International Journal of

Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 7 (3): 63-69, 2012
ISSN 1811-9743 / DOI: 10.3923/ijds.2012.63.69

Effect of Bacterial Interactions on Antimicrobial Compound Production by Lactic Acid Bacteria

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ABSTRACT

In the present study interaction between three lactic acid bacteria species and three food borne pathogenic bacteria species were studied. The objective of the study was to determine the effect of those interactions to the antimicrobial compound producing ability of lactic acid bacteria. Using the three lactic acid bacteria namely, *Lactococcus lactis*, *Lactobacillus acidophilus* and *Lactobacillus casei* and three pathogenic bacteria namely, *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* 56 different culture combinations were prepared and grown in a medium invented by de Man, Rogosa and Sharpe (MRS) broth. Then Cell Free Filtrates (CFF) containing antimicrobial compounds were obtained from all those cultures and tested for antimicrobial activity against the three pathogenic microorganisms. All Cell Free Filtrates (CFF) were found to be having antibiotic activity against tested three pathogenic bacteria. Antibiotic activity of Cell Free Filtrates (CFF) obtained from cultures contained both lactic acid bacteria and some pathogenic bacteria were significantly higher than cultures contained only lactic acid bacteria. However, this stimulating effect of pathogenic bacteria on antimicrobial compound production by lactic acid bacteria was different for different pathogenic bacteria. Stimulation by *Escherichia coli* was not observed and some times *Escherichia coli* shown to be reducing the antimicrobial compound production. Antibiotic activity was found to be higher in mixed cultures of lactic acid bacteria compared to individual cultures. Results of this study confirm the importance of using mixed cultures and effective stimulant bacteria in antimicrobial compound production by lactic acid bacteria.

Key words: Lactic acid bacteria, antimicrobial compounds, bacterial interactions, pathogenic bacteria, cell free filtrates

INTRODUCTION

Consumption of contaminated food leads to food-borne diseases which cause significant morbidity and mortality. As such consumers should be provided with good quality, safe and nutritious food (Tambekar and Bhutda, 2006). Many reports are available on contamination of different dairy products by pathogenic microorganisms from different parts of the world (Tambekar and Bhutda, 2006; Abdalla and El-Zubeir, 2006; Fadel and Ismail, 2009). Different food processing techniques are used to ensure microbial safety. However, most of these techniques are affecting the nutritional, medicinal and sensory properties of food. Hence, there is a growing trend towards less processed foods. As a consequence, bacterial antagonism has received considerable attention in food preservation (Aguilar *et al*., 2011). Lactic acid bacteria are one of the major bacterial groups present in the milk with potential to produce antimicrobial compounds. Antagonistic action of these lactic acid bacteria, over harmful bacteria, makes many traditional and commercial food microbially safe (Aguilar *et al*., 2011). These lactic acid bacteria can be used for the delivery of proteins of interest in foodstuff as well as in the digestive tract because they are considered as food grade bacteria (Kumari *et al.*, 2008). These bacteria have been isolated from different dairy products and studied to be used in food preservation (Mirhosaini *et al.*, 2006, 2010; Ali, 2011; Yateem *et al.*, 2008). Lactic acid bacteria produce lactic acid and other antimicrobial compounds such as diacetyl, acetoin, hydrogen peroxide, acetate, ethanol, carbon dioxide and bacteriocins which are active generally against closely related bacterial species (De Vuyst and Vandamme, 1994; Galvez *et al.*, 2007; Sharma *et al.*, 2010). The antibacterial effect of lactic acid bacteria on pathogenic microbes has been well studied (Mojgani and Amirnia, 2007; Mirhosaini *et al.*, 2006; Feresu and Nyati, 1990; McMullen and Stiles, 1996; Faith *et al.*, 1997; Bredholt *et al.*, 2001; Ogwaro *et al.*, 2002). Number of studies are been conducted to optimize the conditions to obtain higher antimicrobial compound production as well (Sharma *et al.*, 2010; Mojgani and Amirnia, 2007; Kumari *et al.*, 2008; El-Sayed *et al.*, 2008; Vamanu, 2011). Presence of other microorganisms in mixed cultures could effect the ability of antimicrobial compound production by lactic acid bacteria. However, studies on this aspect are very limited (Aguilar *et al.*, 2011). Understanding of the interactions between the involved microorganisms, in different food systems, is very important in successful application of lactic acid bacteria to control these microorganisms. These information are spicily important in selections of lactic acid bacteria for mix-cultures as well as in the selection of stimulant bacteria in antimicrobial compound production using lactic acid bacteria (Leroy *et al.*, 2002). As such, the present study was conducted to investigate the interactions between three lactic acid bacteria and three pathogenic bacteria in relation to antimicrobial compound production.

