Rational evaluation of antimicrobial properties of lactobacilli isolates against some pathogenic microorganisms: a new method comparing the susceptibility of indicator microorganisms

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Key words:
Aspergillus parasiticus, *Candida parapsilosis*, data organization method, *Escherichia coli* O157:H7, *Listeria monocytogenes*

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Received: 14 August 2013
Accepted: 30 September 2013

Abstract:

BACKGROUND: Lactobacilli are known as a valuable source of antimicrobial compounds and have a high potential of use in food biopreservation against food related microorganisms. **OBJECTIVES:** Antimicrobial potency of 63 dairy lactobacilli isolates against four highly important food-related microorganisms were evaluated. In addition, a new way in data organization was introduced, which led to a more informative and rational comparison of indicator microorganisms' susceptibilities to a set of compounds. Correlation of pH and antimicrobial properties was investigated. **METHODS:** Microbroth dilution assay was used to evaluate indicator microorganisms' susceptibility to lactobacilli CFCS (cell free culture supernatant). Results were organized by both the conventional way - demonstrating the minimum inhibitory and lethal concentrations of CFCS - and a new suggested method, representing comparative effectiveness of each CFCS specimen against indicator microorganisms of comparison interest. **RESULTS:** Susceptibilities of tested strains were in the following order: *Escherichia coli* O157:H7 > *Listeria monocytogenes* > *Aspergillus parasiticus* > *Candida parapsilosis*. Despite the high susceptibility of *L. monocytogenes*, it showed the highest resistance to death among the tested microorganisms. Eefficiency of Lactobacilli CFCS in killing the tested strains showed the following susceptibility order: *E. coli* O157:H7 > *A. parasiticus* > *C. parapsilosis* > *L. monocytogenes*. Antimicrobial property was in correlation with the pH value of CFCS. PH had a pronounced impact on susceptibilities of *C. parapsilosis* and *E. coli* in pH values of concentrated CFCS lower than 4 and 4.5, respectively. **CONCLUSIONS:** Potency of lactobacilli isolates in growth inhibition of the indicator microorganisms was found promising, and the suggested data organization method provided additional information, leading to more precise comparison of indicator microorganisms.

Introduction

Employing more effective and less adverse strategies in producing safe foodstuffs is an ever-growing demand. Public claim insists on reducing the additives, chemical preservatives, and physical interventions in food production (Guerrieri et al., 2009; Schnürer and Magnusson, 2005). Biocontroling can be preferably used as a suitable alternative to chemical preservation. Lactic acid bacteria (LAB), including lactobacilli, have a high potential of use in preservation of foods, regarding their long history of usage in foods, possessing GRAS (generally recognized as safe) status, having health promoting effects, and known antagonistic effects against food borne pathogens (Schnürer and Magnusson, 2005; Servin, 2004; Stiles, 1996). LAB also inhibit food spoilage fungi and can even interact with mycotoxins (Dalié et al., 2010).

Microorganisms' specifications may change in adaptation to their environments. Geoecological diversity of Iran as well as pronounced diversity of traditional dairy products among diverse human population with different cultural background raised curiosity of the researchers to evaluate antimicrobial properties of lactobacilli isolates obtained from milk and traditional dairy products throughout the country. The present research aimed at investigating antagonistic potential of the lactobacilli isolates against important food-related bacteria and fungi: *E. coli* O157:H7, *L. monocytogenes*, *A. parasiticus*, and *C. parapsilosis*.

Materials and Methods

63 Lactobacilli strains were isolated from milk and traditionally produced dairy products across the country to evaluate their antimicrobial properties.

Isolation procedure: Maximum recovery diluent was used for dilution of dairy samples to isolate consisting microorganisms from a proper dilution. Cheese samples were homogenized using a bag mixer before preparing the dilutions. 100 microliters of the prepared consecutive dilutions of each dairy sample were surface plated on de Man, Rogosa, and Sharpe (MRS) agar plates and incubated at 37 ^{o}C under microaerophilic condition for three days. All morphologically diverse colonies were picked and cultivated in MRS broth medium after being ensured of colony purity by sub culturing the selected colonies. 25% glycerol stocks of isolates were prepared from 24-hour cultivated microbial suspension. Incubation of slow growing isolates on MRS agar plates was continued for five days, and glycerol stocks were prepared from 72-hour cultured broth.

Preliminary identification of isolates: Probable Lactobacilli isolates were selected among the isolates with regard to gram staining, morphological examination, catalase, and motility tests. Gram positive rods with the growth ability on MRS medium and negative responses in both catalase and motility tests were assumed as lactobacilli.

Preparing the antimicrobial substances source: 100 mL MRS broth was inoculated by second subculture of isolated lactobacilli. Cell free supernatant was collected after 48 h incubation at 35°C by 10 min centrifugation at 10000 ×g at 4°C. Culture supernatants were lyophilized after addition of three mg/mL sucrose as lyoprotectant and filter sterilization through polyethersulfone membrane filters with 0.45 µ pore size. Lyophilized CFCS were resuspended in sterile distilled water to prepare CFCS specimens at 16 fold the initial concentration.

