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Antagonistic effect on *Listeria monocytogenes* and *L. innocua* of a bacteriocin-like metabolite produced by lactic acid bacteria isolated from sucuk

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Abstract

Two *Lactobacilli* and four *Pediococci* strains producing bacteriocin-like metabolities isolated from sucuk were tested with agar spot tests and well diffusion assays for their inhibitory activity against 16 *Listeria* strains, also isolated from sucuk. The production of organic acids and hydrogen peroxide limited, *L. sake Lb 706* (used as a bacteriocin producer strain) and the isolated lactic acid bacteria (LAB) showed inhibitory activity against all of the *Listeria* strains, while *L. sake Lb 706-A* (used as a bacteriocin non-producer mutant) had the same effects against only two *Listeria monocytogenes* strains (51, 52) in agar spot tests. In the well diffusion assays, while *L sake Lb 706* and four Pediococci isolates (413, 416, 419, 446) exhibited inhibitory activity against all of the *Listeria* strains tested, *L. sake Lb 706-A* and two of the Lactobacilli isolates (*77,* 116) showed no effect on the *Listeria* strains tested.

Keywords: Lactobacilli; Pediocci; *L. sake Lb 706*; Well diffusion assay

1. Introduction

One of the oldest and most popular meat products in Turkey is a fermented sausage (Sucuk). It is similar to the fermented sausages produced in Europe and the USA, but has a distinct taste (Gökalp, Kaya, & Zorba, 1994) Starter culture is routinely used in the production of fermented, raw dehydrated, or partially dehydrated bologna and sausage type products throughout the world. However, the use of a starter culture is not common in production of sucuk in Turkey, and sucuk sample, production is done by "change inoculation" (Gökalp et al., 1994; Karakaya & Kılıç, 1994). Due to the use of change inoculation, the commercially produced sucuk exhibits a wide variation in chemical and microbiological composition, depending on the company producing it. Previous studies (Gökalp, Yetim, Kaya, & Ockerman, 1988; Kolsarıcı, Ertaş, & Şahin, 1986; Nazlı, Ugur, & Akol, 1986; Yaman, Gökalp, & Çon, 1998) have found that the majority of samples analyzed were unacceptable based on appearance, chemical and microbiological composition. These studies found *E. coli* and sulphite reducing anaerobic microorganisms along with *Shigella* spp. in the samples analyzed. According to the results of a study conducted by Kaya and Gökalp (1991), 39% of the samples analyzed contained *Listeria* spp. (16% *L. monocytogenes* and 29% *L. innocua*). Their findings correlate well with a study conducted by Çon, Kaya and Gökalp (1996), where 23.33% of the samples analyzed contained *Listeria* spp. (16% *L. monocytogenes* and 23.33% *L. innocua*). The samples analyzed had low counts of *Listeria* spp., but the probability of contamination with *L. monocytogenes* was deemed high. In addition other studies conducted on fermented meat products (Farber, Sanders, & Johnston, 1989; Farber, Tittiger, & Gour, 1988; Johnson, Doyle, Cassens, & Schoeni, 1988) have shown that these products may contain *Listeria* spp. Of all the *Listeria* types, *L. innocua* and *L. monocytogenes* are most commonly found in these fermented meat products. According to Johnson et al. (1988) and Junttila,

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Hirn, Hill, and Nurmi (1990), *Listeria* spp. found in meat products can remain active and viable for long periods. Consumption of meat products contaminated with *L. monocytogenes* can lead to listeriosis (Johnson, Doyle, & Cassens, 1990) which carries a high probability of death (Berry, Hutkins, & Mandigo, 1991; Nielsen, Dickson, & Crouse, 1990; Peterkin, Idziak, & Sharpe, 1991). Therefore, all available technological and hygienic conditions must be utilized to eliminate the presence of this bacteria in meat products, prior the consumption.

Bacteriocin and similar metabolities, propagated by certain lactic acid bacteria (LAB), isolated from different types of fermented meat products, have been shown by numerous researchers (Harris, Daeschel, Stiles, & Klaenhammer, 1989; Kaya, 1992; Lewus, Kaiser, & Montville, 1991; Motlagh, Johnson, & Ray, 1991; Schillinger, & Lücke, 1989; Spelhaugh & Harlander, 1989; Yaman et al., 1998) to inhibit the growth of *L. monocytogenes*. In light of these findings, the antibacterial activities of LAB, isolated from sucuk, on bacteriocin and similar metabolites, also isolated from sucuk, were examined in this study. The determination and identification of those strains possessing antimicrobial activity properties is and the use of appropriate isolates as a sucuk starter culture is planned.

