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Growth of *Listeria monocytogenes* Restricted by Native Microorganisms and Other Properties of Fresh-Cut Spinach†

ISABELLE BABIC, A. E. WATADA,* and J. G. BUTA

Horticultural Crops Quality Laboratory, Agricultural Research Service, United States Department of Agriculture, BARC-West, Beltsville, Maryland 20705-2350, USA

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ABSTRACT

A study was undertaken to investigate the cause of the bacteriostatic activity of fresh-cut spinach leaves against *Listeria monocytogenes*. *L. monocytogenes* was cultivated in pure tryptic soy broth for use as a monoculture, in tryptic soy broth containing 10 mg ml^{-1} of autoclaved or nonautoclaved freeze-dried spinach powder, and in tryptic soy broth in mixed cultures with various microorganisms isolated from fresh-cut spinach, including *Pseudomonas fluorescens* biovar I, *P. fluorescens* biovar III, *Staphylococcus xylosus*, and an undefined culture of mesophilic aerobic microorganisms (MAMs) isolated from freeze-dried spinach powder. These microorganisms were inoculated at 4.4 log CFU ml^{-1} and *L. monocytogenes* was inoculated at 2.4 and 4.4 log CFU ml^{-1}. After 24 h of incubation at 30°C, the populations of the two inoculum levels *L. monocytogenes* increased to 9.0 and 9.6 log CFU ml^{-1} in the tryptic soy broth control, to 5.4 and 7.5 in nonautoclaved spinach powder cultures, and to 8.8 and 9.1 log CFU ml^{-1} in autoclaved spinach powder cultures; In mixed cultures with biovar I of *P. fluorescens*, *L. monocytogenes* increased to 7.4 and 8.6 log CFU ml^{-1}; with biovar III to 7.7 and 9.1, with S. xylosus to 7.8 and 9.2, and with the MAMs to 7.1 and 8.0 CFU ml^{-1} in the low and high listerial inoculum cultures respectively. The $LSD_{(0.05)}$ of the means were 0.5 and 0.6, respectively. The freeze-dried spinach powder had an inhibitory effect on the growth of *L. monocytogenes*. The inhibitory effect was greatly decreased when the native microorganisms were almost eliminated by heating or irradiation. These results indicate that if *L. monocytogenes* is present as a contaminant on fresh-cut spinach, its growth probably will be restricted by native microorganisms.

Key words: *Listeria monocytogenes*, mesophilic microorganisms, fresh-cut spinach

Listeria monocytogenes is a food-borne human pathogen well known to contaminate meat, seafood, and dairy products *(3, 19)*. This bacterium has also been shown to contaminate raw vegetables such as lettuce, broccoli, radishes, cabbages, potatoes, and cucumbers *(10, 14)*. A few cases of human listeriosis have been attributed to the consumption of fresh-cut vegetables such as coleslaw, lettuce *(15)*, and prepacked salads *(22)*. There is great concern about *L. monocytogenes* because it is a psychrotroph that is able to survive and grow on inoculated fresh-cut vegetables at low temperatures (below 10°C) *(2)*.

Listeria spp. have not been isolated from fresh-cut spinach *(1)*. Unpublished preliminary results showed that *L. monocytogenes* inoculated at 4.0 log CFU g^{-1} on fresh-cut spinach leaves stored in air at 10°C or 5°C remained viable but did not grow, whereas the populations of the mesophilic aerobic microorganisms and particularly of *Pseudomonas* spp. increased sharply. Many data exist on the interactions of microorganisms and *Listeria* in food. It is well known that *L. monocytogenes* is inhibited by a wide range of bacteriocins produced by lactic acid bacteria *(13, 21, 23)*. *Pseudomonas fluorescens* cells, are potent inhibitors of *L. monocytogenes* by means of the antagonistic siderophores which they produce *(4)*. Villani et al. *(25)* isolated four strains of *Staphylococcus xylosus* that inhibited the growth of *L. monocytogenes* from Italian sausages.

This paper reports how the growth of *L. monocytogenes* was affected by the fresh-cut spinach and by various microorganisms, including *P. fluorescens*, *S. xylosus*, and an undefined culture of mesophilic aerobic microorganisms that were isolated from the spinach. The isolation involved the physical alteration of fresh-cut spinach by macerating and freeze-drying. The native microorganisms were also controlled by heating and irradiation to attempt to determine their contribution to the restriction of the growth of *L. monocytogenes*.

