Investigation of Antagonistic Effects of Isolated Lactic Acid Bacteria from Different Cheeses of Gorgan City against Main Intestinal Pathogenic Bacteria

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Abstract

Lactic Acid Bacteria (LAB) have been shown to elicit positive health effects particularly in humans. Competitive exclusion of pathogens is one of the most important beneficial health claims of probiotic bacteria. This study was conducted with the aim of isolation of Lactic Acid Bacteria from different Cheeses and their effects on the main pathogenic bacteria in intestine which might provide important information regarding its probiotic potential and its utilization in the future. Morphological, cultural and biochemical characteristics were employed to identify lactic acid bacteria, isolated from different Cheeses in different areas in Gorgan City, Iran. From 9 traditional Cheese and 2 industrial Cheese samples a total of 38 isolates were isolated, 36 isolates from local Cheese and 2 isolates from industrial Cheese. The results showed that Lactobacillus casei has the highest frequency. Also this species showed antagonistic activity against pathogens including Escherichia coli, Staphylococcus aureus, Bacillus cereus and Citrobacter freundii, with an inhibition zone diameter of 17 mm.

Keywords: Lactic Acid Bacteria; Cheese; Pathogenic bacteria; Intestine

Introduction

Now-a-days consumers are concerned about the synthetic chemicals used as preservatives in food, and there is resulting trend towards less processed food. These untreated foods can harbour dangerous pathogens which can multiply under refrigeration and without oxygen. A solution to this dilemma is the use of antimicrobial metabolites of fermentative microorganisms [1]. Many antimicrobial chemicals have been in use for a long time without any known adverse effects. Many of the organic compounds which have stirred interest are antimicrobial metabolites of bacteria used to produce, or associated with fermented foods.

In fermentation, the raw materials are converted by microorganisms (bacteria, yeast and molds) to products that have acceptable qualities of food. In common fermented products such as yogurt, lactic acid is produced by the starter culture bacteria to prevent the growth of undesirable microorganisms [2]. Food fermentations have a great economic value and it has been accepted that these products contribute in improving human health. LAB have contributed in the increased volume of fermented foods worldwide especially in foods containing probiotics or health promoting bacteria [1].

Lactic acid bacteria (LAB) are a group of gram-positive bacteria including the genera Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus. The general description of the bacteria included in the group is gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates [3].

These bacteria are the major component of the starters used in fermentation, especially for dairy products, and some of them are also natural components of the gastrointestinal microflora [4].

Lactic acid bacteria have been used in the production of foods, especially fermented foods because they can produce several compounds that contribute to taste, smell, color, and texture of the foods. In addition, they can produce antimicrobial substances including bacteriocins that have ability to inhibit pathogenic and food spoilage bacteria [3]. The antagonistic activity of such bacteria is known to inhibit a large number of enteric and urinary pathogenic bacteria [5]. The effect of probiotic LAB on competitive exclusion of pathogens has been demonstrated with human mucosal material in vitro, and in vivo in chickens and pigs [6]. Enteric bacterial pathogens must adhere to and penetrate the protective intestinal mucus layer in order to reach and invade the enterocytes and cause clinical infection [7]. The present investigation reports isolation and characterization of LAB species having potential application in the local dairy industry such as cheeses fermentation, source of probiotics and microbial contaminants reducing agents of the product.

Material and Methods

Cheese samples

During the spring of 2014, a total of 11 cheeses (9 traditional and 2 industrial cheese samples) were collected from Gorgan city, Iran. The samples were collected in sterile universal tubes and kept cool until they could be taken to the laboratory, where they were kept at 4°C for further use.

Isolation of lactic acid bacteria

The samples were aseptically weighted and homogenized. From each sample, a 1:10 dilution was subsequently made using peptone

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water followed by making a 10 fold serial dilution. 1 ml from each dilution was then subcultured, in duplicate, into the Plate Count Agar and MRS agars (Merck, Germany) used for isolating LAB [8] and were incubated anaerobically using the Gas Pack system (Merck Anaerocult type A) at 37°C for 2 days.

**Identification of the bacterial strains**

All strains were initially tested for gram reaction, catalase production and spore formation [9]. Colonies were characterized on MRS Broth and M17 Broth. Strains with gram positive and catalase negative reactions were finally used for further identification. Growth at different temperatures (10°C and 45°C) for 2 days, growth in the presence of 6.5% NaCl, were considered to identify the strains.

All strains were also tested for fermentation of L-arabinose, D-xylene, galactose, D-fructose, sorbitol, lactose, mannnitol, saccharose, rhamnose and mannose. *Staphylococcus aureus* (PTCC 1600) were used as indicator culture. As mentioned earlier, the culture broths of both the producer and indicator strains were adjusted to McFarland Index 0.5 (1.5 × 10⁸) to use. The growth of lactic acid bacteria strains isolated from cheeses at 37°C was visually confirmed by the changes in turbidity of Muller Hinton Agar or BHI after 24 h of incubation.

