in antimicrobial activity, i.e. antibacterial genes might be on chromosomal phages. Bacteriophages could be a threat to industrial fermentation foods.

Key words: Lactobacillus plantarum, Plasmid, SDS-PAGE, Phages

Introduction

Lactic acid bacteria are widely used for the fermentation and preservation of meat and dairy products and to improve the aroma and texture (1,2).

There are economic and technological incentives for accelerating and controlling the processing of fermented food. To achieve this, starters may be modified by introducing appropriate genes from other food-grade bacteria with additional peptidase activities to alter or improve the proteolytic properties of lactic acid bacteria (3).

The preservative and aroma enhancing role of Lactobacilli is due largely to the activity of bacteriocins and phages. In lactobacilli the genetic determinant for bacteriocin production can be either plasmid or chromosomally encoded (4), and both plasmid (5) and chromosomal (6, 7) locations have been reported for the bacteriocin gene in Lacto. plantarum.

Plasmid profile analysis of strains (CTC 305, 306) indicated that they harbor several plasmids. Curing experiments with the bacteriocin producing strains CTC 305 and 306 resulted in mutants that have lost the ability to produce bacteriocin but with the same plasmid profile as the parent strain (8).

Since the first phage that attacks dairy lactobacilli was isolated from sewage water in New York City (9), many Lactobacillus phages have been isolated from traditional yogurt starter cultures and from other fermented food starter cultures (10). However, few data are available about phages and bacteriocins of Lacto. plantarum in the sausage sold in food markets. To improve the stability of starter cultures, it is important to test whether some sausage lactobacillus cultures release virulent phages or potent bacteriocins that may attack other sausage lactobacillus strains.

While both phages and bacteriocins may inhibit sensitive dairy lactobacilli, phages normally have a narrower host range than bacteriocins. However, a lytic phage may cause greater damage to dairy starter cultures. Once a sensitive lactobacillus culture encounters a virulent phage, the phage can be rapidly reproduced in the culture, releasing millions of new phages that can soon eliminate the entire sensitive strains. Conversely, an added or contaminating bacteriocin-producing lactobacillus strain may cause only a limited or a slow, adverse effect on the preexistent starter strain because bacteriocins can not be reproduced by target cells and can kill target cells only upon direct contact. Therefore, phages releasing lysogens can be more virulent than bacteriocin producers in attacking other dairy lactobacillus strains (11).

Bacteriophages of lactic acid bacteria are a threat to industrial milk fermentation. Owing to their economical importance, dairy phages became the most thoroughly sequenced phage group in the database (12).

The aim of this study was to screen lactobacillus plantarum with antibacterial activity isolated from sausage for detection of plasmids, protein bands and phages to find a possible linkage of bacteriocin production to genetic location.

Material and Methods

Two Lacto. plantarum isolated from different brands of sausage with activity against indicator strains (L. monocytogenes, Staph. aureus, B. subtilis, Y. enterocolitica and some other lactobacilli without antibacterial activity) were used in this study.

* *Plasmid isolation*

Lactobacillus plantarum strains were grown in MRS broth containing 2% glucose, and plasmid DNA was extracted by alkaline lysis (13). Cells were lysed with lysis solution I (EDTA, glucose, Tris HCl, lysozyme) and lysis solution II (SDS, NaOH), followed by adding solution III (potassium acetate, acetic acid). After centrifugation, the supernatant was extracted by phenol: chloroform, and washed with ethanol. The DNA pellet was resuspended in TE buffer (Tris, EDTA). Electrophoresis was conducted on 1% agarose gels in Tris- EDTA- Boric acid buffer (pH 8.0), using a constant voltage of 80V for 50 minutes. PFG1 (5kb) and recombined RPFG1 (3.5kb) were used as standard markers for molecular weight approximation. The gels were stained with ethidium bromide.

* *Effect of ammonium sulphate precipitation on antagonistic activity*

Cell free culture supernatants were treated with solid ammonium sulphate up to a concentration of 40% (w/v) (14). The mixture was stirred for 4 h, centrifuged and its activity determined.

* *SDS-PAGE*

The estimation of molecular weight of plantaricin was carried out by the SDS-PAGE (sodium dodecyle sulphate polyacrylamide gel electrophoresis) method. A culture of lactobacilli grown in MRS broth without meat extrac and pepton for 24 h at pH 5, was centrifuged for 20 minutes to remove cells and filtered with a 0.45 μm pore size membrane. The bacteriocin present in the supernatant fraction was concentrated by ammonium sulphate precipitation (400g/litre) and dialyzed against deionized distilled water.

Molecular weight standards (Beohringer low molecular weight 12.5 97 KDa and premixed with 29-205 KDa) were dissolved in buffer and loaded onto the gel. After electrophoresis at 22mA for approximately 90 minutes the gel was stained with Coomassie brilliant blue G250.

* *Detection of phages under transmission electron microscope*

Phages of isolated Lactobacillus plantarum strains were induced by mitomycin C (11). Briefly, 0.1 ml of overnight lactobacillus culture in MRS broth supplemented with 10 mM CaCl2 (MRS-c) was transferred to 10 ml of prewarmed fresh MRS-c broth. After 3 h, the culture was divided in two tubes. One tube was used as a control and to the other, mitomycin C (Serva) was added at a final concentration of 2.5 ng/ml. The induction of lactobacillus phages was indicated by a clear lysis of the turbid culture 6-8 h after the addition of mitomycin C. The lysates were centifuged and filtered to remove unlysed cells. The lysates were ultracentrifuged for precipitation of phages and 0.1 ml of precipitate was added to 5 ml of overnight lactobacillus culture (MRS-c). After 15 minutes at 37° C, tubes were centrifuged and fixed by glutaraldehyde in phosphate buffer. Fixation was completed with osmium tetroxide. Pellets were dehydrated through graded concentrations of acetone, embedded in resin and then polymerise with spurr resin at 60° C. Section were cut and placed on electron microscope grids, stained by lead citrate and observed using a transmission electron microscopy.