MATERIALS AND METHODS

Preparation of spinach macerates and freeze-dried spinach powder for microbiological assays

Fresh-cut spinach (*Spinacia oleracea* L.) was obtained from a local supermarket. Spinach leaves (20 g) were macerated in 40 ml of 0.1 M potassium phosphate buffer, pH 7.0, for 1 min with a 400 Lab Stomacher (Seward Medical, London). The macerate (20 ml) was either centrifuged at 10,000 × *g* for 15 min, autoclaved at

* Author for correspondence. Tel: (301)-504-6128; Fax: (301)-504-5107; E-mail: awatada@asrr.arsusda.gov

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120°C for 15 min, or centrifuged and sterilized by vacuum filtration (SVF) using a presterilized Nalgene filter with a 0.2-μm-pore-size cellulose nitrate membrane (Nalge Company, Rochester, NY). The remaining spinach leaves were frozen in liquid nitrogen, freeze-dried, ground to a powder, and stored at −70°C.

Gamma irradiation of spinach powder

Gamma irradiation of spinach powder samples was performed by Irradiation Industries Incorporated (Gaithersburg, MD). The basic equipment used to irradiate the samples was a 3.0 MeV Dynamitron (Radiation Dynamics, Inc., Edgewood, NY). The samples (3 g) were automatically conveyed on a cart into the electron beam where they were irradiated with doses of 2, 3, 4, 7, 10, or 20 kGy.

Microorganisms and inocula

The strain *L. monocytogenes* ATCC 19111 isolated from poultry was provided by the Food Safety Inspection Service (USDA, Beltsville, MD). The strains *Pseudomonas fluorescens* biovar I, *P. fluorescens* biovar III, and *Staphylococcus xylosus* were isolated from fresh-cut spinach leaves as described by Babic et al. *(1)*. All strains were maintained on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) slants at 4°C. An undefined culture of mesophilic aerobic microorganisms (MAMs) were obtained by incubating 10 mg ml^{-1} of spinach powder in TSB at 30°C for 24 h. The culture was centrifuged at 2,000 × *g* for 20 min. An aliquot of the supernatant was inoculated in fresh TSB and incubated at 30°C for 24 h to obtain the culture of MAMs. To produce inocula, microorganisms were subcultured twice in TSB and incubated at 30°C for 24 h.

Microbiological assays

Spinach macerates or TSB were inoculated with a 10^{-3} or 10^{-5} dilution of the *L. monocytogenes* inocula in a sterile NaCl solution (8.5 g l^{-1}) to obtain a final population of around 2.0 log CFU ml^{-1} (low inoculum) or 4.0 log CFU ml^{-1} (high inoculum). Samples were incubated at 30°C for 24 h or at 10°C for 6 days. The initial and final populations of viable microorganisms were determined by plate counts. Fifty microliters of the samples were plated manually on the surface of agar plates of the appropriate media. Plates were incubated at 30°C for 24 to 36 h. Colonies of *L. monocytogenes* were counted on Oxford medium used with Bacto Oxford Antimicrobic supplement (Difco). Colonies of *P. fluorescens, S. xylosus* and MAMs were counted on pseudomonas selective isolation agar *(17)*, Chapman medium *(12)*, and TSA respectively. Colonies of *Listeria* spp. were distinguished from colonies of the other mesophilic aerobic microorganisms by colony morphology and excluded from the counts.

Statistical analysis

In the three experiments to study the inhibitory effect of freeze-dried spinach powder and spinach macerates, the initial and final populations of *L. monocytogenes* were measured on three replicates of six different treatments. A linear model was fitted to the final populations of *L. monocytogenes* with the initial population as a covariate by using the SAS Institute's statistical software procedure PROC MIXED *(20)*. The experiment and the experiment-by-treatment interaction were considered random effects. The initial populations were centered at the overall mean 3.04 CFU ml^{-1}.

The final population = $\alpha + \beta$ (initial population − 3.04), where (the coefficients α and β being allowed to vary for each treatment) the coefficient α represents the mean for the final population at 3.04, and the coefficient β represents the slope of the curve.

For the effect of irradiated freeze-dried spinach powder on the final population of *L. monocytogenes,* the data were analyzed with PROC MIXED as a one-factor model with a covariate. The treatment factor consisted of the control and the six levels of irradiation dosage. The initial inoculum level was the covariate. Pairwise contrasts were performed between the seven treatment levels. Orthogonal polynomial contrasts for the six irradiation dosage levels showed that the *L. monocytogenes* final population values increased as a convex curve for increasing levels of irradiation dosage.

In comparing the growth of *L. monocytogenes* in mixed cultures with the various microorganisms isolated from fresh-cut spinach, the analysis was conducted similarly to that described in A. The initial values were centered at the overall mean 3.44 CFU ml^{-1}, and the final population = $\alpha + \beta$ (initial population − 3.44).