The surface of Muller Hinton Agar plates were evenly streaked with selected indicator strains, with a sterile cotton swab. The culture broth of the producer strains (100 ul) were poured into the wells (7 mm) made in these agar plates with a sterile borer. All plates were stored for 2 h at 4°C prior to incubation at 37°C for 24 h. The antimicrobial activity was recorded as appearance of clear zone around the wells and the zone diameter (Resistance: 7 mm<zone, Inter mediate: 8-9 mm<zone, Sensitive: 10 mm<zone) measured in millimeter. All tests were run in duplicate.

**Results and Discussion**

During the spring of 2014, a total of 11 cheese samples (9 traditional and 2 industrial cheese samples) were collected from Gorgan city (Table 1).

**Microbial count results**

The results showed that the average number of microbial count in Plate count agar is the maximum amount in sheep samples (5/4 × 10⁹ CFU/ml) and minimum amount in water buffalo samples (1 × 10⁷ CFU/ml) (Table 2). Kiai et al. [10] have reported in previous studies, the microbial count in traditional yogurt in Golestan province between CFU/ml 7/4 × 10⁹ and 3/92 × 10⁹ CFU/ml.

Also average number of microbial count in MRS Agar is the maximum amount in cow samples (3/1 × 10⁹ CFU/ml) and minimum amount in goat samples (1/3 × 10⁷ CFU/ml) (Table 3), that has been reported in previous studies on local yogurts, between CFU/ml 3/6 × 10⁷ and 7/2 × 10⁹ CFU/ml [10]. The daily intake of 10⁸ to 10⁹ live bacteria, is the minimum acceptable value, therefore daily intake of 100 grams of probiotic product containing up to 1 × 10⁹ - 5 × 10⁹ CFU live bacteria per gram of product, can provide the optimum desired [11].

**Results of isolation and identification of lactic acid bacteria**

38 species of lactic acid bacteria were isolated and identified from 11 samples of cheese that the types and numbers of these bacteria shown in Tables 4 and 5, according to type of cheese.
According to Table 5, the maximum number of lactic acid bacteria is in sheep and the minimum number is in Industrial cheese. The highest frequency of lactic acid bacteria isolated from the cheese samples, is belonged to the species include Lactobacillus casei, Streptococcus lactis and Enterococcus faecium. It is shown the number and percentage of different species of lactic acid bacteria isolated from cheese samples (Figure 1).

### Results of antagonistic effect of LAB

Lactic acid bacteria isolated from cheese samples showed antagonistic activity against pathogenic bacteria with the production of the antimicrobial compound. The results are shown in Table 6. Lactobacillus casei, Lactobacillus acidophilus, isolated from sheep cheese, inhibit the growth of all pathogenic bacteria. Lactobacillus casei has the highest antibacterial activity against pathogenic bacteria.

*Enterococcus faecium* isolated from cow and goat cheese and also Streptococcus thermophilus and Streptococcus lactis isolated from sheep cheese, have no antimicrobial effect on pathogenic bacteria. So in total, the lactic acid bacteria isolated from sheep cheese, produce greater antimicrobial compounds against pathogenic bacteria [12], in the same study found the similar result and *Lactobacillus acidophilus* showed the highest inhibitory effect with an inhibition zone diameter of 14 mm.

### Conclusion

As we enter in the new millennium, people are aware that for spending a healthy life style diet play a major role in preventing diseases and promoting health. Therefore there is an increasing trend for foods containing probiotic cultures. Probiotics are viable organisms and supportive substances that improve intestinal microbial balance, such as *Lactobacillus acidophilus* and *bioactive proteins* [13]. Antimicrobial effect exerted by the LAB is mainly due to acid production, hydrogen peroxide, fatty acids, aldehydes and other compounds [14]. The empirical evidence that for many years, linked the use of fermented dairy products such as cheese and milk with the promotion of intestinal health is today well supported by modern science. Commercial lactic acid bacterial products have been one of the major health related foods in the world. When a LAB is used as probiotic, it should be safe and possess some basic characteristics, such as the tolerance to acid, ability to adhere to human intestinal epithelium and having antagonistic activity against bacterial pathogens.

This study proved the presence of viable LAB micro flora in cheese samples in Gorgan city. Overall results of this research suggests that the selected LAB strains isolated might be appear to possess probiotic potential, and hence could be exploited further for their use in fermented dairy products.