2. Material and methods

2.1. Bacterial strain and culture conditions

Lactobacillus plantarum 77, L. plantarum 116, Pediococcus acidilactici 413, Pediococcus acidilactici 419, P. acidilactici 446 and *Pediococcus pentosaceus 416* strains were used as the bacteriocin-producing organisms in this research. These strains were isolated from sucuk and identified by Çon and Gökalp (2000). All of the LAB stock cultures were maintained in 11% non-fat milk powder, supplemented with 15% glycerol and stored at −20°C. Working cultures were propagated in de Man, Rogosa, Sharpe agar (Oxoid; MRS) as stab cultures. These cultures were then transferred bimonthly for a maximum of four transfers.

Nine *L. monocytogenes* (isolate nos.: 20,22,38,39,41, 45,50,51 and *52)* and seven *L. innocua* (isolate nos.: 6,9, 19,26,33,42 and 54) isolates used as indicator organisms, were isolated from sucuk and identified by Çon et al. (1996). Additionally, *Lactobacillus sake Lb 706* and *L. sake Lb 706-A* were obtained from the Federal Center for Meat Research, Kulmbach, Germany. All of the *Listeria* spp. were cultivated in Tryptone Soya Broth (Oxoid) (TSB) supplemented with 0.6% Yeast Extract (Oxoid) (TSBYE) at 30°C. *Listeria* spp. were maintained as slant cultures on Tryptone Soya Agar (Oxoid; TSA) supplemented with 0.6% YE (Oxoid; TSAYE) at 4°C. These cultures were subsequently transferred bimonthly for a maximum of four transfers.

2.2. Detection of antagonistic activity

Agar spot tests and well diffusion assays, as described by Schillinger and Lücke (1989), were used to determine the antagonistic activity of the lactic acid bacteria.

Agar spot tests were conducted by placing 0.5 μl of an over-night LAB culture onto the surface of a MRS-0.2 (MRS containing 0.2% glucose) with 1.2% agar plate, and incubating for 24 h at 25°C. Anaerobic conditions were maintained to minimize the formation of H_2O_2 and acetic acid. The plates were overlaid with 7 ml of soft TSAYE (0.7% agar) agar seeded with approximately 5×10^7 cfu of the *Listeria* strain. After anaerobic incubation for 24 h at 25°C, the plates were examined for an inhibition zone surrounding the colonies of the producer strain.

For the well diffusion assay, cell-free supernatants of LAB were obtained from the LAB cultures propagated in MRS broth for 24 h at 25°C under anaerobic conditions. The LAB were centrifuged, and the supernatant adjusted to pH 6.5 with 10N NaOH, and sterilized by filtering through a 0.22 μm-pore-size cellulose acetate filter (Altech Associates, Inc. 2051 Waukagen Road, Baerfleld, IL, 60015, USA). The TSAYE agar plates were then overlaid with 7 ml of soft TSAYE (Soft TSAYE cntaining 0.7% agar) inoculated with 0.3 ml of an overnight culture of the indicator Listeria strain. Wells with a diameter of 3 mm were cut into these agar plates, and 0.03 ml of the cell-free culture supernatant of the LAB strain was placed into each well. The plates were then incubated anaerobically for 24 h at 25°C and subsequently checked for zones of inhibition around the wells. Both of these tests, which were conducted to determine the inhibitory activity due to antimicrobial compounds excreted into the media, *L. sake Lb 706* (as a bacteriocin producer strain), and *L. sake Lb 706-A* (as a bacteriocin non-producer mutant), were used.

3. Results

In this study, the antagonistic activity of six LAB on the 16 *Listeria* spp. strains (nine *L. monocytogenes* and seven *L. innocua),* all isolated from sucuk and the two control *L. sake* strains (*Lb 706* and *Lb 706-A*), was determined using the agar spot test and the well diffusion assay (Table 1).

As can be seen from Table 1, *L. sake Lb 706,* which was used as the positive control, exhibited inhibitory effects on all of the *Listeria* spp. in both the agar spot and the well diffusion test.

L. sake Lb 706-A, used as the negative control, in which its bacteriocin producing plasmid has been cured

Table 1
Antagonistic activity of lactic acid bacteria against *Listeria* spp[a]

