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Competitive Growth and Attachment of *Listeria monocytogenes* and *Lactococcus lactis* ssp. *lactis* ATCC 11454

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The effect of a nisin-producing *Lactococcus lactis* spp. *lactis* (*L. lactis*) on the growth and attachment of *Listeria monocytogenes* Scott A and Brie 1 on stainless steel and their growth in Brain Heart Infusion broth was determined. Viable cells of *Listeria* decreased rapidly after 9~12 hr of incubation at 21°C and after 6~9 hr of incubation at 32°C in the presence of *L. lactis*. The number of *L. monocytogenes* Scott A attached to stainless steel in pure culture was 2.5×10^3 /cm² at 21°C and 2.3×10^3/cm² at 32°C after 48 hr of incubation, but was only 10/cm² at 21°C and 1.1×10/cm² at 32°C in the presence of *L. lactis*. Results from *L. monocytogenes* strain Brie 1 were similar to those from strain Scott A. The population of *L. monocytogenes* Scott A which attached to stainless steel with previously adherent *L. lactis* was 1.8×10^2/cm² at 21 °C and 8.2×10^2/cm² at 32°C, whereas the population attached to sterile stainless steel was 1.2×10^3/cm² at 21°C and 2.1×10^2/cm² at 32°C. For *L. monocytogenes* Brie 1, the attached population of the control was 1.6×10^4/cm² at 21°C and 3.2×10^2/cm² at 32°C, and on stainless steel with adherent *L. lactis*, it was 1.1×10 /cm² at 21°C and 6.9×10/cm² at 32°C. Surface adherent *L. lactis* was less inhibitory to attachment of *L. monocytogenes* on stainless steel than a liquid culture inoculum. *Listeria* attached to stainless steel survived dry storage for 20 days both in the presence and absence of adherent lactococci.

Listeria monocytogenes is a pathogenic bacterium which has been associated with food-borne illness. In addition, its presence in food has resulted in numerous product recalls. *L. monocytogenes* can be found on inadequately cleaned food contact surfaces and environmental surfaces within food processing plants (2, 11). *L. monocytogenes* can grow on moist surfaces under low nutrient conditions and form adherent microcolonies which are resistant to various sanitizing agents (5, 10). Food safety considerations require the prevention of contamination and growth of *L. monocytogenes* on surfaces in food processing environments. Recent researches have reported that the growth of *L. monocytogenes* was inhibited by lactic acid bacteria (1, 6, 13, 14). However, the inhibition of surface attachment of *L. monocytogenes* by lactic acid bacteria has not been previously studied. The purpose of this research was to investigate the effects of a nisin producing *Lactococcus lactis* spp. *lactis* (*L. lactis*) on the attachment of *L. monocytogenes* to a stainless steel surface.

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MATERIALS AND METHODS

Cultures and Maintenance

L. monocytogenes strains Scott A and Brie 1 were obtained from the culture collection of the Department of Food Science and Technology, Georgia Agricultural Experiment Station, Griffin, and were maintained on tryptic soy agar (Difco) at 4℃. *L. lactis* ATCC 11454 was obtained from American Type Culture Collection (Rockville, Maryland) and maintained on deMan, Rogosa and Sharp (MRS) agar (3) at 4℃. All cultures were grown at 32℃ for 24 hr in brain heart infusion broth (BHI, Difco) before use in experiments.

Competitive Growth of *Listeria* and *Lactococcus* in Broth

Equal quantities (0.05 ml) of active culture were transferred to six flasks containing 100 ml of BHI broth. Two flasks were inoculated with *Listeria*, another two with

Listeria and *Lactococcus* and the last two with *Lactococcus* culture. Each three kinds of flasks was incubated at 21℃ and 32℃ for 48 hr, respectively.

During incubation, 1 ml samples were taken every 3 hr and diluted in 0.1% peptone solution for enumeration. Appropriately diluted samples were surface-plated on McBride Listeria Agar (Difco) for enumeration of *L. monocytogenes*, and were pour-plated on MRS agar for *L. lactis*. Media were incubated at 32℃ for 48 hr before colony enumeration. These experiments were replicated twice. The pH of the culture media was measured with a Corning pH meter (Model 10).

Preparation of Stainless Steel Slides

Stainless steel slides (2×5 cm, type 304, finish No. 4) were cleaned by boiling in 2% Micro solution (International Products Corp., Trenton, NJ) followed by rinsing in distilled water as previously described (10).

