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ORIGINAL ARTICLE

Isolation and Identification of Lactic Acid Bacteria from a Traditional Fermented Fish Sauce (Mahyaveh) in Fars Province, Iran

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ABSTRACT

Background: Mahyaveh is a fermented fish sauce in southern parts of Iran. Lactic acid bacteria (LAB) are commonly dominant microorganisms in fermented fish products. These bacteria develop organoleptic characteristics of fermented foods and play a significant role in promoting their quality and safety. The present study aimed to identify LAB isolated from Mahyaveh using 16SrDNA gene sequences.

Methods: Mahyaveh samples were collected from different regions of Fars province, southern Iran. Then, LAB colonies were isolated using specific media and identified by microscopic observations and biochemical tests. Afterwards, DNA was extracted, PCR was done by general primers of 16S rDNA, and the bacteria were recognized.

Results: The 16S rDNA sequence of all isolates was related to *Lactobacillus plantarum* and *Enterococcus faecium* type strains.

Conclusion: *L. plantarum* and *E. faecium* were shown to be prevalent LAB strains that could be used as starters in Mahyaveh fermentation in southern Iran.

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Introduction

Traditional fermentation is known as a processing and preserving method in developing countries, which is essential in extending shelf-life and enhancing organoleptic properties of end products (1). There are a number of fermented traditional fishery products, such as fish sauce, which are commonly used as seasoning, condiment for cooking, flavor enhancer, or main dish(2). Mahyaveh is a fermented fish sauce in southern parts of Iran, especially in Hormozgan province and Larestan county. It is commonly made from Sardines (*Sardinella* sp.) or Anchovies (*Stelophorus* sp.), salt, mustard (*Brassica juncea*) and water (3, 4).

Lactic acid bacteria (LAB) are commonly the dominant microorganisms in various types of fermented fish products (5). They are considered to be gram-positive, non-spore forming, and cocci or rod shaped bacteria (6). During fermentation, LAB

produce organic acids particularly lactic acid as the main metabolites of glucose fermentation. These acids not only develop organoleptic characteristics of fermented foods, such as taste, aroma, and texture, but they also decrease the product's pH that plays a significant role in promoting its quality and safety (7).

Because of the significant impacts of LAB species on Mahyaveh quality, it is necessary to detect a simple and effective method for their identification. Due to similar nutritional and growth requirements in many LAB species, biochemical methods are not conclusive for their characterization (8). Thus researches are continuously being performed to distinguish and characterize new LAB. For example, Savadogo et al. in Nigeria, Singh et al. in India, Madoroba et al. in South Africa, Kopermsub et al. in Thailand, and many other researchers have made attempts to isolate LAB species from fermented foods (7, 9-11).

Recent years have seen a revolution in development of molecular techniques for recognizing microorganisms and analyzing their activity. In fact, a great number of available methods for identification and characterization of LAB are based on molecular methods (12). However, no research has been carried out on isolation and characterization of LAB from Mahyaveh so far. Considering the above premises, the present study aims to identify LAB isolated from Mahyaveh using 16SrDNA gene sequences.

Materials and Methods

Sample collection

The mahyaveh samples were collected from different regions of Fars province, including Lar, Lamerd, Khonj, and Zarindasht. The samples were placed in sterilized containers and transferred to laboratory under aseptic conditions.

The LAB isolation

Ten gr of each sample were added to 90 ml of sterile saline (85% NaCl) and shaken for 1 min. Then, decimal serial dilutions (10^{-1}– 10^{-5}) were prepared. Afterwards, 1 ml of each dilution was inoculated to de Man, Rogosa and Sharpe (MRS) broth (Merck-Darmstadt, Germany) and was incubated anaerobically at 30 ˚C for 24-48 hr (13). The suspected colonies were stained by gram staining method and tested for catalase reaction (14). The selected colonies were isolated on MRS agar (Merck-Darmstadt, Germany) (13). The isolates were placed in MRS broth supplemented with 15% glycerol at 15 ˚C until further analysis.

Extraction of DNA

DNA was extracted from the bacteria using DNA extraction kit (Cinnagen, Tehran, Iran).

PCR

In order to strain identification, the partial 16S rDNA gene (1500 bp) was amplified by the use of primers LAB-F (5-AGTTTGATCCTGGCTCAG-3) and LAB-R (5-GTTACCTTGTTACGACTT-3) (15). Each PCR consisted of 2.5 µl of 10x buffer, 0.75 µl of $MgCl_2$, 0.75 of dNTP, 1 µl of each primer, 0.2 µl of Taq enzyme, 3 µl of the DNA sample, and sterile water to a volume of 25 µl. PCR amplification was started by DNA denaturation at 94 ˚C for 10 min followed by 35 cycles of denaturation at 94 ˚C for 90 seconds, annealing at 52 ˚C for 90 seconds, and a final extension at 72 ˚C for 2 min. The PCR products were then purified using gel extraction kit (Cinnagen, Tehran, Iran) and sequenced. The 16S rDNA sequencing results were aligned using gen bank (http://blast.ncbi.nlm.nih.gov/Blast).