PH metery: Electrode pH meter was used to determine the pH values of 16 fold concentrated CFCS. PH was recorded after getting stable.

Indicator microorganisms: An O157:H7 serotype of *E. coli* (ATCC 700728), *L. monocytogenes* (ATCC 19115), *C. parapsilosis* (ATCC 22019), and *A. parasiticus* (ATCC 15517) were selected among Gram negative and Gram-positive bacteria, yeasts, and molds to be used as indicator microorganisms. They were selected with respect to their superior hygienic importance and their employment history as the standard strains in antimicrobial susceptibility tests.

Antimicrobial test procedure: Antimicrobial properties of isolated lactobacilli were evaluated using microbroth dilution assay according to M27-A3, M38-A2, and M07-A8 standards of Clinical and Laboratory Standards Institute (CLSI) with the following minor modifications.

RPMI-1640 and Cation-Adjusted Mueller- Hinton broth (CAMHB) media were prepared in double strength to provide the required concentration of media components after introducing to the equal

amount of the diluted CFCS within the wells. No buffer was added to the culture media; however, pH was adjusted to 7 and 7.2 for fungi and bacteria culture media, respectively.

Double strength culture media were inoculated by indicator microorganisms to provide final inoculum sizes of 2×10^5 to 8×10^5, 0.5×10^3 to 2.5×10^3 and 0.4×10^4 to 5×10^4 in the wells related to bacteria, yeast, and mold, respectively, according to CLSI standards of M07-A8, M27-A3, and M38-A2 (CLSI, 2008a; CLSI, 2008b; CLSI, 2009).

All plates were incubated at 35 ^{o}C without agitation, and the growth was examined after 48 hours except for *E. coli* whose growth was checked at hour 24 of incubation.

Round bottom 96 wells microplate was used to perform the assay.

MIC and MLC determination: MIC was defined as the lowest concentration to prevent any distinguishable growth.

For MLC determination, specimens were taken from wells with complete growth inhibition, after 48 hours of incubation. The sampling was done from the latest growth positive well as the last tolerated concentration and growth control well as controls. The specimens of *E. coli* related tests were taken 24 hours after the incubation. 10, 20, and 100 microliters samples were taken from the wells of bacteria, mold, and yeast assays, respectively. Specimens of *L. monocytogenes*, *E. coli*, and both fungi tests were cultured on Triptic Soy Agar-Yeast Extract (TSAYE), nutrient agar, and Sabouraud Dextrose Agar (SDA) plates, respectively. All inoculated plates were incubated at 35 ^{o}C until growth was seen in growth control related plates. The minimum CFCS concentration leading to a maximum two colonies formation on bacteria and mold related plates and one colony on yeast related plates were considered as MLC against the related indicator microorganism. The defined MLC represents killing potency of 97.5% to 99.8% against mold, 98% to 99.6% against yeast, and 99.9 to 99.95% against bacteria.

New method in comparing indicator microorganisms' susceptibility: MIC of each CFCS specimen against *C. parapsilosis* and *L. monocytogenes* was apportioned to the MIC against *A. parasiticus* and *E. coli*, respectively, to find out that the MIC against one fungus or bacterium strain how many times the other one is. Because the performed microbroth dilution assay had a twofold dilution order, the possible MIC ratios would have the same order. All probable MIC ratios and frequency of each observed ratio were tabulated. The CFCS specimens were clustered to different pH ranges to find out the pH impact on alteration of indicator microorganisms' susceptibility. Geometric averages of the MIC ratios for each pH range and for all tested lactobacilli CFCS were calculated to compare indicator micro-organisms' total susceptibilities and in each pH range.

The method was also employed to compare indicator microorganisms' susceptibility from MLC aspect.

Results

Table 1 represents consensus overview on potency of lactobacilli isolates CFCS in inhibiting the four nominated indicator microorganisms, gives a rough preliminary estimate of their susceptibility to the lactobacilli metabolites and also provides a comparison among them. Higher susceptibilities of *A. parasiticus* and *L. monocytogenes* than *C. parapsilosis* and *E. coli*, respectively, were presumable regarding to more lactobacilli CFCS capable in inhibiting them even with lower concentrations. The bacteria were more susceptible than the fungi according to independent samples T-test ($p<0.01$).

Table 1 also indicates average pH of CFCS specimens with similar MIC against each indicator microorganism. MIC values were in agreement with pH values as is seen in table 1, and the correlation was significant in 99% level for all the indicator microorganisms.

The figures 1 and 2 show the effectiveness of isolates CFCS, belonging to each pH, ranges in growth inhibition of tested microorganisms. *A. parasiticus* was generally more susceptible than *C. parapsilosis* with significant difference at 95% level in pH ranges below 5 (Figure 1a & 1b), while slightly more susceptibility of *E. coli* than *L. monocytogenes* was not found to be significant, according to independent samples T-test results (Figure 2a & 2b). There was no growth inhibition by CFCS specimens belonging to pH ranges higher than 5.5, 6, and 6.5 on the yeast, mold, and bacteria respectively.