MATERIALS AND METHODS

Preparation of pathogenic bacterial cultures: *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* were cultured for 6 h at 36°C using universal culture medium, Tryptic Soy Broth Yeast Extract medium (TSBYE). Then sample of each culture were kept in refrigerated conditions. The cultures were then serially diluted (10^{-1}-10^{-10}) in sterile distilled water and enumerated in Tryptic Soy Agar (TSA) plates. Bacterial concentration was estimated by calculating the average number of colonies on plates containing 30-300 colonies. Then cultures containing 10^{3} CFU mL^{-1} bacterial concentrations were prepared using the each bacterial culture kept in refrigerated conditions. Those prepared cultures were used to study the effect of interactions between lactic acid bacteria and pathogenic bacteria for the antimicrobial compound production by antibacterial activity assays.

Preparation of lactic acid bacterial cultures: Three bacteria with antibacterial potential namely, *Lactococcus lactis*, *Lactobacillus acidophilus* and *Lactobacillus casei* were used in this study. Different combinations (56) of those lactic acid bacteria and pathogenic bacteria (Table 1) were cultured in MRS broth at 35°C for 24 h. One milliliter of each bacterial solutions of 10^{3} CFU mL^{-1} bacterial concentrations were used to inoculate 250 mL of MRS broth in each case. After incubation, incubated cultures were used to obtain Cell Free Filtrates (CFF) to test for antimicrobial activity.

Assessment of antibacterial activity: By centrifugation of incubated cultures (56) for 12000 g at 4°C for 10 min, cell-free culture supernatants were obtained. Then those supernatants were filtered using membrane filter with membrane of 0.45 μm pore size and Cell Free Filtrates

Table 1: Prepared culture combinations of lactic acid and pathogenic bacteria

	Pathogenic bacteria							
Lactic acid bacteria		Pa-1+Pa-2+Pa-3	Pa-1+Pa-2	Pa-1+Pa-3	Pa-2+Pa-3	Pa-1	Pa-2	Pa-3
La-1+La-2+La-3	C1	C8	C15	C22	C29	C36	C43	C50
La-1+La-2	C2	C9	C16	C23	C30	C37	C44	C51
La-1+La-3	C3	C10	C17	C24	C31	C38	C45	C52
La-2+La-3	C4	C11	C18	C25	C32	C39	C46	C53
La-1	C5	C12	C19	C26	C33	C40	C47	C54
La-2	C6	C13	C20	C27	C34	C41	C48	C55
La-3	C7	C14	C21	C28	C35	C42	C49	C56

La-1 to La-3 are *Lactococcus lactis, Lactobacillus acidophilus* and *Lactobacillus casei* respectively, Pa-1 to Pa-3 are *Salmonella enterica, Escherichia coli* and *Listeria monocytogenes*, respectively, C1-C56 are cultures prepared by different combinations of lactic acid and pathogenic bacteria

(CFF) were obtained. The obtained Cell Free Filtrates (CFF) were then stored at 4°C until use which is less than 30 min. Then those cell free filtrates were tested for antibacterial activity against prepared 10^{3} CFU mL^{-1} concentration cultures of *Salmonella enterica, Escherichia coli* and *Listeria monocytogenes*. From each above cultures, one milliliter were cultured on nutrient agar plates. Then, with the help of a sterile cork borer wells of 4 cm diameter were prepared on those plates. Fifty micro liter of each prepared cell free filtrate were added to those wells and incubated at 37°C for 24 h. After incubation, clear circular zone of inhibition were observed around the wells. Diameters of those inhibition zones were measured and those measurements were used to evaluate the antibacterial activity of each CFF.

