Original Research Article

Antifungal effects of *Lactobacillus* species isolated from local dairy products

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Abstract

Objective: The *Lactobacillus* is a genus of lactic acid bacteria which are regularly rod-shaped, nonspore, Gram-positive, heterogeneous, and are found in a wide range of inhabitants such as dairy products, plants, and gastrointestinal tract. A variety of antimicrobial compounds and molecules such as bacteriocin are produced by these useful bacteria to inhibit the growth of pathogenic microbes in the food products. This paper aims to examine the isolation of *Lactobacillus* from local dairies as well as to determine their inhibition effect against a number of pathogens, such as two fungi: *Penicillium notatum* and *Aspergillus fulvous*.

Materials and Methods: Twelve *Lactobacillus* isolates from several local dairies. After initial dilution (10^{-1}–10^{-3}) and culture on the setting, de Man, Rogosa and Sharpe-agar, the isolates were recognized and separated by phenotypic characteristics and biochemical; then their antifungal effect was examined by two methods.

Results: Having separated eight *Lactobacillus* isolates, about 70% of the isolates have shown the inhabiting areas of antifungus on the agar-based setting, but two species *Lactobacillus alimentarius and Lactobacillus delbrueckii* have indicated a significant antifungal effect against *P. notatum* and *A. fulvous*. Except bacteriocin, lactic acid, and acetic acid, the inhibitor substance is produced by these bacteria.

Conclusion: Given the vitality of *Lactobacillus* in human health, recognition and isolation of the species producing compound in antagonist to the pathogens existing in the food products can be a helpful and effective step toward maintaining the valuable native *Lactobacillus* and using them in the dairy industries.

Keywords: Antifungal effects, bacteriocin, dairy products, *Lactobacillus*

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INTRODUCTION

During storage and maintenance of food products, the spoilage and toxicity will occur by fungi such as *Fusarium* and *Aspergillus*. However, a major problem of such spoilage is induced by *Penicillium fungus* which has resulted in huge economic losses around the world.[1] In addition to it, the *Penicillium* produces the allergen spores and mycotoxin that threats seriously the human life.[1] The controlling measures to prevent the fungus growing in the grains, foodstuff, and storage and to inhibit the food contamination and reducing the public health hazards are of special importance.[2] Over the past few years, the interests in using of natural

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products in prevention and treatment of diseases have increased.[3-8] Researchers have also tried to prepare natural drugs.[9-14] The use of microorganism or their metabolites for biological protection and avoiding foodstuff spoilage and extending the time of food products retention has also been increased. The lactic acid bacteria (LAB) include a wide range of genus as well as substantial species.[15] The lactic acid bacteria are generally recognized as secure microorganisms in which they have been used in the fragmented food product and for protection for centuries.[2] These bacteria are utilized a main starter compound for fragmentation, especially for dairy products or some of them. They also are the natural flora of digestive tract.[1] The LAB play an important role as a culture starter in the food industries in producing flavor, odor, color, and texture and natural characteristics.[2] The interest and attention to LAB in biological protection of foodstuff have been grown because they generate antimicrobial compound and products such as lactic acid and bacteriocin.[15]

The LAB are a family comprised a group of heterogenic, Gram-positive, nonspore, catalase, and negative cytochrome, anaerobe bacteria. The recent taxonomical studies suggest that LAB are consisted of 13 genera. The antimicrobial effects and immunity of some of the genera such as *Lactobacillus* and *Lactococcus i*n the protection of foodstuff have been widely accepted.[16] Some useful effect of probiotics on health includes the development of natural microflora, prevention of infectious diseases and allergy, reduction of serum cholesterol, anticancer activity, reinforcement of immune system, and improvement of lactose digest in the host.[17] Some genus used as probiotics are *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and some fungi and yeast species.[17] *Lactobacillus* is one of the most important genera of LAB.[1] The lactobacilli homofragmentation fragments the hexose to lactic acid alone or accompanied with acetic acid, ethanol, and formic acid during glucose deficiency. The lactobacilli heterofragmentation fragments the hexose to *Lactobacillus*, acetic acid, ethanol CO_2.[18] There is a large number of these bacterial species and some dominate species that can produce lactic acid from carbohydrate and that the LAB not only are desirable for this reaction but also are necessary for its promotion. The bacteria and fungi are two microorganism groups which can produce lactic acid. Although most researches investigating the production of lactic acid have focused on LAB and filamentous fungi such as rhizopus, use of glucose under aerobic condition has produced lactic acid as well.[19]