Indicator microorganisms' susceptibilities to lactobacilli CFCS were also compared in a new way

to obtain more detailed information by comparing the effectiveness of each individual CFCS separately against either both bacteria or both fungi strains.

70 % of isolates CFCS showed a higher inhibitory effect on *A. parasiticus* than *C. parapsilosis*, while *C. parapsilosis* was more susceptible in 2.5 percent of CFCS specimens. Geometric mean of the MIC ratios indicated that 1.866 times the minimum required concentration to inhibit the mold growth was necessary for inhibiting the yeast strain, averagely (table 2). As shown in table 3, about 33.3% and 19.3% of CFCS specimens were more effective on *E. coli* and *L. monocytogenes*, respectively. Averagely, 1.157 times the minimum required concentration to inhibit the *E. coli* growth was necessary for inhibiting the *L. monocytogenes* (table 3). *A. parasiticus* and *E. coli* were significantly more susceptible than *C. parapsilosis* and *L. monocytogenes* in 99% and 95% levels, respectively, according to one sample T-test statistical analysis.

According to the introduced new data organization method, the killing potencies of each CFCS specimen against the bacteria were individually compared, and the results were stated in table 5 as MLC ratios. The same comparison was done between the killing potencies of each CFCS specimen against the fungi, and the obtained MLC ratios were stated in table 6. About 48.1% and 7.4% of the evaluated CFCS specimens had more killing potency on *A. parasiticus* and *C. parapsilosis*, respectively, than on the other indicator fungi. Averagely, about 1.508 times MLC against *A. parasiticus* was required to kill *C. parapsilosis* (table 5). About 84.4% of the evaluated CFCS were more potent in killing the *E. coli* than killing the *L. monocytogenes*. Only in 3.1% of CFCS specimens, higher concentrations of CFCS were needed to kill *E. coli*. Averagely, about 2.378 times MLC against *E. coli* was required to kill *L. monocytogenes* (Table 6). Higher susceptibilities of *A. parasiticus* and *E. coli* against killing concentrations of CFCS specimens than *C. parapsilosis* and *L. monocytogenes*, respectively, were found significant at 99% level using one sample T-test statistical analysis.

Discussion

Lactobacilli are known as a valuable source of antimicrobial compounds and have a high potential of use in biocontrolling the microorganisms of concern, in food industry and public health aspects.

A. parasiticus and *C. parapsilosis* were selected among fungi to be used as indicator microorganisms to evaluate antimicrobial properties of isolated lactobacilli. They were chosen because of their significant role in food spoilage and their importance as the life threatening fungi. *A. parasiticus* and *A. flavus* are the only aflatoxigenic fungi in food. Although *A. parasiticus* is less widespread than *A. flavus*, almost all its isolates are aflatoxigenic and capable of producing all naturally occurring aflatoxins; B1, B2, G1, and G2. *Candida parapsilosis* is an emerging and high ranked prevalent nosocomial pathogen, which is considered as an important etiology to invasive mycoses, especially in immune compromised patients and cases with implantation history (Hocking and Blackburn, 2006; Loureiro and Querol, 1999; Trofa et al., 2008).

E. coli O157:H7 and *L. monocytogenes* were the next indicator microorganisms, which were nominated among bacteria, because of their high importance as food-borne pathogens with emerging outbreaks and high fatality rate. Shiga toxin producing *E. coli* (STEC) and *L. monocytogenes* were respectively the causes of 308 and 25 outbreaks in US since 1998 till 2008. While they caused about 4 and almost zero percents of the food-borne outbreaks, they were respectively recognized as the causes of 11 and 25 percents of deaths occurred by food-borne pathogens in the same period of time (Gould et al., 2013b). Among the 23 deaths of food-borne outbreaks in US in 2009 and 2010, 9 were attributed to *L. monocytogenes* and 4 to STEC O157 (Gould et al., 2013a).

Antifungal property: Lactobacilli have been reported to be effective against *A. parasiticus* and *C. parapsilosis*. Lactobacilli from a commercial silage inoculum inhibited growth and aflatoxin production of *A. parasiticus* (Gourama and Bullerman, 1995). *Lactobacillus rhamnosus* RC007 and *Lactobacillus plantarum* RC009 reduced the *A. parasiticus* growth rate especially at 0.99 aw (Dogi et al., 2013). A mixed culture of lactobacilli and propionibacteria yield a total inhibition of *C. parapsilosis* (Schwenninger and Meile, 2004). 25% and less than 10% of lactobacilli isolated from vaginal tract of healthy women and