RESULTS AND DISCUSSION

Antagonistic activity of food grade microorganisms on food pathogen is been recognized as a method of food preservation. This method is considered as a better method over other food preservation techniques as other techniques affect important food qualities. These food grade microorganisms which produce antimicrobial compound can be directly added to the food or the antimicrobial compounds that they produce can be extracted and added to the foods. Some times group of two or three of these microorganisms are added to food expecting higher antibacterial activity. When those microorganisms are added to the food they have to grow with autochthonous microorganisms in food. These other microorganisms in food can have inhibitory or inducing effect to a particular antimicrobial compound producing microorganism as well as to its antimicrobial compound production. Most of the intrinsic extrinsic and implicit factors that effect to the production of antimicrobial compounds by microorganisms been well studied (Aguilar *et al.*, 2011). However, effect of microbial interaction in mix-cultures to the production of antimicrobial compound has not been well studied. In the present study, the effect of interaction between different lactic acid bacteria and interaction between pathogenic bacteria and lactic acid bacteria to the antimicrobial compound production was investigated. The antimicrobial effect of CFF obtained from 56 different culture combinations on three pathogenic bacteria and three lactic acid bacteria was studied and results are shown in the Table 2.

All 56 CFFs showed antimicrobial activity against three pathogenic bacteria tested *Salmonella enterica, escherichia coli* and *listeria monocytogenes*. This shows the ability of three lactic acid bacteria to produce antimicrobial compound in all tested culture types, individual lactic

Table 2: Results of antimicrobial assay for CFF obtained from prepared 56 culture combinations

	Pathogenic bacteria used to test antimicrobial activity		
Culture used to obtain CFF	*Salmonella enterica*	*Escherichia coli*	*Listeria monocytogenes*
C1	++	++	++
C2	++	++	++
C3	++	+	++
C4	++	++	++
C5	+	+	+
C6	+	+	+
C7	+	+	+
C8	+++++	++++	+++++
C9	+++++	+++++	+++++
C10	+++++	+++++	+++++
C11	+++++	++++	+++++
C12	++++	++++	++++
C13	++++	+++	++++
C14	++++	++++	++++
C15	++++	++++	++++
C16	++++	++++	++++
C17	++++	+++	++++
C18	++++	+++	++++
C19	++++	++++	++++
C20	++++	++++	++++
C21	++++	++++	++++
C22	+++++	+++++	+++++
C23	+++++	++++	+++++
C24	+++++	+++++	+++++
C25	+++++	+++++	+++++
C26	+++++	++++	+++++
C27	+++++	+++++	++++
C28	+++++	+++++	+++++
C29	++++	+++	++++
C30	++++	++++	++++
C31	++++	++++	++++
C32	++++	++++	++++
C33	++++	+++	++++
C34	++++	+++	++++
C35	++++	++++	++++
C36	+++	+++	+++
C37	+++	+++	+++
C38	+++	++	+++
C39	+++	++	+++
C40	+++	+++	+++
C41	+++	++	+++
C42	+++	+++	+++
C43	+++	+++	+++
C44	++	++	++
C45	++	++	++
C46	++	+	++
C47	++	++	++

Table 2: Continued

	Pathogenic bacteria used to test antimicrobial activity		
Culture used to obtain CFF	*Salmonella enterica*	*Escherichia coli*	*Listeria monocytogenes*
C48	++	+	++
C49	++	++	++
C50	+++	++	+++
C51	+++	+++	+++
C52	+++	+++	+++
C53	+++	+++	+++
C54	+++	+++	+++
C55	+++	++	+++
C56	+++	++	+++

+, ++, +++, ++++ and +++++ zone diameter less than 5 mm, zone diameter 5-10 mm, zone diameter 10-15 mm, zone diameter 15-20 mm and zone diameter higher than 20 mm, respectively

acid bacteria cultures, mixed cultures of lactic acid bacteria as well as mixed cultures of lactic acid bacteria and pathogenic bacteria. These results are in agreement with the results of previous studies conducted by Mirhosaini *et al*. (2006), Yateem *et al*. (2008) and Sharma *et al*. (2010).