Mold of fungi and yeasts are the organisms that produce substantial spoilage in the food products. In addition, generation of cancer-bearing and toxic mycotoxins potentially by molds is a concern. It is estimated that about 5%–10% of foodstuff are spoiled by these organisms. The LAB are commonly found in the nutrient-rich settings. They exist naturally in many food systems producing livestock meal. These bacteria can protect the foodstuff from microbial decay through generating the antagonist metabolic products or establishing the other antimicrobial compounds.[20]

In addition to the publications, in which the active antifungal substances are recognized, the studies on antifungal products of LAB showed that the substances inhibiting the fungi are different among species. Those species belonging to *Lactobacillus* genus are reported in most of studies. Since the fungi are eukaryote organism, the common target mechanism of effective material for their growth inhibition is cellular walls, cellular membrane, protein synthesis, and cellular division.[2,20]

The purpose of this study was to investigate the effects of antifungal activity of probiotic bacteria *Lactobacillus* isolated from various local dairy Ahvaz.

MATERIALS AND METHODS

Sampling

In this study, twenty local diary samples (yogurt, milk, and cheese) were gathered from different districts of Ahvaz. The samples were stored in the tubes then, they were enriched in the culture setting de Man, Rogosa, and Sharpe (MRS)-broth (Merck, Germany) at temperature 37°C for 24 h. After enrichment, they were incubated on the culture setting MRS-Agar (Merck, Germany) at temperature 37°C for 48 h. The observed colonies on setting MRS-Agar (Merck, Germany) were subcultured for purification.[21] The isolates at genus levels were identified using morphological, phenotype, and biochemical tests characteristics such as: (Gram-staining, catalase, (mobility examination) SIM, indale test, oxidase, carbohydrate fermentation (Lactose-Socrose-Arabinose-Trehalose-Salissin), then the effects of their antifungal were examined by two methods: (I) Use of extract on bacteria culture (II) sonication (using ultrasonic waves).[20]

Biochemical tests such as Gram-stain, catalase, oxidase, indole production and motion study, growth at different temperatures (15°C-37°C-45°C) and various fermentation of sugars (sucrose, lactose, maltose, trehalose, galactose, arabinose, mannitol, fructose, and salicin) were studied.[22]

The antifungal effect was assessed by two methods:

In the first method using supernatant bacteria (supernatant culture antifungal effect of lactobacilli were investigated in

this way that the isolation and identification of bacteria, the bacterial inoculation at 37°C for 24 h were incubated until turbidity equal half McFarland MRS broth in culture were centrifuged. Then, for preparation supernatant culturing bacteria for 25 min at 4°C at around 3500 then drives dipped in the supernatant probiotics with specified distance on the plates (cultured mushrooms to) the agar was placed for 24 h were incubated at 37°C, then were measured by the diameter created with the ruler.[2]

In the second method using ultrasonic waves, cell wall of bacteria (24 h culture bacteria) and then destruction of the disk impregnated with the extract of the plates (cultured the fungi) on agar (Merck, Germany) was placed were incubated for 24 h at 37°C. Then, started diameter was measured by a ruler.[20] Moreover, the results with the results of plates prepared discs containing antifungal (miconazole, terbinafine, and fluconazole) on two species of fungi (*Penicillium notatum Aspergillus fulvous*) were compared.

RESULTS

Having separated eight *Lactobacillus* isolates, about 70% (more than half of them) of the isolates have shown the inhabiting areas of antifungus on the Agar-based setting, but two species *Lactobacillus alimentatus and Lactobacillus delbrueckii* have indicated a significant antifungal effect against *Penicillium* and *A. fulvous*.

In the second method (using ultrasonic waves), *L. alimentarus* created a halo 12 mm diameter against *A. fulvous* fungus and did a halo 10 mm diameter against *P. notatum*, and the species *L. delbrueckii* produced a halo 11 mm against *A. fulvous* fungus and a halo 13 mm against *P. notatum* [Table 1]. To compare the prepared 3 discs, the antibiotic myconazole, terbinafin, and fluconazole were examined, respectively. The micoazole disc produced a halo 20 mm diameter on average on both fungi, i.e., *Penicillium* and *A. fulvous* and terbinafin did a halo 38 mm on average on both above fungi, but the fluconazole did not produce any halo against the two fungi [Table 2 and Figures 1, 2].

DISCUSSION

The use of biological methods to eliminate and reduce fungal toxin is highly regarded. One of these methods is to use LAB such as lactobacilli are effective Studies have shown that LAB in the fermentation process to produce bacteriocin-like compounds and organic acids can inhibit the growth of mold and thereby preventing aflatoxin B1 production.