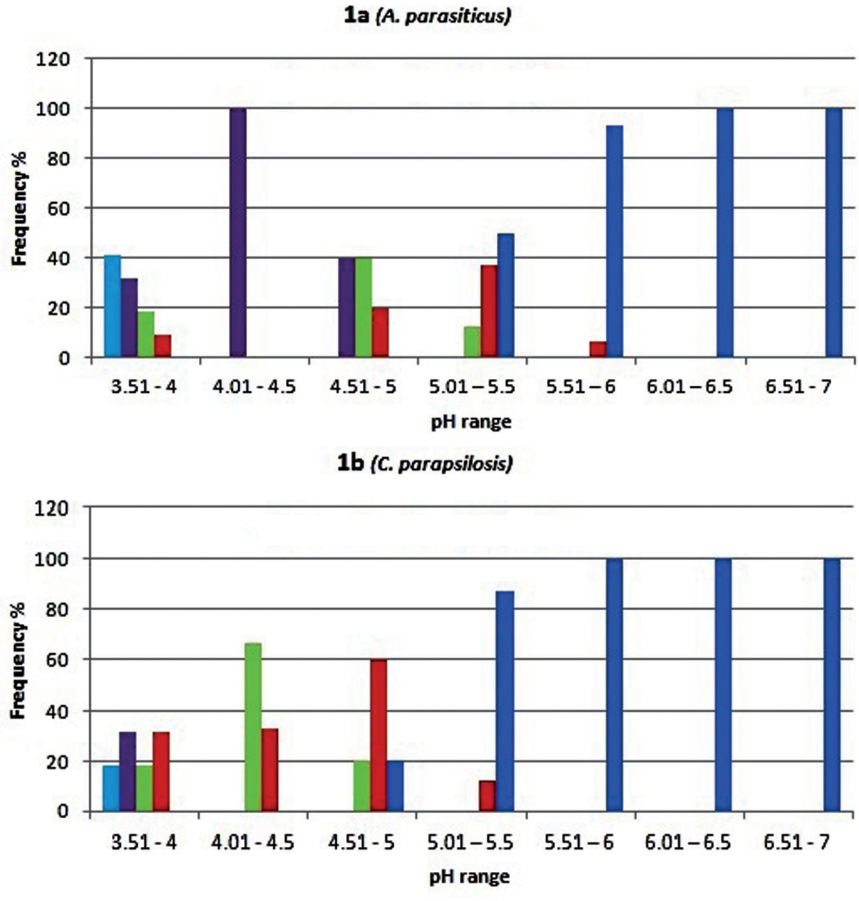

Figure 1. MIC (minimum inhibitory concentration) distribution of lactobacilli CFCS (cell free culture supernatant) belonging to each pH range against indicator fungi. X/2 1X 2X 4X ≥8X

women with bacterial vaginitis, respectively, showed antagonism against *C. parapsilosis* ATCC 22019 (Branco et al., 2010).

There are also several reports comparing susceptibilities of *C. parapsilosis* and *A. parasiticus*; however, their results are not comparable to our results because of undefined test conditions or several deviations in used culture media, incubation condition, inoculum size, and the tested strain. *Candida parapsilosis* ATCC 22019 was found to be more susceptible to hydroxychavicol than *A. parasiticus* MTCC 2796 (Ali et al., 2010). Anteiso-C17 mycosubtilin was more effective against *C. parapsilosis* than *A. Parasiticus* (Fickers et al., 2009). Susceptibilities of *C. parapsilosis* ATCC 22019 and *A. parasiticus* KCTC 6598 were compared in another study, and the results showed equal susceptibility to melittin and higher susceptibility of *C. parapsilosis* to papiliocin (Lee et al., 2010).

Our performed antifungal susceptibility test, meeting the CLSI recommendations, showed higher susceptibility of *A. parasiticus* ATCC 15517 than *C. parapsilosis* ATCC 22019 to the tested lactobacilli CFCS, especially by employing the new data organization method (Tables 1, 2, 4 & 5).

Antibacterial property: There are several reports of *E. coli* O157:H7 and *L. monocytogenes* inhibition by lactobacilli.

Ogawa et al. (2001) reported growth inhibition and killing activity of two probiotic lactobacilli against *E. coli* O157:H7 and suggested a correlation between lactic acid production level, pH, and antimicrobial effects (Ogawa et al., 2001). Lactobacilli strains showed reduction in *E. coli* O157:H7 population in both exposing in broth and vacuum-packaged fresh ground beef during storage at 5°C (Smith et al., 2005). In another study, all five indigenous *Lactobacillus sakei* strains inhibited growth of both *L. monocytogenes* and *E. coli* O157:H7 (Bredholt et al., 1999). In another study, four of the five lactobacilli strains isolated from retail meat cuts had inhibitory activity against all four