In the two experiments (low inoculum and high inoculum levels) to study the effect of the initial population of MAMs on the growth of *L. monocytogenes,* a completely randomized one-factor mixed model with a covariate was used. The final *L. monocytogenes* population values were analyzed with PROC MIXED. The initial level of MAMs was the treatment and the initial level of *L. monocytogenes* was the covariate.

RESULTS

Inhibitory effect of spinach macerates and spinach powder on the growth of L. monocytogenes

The final population of *L. monocytogenes* differed from that in TSB when cultures were incubated with different treatments of spinach leaves for 24 h at 30°C (Table 1). Populations were in the order raw spinach < centrifuged < autoclaved < SVF centrifuged macerates ($P \leq 0.05$). In raw macerates, the final population of *L. monocytogenes* remained at a low level, ranging from 3.9 to 5.8 log CFU ml^{-1}, depending on initial inoculum level. The population was 1.0 to 2.0 logs greater in centrifuged macerates and more than 2.0 logs greater in the autoclaved macerates than in raw macerates. The population was 3.0 to 5.0 logs greater in SVF centrifuged macerates than in the raw macerates and similar

TABLE 1. *Estimated final populations of* Listeria monocytogenes *ATCC 19111 after 24 h at 30°C in tryptic soy broth with or without freeze-dried spinach powder or in raw or treated spinach macerates*

	Final population[a] of *L. monocytogenes* (log CFU ml^{-1}) from initial population of:				
Sample[b]	1.04	2.04	3.04	4.04	5.04
Control TSB	9.4 a[c]	9.2 a	9.1 a	8.9 a	8.7 a
TSB + spinach powder	5.6 c	5.9 c	6.1 d	6.3 c	6.6 b
Raw spinach macerate	3.9 d	4.4 d	4.9 e	5.4 d	5.8 b
Autoclaved macerate	6.6 b	7.0 b	7.3 c	7.6 b	8.0 a
Centrifuged macerate	6.0 bc	6.1 c	6.3 d	6.4 c	6.6 b
SVF macerate	8.8 a	8.6 a	8.5 b	8.3 a	8.2 a

[a] Final populations of *L. monocytogenes* were calculated for six initial (abscissa) values on three replicates in three experiments.

[b] TSB, tryptic soy broth; spinach powder, freeze dried, 10 mg ml^{-1}; SVF, centrifuged and sterilized by vacuum filtration.

[c] Values in the same column that are followed by the same letter are not significantly different at the 0.05 level.

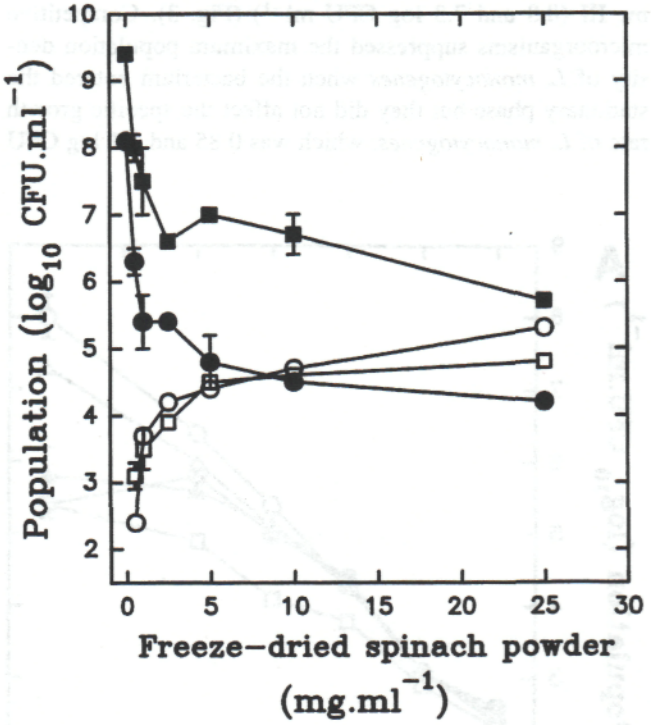

FIGURE 1. *Effect of different concentrations of freeze-dried spinach powder on the final population of* Listeria monocytogenes *ATCC 19111 and on the initial population of native MAMs in tryptic soy broth after 24 h at 30°C.* L. monocytogenes (●) *and MAMs* (○) *in low-inoculum cultures;* L. monocytogenes (■) *and MAMs* (□) *in high-inoculum cultures. Means and standard deviations were calculated on 3 replicates.*

to that in the control TSB. When spinach powder was added to TSB, the growth of *L. monocytogenes* was reduced by 3.8 to 2.1 logs compared to TSB alone. The inhibitory effect of raw spinach macerate or spinach powder was greater with lower listeria inoculum levels. The concentration of the spinach powder (1.0 to 5.0 mg ml^{-1}) did not have an effect on the degree of inhibition (Fig. 1). In the same time period, the initial population of native MAMs increased sharply and then remained relatively stable. When spinach powder was given increased dosages of irradiation, the final population of *L. monocytogenes* in TSB containing irradiated spinach