Preparation of Surface-adherent *L. lactis* for Attachment Inhibition Experiment

To obtain surface-adherent *L. lactis*, cleaned slides were placed in test tubes (25 mm×180 mm) containing 25 ml of BHI broth and autoclaved. A 24 hr *L. lactis* culture (0.05 ml) was inoculated into the tube and incubated at 32℃. Every two days of incubation, each slide was aseptically removed and transferred to a fresh medium. Total incubation was for eight days.

To investigate the effects of adherent *L. lactis* on attachment of *L. monocytogenes*, stainless steel slides with adherent *L. lactis* were transferred to a 24 hr *L. monocytogenes* culture grown at 21℃ for 4 hr. Additional slides were incubated in broth at 21 and 32℃ for 48 hr to investigate growth effects. Sterile stainless steel slides treated in a similar manner served as controls. After incubation, slides were rinsed and attached cells were removed from each slide by using a teflon scraper and calcium alginate swab (Frank and Koffi 1990). Phosphate buffer (20 mM) was used for the cell removal process. The swab and rinsings were placed in a sterile screw cap tube and vortexed for 1 min. Viable listeriae and lactococci were enumerated as previously described. These experiments were replicated four times.

Concurrent Attachment and Growth of *L. monocytogenes* and *L. lactis*

L. monocytogenes and *L. lactis* were inoculated (0.05 ml) together and individually into tubes of sterile BHI broth containing stainless steel slides and incubated at 21 and 32℃ for 48 hr, respectively. The pure culture inoculations served as controls. Attached cells of each organism were enumerated by scraping the surface as previously described. These experiments were replicated four times.

Survival of *Listeria* and *Lactococcus* on a Dry Surface

Stainless steel slides with adherent *L. lactis* prepared as previously described and sterile stainless steel slides were immersed into a 24 hr *Listeria* culture for 30 seconds, rinsed with sterile phosphate buffer, allowed to air dry, and then were placed in sterile screw cap test tubes. The slides were stored at 21℃ for 20 days at ambient humidity. Sterile stainless steel slides dipped into *Listeria* culture served as controls. Changes in the number of viable cells were determined by scraping the surfaces as previously described. These experiments were replicated four times.

RESULTS

Inhibition of *L. monocytogenes* by *L. lactis* in Broth

Growth of *L. monocytogenes* Scott A and Brie 1 at 21 and 32℃ was inhibited to a similar extent due to the presence of *L. lactis* (Fig. 1 and 2). Viable cells of both *Listeria* declined rapidly after 9 hr of incubation at 21℃ and 6 hr at 32℃ in mixed culture. The pH of this time was 5.92 and 5.90 in the Scott A mixed

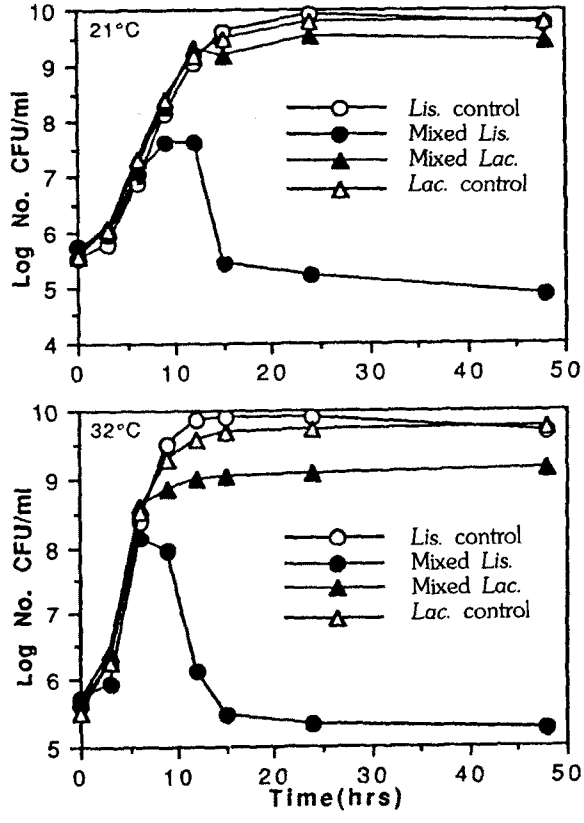

Fig. 1. Inhibition of *Listeria monocytogenes* Scott A by *Lactococcus lactis* spp. *lactis* ATCC 11454 in brain heart infusion broth incubated at 21 and 32°C for 48 hr.