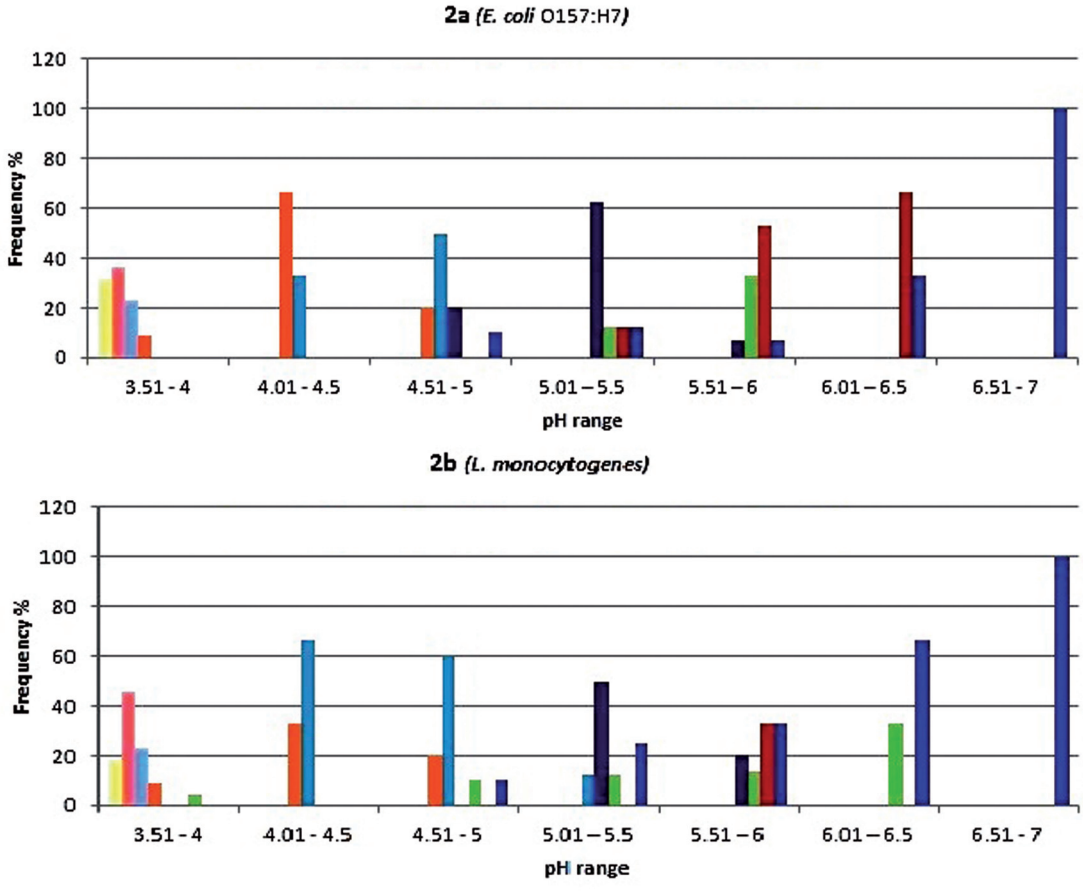

Figure 2. MIC (minimum inhibitory concentration) distribution of lactobacilli CFCS (cell free culture supernatant) belonging to each pH range against indicator bacteria. ≤ X/32 X/16 X/8 X/4 X/2 1X 2X 4X ≥8X

tested *L. monocytogenes* strains (Lewus et al., 1991). A strain of *Lactobacillus sakei* inhibited growth of 10^3 cfu/g of a cocktail of three rifampicin resistant mutant *L. monocytogenes* strains both at 8°C and 4°C (Bredholt et al., 2001). The isolates *Lactobacillus casei* AP8 and *Lactobacillus plantarum* H5 isolated from the intestinal flora of Sturgeon fish showed activity against *L. monocytogenes* ATCC 19115 (Ghanbari et al., 2013).

Our results represented in tables 3 and 6, indicating higher sensitivity of *E. coli* O157:H7 than *L. monocytogenes*, are in accordance with several reports.

Santos et al. in evaluation of antimicrobial activity of 58 lactobacilli isolated from Kefir found that 75% of isolates were effective against *E. coli* CECT 4076, while only 50 % could inhibit *L. monocytogenes* CECT 4032 (Santos et al., 2003). In determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of vanillin and mixtures with clove and cinnamon against *L. monocytogenes* Scott A and *E. coli* O157:H7, higher susceptibility of *E. coli* O157:H7 was revealed in various conditions, including different culture media with various pH and various incubation and storage temperatures (Cava-Roda et al., 2012). *Escherichia coli* O157:H7 showed also higher susceptibility to γ-irradiation than *L. monocytogenes* and 3 kGy dose could effectively eliminate these bacteria by more than 4 log and 3 log units, respectively (Badr, 2005).

There were lactobacilli CFCS with higher effectiveness against either of the tested indicator bacteria. Although Gram negative bacteria are usually more resistant to antimicrobial substances because of their rigid outer membrane, *Escherichia coli* ATCC 700728 was found to be more sensitive to isolated lactobacilli CFCS than *L. monocytogenes* ATCC 19115 in average, according to tables 3 and 6.

Antimicrobial effect - pH correlation: The observed MIC-pH relation in table 1 was further investigated by clustering the isolates CFCS specimens to different pH ranges and comparing the

Table 1. Consensus overview on growth inhibitory and pH characteristics of CFCS (cell free culture supernatant) of tested lactobacilli isolates. (*) X-times concentrated CFCS; Mold (*Aspergillus parasiticus*, ATCC 15517); Yeast (*Candida parapsilosis*, ATCC 22019); GNB (*Escherichia coli* O157:H7, ATCC 700728); GPB (*Listeria monocytogenes*, ATCC 19115).