TABLE 2. *Effect of irradiated freeze-dried spinach powder on the final population of* Listeria monocytogenes *ATCC 19111 in tryptic soy broth after 24 h at 30°C*

Dosage of irradiation of spinach powder (kGy)	Final population of *L. monocytogenes* (log CFU ml^{-1})[a]	
	Low inoculum	High inoculum
0	5.5 a[b]	7.0 a
2	6.4 b	7.5 b
4	6.8 c	8.0 c
7	6.9 cd	8.2 d
10	6.9 cd	8.2 d
20	7.0 d	8.2 d
Control: no spinach powder	8.2 e	9.5 e

[a] The initial population of *L. monocytogenes* was 2.3 log CFU ml^{-1} in the low-inoculum culture and 4.3 log CFU ml^{-1} in the high-inoculum culture.

[b] In each column, values that are followed by different letters are statistically different at the 0.05 level.

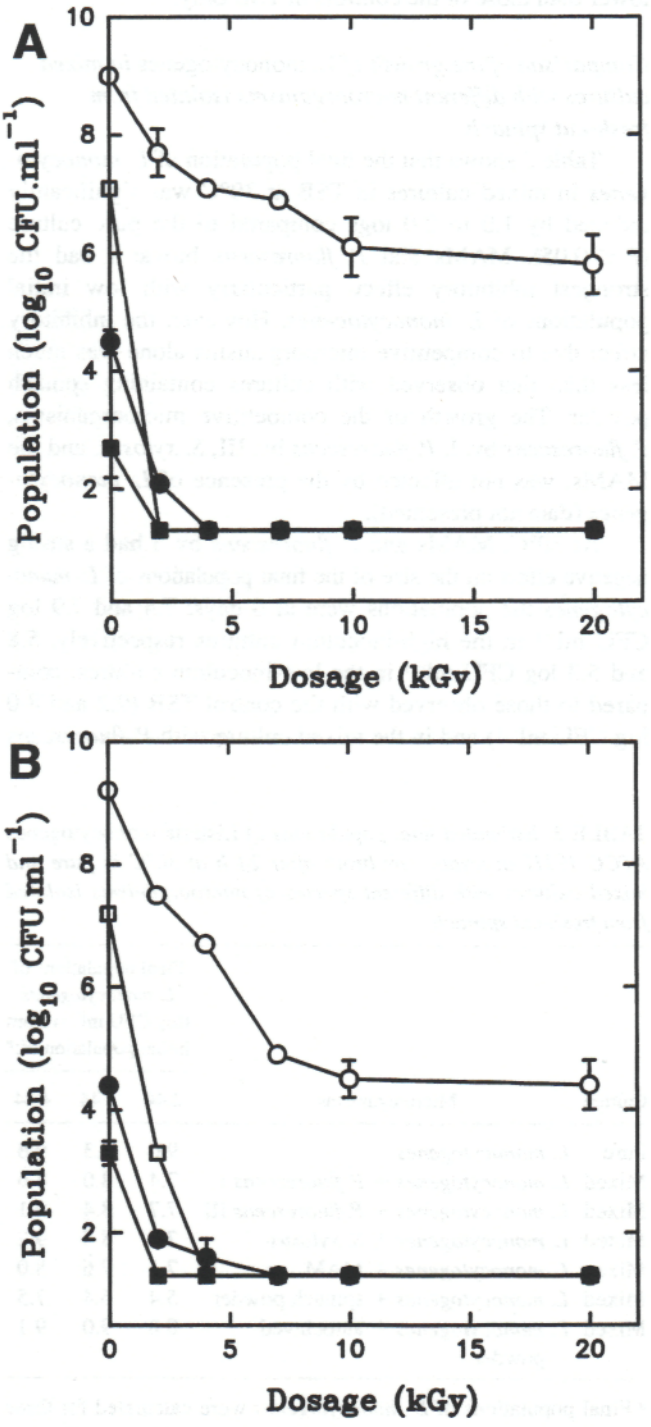

FIGURE 2. *Effect of different dosages of gamma irradiation of freeze-dried spinach powder on the initial population of MAMs* (●) *and pseudomonads* (■) *and on the final population of MAMs* (○) *and pseudomonads* (□) *in low (A) and high (B) inoculum cultures of* L. monocytogenes *after 24 h at 30°C. Means and standard deviations were calculated on 3 replicates. Limit of detection of microorganisms was 1.3 log CFU ml^{-1}.*

powder increased significantly by 1.0 to 1.5 log (Table 2). In these experiments with the irradiated spinach powder, the initial population of native MAMs and *P. fluorescens* decreased sharply to below the limit of detection and the final population of surviving cells was strongly reduced (Fig. 2). However, all populations remained significantly lower than those of the controls in TSB only.