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### References

1. Soomro A, Masud T, Anwaar K (2002) Role of lactic acid bacteria (LAB) in food preservation and human health–a review. Pakistan Journal of Nutrition 1:20-24.
2. Ray B, Daeschel M (1992) Food biopreservatives of microbial. Origin CRC Press, In. Boca Raton, Florida.
3. Rattanachaikunsopon P, Phumkhachorn P (2010) Lactic acid bacteria: their

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**Table 5:** Types and number of lactic acid bacteria isolated from cheeses in Gorgan city on the type of livestock.

| No. | Type of livestock | Number of samples | Lactic acid bacteria | Number | Type |
|-----|------------------|-------------------|---------------------|--------|------|
| 1   | Cow              | 3                 | L. casei            | 3      |      |
|     |                  |                   | L. delbruecki       | 2      |      |
|     |                  |                   | E. faecalis         | 1      |      |
| 2   | Sheep            | 3                 | L. casei            | 2      |      |
|     |                  |                   | L. acidophilus      | 2      |      |
|     |                  |                   | L. delbruecki       | 1      |      |
|     |                  |                   | S. lactis           | 5      |      |
|     |                  |                   | S. thermophilus     | 2      |      |
|     |                  |                   | E. faecalis         | 1      |      |
| 3   | Water buffalo    | 2                 | L. casei            | 6      |      |
|     |                  |                   | L. acidophilus      | 1      |      |
| 4   | Goat             | 1                 | E. faecium          | 4      |      |
| 5   | Industrial       | 2                 | L. casei            | 1      |      |
|     |                  |                   | L. acidophilus      | 1      |      |

**Table 6:** Results in inhibition of lactic acid bacteria isolated from cheeses against pathogenic bacteria.

| LAB                  | *Escherichia coli* | *Bacillus cereus* | *Citrobacter freundii* | *Staphylococcus aureus* |
|----------------------|--------------------|-------------------|------------------------|------------------------|
| L. casei from water buffalo sample | -                  | -                 | 9 ± 0*                 | -                      |
| L. casei from cow sample         | 12 ± 0*            | 12 ± 0*           | 11 ± 0*                | -                      |
| L. casei from sheep sample       | 15 ± 3.46*         | 13.66 ± 0.57*     | 18 ± 1.73*             | 16 ± 0*                |
| L. acidophilus from sheep sample | 16 ± 0*            | 15 ± 0*           | 18 ± 2.82*             | 15.33 ± 1.15*          |
| L. delbruecki from sheep sample  | 12.5 ± 4.94*       | 12 ± 1.73*        | 14 ± 3.46*             | -                      |
| L. delbruecki from water buffalo sample | 9 ± 0*            | 14 ± 5.19*       | 17 ± 0*                | 12 ± 0*                |
| L. rhamnosus from cow sample     | 9.33 ± 0.57*       | -                 | 11 ± 0*                | -                      |
| S. thermophilus from sheep sample | -                  | -                 | -                      | -                      |
| L. casei from sheep sample       | -                  | -                 | -                      | -                      |
| E. faecalis from sheep sample    | -                  | -                 | -                      | -                      |
| E. faecium from goat sample      | -                  | -                 | -                      | -                      |
| L. faecium from cow sample       | -                  | -                 | -                      | -                      |
antimicrobial compounds and their uses in food production. Annals of Biological Research 1: 218-228.
4. Coeuret V, Dubernet S, Bernarden M, Gueguen M, Vernoux JP (2003) Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Dairy Science and Technology 83: 269-306.
5. Aslam S, Qazi JI (2010) Isolation of acidophilic lactic acid bacteria antagonistic to microbial contaminants. Pakistan Journal of Zoology 42: 567-573.
6. Rinkinen M, Westermark E, Salminen S, Ouwehand AC (2003) Absence of host specificity for in vitro adhesion of probiotic lactic acid bacteria to intestinal mucus. Veterinary microbiology 97: 55-61.
7. Sylvester FA, Philpott D, Gold B, Lastovica A, Forstner JF (1996) Adherence to lipids and intestinal mucin by a recently recognized human pathogen, Campylobacter upsaliensis. Infection and immunity 64: 4060-4066.
8. Guessas B, Kihal M (2004) Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats' milk. African Journal of Biotechnology 3: 339-342.
9. Harrigan WF, McCance ME (1976) Laboratory methods in food and dairy microbiology. Rev. Edn., London, New York, Academic Press. PP: 33-200.
10. Kiaie E, Mozafari N, Samioladah H, Jandaghi N, Ghaemi E (2006) Antagonistic effect of lactic acid bacteria isolated from yoghurt against pathogenic bacteria. Scientific Journal of Gorgan University of Medical Sciences 8: 28-33.
11. Gibbon S, Fuller R (1989) Probiotics in man and animals. Journal of Applied Bacteriology 66: 365-378.
12. Kazemi R, Ghaemi N, Mirpour MS (2010) Antimicrobial activity of lactic acid bacteria isolated from probiotic products (Lactobacillus and Bifidobacterium). Scientific journal of Microbial Biotechnology in Islamic Azad University 2: 29–36.
13. Fuller R (1991) Probiotics in human medicine. Gut 32: 439–442.
14. Daeschel MA (1989) Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food technology 43: 164-167.