	Minimum Inhibitory Concentration (CFCS concentrations)(*)									**Indicator microorganism**
	≥ 8 X	**4 X**	**2 X**	**1 X**	**X/2**	**X/4**	**X/8**	**X/16**	**≤ X/32**	
Frequency of isolates CFCS with related MIC	23	8	9	14	9	0	0	0	0	Mold
Avg. pH±SD	5.81± 0.4	4.81± 0.6	4.40± 0.5	4.16± 0.4	3.87± 0.1	-	-	-	-	
Frequency of isolates CFCS with related MIC	29	15	8	7	4	0	0	0	0	Yeast
Avg. pH±SD	5.67± 0.4	4.33± 0.4	4.18± 0.3	3.82± 0.1	3.84± 0.1	-	-	-	-	
Frequency of isolates CFCS with common MIC	23	3	2	3	3	0	0	0	0	Both fungi
Avg. pH ±SD	5.81± 0.4	4.30± 0.7	4.30± 0.5	3.85± 0.1	3.92± 0.	-	-	-	-	
Frequency of isolates CFCS with related MIC	6	11	6	8	6	6	5	8	7	GNB
Avg. pH ±SD	5.94± 0.8	5.80± 0.3	5.61± 0.2	5.12± 0.2	4.66± 0.1	4.32± 0.3	3.85± 0.1	3.82± 0.1	3.86± 0.1	
Frequency of isolates CFCS with related MIC	12	5	6	7	9	5	5	10	4	GPB
Avg. pH±SD	5.81± 0.6	5.86± 0.1	5.28± 0.7	5.34± 0.2	4.72± 0.2	4.27± 0.4	3.85± 0.1	3.83± 0.1	3.87± 0.1	
Frequency of isolates CFCS with common MIC	6	4	1	3	5	2	2	6	4	Both bacteria
Avg. pH±SD	5.94± 0.8	5.86± 0.2	5.79	5.12± 0.1	4.66± 0.2	4.48± 0.1	3.92± 0.1	3.83± 0.1	3.87± 0.1	

Table 2. Frequency of lactobacilli CFCS (cell free culture supernatant) representing the related MIC (minimum inhibitory concentration) ratio against the indicator fungi in each pH range. (*) CFCS specimens with no inhibition on both fungi were not included in the table; (**) sign ≥ was used wherever some CFCS didn't show inhibition on yeast and MIC value of ≥ 8 was considered for them; µg (geometric mean); Mold (*Aspergillus parasiticus*, ATCC 15517); Yeast (*Candida parapsilosis*, ATCC 22019).

PH ranges of CFCS	**Frequency in each pH range(*)**	**MIC against Yeast / MIC against Mold**							**μ_g in each pH range**	**Total μ_g**
		8	**4**	**2**	**1**	**1/2**	**1/4**	**1/8**		
3.51 - 4	22	1	2	9	9	1	0	0	1.604	
4.01 - 4.5	3	0	1	2	0	0	0	0	2.519	
4.51 - 5	10	0	3	6	1	0	0	0	≥ 2.297(**)	
5.01 - 5.5	4	0	1	2	1	0	0	0	≥ 2	≥ 1.866
5.51 - 6	1	0	0	1	0	0	0	0	≥ 2	
6.01 - 6.5	0	0	0	0	0	0	0	0	-	
6.51 - 7	0	0	0	0	0	0	0	0	-	

antimicrobial effectiveness of the specimens belonging to each cluster. Frequencies of various observed MIC values of specimens belonging to each cluster against the indicator microorganism were demonstrated in figures 1 & 2. Susceptibilities of the indicator strains in association to pH value are also comparable in these figures. The lower the pH, the higher the susceptibility of all tested strains, according to Kruskal-Wallis statistical analysis ($p<0.01$). In comparison of tested fungi, *A. parasiticus* was generally more susceptible than *C. parapsilosis*, and there was an increasing difference in susceptibility by decreasing the pH till 4 (Figure 1a & 1b). A reduction in fungi susceptibility difference to the CFCS specimens belonging to the lowest pH range was seen (Figure 1a & 1b), which was confirmed by comparison of indicator fungi susceptibility, using the new data organization method (Table 2). Down shifting the increasing susceptibility differences of fungi strains at the lowest pH range may be due to reduction of the yeast cells' resistance to acidic condition. Meeting the critical point of acid tolerance could be the reason of the yeast growth suppression in lower pH values.

Candida parapsilosis was shown to be able to grow at pH 2.5 at 22°C in the culture medium (Betts

Table 3. Frequency of lactobacilli CFCS (cell free culture supernatant) representing the related MIC (minimum inhibitory concentration) ratio against the indicator bacteria in each pH range. (*) CFCS specimens with no inhibition on both bacteria were not included in the table; (**) sign ≥ was used wherever some CFCS didn't show inhibition on GPB and MIC value of ≥ 8 was considered for them; μg (geometric mean); GNB (*Escherichia coli* O157:H7, ATCC 700728); GPB (*Listeria monocytogenes*, ATCC 19115).