As per these results, no need of an external stimulus provided by additional bacterial population in the same growth medium to start the production of antimicrobial compounds. However, quantitative deference in antimicrobial compound production between individual cultures and mixed cultures of lactic acid bacteria were observed. These results are in agreement with the results of studies conducted by Maldonado *et al*. (2004) and Rojo-Bezares *et al*. (2007), on the effect of externally added stimulant bacterium on antimicrobial compound production. Individual cultures had lowest antimicrobial compound production and mixed cultures showed comparatively higher antimicrobial compound production (compare the results of culture 5, 6 and 7 with culture 1, 2, 3 and 4). These results indicate that there is a stimulating effect for the antimicrobial compound production by particular lactic acid bacteria from other lactic acid bacteria in the same medium. Hence, mixed cultures of lactic acid bacteria found to be more effective compared to individual cultures in antimicrobial compound production.

Highest antimicrobial compound production was observed in mixed cultures of lactic acid bacteria and pathogenic bacteria (results of culture 8, 9, 10, 11, 22, 23, 24, 25, 26, 27 and 28). This indicates that, the stimulation of antimicrobial compound production by particular lactic acid bacteria by pathogenic bacteria is higher than that of other types of lactic acid bacteria. Hence, even for mixed cultures of lactic acid bacteria, pathogenic bacteria can be used as a stimulant to increase the antimicrobial compound production. This information would be useful in industrial production of antimicrobial compounds.

The results of this study confirmed the importance of external stimulus provided by additional bacterial population to increase antimicrobial compound production by lactic acid bacteria as previously demonstrated by Maldonado *et al*. (2004) and Rojo-Bezares *et al*. (2007). Hence, in the selection of bacteria to produce commercial mixed cultures, information about the interactions between bacteria related to antimicrobial compound production should be considered to have effective commercial mixed cultures.

Compared to *Escherichia coli*, higher antimicrobial effect of all CFFs against *Salmonella enterica* and *Listeria monocytogenes* was observed. In some previous studies antimicrobial effect of lactic acid bacteria on *Listeria monocytogenes* was found to be higher than that of other bacteria (Mirhosaini *et al.*, 2006). Hence, the results of this study are in agreement with previous studies but provide comparatively more information. Further, the antimicrobial effect of CFFs obtained from some *Escherichia coli* added cultures was lower than that of other cultures (compare the results of cultures 36-42 and 50-56 with culture 43-49). Similar results were observed in cultures where they added with some other pathogenic microorganisms as well (compare the results of culture 15-21 and 29-35 with culture 22-28). This shows that there is a resistance for antimicrobial compound and competitive affect on antimicrobial compound production by *Escherichia coli*. So, *Escherichia coli* should not be selected as stimulant for antimicrobial compound production by tested three lactic acid bacteria. Further, interaction between lactic acid bacteria and bacteria used as stimulant should be well studied in the selection of stimulant bacteria for industrial antimicrobial compound production. In some cases, targeted pathogenic bacteria might not be the suitable stimulant in antimicrobial compound production as in the case of *Escherichia coli* in this study.

Results of this study confirmed the importance of information on bacterial interaction in mixed cultures to select the best combination of lactic acid bacteria and best stimulant bacteria in antimicrobial compound production. However, other factors such as medium composition, medium pH, incubation temperature, incubation time etc. are also effect the antimicrobial compound production. As such, in the selection of bacteria for commercial mixed cultures for antimicrobial compound production, effect of above factors in different mixed cultures also should be studied.

CONCLUSION

Results of this study confirmed that there is an effect of microbial interaction in mixed cultures for the antimicrobial compound production by lactic acid bacteria. When antimicrobial compound production is concerned, this is a stimulatory effect. So mixed cultures of lactic acid bacteria are more effective in antimicrobial compound production compared to individual cultures. Stimulation ability of some pathogenic bacteria was found to be higher than that of lactic acid bacteria. Hence, mixed cultures of some selected lactic acid bacteria and pathogenic bacteria would be more effective in industrial antimicrobial compound production.

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