Antimicrobial Activity of Lactobacilli And Bifidobacteria Isolates Against Pathogenic Bacteria In Dairy Products

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Abstract

Four lactic acid bacteria were isolated from a traditional Egyptian fermented dairy product. The isolates were identified using genus-specific PCR technique. These isolates showed different antibacterial agents against pathogens. The antimicrobial agents were bacteriocin and organic acids. HPLC analysis indicated that the isolates produced lactic and acetic acids. Propionic acid was produced only by bifidobacteria isolates. All isolates behave a good antagonistic activity against the tested indicator strains. Supernatant and cell pellets of isolates were used as biopreservation system in yoghurt and Karish soft cheese. Data showed that no apparent or detectable of pathogenic bacteria growth to the end of the storage period 21 days. Supernatant and cell pellets at concentration of 1:1, enhanced the antimicrobial activity and improved the organoleptic properties and the viability of starter culture of the resulted dairy products.

Introduction

Lactic acid bacteria (LAB) are widely used as starter cultures in dairy fermentations. One of the major reasons for their wide use is the wide range of antimicrobial substances they are able to produce which efficiently contribute to the preservation of the fermented products (De Vuyst and Vandamme, 1994). The antibacterial activity of probiotic Lactobacillus strains appears to be multifactorial (Servin 2004). In particular, by producing metabolites such as acetic and lactic acid and thus lowering the pH, *Lactobacillus* strains inhibit the growth of bacterial pathogens (Richard *et al*, 2006).The aim of this study was to examine the antagonistic activity by the Lactobacillus and bifidobacteria.

Methods

Isolation: *Lactobacillus and Bifidobacterium* were isolated from a traditional Egyptian fermented dairy product (Soft cheese).**Identification:** Bifidobacteria and lactobacilli isolates were identified by amplified with T7 and SP6 sequencing primers, PCR products were purified and applied for sequencing. **Antibacterial activity:** The antibacterial spectrum of the supernatants from the isolates was determined using disc fusion method Indicator strains: *Staphylococcus aureus* (ATCC 6538), *Salmonella* (DSM12443), *Listeria monocytogenes* (DSM 12464) and *E.coli* O157:H7(ATCC 11775).

Results

Isolates *B. bifidum* B11, B12, *L.plantarum HM1* and *L. rhamnosus* HM2 showed their antimicrobial activity against the used pathogen's strains by using cell filtrates. *L. rhamnosus* HM1 did not show any antimicrobial activity against all used pathogens as shown in Table 1. Our isolate strains showed different diameters of inhibition zones. The inhibition zone's diameters were ranged between 1.3 to 3.5 cm according to the isolate and the indicator strains. *Listeria monocytogenes* DSM 12464 and *Staphylococcus aureus* ATCC 6538 were highly affected with antimicrobial substance in lactobacilli and bifidobacteria cell filtrates.

Table (1): Inhibition zone diameter (cm) using culture cell filtrate of *B. bifid*um B11, B12, ,*L.plantarum* HM1 *and L. rhamnosus* HM2 strains without adjust the pH

Indicator strains	*B.*B11	*B.*B12	*Lp.*HM1	*Lc.*HM2
Staphylococcus aureus (ATCC 6538)	3.0	2.7	0	3.2
Salmonella (DSM 12443)	1.7	2.4	0	2.4
Listeria monocytogenes (DSM 12464)	1.8	1.9	0	1.6
E.coli O157:H7 (ATCC 11775)	3.1	3.7	0	3.7

Table (2): Effect of neutral pH culture cell filtrate of *B. bifidum* B11, B12 and *L. plantarum* HM1 and *L. rhamnosus* HM2 strains on the inhibition zone diameter (cm)

Indicator strains	*B.*B11	*B.*B12	*Lp.*HM1	*Lr.*HM2
Staphylococcus aureus (ATCC 6538)	2.8	2.7	2.4	3.0
Salmonella (DSM 12443)	1.7	2.4	2.0	2.4
Listeria monocytogenes (DSM 12464)	1.8	1.5	1.3	1.6
E.coli O157:H7(TCC 11775)	2.9	3. 7	3.1	3.7

Table 2: shows the effect of neutral pH of cell filtrate on indicator strains; neutral pH of culture cell filtrate of *B. bifidum* B11*, L. plantarum* HM1 and *L. rhamnosus* HM2 strains reduced inhibition zones' diameters of *L. monocytogenes* DSM 12464. However, neutralized pH of culture cell filtrates of *B. bifidum* B11, B12 and *L. rhamnosus* HM2 strains reduced inhibition zones' diameters of *S. aureus* ATCC 6538 and *E.coli* O157:H7(ATCC 11775), respectively, reduction in inhibation zones' diameters was ranged by 0.2 to 0.3 cm.

