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Research Article

Effect of *Lactobacillus sakei* Lb706 on Behavior of *Listeria monocytogenes* in Vacuum-Packed Rainbow Trout Fillets

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Abstract: The effect of a bacteriocin-producing strain, *Lactobacillus sakei* Lb706, on the behavior of *Listeria monocytogenes* in rainbow trout fillets stored under vacuum at 4 ºC for 14 days and at 10 ºC for 5 days was investigated.

At 4 ºC, growth of *L. monocytogenes* was significantly inhibited ($P < 0.05$) by *L. sakei* Lb706 during 10 days of storage while bacteriocin–negative Lb706-B did not affect the growth of *L. monocytogenes* during 14 days of storage. In the presence of the sakacin A-producing strain of *L. sakei* (Lb706), the growth of *L. monocytogenes* was significantly inhibited ($P < 0.05$) in the first 3 days of storage at 10 ºC, after which its count increased to 10^7 CFU/g. The control group and the group containing *L. sakei* Lb706-B showed similar growth patterns of *L. monocytogenes*. Significant increases ($P < 0.05$) were observed in all groups in terms of psychrotrophic bacteria counts. No significant ($P > 0.05$) changes in pH values during storage at 4 ºC and 10 ºC were determined.

Consequently, the results of the present study showed that *L. sakei* Lb706 can be used as a protective culture in freshwater reared rainbow trout, especially for preventing possible contaminations of Gram-positive food-borne pathogenic bacteria such as *L. monocytogenes* during filleting.

Key Words: Rainbow trout, vacuum packaging, *Listeria monocytogenes*, bacteriocin, *Lactobacillus sakei* Lb706

Vakum Paketlenmiş Gökkuşağı Alabalığı Filetolarında *Lactobacillus sakei* Lb706'nın *Listeria monocytogenes*'in Davranışı Üzerine Etkisi

Özet: Bakteriyosin üreten *Lactobacillus sakei* Lb706 suşunun vakum uygulanarak ambalajlanan gökkuşağı alabalık filetolarında *Listeria monocytogenes*'in davranışına etkisi 4 ºC'de 14 gün ve 10 ºC'de 5 günlük depolama süresince incelenmiştir.

4 ºC'de *L. monocytogenes*'in gelişimi *L. sakei* Lb706 tarafından 10 gün süreyle önemli derecede ($P < 0,05$) inhibe edilirken bakteriyosin negatif Lb706-B 14 günlük depolama süresince *L. monocytogenes*'in gelişimini engelleyememiştir. 10 ºC'de sakasin–A üreten *L. sakei* suşunun (Lb706) varlığında *L. monocytogenes*'in gelişimi ilk üç gün önemli derecede inhibe olmuş ($P < 0,05$), sonra sayı 10^7 kob/g'a kadar artmıştır. Kontrol grubu ile *L. sakei* Lb706-B içeren grupta *L. monocytogenes* gelişimleri benzerlik göstermiştir. Hem 4 ºC ve hem de 10 ºC'deki depolamalarda psikrotrofik bakteri sayısı açısından tüm gruplardaki artış önemli ($P < 0,05$) bulunmuş, pH'lardaki değişimler ise önemsiz bulunmuştur ($P > 0,05$).

Sonuç olarak, bu çalışmanın bulguları *Lactobacillus sakei* Lb706'nın tatlı suda yetiştirilen gökkuşağı alabalığında koruyucu kültür olarak, özellikle filetolama esnasında *L. monocytogenes* gibi Gram pozitif gıda-kaynaklı bakterilerin muhtemel kontaminasyonlarını önlemede kullanılabileceğini göstermektedir.

Anahtar Sözcükler: Gökkuşağı alabalığı, vakum ambalajlama, *Listeria monocytogenes*, bakteriyosin, *Lactobacillus sakei* Lb706

Introduction

It has been reported that food-borne pathogenic bacteria may contaminate fish during faulty rearing, harvesting and processing practices (1). These were isolated from fish products (2-5), especially from cold-smoked fish and lightly preserved fish products (6-8).

