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Effect of *Streptococcus thermophilus* supernatant and inulin on *Listeria monocytogenes* in soft cheese

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Abstract
The antilisterial activity of 0.1, 0.5 and 1 ml of both *Streptococcus thermophilus* supernatant (as derived probiotics) and inulin (as prebiotics) in soft cheese for three co-incubation periods (24,48 and 72 h) was evaluated. The results showed that the supernatant of *S. thermophilus* inhibited growth of *Listeria monocytogenes* at all co-incubation periods, especially when used 1 ml of supernatant (3 log reduction at 24 h and 2 log reduction at 48 and 72 h) compared with control (*L. monocytogenes* only). The *S. thermophilus* supernatant at 0.5 and 1 ml was more effective in reducing growth of *L. monocytogenes* compared with 0.1 ml of supernatant. No inhibition activity of inulin against *L. monocytogenes* was observed at all co-incubation periods. These results indicate that *S. thermophilus* supernatant has a protective action against *L. monocytogenes* in soft cheese.

Keywords: *Streptococcus thermophilus*, *Listeria monocytogenes*, Antilisterial activity, Inulin, Soft cheese.

1.Introduction

Streptococcus thermophilus is a Gram-positive facultative anaerobe, oxidase- and catalase-negative organism that is nonmotile, non-spore forming and homofermentative. It is also classified as a lactic acid bacteria (LAB) (Hardie & Whiley 1995; Robinson *et al*.2002). These bacteria may have probiotic properties and they have a long history of safe use as starter culture bacteria(Akpinar *et al*.2011).Probiotics are defined as live microorganisms in food stuffs which, when consumed at certain levels in nutrition stabilizes the gastrointestinal tract microflora thereby conferring health benefits on the consumer (FAO/WHO 2001). Beneficial bacteria (probiotics) and probiotic-derived factors have the potential to ameliorate disorders of the intestine. Recently, advantageous effects of probiotics have been reported in inflammatory bowel disease, colitis, diarrhea, enteric infection, irritable bowel syndrome, colon cancer and chemotherapy-induced mucositis (Whitford *et al*.2009).

Antimicrobial substances are produced by a wide range of bacteria, including dairy starter cultures. LAB can produce antimicrobial substances with the capacity to inhibit the growth of pathogenic and spoilage microorganisms. Lactic acid, acetic acid, formic acid, fatty acids, ethanol, hydrogen peroxide, diacetyl and bacteriocins are included among these compounds (Šušković *et al*.2010 ; De Vuyst & Leroy 2010 ; Ukeyima *et al*.2010). LAB isolates showed antagonistic activity against *Listeria monocytogenes* (Anna *et al*.2012). Mantovani and Russell (2003) reported that *L. monocytogenes* is sensitive to a variety of bacteriocins produced by LAB (e.g. nisin, pediocin, lactococcin, etc.) .

S. thermophilus is used primarily as starter cultures to counter the harmful bacteria grow in cheese and yogurt making/preservation processes. These bacteria produce bacteriocins having the antimicrobial activities against both Gram positive and Gram negative bacteria. *S. thermophilus* bacteriocins have been reported to have a broad inhibitory spectrum against several bacteria; *L. monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Yersinia pseudotuberculosis* , *Yersinia enterocolitica* and *Clostridium tyrobutyricum* (Aslam *et al*.2011). Also the lactic acid in yoghurt produced by *Lactobacillus bulgaricus* and *S. thermophilus* decreases pH, so inhibiting diarrhea-causing pathogenic bacteria (Nurhajati *et al*.2008).

The inulin acts as "prebiotic", prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health.Inulin type fructans naturally occur in many edible plants as storage carbohydrates. They are present in leek, onion, garlic, wheat, chicory, artichoke, and banana (Caralampopoulos & Rastall 2009).

L. monocytogenes is a food-borne human pathogen that can cause the highly fatal infection listeriosis. The number of listeriosis cases has increased in recent years (Christensen *et al*.2011).Since *L. monocytogenes* exhibits resistance towards heat and cold stress it can proliferate in food processing environments and thus colonize dairy and meat products which have caused several outbreaks as well as sporadic cases of listeriosis (Kuenne *et al*.2010) . Because *L. monocytogenes* has been used as a model organism to study the efficacy of many different preservation methods, this study was aimed to evaluate the antilisterial activity of *S. thermophilus*

supernatant and inulin against it in soft cheese.

2.Materials and Methods

Bacterial isolates and culture media:

The bacterial isolates, *Listeria monocytogenes* (isolated from Iraqi cheese) and *Streptococcus thermophilus* that were used in this study were obtained from Department of biology/College of Science/Al-Mustansiriya University/Baghdad/ Iraq. *L. monocytogenes* was grown in brain heart infusion broth(Hi-Media / India) , while *S. thermophilus* was grown in de Man Rogosa and Sharpe (MRS) broth (Hi-Media / India).