Comparison of the growth of L. monocytogenes *in mixed cultures with different microorganisms isolated from fresh-cut spinach*

Table 3 shows that the final population of *L. monocytogenes* in mixed cultures in TSB at 30°C was significantly reduced by 1.0 to 2.0 logs compared to the pure culture ($P \leq 0.05$). MAMs and *P. fluorescens* biovar I had the strongest inhibitory effect, particularly with low initial populations of *L. monocytogenes*. However, the inhibitory effect due to competitive microorganisms alone was much less than that observed with cultures containing spinach powder. The growth of the competitive microorganisms, *P. fluorescens* bv. I, *P. fluorescens* bv. III, *S. xylosus*, and the MAMs, was not affected by the presence of *L. monocytogenes* (data not presented).

At 10°C, MAMs and *P. fluorescens* bv. I had a strong negative effect on the size of the final populations of *L. monocytogenes* the populations were at 6 days; 7.4 and 7.9 log CFU ml^{-1} in the high-inoculum cultures respectively, 5.8 and 5.3 log CFU ml^{-1} in the low-inoculum cultures, compared to those observed with the control TSB (9.2 and 8.0 log CFU ml^{-1}) and in the mixed culture with *P. fluorescens* bv. III (8.8 and 7.3 log CFU ml^{-1}) (Fig. 3). Competitive microorganisms suppressed the maximum population density of *L. monocytogenes* when the bacterium entered the stationary phase but they did not affect the specific growth rate of *L. monocytogenes*, which was 0.85 and 1.1 log CFU

TABLE 3. *Estimated final populations of* Listeria monocytogenes *ATCC 19111 in tryptic soy broth after 24 h at 30°C in pure and mixed cultures with different species of microorganisms isolated from fresh-cut spinach*

Culture	Microorganisms	Final population[a] of *L. monocytogenes* (log CFU ml^{-1}) from initial population of:[b] 2.44	3.44	4.44
Pure	*L. monocytogenes*	9.0	9.3	9.6
Mixed	*L. monocytogenes* + *P. fluorescens* I	7.4	8.0	8.6
Mixed	*L. monocytogenes* + *P. fluorescens* III	7.7	8.4	9.1
Mixed	*L. monocytogenes* + *S. xylosus*	7.8	8.5	9.2
Mixed	*L. monocytogenes* + MAM	7.1	7.6	8.0
Mixed	*L. monocytogenes* + spinach powder	5.4	6.4	7.5
Mixed	*L. monocytogenes* + autoclaved powder	8.8	9.0	9.1

[a] Final populations of *L. monocytogenes* were calculated for three initial (abscissa) values. Means and least significant difference were calculated for replicates in three experiments. $LSD_{(0.05)}$ of the final populations for the starting populations 2.44, 3.44, and 4.44 log CFU ml^{-1} were respectively 0.5, 0.3, and 0.6.

[b] Initial populations of microorganisms other than *Listeria* were: *P. fluorescens* biovar I, 3.8 ± 0.1; *P. fluorescens* biovar III, 4.1 ± 0.1; *S. xylosus*, 3.1 ± 0.1; MAMs, 4.2 ± 0.2; and native MAMs of freeze-dried spinach powder, 4.9 ± 0.1.