Fresh rainbow trout purchased on the retail market in the USA was found to be heavily contaminated with *Listeria monocytogenes*, i.e. 54% tested positive (9).

L. monocytogenes, a Gram-positive, rod-shaped and food-borne pathogenic bacterium, has been identified as a causative agent in sporadic cases of seafood-borne listeriosis (8), and the first rainbow trout-borne outbreak of listeriosis was reported by Ericsson et al. (10). Furthermore, 6 to 9 cases (3 pregnancy-related cases and 6 non-pregnancy-related cases, including 2 deaths) were linked to the consumption of 'gravad' rainbow trout in Sweden (11). Church and Parsons (12) reported the possibility of *L. monocytogenes* growth in vacuum-packed fish, depending on the finding of Church (2) of an increase in *L. monocytogenes* count at 5 °C in an aerobically stored pack of rainbow trout.

It has been reported that *L. monocytogenes* is widely distributed in the general environment, including fresh water and coastal water and live fish from these areas (13). The growth, survival and/or death of *L. monocytogenes* in fish products may be affected by temperature, pH, organic acid concentration, water activity, gaseous atmosphere and smoke components (14). The bacteria can grow at refrigeration temperatures and at relatively low pH values (15). However, there is no model that includes all the mentioned variables that may affect the fate of *L. monocytogenes* in fish products (14).

The risk of contamination by *L. monocytogenes* can be reduced, but the organism cannot always be eradicated from the finished product or the environment (16). Some protective microflora, especially bacteriocin producing lactic acid bacteria (LAB) or their bacteriocins, have been applied successfully in fresh fish and lightly preserved fish products (8,17). LAB bacteriocins are biologically active proteins or protein complexes that have the potential to prevent microbial food spoilage and to inhibit the growth of pathogens, usually closely related to the producer strain (18,19).

The aim of the present study was to determine the behavior of *L. monocytogenes* in vacuum-packed trout fillets sinking-surface-inoculated with a bacteriocin-producing *Lactobacillus sakei* Lb706. The effects of strains *L. sakei* Lb706 (bacteriocin-positive) and *L. sakei* Lb706-B (bacteriocin-negative) on *L. monocytogenes* were determined in vacuum-packed freshwater-reared rainbow trout fillets stored at 4 ± 1 °C and 10 ± 1 °C.

Materials and Methods

Bacterial cultures

L. monocytogenes (Li 6), *L. sakei* Lb706 (bacteriocin-positive) and *L. sakei* Lb706-B (bacteriocin-negative) were obtained from the Federal Center for Meat Research, Kulmbach, Germany. *L. monocytogenes* was inoculated in TSA Broth (Merck, 1.05458) and incubated at 37 °C for 24 h. *L. sakei* Lb706 and its plasmid-cured non-bacteriocin-producing derivative Lb706-B were cultivated in MRS Broth (Merck, 1.10661) and incubated at 30 °C for 24 h prior to inoculation.

Preparation and storage of samples

Rainbow trout with an average weight of 200 g reared in a freshwater farm, located at the Research and Extension Center of the Fisheries Department, Agricultural Faculty, Erzurum, were transferred to the laboratory, decapitated and filleted by hand. Two fillets were obtained from each fish. The fillets were divided into 3 groups. Control fillets inoculated with *L. monocytogenes* (without lactobacilli) were dipped into 1500 ml of sterile physiological saline solution (0.85% NaCl), whereas the other fillets were dipped into 1500 ml of sterile physiological saline solution (0.85% NaCl) containing *L. monocytogenes* - *L. sakei* Lb706 and *L. monocytogenes* - *L. sakei* Lb706-B.