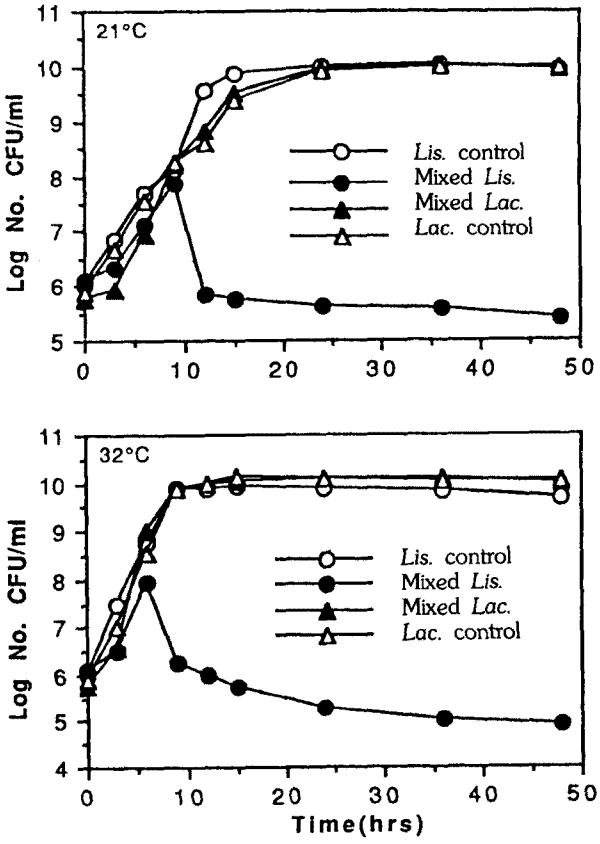

Fig. 2. Inhibition of *Listeria monocytogenes* Brie 1 by *Lactococcus lactis* spp. *lactis* ATCC 11454 in brain heart infusion broth incubated at 21 and 32°C for 48 hr.

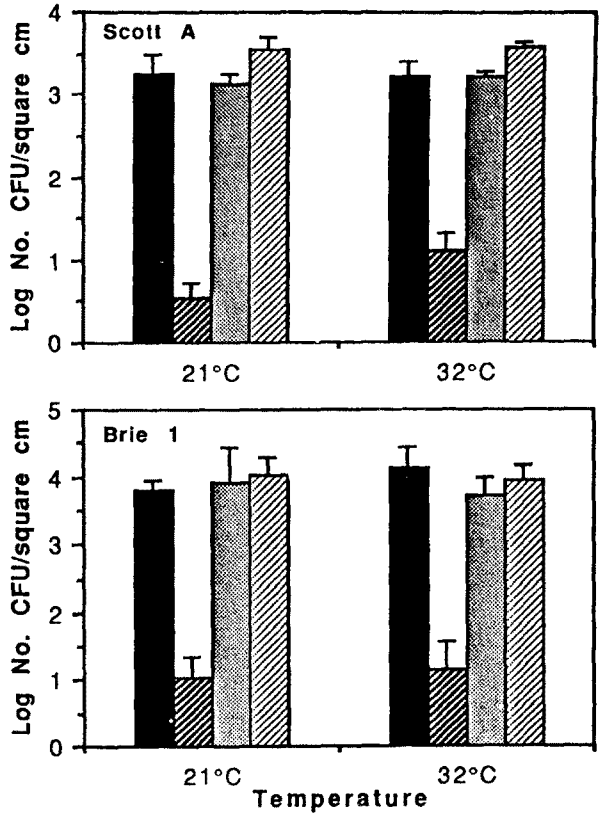

Fig. 3. Attachment of *Listeria monocytogenes* Scott A and Brie 1 on stainless steel surface with concurrent inoculation of *Lactococcus lactis* spp. *lactis* ATCC 11454.

Incubation was at 21 and 32 ℃ for 48 hr.
■ *Listeria* control ▨ *Listeria* in mixed culture
□ *Lactococcus* control ▧ *Lactococcus* in mixed culture

culture, 5.81 and 5.92 in the Brie 1 mixed culture. The growth of both listeriae did not declined at this pH value in *Listeria* spp. pure culture. The pH of each culture medium after 48 hr of incubation was 4.95~5.05 for the *Listeria* spp. in pure culture, 5.05~5.1 for the mixed culture and 5.05~5.1 for the *L. lactis* in pure culture. Inactivation of *Listeria* occurred while *L. lactis* was in the exponential growth phase.