PH ranges of CFCS	Frequency in each pH range(*)	MIC against GPB / MIC against GNB							μ_g in each pH range	Total μ_g
		8	4	2	1	1/2	1/4	1/8		
3.51 - 4	22	1	0	7	12	2	0	0	1.287	
4.01 - 4.5	3	0	0	1	2	0	0	0	1.260	
4.51 - 5	9	0	0	2	5	2	0	0	1	
5.01 - 5.5	7	1	0	0	3	3	0	0	≥ 1(**)	≥1.157
5.51 - 6	14	0	0	6	5	3	0	0	≥ 1.160	
6.01 - 6.5	2	0	0	1	0	1	0	0	≥ 1	
6.51 - 7	0	0	0	0	0	0	0	0	-	

Table 4. Frequency of MLC (minimum lethal concentration) values of lactobacilli CFCS (cell free culture supernatant) with inhibitory effect against indicator microorganisms. (*) The number of times concentrated CFCS; (**) Signs ≥ and ≤ were used wherever some CFCS didn't eliminate indicator microorganisms at the concentration 8X and eliminate them at the concentration X/32, respectively;. Mold (*Aspergillus parasiticus*, ATCC 15517); Yeast (*Candida parapsilosis*, ATCC 22019); GNB (*Escherichia coli* O157:H7, ATCC 700728); GPB (*Listeria monocytogenes*, ATCC 19115).

	Minimum Lethal Concentration (CFCS concentrations)(*)									Avg. MLC
	≥8 X	4 X	2 X	1 X	X/2	X/4	X/8	X/16	≤X/32	
Frequency of observed MLC values (out of 40 effective isolates CFCS against Mold)	10	11	4	11	4	-	-	-	-	≥3.62X(**)
Frequency of observed MLC values (out of 34 effective isolates CFCS against Yeast)	5	16	8	2	3	-	-	-	-	≥ 3.63X
Frequency of observed MLC values (out of 57 effective isolates CFCS against GNB)	17	9	3	2	5	5	6	7	3	(≥;≤) 3.25X
Frequency of observed MLC values (out of 51 effective isolates CFCS against GPB)	19	6	3	2	6	7	6	2	0	≥ 3.72X

Table 5. Frequency of lactobacilli CFCS (cell free culture supernatant) representing the related MLC (minimum lethal concentration) ratio against the indicator fungi in each pH range. (*) CFCS specimens with no defined MLC against either of fungi were not included in the table; μg (geometric mean); Mold (*Aspergillus parasiticus*, ATCC 15517); Yeast (*Candida parapsilosis*, ATCC 22019).

PH ranges of CFCS	Frequency in each pH range(*)	MLC against Yeast / MLC against Mold							μ_g in each pH range	Total μ_g
		8	4	2	1	1/2	1/4	1/8		
3.51 - 4	21	0	2	7	10	2	0	0	1.346	
4.01 - 4.5	3	0	2	0	1	0	0	0	2.520	1.508
4.51 - 5	3	0	1	1	1	0	0	0	2	

Table 6. Frequency of lactobacilli CFCS (cell free culture supernatant) representing the related MLC (minimum lethal concentration) ratio against the indicator bacteria in each pH range. (*) CFCS specimens with no defined MLC against either of bacteria were not included in the table; μg (geometric mean); GNB (*Escherichia coli* O157:H7, ATCC 700728); GPB (*Listeria monocytogenes*, ATCC 19115).

PH ranges of CFCS	Frequency in each pH ranges	MLC against GPB / MLC against GNB							μ_g in each pH range	Total μ_g
		8	4	2	1	1/2	1/4	1/8		
3.51 - 4	22	1	8	9	4	0	0	0	2.416	
4.01 - 4.5	2	0	1	1	0	0	0	0	2.828	
4.51 - 5	7	1	2	4	0	0	0	0	2.972	2.378
5.01 - 5.5	0	0	0	0	0	0	0	0	-	
5.51 - 6	1	0	0	0	0	0	1	0	0.25	

et al., 1999). Aspergillus strains were reported to be able to grow over the pH range 2 to 11 (Wheeler et al., 1991).

Although the susceptibility difference of the tested bacteria was not significant according to table 1, *E. coli* seemed to be slightly more susceptible than *L. monocytogenes*, especially in pH values lower than 4.5 (Figure 2a & 2b). This was confirmed and was found significant ($p<0.05$) by the results gathered from comparison of antibacterial effectiveness of each individual CFCS specimen against the tested bacterial strains, according to the new data organization method (Table 3). It may be due to higher susceptibility of *E. coli* to acidic condition than *L. monocytogenes*. Although both bacterial species tolerate high acidic conditions, it has been reported

that *L. monocytogenes* has higher tolerance to acidic condition. Minimum pH values for the growth of *E. coli* O157:H7 and *L. monocytogenes* are 4.5 and 4.1 respectively (Jay et al., 2005).