After inoculation, the fillets were vacuum packed by using a Multivac packaging machine (Multivac A 300/16, Sepp Haggenmuller, D 87787 Wolfertschwenden, Germany). Packaging material was a film bag of 18 x 25 cm PETM/PE80-PETX/PE 80 (Südpack Verpackungen Memminger Strasse D-88418 Ochsenhausen) having low gas permeability at 23 °C (oxygen: 10 cm^3/m^2 daybar, nitrogen: 6 cm^3/m^2 daybar, carbon dioxide: 35 cm^3/m^2 daybar and water vapor: < 2 g/m^2 day).

All of the fillets, including the control group, were stored at 4 ± 1 °C for 14 days or 10 ± 1 °C for 5 days. The fillets were subjected in duplicate to microbial analyses and pH measurements during the storage periods.

Microbial analysis

Each fish muscle (25 g) was removed aseptically and homogenized for 1 min in a Stomacher 400 (Lab Stomacher Blender 400-BA7021, Sewardmedical) bag containing 0.85% NaCl solution. Further dilutions were made and then 0.1 ml of each dilution was transferred

onto the surface of plate count agar (Merck, 1.05463) for psychrotrophic bacteria and onto Palcam agar (Merck, 1.1117550500) for *L. monocytogenes*. PCA plates were incubated for 7 days at 10 °C for the psychrotrophic bacteria count. Palcam plates were incubated for 2 days at 37 °C for *L. monocytogenes* and enumerated (20).

pH value

The pH values were recorded with a Schott model pH meter (Schott, Lab Star pH) after homogenization of a 10-g fish muscle in 100 ml of distilled water from each sample (20).

Statistical analysis

All of the data were checked for normal distributions with normality plots prior to one-way analysis of variance (ANOVA), and followed by Duncan's multiple range test to determine significant differences among means at α = 0.05 level (21).

Results

Behavior of *L. monocytogenes*, counts of psychrotrophic bacteria and changes in pH values of fillets inoculated with 2 different *L. sakei* strains at 4 °C for 14 days and 10 °C for 5 days are shown in Figures 1a-c and 2 a-c.

In the absence of *L. sakei* (in control group fillets), *L. monocytogenes* grew from an initial level of approximately 10^4 to 10^7 CFU/g by the end of the 14-day storage period. Growth of *L. monocytogenes* was significantly inhibited ($P < 0.05$) by Lb706, which is a bacteriocin-producing strain, during 10 days of storage, while Lb706-B, a non-bacteriocin producing strain, did not inhibit the growth of *L. monocytogenes* during 14 days of storage at 4 °C. Psychrotrophic bacteria counts significantly increased ($P < 0.05$) in control fillets and lactobacillus treated group fillets. pH values did not significantly change during the storage in any of the groups ($P > 0.05$)

At 10 °C, rapid growth of *L. monocytogenes* was observed in the control group. The number of viable *L. monocytogenes* cells increased from 10^4 to 10^7 CFU/g within the first 5 days. In the presence of *L. sakei* Lb706-B, growth of *L. monocytogenes* was unaffected and *L. monocytogenes* counts were similar to those of control samples. In the presence of the sakacin A-producing strain of *L. sakei* (Lb706), the growth of *L. monocytogenes* was significantly inhibited ($P < 0.05$) in the first 3 days of storage; however, it increased to 10^7 CFU/g by the end of storage. Significant increases ($P < 0.05$) were observed in all groups in terms of psychrotrophic bacteria counts. However, no significant changes were observed in pH ($P > 0.05$) during storage.