Tajabady *et al*. in 2008, ten strains of lactobacilli from our local separate and properties of probiotics then examined since the bacteria from traditional dairy products were isolated as reservoirs of microbial native Iran and in side effects such as reduced fungal aflatoxins are used in the industry in good taste.[23] In the current study of *Lactobacillus probiotic* dairy different locations in the city of Ahvaz, such as yogurt, milk, cheese, and continued isolation and identification of antifungal effects were observed. Several studies also differences in the structure of the bacterial cell wall and cell populations LAB as a factor in reducing the toxins aflatoxin fungus and fungal expression.[24]

Our study isolates of *Lactobacillus* bacteria, inhibition zone of inhibition in both technique and anticancer, antifungal effects. In the second method, especially with L. alimentarus and *L. delbruecki*, while two species of fungi were negligible destruction of the walls and exit intracellular antimicrobial and antifungal activity showed signifi cant inhibitory. Similar[25] increased inhibitory activity in the year 2012 Gerbaldo *et al*. results of study. Antifungal lactobacilli may be due to the production of secondary metabolites such as bacteriocin, lactic acid, and hydrogen peroxide.[26] The study also inhibits fungal growth desired effect was also observed by the two species. Similar studies indicated

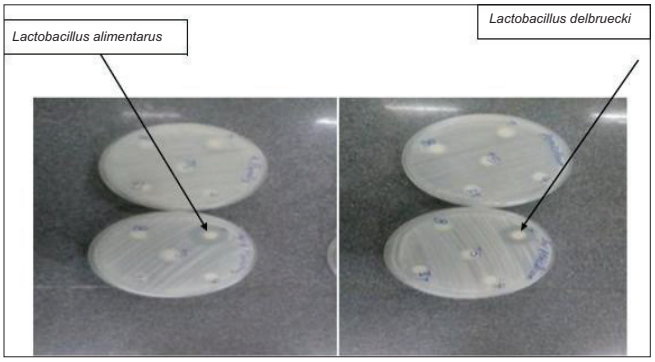

Figure 1: Zone of inhibition (mm). Inhibition caused by antifungal activity of two species of lactobacilli isolated. *Lactobacillus alimentarus*: Left side. *Lactobacillus delbruecki*: Right side

Table 1: Diameter caused by two strains of *Lactobacillus* in both

Bacteria	Fungi			
	Penicillium notatum		*Aspergillus fulvous*	
	Centrifuges	Sonication (mm)	Centrifuges	Sonication (mm)
Lactobacillus alimentarius	-	10	-	12
Lactobacillus delbrueckii	-	13	-	11

Table 2: Created by ready-diameter discs of antibiotics

Antibiotics	Fungi	
	Penicillium notatum (mm)	*Aspergillus fulvous* (mm)
Myconazol	20	20
Terbinafin	38	38
Fluconazole	-	-

Figure 2: Zone of inhibition (mm). Inhibition caused by the discs ready antifungal activity (fluconazole, terbinafine, and miconazole) on two species of fungi (*Penicillium notatum* the left side) and (right side *Aspergillus flavus*). (Shape 2) Zone of inhibition (mm)

which Lactobacillus delbruecki, *L. alimentarus* Lactobacillus fermentum, *Lactobacillus* delbruecki was observed they had antifungal effects. Aryantha and Langgani The fungal inhibitory activity of LAB is very complex. Hopefully, this thesis has contributed to clarify some aspects of this activity. That LAB with fungal inhibitory properties can be useful in biopreservation of both food and feed, alone or in combination with other microorganisms.[27]

In the present study, the characteristics of LABs were investigated. There are a large number of reports that utilization of antifungal characterized *Lactobacillus* bacteria in biological protection is possible. They are recently employed by Suchnurer and Magnusson. The LAB with antifungal property probably can be used in the food and meal systems for increasing the quality, for example, by reducing the use of the adding chemical substances and inhibiting the growth of fungi, yeast, and mycotoxcins.[8] Since LAB occur naturally in many food systems and they have been a part of the human diet for centuries, they can be regarded as safe organisms to consume. They have a great potential for extended use in biopreservation of both food and feed product.[17]

Although the consumers are becoming aware more and more on immunity of foodstuff, setting up a dialog between researchers, users, and regulatory authorities for new application is necessary.[20] In the recent years, special attention has paid on the identification of LABs in particular lactobacilli because their bacteriocin is useful in food industries as well as in biological protection of foods produced by them.[17] The LABs are secure organisms that can be replaced by antibiotics.[16]

CONCLUSION

According to the results, by comparing the results, we can say that by destroying the cell wall of bacteria, lactobacilli and exit antimicrobial (antifungal) that the substances secreted out of the cell wall of probiotic lactobacilli defense mechanisms against bacterial and fungal pathogens due to the favorable effects of antimicrobial effects, and they Vmfyd these bacteria, Bakarbrd most of them in the food industry can be used to further the health of these new methods and natural step.

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Conflicts of interest
There are no conflicts of interest.

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