The suggested new method to data organization: Both the conventional and new suggested data organization methods were useful in assessing the results of antimicrobial tests and helped to have a more precise interpretation. While the conventional way of data organization provides useful information like MIC and MLC values, indicating the tested antimicrobial substances potency, the suggested data organization method is more beneficial in comparing the indicator microorganisms' susceptibility to various sets of antimicrobial substances. Whereas tables 1 and 4 represented the test results using conventional data organization method, tables 2, 3, 5, and 6 were consequences of the new suggested way in data organization. The new method provided some additional information such as the extent of the indicator strains' susceptibility difference to antimicrobial substances. In addition, it indicated the frequency of antimicrobial substances causing each degree of susceptibility differences between indicator microorganisms of comparison interest. In the new method, instead of averaging the observed MIC values, MIC ratios of antimicrobial substances against two indicator microorganisms were geometrically averaged, and the obtained value gave an immediate comparison of the strains' susceptibility.

This method is also applicable in analyzing the results of hurdle technology in evaluation of the effect of different variables such as pH and culture conditions, including culture media, incubation temperature, and presence of additives, on susceptibility changes of the indicator microorganisms. This method makes it easier to track susceptibility changes of the indicator microorganism in response to introduced variables.

Akcnowledgments

A very Special thank is offered to Fourough Ranaei for valuable assistance during the project.

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مجله طب دامی ایران، ۱۳۹۲، دوره ۷، شماره ۴، ۲۵۲-۲۴۳

بررسی مدل خصوصیات ضد میکربی جدایه‌های لاکتوباسیل علیه برخی از میکروارگانیسم‌های بیماریزا؛ روشی جدید جهت مقایسه حساسیت میکروارگانیسم‌های شاخص

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(دریافت مقاله: ۲۳ مرداد ماه ۱۳۹۲ ، پذیرش نهایی: ۸ مهر ماه ۱۳۹۲)

چکیده

زمینـه مطالعه: لاکتوباسیل‌ها بعنوان منبعی ارزشمند برای ترکیبات ضد میکربی شناخته می شوند و از پتانسیل بالایی جهت محافظت بیـولـوژیـک غـذا در بـرابـر میکـروارگـانیسم‌های مرتبط با آن برخوردارند. **هدف:** توان ضد میکربی ۶۳ جدایه لاکتوباسیل لبنی علیه چهار میکروارگانیسم مهم مرتبط با غذا سنجیده شد. همچنین روش جدیدی به منظور سازماندهی داده‌های حاصل از آزمون میکربی معرفی گردید که نتیجه‌آن مقایسه‌ای مستدل تر و بر پایه اطلاعات بیشتر از حساسیت میکروارگانیسم‌های شاخص به یک گروه از ترکیبات بود. ارتباط pH و خصوصیات ضد میکربی نیز سنجیده شد. **روش کار:** روش microbroth dilution assay جهت ارزیابی حساسیت میکروارگانیسم‌های شاخص به سوپ رویی عاری از سلول کشت مایع (cell free culture supernatant; CFCS) لاکتوباسیل‌ها مورد استفاده قرار گرفت. سازماندهی و پردازش نتایج حاصل از آزمون‌های میکربی به هر دو روش سنتی (بیان مقادیر حداقل غلظت ممانعت کننده رشد و حداقل غلظت کشنده) و روش پیشنهادی جدید که قدرت اثر مقایسه‌ای هر نمونه CFCS علیه میکروارگانیسم‌های شاخص مقایسه شونده را نشان می دهد، صورت گرفت. **نتـایـج:** حساسیت سویه های تست شده از ترتیب زیر تبعیت کرد: *E. coli* O157:H7 > *L. monocytogenes* > *A. parasiticus* > *C. parapsilosis*. لیستریا مونوسایتوژنز برخلاف حساسیت بالا، بیشترین مقاومت را نسبت به مرگ در میان میکروارگانیسم‌های آزمایش شده از خود نشان داد. میزان کارامدی CFCS لاکتوباسیل‌ها در کشتن سویه های تست شده، ترتیب حساسیت زیر را نشان داد: *E. coli* O157:H7 > *A. parasiticus* > *C. parapsilosis* > *L. monocytogenes*. بین میزان pH و خصوصیت ضد میکربی ارتباط وجود داشت. PHهای CFCS غلیظ شده با مقادیر کمتر از ۴ و ۴/۵ اثر مشخصی را به ترتیب بر روی حساسیت *C. parapsilosis* و *E. coli* داشتند. **نتیجه‌گیری نهایی:** توانایی لاکتوباسیل‌ها در ممانعت از رشد میکروارگانیسم‌های شاخص امیدوارکننده بود و روش پیشنهادی برای سازماندهی داده‌ها، با فراهم نمودن اطلاعات بیشتر موجب مقایسه دقیق‌تر میکروارگانیسم‌های شاخص گردید.

واژه‌های کلیدی: آسپرژیلوس پارازیتیکوس، کاندیداپاراپسیلوسیس، روش سازماندهی اطلاعات، اشریشیاکولای O157:H7، لیستریا مونوسایتوژنز

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