Attachment and Growth Inhibition of *L. monocytogenes* on Stainless Steel after Concurrent Inoculation with *L. lactis*

Accumulation of *Listeria* on a stainless steel surface after 48 hr incubation at either 21 or 32℃ was substantially reduced when *L. lactis* was added with the inoculum (Fig. 3). This may have resulted from the inhibition of either growth or attachment of the *Listeria* due to presence of the *Lactococcus* culture. Pure cultures of *Listeria* strain Scott A attached at a level of 2×10^3 cfu/cm^2, but in the presence of the *L. lactis*, attachment was at a mean level of 10 cfu/cm^2 or less. Strain Brie 1 attached at a mean level of 6.7×10^3 cfu/cm^2 at 21℃ and 1.8×10^4 cfu/cm^2 at 32℃ in pure culture, but in the presence of the *L. lactis* culture, at mean levels of 12~14 cfu/cm^2 were observed. The number of *L. lactis* attached to the stainless steel slide was not influenced by the presence of *Listeria*.

Effect of Adherent *L. lactis* on Attachment of *L. monocytogenes* to Stainless Steel

Attachment of *L. monocytogenes* on stainless steel was only slightly inhibited by the presence of adherent *L. lactis* (Fig. 4). The 4 hr incubation at 21℃ was used to provide the adherent *L. lactis* with insufficient time to grow and produce inhibitors. Consequently, the results indicate that the inhibition of attachment was separate from the effects of growth inhibitors. The results of this experiment imply that the low level of attachment observed in the previous experiments with the mixed culture (Fig. 3) was due primarily to inhibitors produced during the growth of *L. lactis* rather than competition for attach-

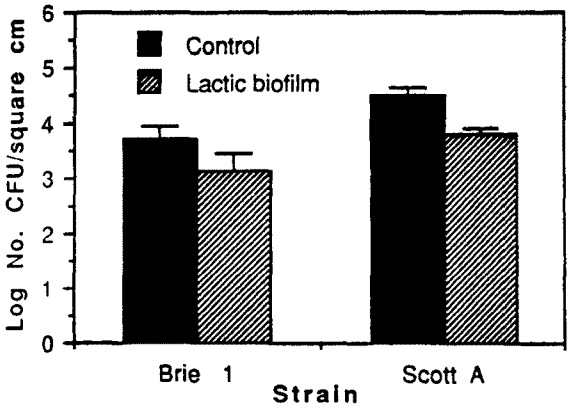

Fig. 4. Attachment of *Listeria monocytogenes* to stainless steel in the presence of adherent *Lactococcus lactis* spp. *lactis* ATCC 11454.
Incubation for attachment was for 4 hr at 21℃.

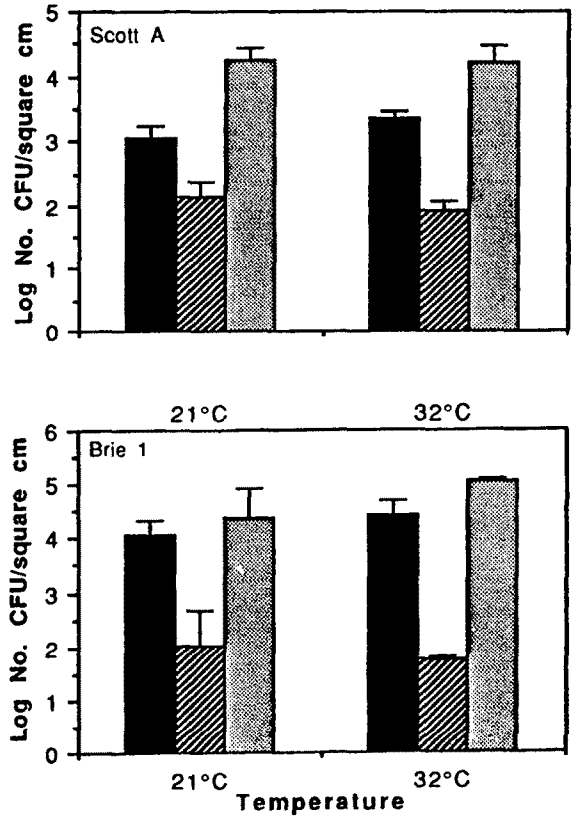

Fig. 5. Attachment of *Listeria monocytogenes* to stainless steel in the presence of adherent *Lactococcus lactis* spp. *lactis* ATCC 11454 with incubation at 21 and 32°C for 48 hr.
■ *Listeria control*
▨ *Listeria* with adherent *Lactococcus*
□ Adherent *Lactococcus*