Results

When comparison was made with a standard plasmid as marker, one plasmid band of about 4.5 kbp was detected in one strain of Lactobacillus plantarum by electrophoresis on agarose gel. It was not possible to detect any plasmid in the other strain of Lacto. plantarum.

A comparison of the protein bands in the stained gel with the marker showed that Lacto. plantarum without plasmid had 2 protein bands with 30 KDa and 105 KDa. Lacto. plantarum with plasmid had 2 protein bands with 22 KDa and 26 KDa.

Phage particles were observed on the cell wall of one strain of Lacto. plantarum. Plasmid was not detected in this strain. Thus it seemed that antibacterial activity gene is located on the phage of this strain.

Discussion

In the present study, two lactobacillus isolated from sausage with antibacterial activity were screened for plasmids by electrophoresis, protein bands by SDS-PAGE and the existence of phages. Plasmid profile analysis of strains indicated that one plasmid of about 4.5 kbp may be involved in bacteriocin production and immunity to this antibacterial compound. It has previously been shown that there is a relationship between the existence of plasmid and production of bacteriocin. A plasmid profile study of strains LPC10 with antimicrobial activity showed the presence of nine plasmids of sizes 49, 35, 27, 18, 16.5, 12.0, 8.4, and 2.4 kb (15). Plasmid profile analysis of two bacteriocin-producing Lacto. plantarum strains indicated that they harbor several plasmids ranging from 2kb to 55kb (8). Genetic analysis showed that Lacto. plantarum C-11 contained two cryptic native plasmids of approximately 4.3 and 6.5 MDa (16). Our

results showed only one plasmid in one strain with 4.5 kb. It is obvious that different strains have different types and number of plasmids.

Previous estimates of the molecular weight of lactic acid bacteria bacteriocin have ranged from 3.4 and 5.6 KDa (17) to 10 KDa (18) and 45 KDa (19). The higher molecular weight of proteins (band of 22Kda to 105 Kda) with a wide spectrum effect on bacteria observed in the present report may indicated chromosome coded bacteriocin. Also, plasmid was not detected in this strain with antimicrobial activity. This is in agreement with the results of Olasupo et al (20).

In this study it was interesting that a phage was detected in one Lacto. plantarum. In this strain, plasmid was not detected, but the molecular weights of protein bands were higher than in the other one from a different brand of sausage. It seemed the antibacterial activity genes are on phages. This needs further investigation.

Plasmid analysis of Lacto. plantarum strain BN showed that plasmid DNA was not detected in the producer strain but a chromosomal DNA band was observed, suggesting possible linkage of bacteriocin. production to the chromosome (18).

In the present study, since the phage particle was observed on the cell wall of lactobacilli, it might be possible that phages can play a role in destruction of other lactobacilli or other food bacteria. Phages normally have a narrower host range than bacteriocins. However, a lytic phage may cause greater damage to other lactobacilli (11). Once a sensitive lactobacilli is in a fermented sausage, phages can be rapidly reproduced in culture releasing millions of new phages that can soon eliminate the entire fermenting lactobacilli. On the other hand bacteriocins have a slow antibacterial activity on target cells. Therefore, phage releasing lysogenic lactobacilli can be more virulent than bacteriocin producing lactobacilli in attacking sausage fermenting lactobacilli.

Because phages and bacteriocins are remarkably similar in their induction mechanisms, and their lytic processes (21), the bacteriocin producers might be incompatible with phages and thus unable to survive attacks by lysogens produced by phages. Therefore, a bacteriocin-producing strain in a dairy starter culture could be unstable because it can be eliminated by a phage (11).

Bacterophages of lactic acid bacteria are a threat to industrial milk fermentation (12).

The risk of a lactobacillus starter culture being attacked by phages may be evaluated as follows:

a- If a starter culture is already a lysogen, it may be immune from further infection by the same type of phages, but the culture itself may be a source of an infective phage and thus be hazardous.

b- If a culture is a bacteriocin producer but a nonlysogen, this culture does not release phages, but it may be sensitive to phage attacks. Therefore, to competely prevent phage attacks in a starter culture and to ensure the safety of a product, it is important to isolate or develop ideal lactobacillus strains that are both phage free and phage resistant. This was confirmed by Kilic (11).

The role of phages in lactobacilli could be a factor which inhibit meat products starter culture, i.e. bacterial genes might be on chromosome phages. This needs further investigation. However, in this study, the current starter culture of a particular brand of sausage was identified as releasing phages. This is a cause for concern about the safety and stability of this bacterial culture in this sausage.

Industrial use of lactic acid bacteria genetically modified to produce broad spectrum bacteriocins with different mode of action may overcome the bacterial resistance problem, as well as be very effective against spoilage and food borne pathogenic bacteria, such as L. monocytogenes, and Staph. aureus.

Bacteriocin producing lactobacilli can prevent contamination by other non-significant lactobacillus strains and can inhibit pathogenic microorganisms. Therefore, these lactobacilli have been considered advantageous both in maintaining the purity of cultures and in promoting health in humans. As a result, bacteriocin producing strains have been selected by certain manufacturing plants to be used in their products.

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