Cheese sample:

sample of soft cheese was collected from the local market inside ice box and transferred immediately to the laboratory .

Preparation of *Streptococcus thermophilus* supernatant :

Streptococcus thermophilus was cultivated overnight in MRS broth at 37°C. After centrifugation at 6000 rpm for 20min, the cell-free supernatant was filtered through 0.22µm filters and concentrated threefold (Kang & Lee 2005).

Antilisterial activity:

Five grams of cheese sample was inoculated into distilled water and sterilized for 15 min , after cooling, it was inoculated with *L. monocytogenes* (10^5cell/ml) and homogenized for 15 min. The antilisterial activity of *S.thermophilus* supernatant (as derived probiotics) and inulin(10% w/v) (as prebiotics) was quantified by adding 0.1, 0.5 and 1 ml from each of both , separately to *L. monocytogenes* homogenizer. The homogenizer of *L. monocytogenes* without *S.thermophilus* supernatant or inulin was served as control. All the treatments and control were incubated at 37^{o}C for 24,48 and 72 h. After each incubation period, serial ten-fold dilutions (up to 10^{-6})was made, 100 µl of each dilution was cultured on nutrient agar (Hi-Media / India) and incubated at 37°C for 24 – 48 h. The log10 CFU/ml was counted and the inhibition activity was evaluated for all treatments at all three incubation periods.

3.Results and Discussion

Antilisterial activity of *S. thermophilus* supernatant and inulin was assayed by co-incubation of *L. monocytogenes* with both of them , separately in soft cheese for three different periods. The results showed that 1 ml of bacterial supernatant reduced the viable count of *L. monocytogenes* by 3 log units at 24 h and 2 log units at 48 and 72 h, respectively comparison with control. 0.5 ml of the supernatant reduced the viable count of *L. monocytogenes* by 3 log units at 24 h and 1 log unit at 48 and 72 h, respectively comparison with control. On the other hand , 0.1ml of *S. thermophilus* supernatant resulted in a reduction of the viable count of *L. monocytogenes* by one log unit at 24 h , no inhibition of bacterial growth was observed with 0.1 ml of the supernatant at 48 and 72 h of co-incubation (Figures 1,2 and 3). In this regard, some studies have shown that the growth of *L. monocytogenes* was only reduced at a level of 10^3 CFU / ml in taleggio and in milk in the presence of bacteriocinogenic strains (Benkerroum *et al*.2000) .

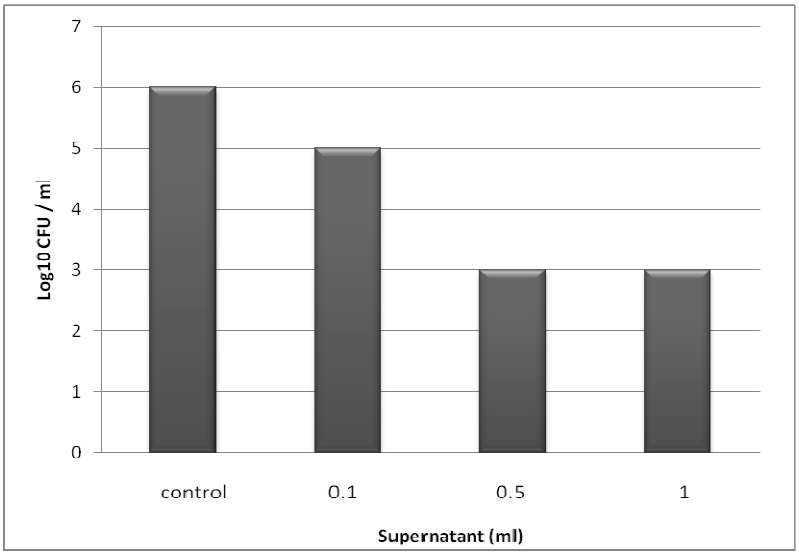

Figure 1. Effect of *Streptococcus thermophilus* supernatant on *Listeria monocytogenes* growth in soft cheese at 37ºC for 24 hours.