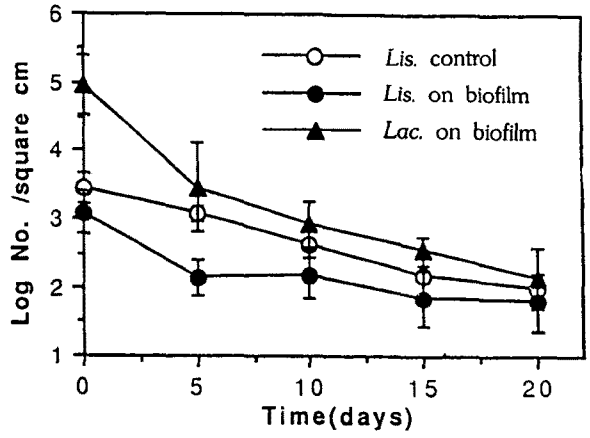

Fig. 6. Survival of *Listeria monocytogenes* and *Lactococcus lactis* spp. *lactis* ATCC 11454 on dry stainless steel incubated at 21°C for 21 days.

ment sites. When the incubation time was increased from 4 to 48 hr, the numbers of *Listeria* on the surface decreased (Fig. 5). Under these conditions, levels of lactics attached to the stainless steel were greater (over the 10^4 cfu/cm^2) than levels obtained when lactic culture was concurrently inoculated with *Listeria* (under 10^4 cfu/cm^2, Fig. 3). However, the inhibition of *Listeria* attachment observed in the presence of preattached *L. lactis* was not as great as that observed in the concurrent inoculation experiment (compare data in Fig. 3 with that in Fig. 5). One explanation is that concurrent inoculation resulted in a greater production of inhibitors by *L. lactis* in the broth than as compared to the attached *L. lactis*. Inhibitors in the broth (nisin and lactic acid) could inactivate both free living and attached *Listeria*.

Influence of Attached *L. lactis* on Survival of *Listeria* on Dried Stainless Steel

Viable cells of *Listeria* on dry stainless steel gradually decreased during storage, dropping in number by about one order of magnitude in 20 d (Fig. 6). Viable cells of *Listeria* attached to stainless steel which also contained adherent lactococci initially decreased more rapidly than the pure culture control population, but at 10~20 d of storage, the rate of decrease were similar.

DISCUSSION

Various studies have demonstrated that bacteriocin produced by lactic acid bacteria are antagonistic to *L. monocytogenes* (8, 13, 1, 6, 16). *L. lactis* ATCC 11454 has previously been shown to inhibit *Listeria* spp. by the production of nisin (6, 16). The growth inhibition of *Listeria monocytogenes* Scott A and Brie 1 occurred during the exponential growth phase of *L. lactis*. The

pH was 5.8~5.9 in mixed culture and 5.78~5.82 in *Listeria* pure culture at that time. The growth of both *Listeria* spp. was not inhibited in *Listeria* pure culture at this pH. It was found that the nisin synthesis does not take place during the early growth phase and gradually increases after lag phase (7). Lee and Kim (1985) reported that the synthesis was slowly increased up to 4 hr of incubation and rapidly increased during the exponential growth phase. Data reported in this study indicate that *L. lactis* ATCC 11454 inhibited the growth of *Listeria* by production of nisin.

Cox *et al.* (1989) and Nelson (1990) have isolated *Listeria* at various sites in food processing environments. Most isolations are from wet areas which are difficult to clean. These areas also contain a multitude of microbial species, which implies the possibility for microbial interactions (5). Data reported in this study indicate that if a species antagonistic for *Listeria* is growing at a contaminated site, growth of *L. monocytogenes* at that site could be inhibited in both the liquid phase and at the surface. Additional research is presently underway to isolate processing plant contaminants with antagonistic abilities toward *L. monocytogenes* in a biofilm environment.

Listeria-contaminated sites in a processing plant may at times dehydrate before *Listeria* has been inactivated. Palumbo and Williams (1990) reported survival of *L. monocytogenes* dried on coverslips which were stored at different relative humidities and in various food component systems. They did not observe evidence of cell injury during the dry storage period. Data reported in this study indicate that *L. monocytogenes* will survive on dry stainless steel surfaces to a sufficient extent to be of concern in plant sanitation.

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