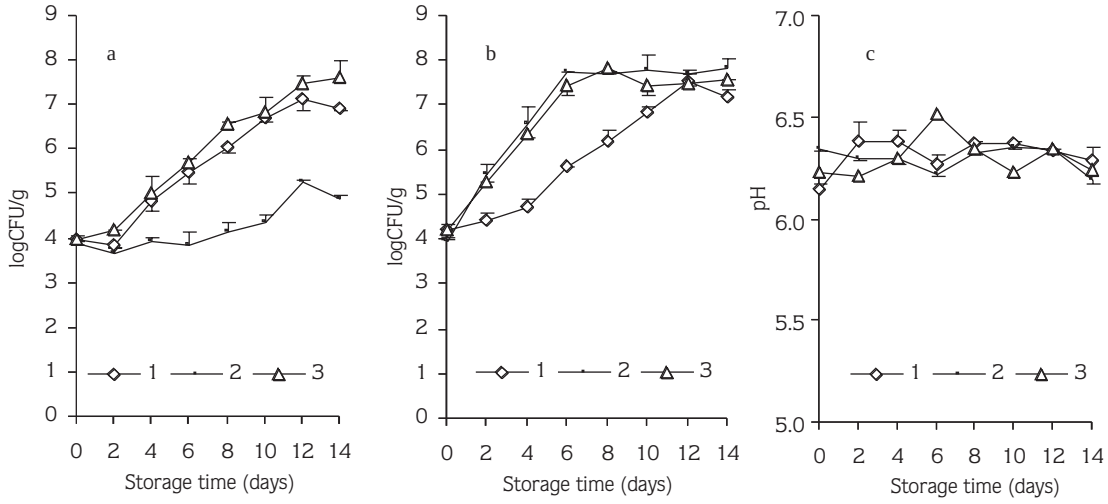

Figure 1. Changes in *L. monocytogenes* (a), psychrotrophic bacteria counts (b) and pH values (c) of vacuum-packed rainbow trout fillets inoculated with *L. monocytogenes* (control) (1), *L. monocytogenes* + *L. sakei* Lb 706 (2) and *L. monocytogenes* + *L. sakei* Lb 706-B (3) stored at 4 °C for 14 days.

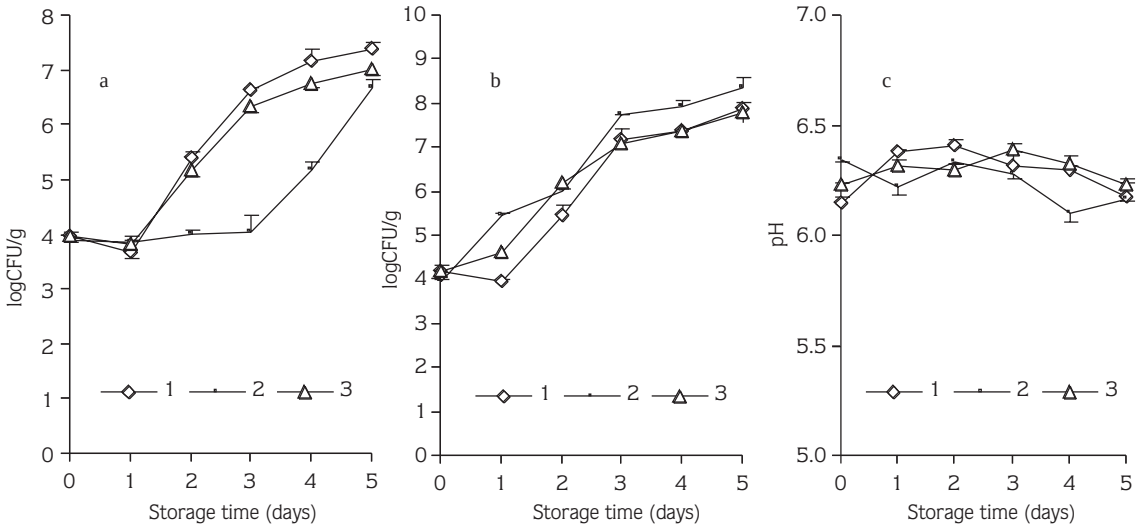

Figure 2. Changes in *L. monocytogenes* (a), psychrotrophic bacteria counts (b) and pH values (c) of vacuum-packed rainbow trout fillets inoculated with *L. monocytogenes* (1), *L. monocytogenes* + *L. sakei* Lb 706 (2) and *L. monocytogenes* + *L. sakei* Lb 706-B (3) stored at 10 °C for 5 days.