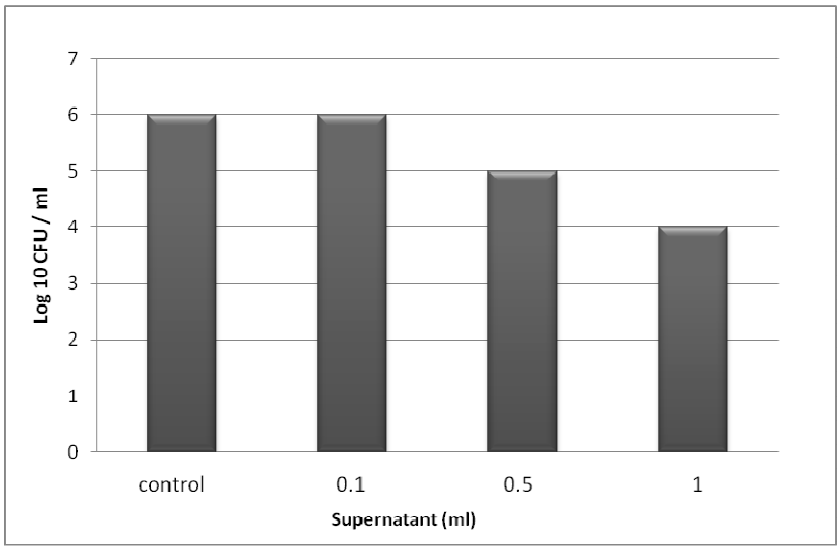

Figure 2. Effect of *Streptococcus thermophilus* supernatant on *Listeria monocytogenes* growth in soft cheese at 37ºC for 48 hours.

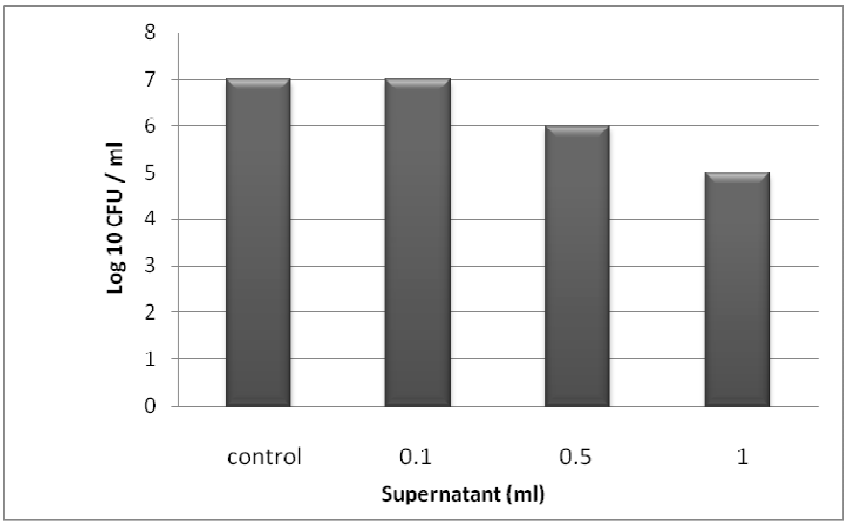

Figure 3. Effect of *Streptococcus thermophilus* supernatant on *Listeria monocytogenes* growth in soft cheese at 37ºC for 72 hours.

One explanation of the inhibition activity of supernatant against *L. monocytogenes* is the synergistic effect of lactic acid and bacteriocin in supernatant which caused the reduction of the pathogen viable count. Mezani *et al* .(2009) observed that the supernatant of *S. thermophilus* had broad spectrum activity against Gram-positive bacteria. Salman *et al* .(2009) showed that the concentrated supernatant of *S. thermophilus* had inhibitory activity against pathogenic bacteria; *Pseudomonas aeruginosa* , *Klebsiellasp, Staphylococcus aureus* and *Escherichia coli.*

S. thermophilus displays an inhibitory spectrum toward related Gram-positive bacteria, including pathogens such as *L.monocytogenes* (Fontaine & Hols 2008). *S. thermophilus* isolated from yogurt had shown antimicrobial activity against some foodborne pathogen and spoilage microorganisms especially *L. monocytogenes*, *E. coli*, *K. pneumonie* and *P. fluorescens* (Akpinar *et al*.2011).

No inhibition of *L. monocytogenes* was observed by inulin at all co-incubation periods (data not shown). Few studies investigated the antimicrobial activity of inulin, but no studies investigated the antilisterial activity of inulin. Gibson(1999) showed that the inulin reduced the amount of harmful bacteria such as Bacteroides, Fusobacteria and Clostridia. Salman *et al* .(2013) observed the antimicrobial activity of Inulin (10 %) against some pathogenic bacteria; *E. coli* , *Proteus mirabilis* , *Klebsiella sp* , *Pseudomonas aeruginosa* and *Serratia marcescens*. However, inulin has been shown to decrease the numbers of pathogenic bacteria such as *E.* coli , Clostridia and Staphylococci (Mantovani & Russell 2003).

Conclusion:

Depending on the results of this study, it may be concluded that *S. thermophilus* supernatant has a good antilisterial activity, so it can be used as a protection factor against *L. monocytogenes* in the soft cheese processing.

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