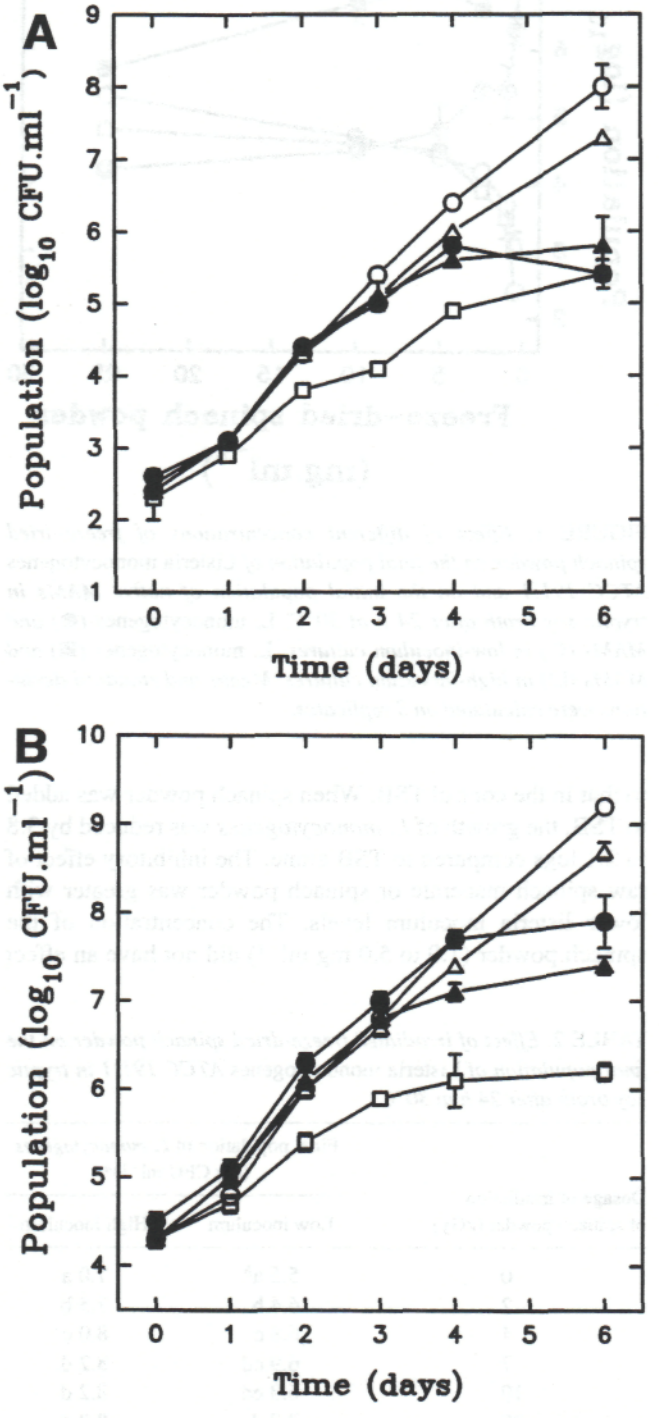

FIGURE 3. *Growth of* Listeria monocytogenes *ATCC 19111 in tryptic soy broth at 10°C in pure (○) and mixed cultures with microorganisms isolated from fresh-cut spinach,* P. sluorescens *biovar I (●),* P. fluorescens *biovar III (△), and MAMs (▲), and in culture containing 10 mg ml^{-1} of freeze-dried spinach powder (□). (A) Low-inoculum cultures, (B) high-inoculum cultures. Means and standard deviations were calculated on 3 replicates.*

TABLE 4. *Effect of the initial population of MAMs on the final population of* Listeria monocytogenes *ATCC 19111 in tryptic soy broth after 24 h at 30°C*

Initial population of MAMs (log CFU ml^{-1})	Final population of *L. monocytogenes* (log CFU ml^{-1}) from initial population: Low inoculum (2.3 log CFU ml^{-1})	High inoculum (4.3 log CFU ml^{-1})
0	9.0 a[a]	9.5 a
2	7.7 b	9.4 a
4	6.6 c	8.2 b
6	5.8 d	7.3 c

[a] In each column, means that are followed by different letters are statistically different at the 0.0001 level.

ml^{-1} day^{-1} in all high- and low-inoculum cultures respectively. The growth curve of *L. monocytogenes* in mixed culture with *S. xylosus* was similar to that obtained with *P. fluorescens* bv. III (data not presented).

The growth kinetics of *L. monocytogenes* in TSB containing spinach powder had a different profile. The growth was strongly reduced from the second day. The stationary phase was observed after 3 days in high-inoculum cultures but only after 4 days in low-inoculum cultures. Final populations of *Listeria* were 5.3 and 6.2 log CFU ml^{-1} after 6 days in low- and high-inoculum cultures respectively. The specific growth rate in the presence of spinach powder was much lower (0.6 log CFU ml^{-1} day^{-1}) than when *L. monocytogenes* was cocultured with competitive microorganisms. These competitive microorganisms (MAMs, native MAMs, in spinach powder, *P. fluorescens* bv. I and bv. III, and *S. xylosus*) grew faster at the specific growth rate of 1.6 log CFU ml^{-1} day^{-1}.

Effect of the initial population of MAMs on the growth of Listeria monocytogenes *in TSB at 30°C*

For the two experiments, low listeria inoculum and high listeria inoculum, the effect of the initial population of MAMs on the final population of *L. monocytogenes* was statistically different. Whenever the initial population of MAMs increased by 2 logs in low listeria inoculum cultures, the final population of *L. monocytogenes* decreased significantly by 1 log ($P \leq 0.0001$) (Table 4). In high-inoculum cultures, a similar effect was only observed for an initial population of MAMs of 4 log CFU ml^{-1} or higher.