Listeria isolate number	Lactic acid bacteria isolates															
	L. sake Lb 706		*L. sake Lb 706-A*		*L. plantarum 77*		*L. plantarum 116*		*P. acidilactici 413*		*P. acidilactici 419*		*P. acidilactici 446*		*P. pentosaceus 416*	
	Spot	Well	Spot	Well	Spot	Well	Spot	Well	Spot	Well	Spot	Well	Spot	Well	Spot	Well
6	7.0	4.0	–	–	1.0	–	0.5	–	4.2	3.5	5.0	1.5	4.0	4.0	5.0	3.5
9	7.5	4.5	–	–	1.5	–	1.0	–	4.5	3.7	4.2	4.0	4.5	3.8	4.5	4.5
19	7.5	3.0	–	–	0.7	–	0.7	–	4.0	4.0	4.0	3.0	4.0	3.0	3.5	3.5
20	6.0	3.0	–	–	1.0	–	1.0	–	4.5	3.0	4.5	2.0	5.0	3.0	4.5	4.5
22	6.0	4.0	–	–	1.0	–	1.0	–	5.5	2.5	4.5	4.0	5.0	2.0	5.5	3.0
26	6.0	4.0	–	–	0.6	–	1.0	–	4.5	3.5	3.5	3.0	4.7	3.5	3.8	3.5
33	7.5	4.5	–	–	1.0	–	1.0	–	5.2	2.0	4.5	4.0	5.0	4.0	5.3	4.0
38	7.0	4.0	–	–	1.5	–	1.0	–	5.0	2.5	4.7	4.0	3.8	3.0	5.0	4.5
39	7.0	5.0	–	–	1.2	–	0.5	–	5.0	3.0	4.5	3.0	4.5	2.5	5.0	4.5
41	7.0	4.5	–	–	0.7	–	0.5	–	4.5	4.0	4.5	3.0	4.5	4.5	4.5	3.5
42	6.0	4.0	–	–	1.5	–	0.5	–	5.0	4.5	4.5	3.5	5.5	4.5	5.2	5.0
45	6.0	5.0	–	–	1.0	–	1.5	–	5.7	5.0	5.0	4.2	6.0	4.0	6.0	5.0
50	6.5	4.5	–	–	1.0	–	1.0	–	6.0	3.5	4.5	4.0	5.0	4.0	5.8	4.0
51	7.0	4.5	0.7	–	2.0	–	1.5	–	5.5	4.5	4.5	2.5	4.0	3.0	5.0	5.0
52	4.0	3.0	0.5	–	1.0	–	1.0	–	5.0	5.0	5.0	3.2	4.8	3.5	5.0	5.0
54	6.5	4.5	–	–	1.0	–	1.5	–	5.0	3.8	5.0	2.0	4.8	4.0	5.0	5.0

[a] All of which were isolated from sucuk (inhibition zone; mm). – No inhibition, 0.5–1.0: low inhibition, 1.1–2.0; medium inhibition, 2.1–3.5: high inhibition, > 3.5: extremely high inhibition.

of acriflavine exhibited no inhibitory effect overall, except for a small inhibitory effect against two isolates (nos.: 51 and 52). However, the well diffusion test results showed no inhibitory activity on all the *Listeria* spp. isolates.

L. plantarum 77 and *116*, isolated from sucuk, exhibited low to medium inhibitory activity against *Listeria* spp. isolates on the agar spot test. The well diffusion test on the other hand, showed no inhibitory effects on any of the *Listeria* spp. isolates (Table 1).

P. acidilactici 413 419 and *446* and the P. *pentosaceus 416* isolates showed high to extremely high inhibitory activity against all of the *Listeria* spp. isolates as judged by the agar spot test. The results of the well diffusion test showed medium to extremely high inhibitory activity against all of the *Listeria* spp. isolates (Table 1).

4. Discussion

A significant inhibitor effect of the LAB isolates: *P. acidilactici 413, 419* and *446 P. pentosaceus 416* strains, on the *L. monocytogenes* and *L. innocua and L. plantarum 77* and *116* was seen. By keeping the glucose content of the culture medium constant at 0.2% the production of lactic acid was limited and as the anerobic conditions inhibited formation of H_20_2 , it was concluded that the inhibitory effects were due to the presence of bacteriocin-like metabolities. Additionally, bacteriocin producing strains L. *sake Lb 706,* and *L. sake Lb 706-A* (a derivate of *L. sake Lb 706*) which has similar biochemical properties, but with its bacteriocin producing capabilities, was used to confirm that these inhibitory effects were not due to presence of other metabolities. *L. sake Lb 706* exhibited high to extremely high range inhibition of all the *Listeria* isolates; while the *L. sake Lb 706-A* derivate showed only a low inhibitory effect on *L. monocytogenes 51* and *52* in the agar spot test. This supports the hytpothesis that the antimicrobial effects of the LAB used in this study are due to presence of bacteriocin-like metabolities. As the neutralized supernatant was used in the well diffusion test the results can not be due to lowering of the pH.

L. plantarum 77 and *116* showed inhibitory activity in the agar spot test but was shown to be ineffective in the well diffusion test. These findings are supported by studies conducted by Schillinger and Lücke (1989), Geis, Singh, and Teuber (1983), and Kaya (1992).

According to this study, *P. acidilactici 413, 419* and *446,* and *P. pentosaceus 416* strains have the best potential for use as sucuk starter cultures since these strains are very adaptable to the environment found in sucuk. Therefore, they can be used as a protective culture in this product.

In this study, the isolates were studied to produce bacteriocin-like metabolities in a model system. However, the ability of these isolates to produce bacteriocin-like metabolites in sucuk and/or the degree to which they can exhibit inhibitory activity on *Listeria* spp. were not determined. The effectiveness of these isolates against *L. monocytogenes* and other bacteria present in sucuk will be studied later.

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