Discussion

The bacteriocin-positive strain *L. sakei* Lb706 inhibited growth of *L. monocytogenes* for up to 10 days at 4 °C. In the presence of *L. sakei* Lb706-B (a derivate of Lb706 with same growth rate but lacking the ability to produce sakacin A) a 3-log increase was observed in counts of *L. monocytogenes* in vacuum-packed fillets during 14 days of storage at 4 °C.

Growth of *L. monocytogenes* was inhibited by *L. sakei* Lb706 for the first 3 days at 10 °C. After that its count increased 1 log-unit on the fourth day and reached 3 log-units on the fifth day of storage compared to the initial count (10^4 CFU/g). On the other hand, *L. monocytogenes* started to grow in fillets with *L. sakei* Lb706-B and control fillets after the first day of storage at 10 °C. The anti-listerial activity of lactic acid bacteria, which are able to produce bacteriocins, is quite well known and this may be one reason for the reduced growth rate of *L. monocytogenes* in vacuum-packed cold smoked salmon. The mechanism of this inhibition has not been elucidated (14).

Inhibition of *L. monocytogenes* by bacteriocin-producing LAB has been reported by other researchers. It has been shown that the bacteriocins (piscicocin V1 and divercin V41) of *Carnobacterium piscicola* and *C. divergens* isolated from fish inhibited the growth of *L. monocytogenes* (22). It has been reported that inoculation of vacuum-packed smoked salmon with 2 x 10^6 CFU/g of *C. piscicola* strongly suppressed the growth of *L. monocytogenes* (23).

Harrison et al. (24) found no increase in the *L. monocytogenes* count of film-over-wrapped and vacuum-packed fish and shrimp when stored on ice. Church (2) reported no significant differences in the inhibition of *L. monocytogenes* between aerobic (40% CO_2 / 30% O_2 / 30% N_2) and anaerobic (60% CO_2 / 40% N_2) atmospheres examined for cod. Similarly, Lyver et al. (25) reported no inhibition of *L. monocytogens* in raw and cooked seafood nuggets in 100% CO_2 conditions. However, it was reported that addition of nisin to CO_2-packed cold-smoked salmon resulted in a 1 to 2 log reduction in *L. monocytogenes* (26). It was reported that 100% CO_2 was needed to effectively inhibit the growth of *L. monocytogenes* in smoked cod packed with modified atmosphere (MAP) (27). In order to completely inhibit *L. monocytogenes* in cold smoked salmon 70% CO_2 was found to be insufficient (26). An 8-day extension of the lag phase of *L. monocytogenes* in samples of crayfish tail meat treated with 1% lactic acid and packed under modified atmosphere (75% CO_2, 10% O_2 and 15% N_2) conditions as compared with that air or vacuum-packed was reported by Pothuri et al. (28).

Pelroy et al. (29) showed that total inhibition of *L. monocytogenes* was possible by the addition of 2% sodium lactate to cold-smoked salmon. However, there was little or no effect in controlling the growth of *L. monocytogenes* in crayfish tail meat by a spray application of 0.03 g/kg potassium sorbate or citric acid (30).

Consequently, considering the studies mentioned above, the complete elimination of *L. monocytogenes* from all aquatic products seems impossible. However, some protective microflora, especially bacteriocin producing LAB or their bacteriocins, can be expected to minimize growth of *L. monocytogenes*.

The results of the present study suggested that *L. sakei* Lb706 can be used as a protective agent in freshwater-reared rainbow trout, especially for preventing possible contamination by Gram-positive food-borne pathogenic bacteria such as *L. monocytogenes* during filleting.

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