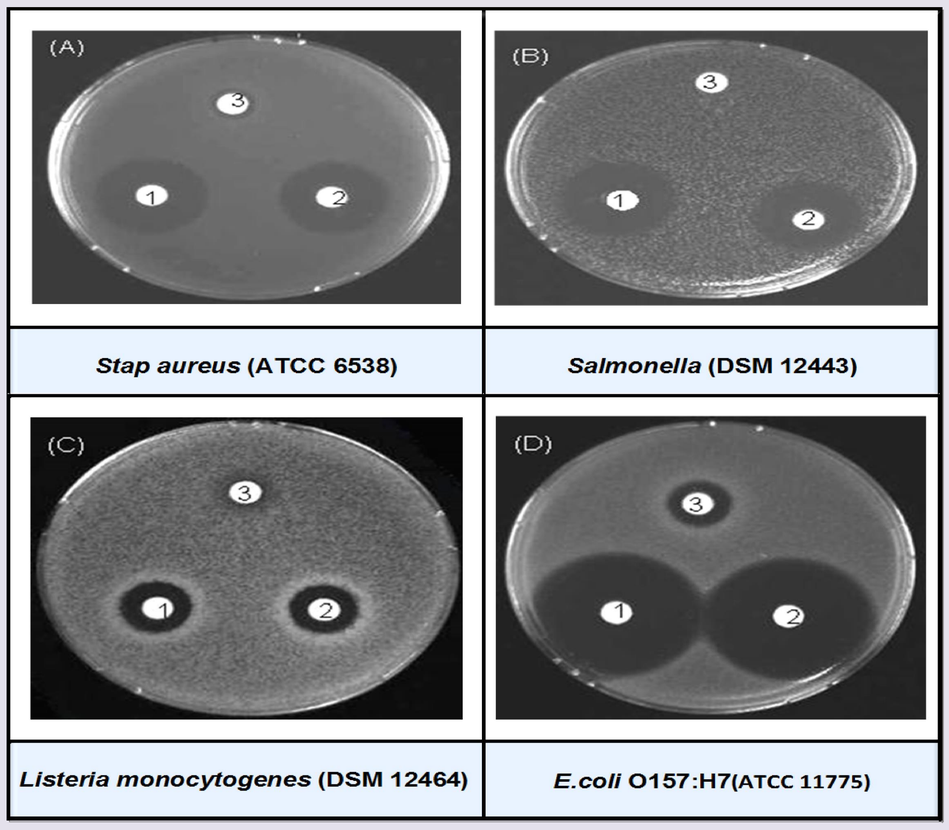

Fig (1):Effect of addition of proteinase K and neutralization of pH on the inhibition zones' diameter; 1) cell filtrate; 2) neutral pH cell filtrate and 3) digested cell filtrate with proteinase K.

Table 2 and Figure 1 show the effect of proteinase K on the inhibition zones' diameters. Observed inhibition zones' diameters were ranged from 0.7 to 3.7 cm. These differences in inhibition zones' diameters indicated that the cell filtrates contained different substances produced by the isolates.

Conclusion

These results indicate that isolated lactic bacteria can be used as control agent for food contamination by pathogenic bacteria as bio-preservation system.

We can conclude that, the application of antimicrobial compounds-producing protective cultures may provide an additional parameter of processing in order to improve the safety and ensure food keeping quality. The three *B. bifidum* (B11 and B12) *L.plantarum* (HM1) and *L. rhamnosus* HM2 strains used in this study should be considered as prebiotic strains with interesting potential for preventing contamination in food and enteric infections in humans.

References

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