DISCUSSION

Our results showed that native mesophilic aerobic microorganisms on fresh-cut spinach could reduce the growth of *L. monocytogenes* ATCC 19111 in tryptic soy broth at 30°C and 10°C. Jeong and Frank *(16)* also reported that, in biofilms, *L. monocytogenes* grew more slowly in the presence of competing microorganisms than in monoculture. In our study, temperature (10°C or 30°C) did not affect the final population of *L. monocytogenes*. The degree of reduction was dependent on both the initial population of mesophilic microorganisms and the initial population of *L. monocytogenes*. This result differs from that of Tran et al. *(24)* who demonstrated that the initial population of mesophilic aerobic microorganisms did not influence the isolation of *L. monocytogenes* in enrichment broths. Duffy et al. *(9)* also concluded that changes in the initial population of meat microorganisms and in the initial population of *L. monocytogenes* had no effect on the growth of *L. monocytogenes* in enrichment broths. In our study, among the different microbial species tested, *Pseudomonas fluorescens* biovar I had the strongest inhibitory effect against *L. monocytogenes* in mixed TSB cultures. *P. fluorescens* biovar III and *Staphylococcus xylosus* had a slight negative effect only on low listeria inoculum levels. All of the competitive microorganisms tested in this study had the same specific growth rate at 10°C. They grew faster and might compete with *L. monocytogenes* on fresh-cut spinach during storage. The role *Pseudomonas* spp. might play in the growth of *Listeria* spp. is not perfectly clear. Farrag and Marth *(11)* reported that *P. fluorescens* had a slight negative effect on *L. monocytogenes* at 7°C and 13°C but that some other *Pseudomonas* spp. could stimulate the growth of *Listeria* spp. Our study showed that even different biovars of the same species, *P. fluorescens,* could differ in activity against *L. monocytogenes*. Therefore, competition between *L. monocytogenes* and mesophilic microorganisms is probably due to specific microbial competitors.

MAMs had the strongest inhibitory effect on the growth of *L. monocytogenes*. The undefined culture of mesophilic aerobic microorganisms used in this study included gram-positive cocci (other than species of Micrococcaceae), which might play a role as competitors *(1)*. Chung and Murdock *(5)* isolated a gram-positive coccus from milk that inhibited the growth of *L. monocytogenes,* probably by producing inhibitory substances. Dallas et al. *(6, 7)* also reported that some strains of *Enterococcus* spp. could compete with *L. monocytogenes* during enrichment culture. Our study was not designed to elucidate the mode of action of mesophilic aerobic microorganisms isolated from fresh-cut spinach. However, they did not produce detectable inhibitory substances in TSB and did not change the pH, which was 7.0 for all experiments (results not shown). At 10°C, they induced an earlier stationary phase in *L. monocytogenes* growth, thereby reducing the maximum population size, but they had no effect on its specific growth rate. Recently, Rees et al. *(18)* highlighted the identification of a central regulator of stationary-phase gene expression which is responsible for the induction of specific bacterial genes under stress conditions. We can postulate that the population density of MAMs could induce the synthesis of this central regulator in listeria cells, thereby inducing an early stationary phase.

Our study demonstrated that the microbial component played a major role in the inhibitory effect of fresh-cut spinach on the growth of *L. monocytogenes*. However, there may be a second component involved in this inhibitory effect, as microorganisms alone were less effective than spinach powder. Preliminary investigations were conducted

to determine the nature of this second inhibitory spinach component. It is not extractable with a series of solvents ranging in polarity from hexane through water (unpublished data). It can be postulated that the inhibitory component is related to the physical structure or matrix of spinach leaves, since the inhibitory properties against *L. monocytogenes* were largely lost when the spinach macerate was filtered, while the autoclaved macerate retained partial activity. Attempts to isolate active proteins were not successful and treatments with detergents and enzymatic digestion with cellulysin did not change the inhibitory effect (unpublished data). Donald et al. *(8)* previously pointed out that *L. monocytogenes* survival in grass silage depended on the establishment of a fine balance between the physicochemical and microbiological characteristics. We can also speculate that the growth of *L. monocytogenes* on fresh-cut spinach may depend on the interaction between native mesophilic aerobic microorganisms and the physical characteristics of spinach leaves.