Technological Characterizations of LAB isolates

Proteolytic, Amylolytic and Lipolytic activity of isolates were determined as described by Thapa *et al.* (2006) (16).

Results

The results showed increased bond strip containing 1500 nucleotides, which have been depicted in Figure 1. Moreover, the results indicated that the 16S rDNA sequence of all isolates was related to *Lactobacillus plantarum* and *Enterococcus faecium* type strains. However, no proteolytic, lipolytic, and amilolytic activity was detected in these strains.

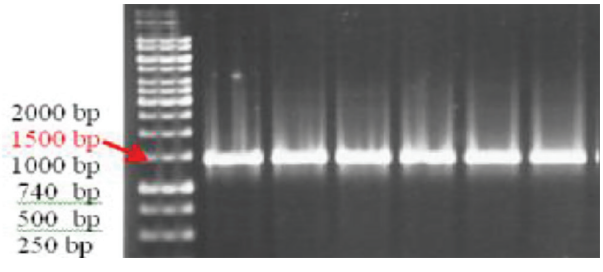

Figure 1: PCR results.

Discussion

Up to now, not much attention has been paid to screening of LAB isolates obtained from Mahyaveh fermentation. To the best of our knowledge, this research reports for the first time on predominant LAB species during Mahyaveh fermentation using PCR method. Normally, starter cultures are involved in development of the typical characteristics of fermented foods, such as taste, flavor, texture, shelf-life, and nutritional quality and safety (5). Additionally, abiotic and biotic conditions of food matrix have a pronounced influence on growth of specific microbial populations during fermented

foods production (7).

In the case of Mahyaveh, the concentrations of salt, protein, carbohydrates, and water ranged from7.48-17.1%, 6-12%, 0-8%, and 55-78%, respectively (4), making a suitable environment for growth of LAB. In fact, LAB dominate many fermented fish products mostly owing to production of lactic acid, thus reducing pH that inhibits the growth of food borne pathogens (5). The pH of Mahyaveh has been reported to range from 4.89 to 7.55 (4). Several researchers have reported *Lactobacillus*as the major member of LAB population present in fish viscera or meat (17). *L. plantarum* has been found in various sources, especially in fermented food products (18).

This result is in accordance with that of the study by Moe et al. (2015) who isolated *L. plantarum* from small fish fermented with boiled rice (19). Moreover, Kheng Yuen et al. (2009) demonstrated that *L. plantarum* was a part of the dominant microflora involved in Budu (an indigenous Malaysian fish sauce) fermentation that showed potential probiotic activities (20). Production of antimicrobial substances against pathogens are among the most important technological properties of LAB in fermented meat products (21). Similarly, Lash et al. (2005) described a bacteriocin produced by *L. plantarum* that showed antibacterial activities towards S. aureus, *E. coli*, *L. innocua*, and *P. aeruginosa* (22).

Liasiet al. (2009) also examined the fermentation of Budu (the fermented fish product) and clarified that the highest population was *L. plantarum* (23). They also showed that this strain produced an antibacterial agent, which inhibited the growth of a range of Gram-positive and Gram-negative microorganisms. Similar experiments have also been performed with som-fak (a fermented fish product) (24). *E. faecium* is isolated from various fermented foods, such ascheese, sausage, fermented vegetables, and fermented milk (25). Moreover, Enterococci could contaminate fishery products during handling and processing. Contamination might occur from intestine within fish evisceration, from environmental sources within handling and processing, or both (26).

These findings are consistent with those reported by Lee and Kim (2010) who identified *E. faecium* from Korean gajami-sikhae (a lactic fermented flat fish) (27). The isolation of this species from Thai fermented fish has been recently demonstrated, as well (28). In addition, Miyashita et al. (2012) concluded that *E. faecium* and *L. plantarum* were a part of the dominant microflora involved in fermented foods in Thailand (29). In the same line, Barbosa et al. (2014) recommended the potential application of *E. faecium* isolates in food preservation via inhibition of pathogens growth (30). Sonsa-Ard et al. (2015) similarly reported that *E. faecium* was isolated from som-kai-pla (a Thai fermented fish roe) which had an inhibitory impact on the growth of *L. monocytogenes* via production of bacteriocin. Moreover, due to lack of virulence factors and antibiotic resistance, the isolate was considered to be safe (25).

Conclusion

L. plantarum and *E. faecium* were prevalent LAB strains that could be used as starters in Mahyaveh fermentation. Yet, further studies for the selection of suitable strains which could act as starter for better control of fermentation are needed. This research could improve the safety and quality of final fermented products.

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Conflict of Interest

None declared.

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