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REFERENCES

1. Babic, I., S. Roy, A. E. Watada, and W. P. Wergin. 1996. Changes in microbial population on fresh-cut spinach. Int. J. Food Microbiol. 31:107–119.
2. Beuchat, L. R., M. E. Berrang, and R. E. Brackett. 1990. Presence and public health implications of *Listeria monocytogenes* on vegetables, ch. 25. *In* A. J. Miller, J. L. Smith, and G. A. Somkuti (ed.), Foodborne listeriosis. Elsevier Scientific Publishing Co., Amsterdam.
3. Brackett, R. E. 1988. Presence and persistence of *Listeria monocytogenes* in food and water. Food Technol. 42(4):162–178.
4. Cheng, C. M., M. P. Doyle, and J. B. Luchansky. 1995. Identification of *Pseudomonas fluorescens* strains isolated from raw pork and chicken that produce siderophores antagonistic towards pathogens. J. Food Prot. 58:1340–1344.
5. Chung, K. T., and C. A. Murdock. 1991. Natural systems for preventing contamination and growth of microorganisms in foods. Food Struct. 10:361–374.
6. Dallas, H. L., and A. D. Hitchins. 1993. Antagonism of bacterial competitors towards *Listeria monocytogenes* in enrichment culture. J. Food Safety 13:113–123.
7. Dallas, H. L., T. T. Tran, C. E. Poindexter, A. D. Hitchins, and L. J. Romanell. 1991. Competition of food bacteria with *Listeria monocytogenes* during enrichment culture. J. Food Safety 11:293–301.
8. Donald, A. S., D. R. Fenlon, and B. Seddon. 1995. The relationship between ecophysiology, indigenous microflora and growth of *Listeria monocytogenes* in grass silage. J. Appl. Bacteriol. 79:141–148.
9. Duffy, G., J. J. Sheridan, R. L. Buchanan, D. A. McDowell, and I. S. Blair. 1994. The effect of aeration, initial inoculum and meat microflora on the growth kinetics of *Listeria monocytogenes* in selective enrichment broths. Food Microbiol. 11:429–438.
10. Farber, J. M., G. W. Sanders, and M. A. Johnston. 1989. A survey of various foods for the presence of *Listeria* species. J. Food Prot. 52:456–458.
11. Farrag, S. A., and E. H. Marth. 1989. Behavior of *Listeria monocytogenes* when incubated together with *Pseudomonas* species in tryptose broth at 7 and 13°C. J. Food Prot. 52:536–539.
12. Guiraud, J. P., and P. Galzy. 1980. L'analyse microbiologique dans les industries alimentaires. Collection Génie Alimentaire. Les Éditions de l'Usine Nouvelle, Paris.
13. Harris, L. J., M. A. Daeschel, M. E. Stiles, and T. R. Klaenhammer. 1989. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. J. Food Prot. 52:384–387.
14. Heisick, J. E., D. E. Wagner, M. L. Nierman, and J. T. Peeler. 1989. *Listeria* spp. found on fresh market produce. Appl. Environ. Microbiol. 55:1925–1927.
15. Ho, J. L., K. N. Shands, and D. W. Fraser. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. 146:520–524.
16. Jeong, D. K., and J. F. Frank. 1994. Growth of *Listeria monocytogenes* at 10°C in biofilms with microorganisms isolated from meat and dairy processing environments. J. Food Prot. 57:576–586.
17. Krueger, C. L., and W. Sheik. 1987. A new selective medium for isolating *Pseudomonas* spp. from water. Appl. Environ. Microbiol. 53:895–897.
18. Rees, C. E. D., C. E. R. Dodd, P. T. Gibson, I. R. Booth, and G. S. A. B. Stewart. 1995. The significance of bacteria in stationary phase to food microbiology. Int. J. Food Microbiol. 28:263–275.
19. Rodriguez, J. L., P. Gaya, and M. Nunez. 1994. Incidence of *Listeria monocytogenes* and other *Listeria* spp. in ewes' raw milk. J. Food Prot. 57:571–575.
20. SAS Institute Inc., SAS/STAT Software. 1996. Changes and enhancements through release 6.11. SAS Institute Inc., Cary, NC.
21. Schillinger, U., M. Kaya, and F. K. Lücke. 1991. Behavior of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. 70:473–478.
22. Sizmur, K., and C. W. Walker. 1988. *Listeria* in prepacked salads. Lancet 1167.
23. Stecchini, M. L., V. Aquili, and I. Sarais. 1995. Behavior of *Listeria monocytogenes* in Mozarella cheese in presence of *Lactococcus lactis*. Int. J. Food Microbiol. 25:301–310.
24. Tran, T. T., P. Stephenson, and A. D. Hitchins. The effect of aerobic mesophilic microfloral levels on the isolation of inoculated *Listeria monocytogenes* strain LM82 from selected foods. 1990. J. Food Safety 10:267–275.
25. Villani, F., O. Pepe, G. Mauriello, G. Salzano, G. Moschetti, and S. Coppola. 1994. Antimicrobial activity of *Staphylococcus xylosus* from Italian sausages against *Listeria monocytogenes*. Lett. Appl. Microbiol. 18:159–161.