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THE EFFECT OF *LACTOCOCCUS LACTIS* ON THE GROWTH CHARACTERISTICS OF *LISTERIA MONOCYTOGENES* IN ALFALFA SPROUT BROTH

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Lactic acid bacteria isolated from commercially produced alfalfa sprouts were screened for activity against *Listeria monocytogenes* F4258. Most active isolates were identified as *Lactococcus lactis* subsp. *lactis*. The isolates fell into two categories, strains that inhibited by acid production only, and strains that appeared to have additional inhibitory activity.

An acid-only isolate, SP26, was used to evaluate the effect of initial pH (5, 6, 7, 8) and temperature (10, 20, and 30 °C) on the interaction between the lactic acid bacterium and *L. monocytogenes* using "sprout juice" as a model system. The model system was inoculated with an initial level of approx. 10^3 CFU ml^{-1} *L. monocytogenes* in both mono-culture controls and the co-cultures and the co-cultures with *L. lactis* (10^3–10^4 CFU ml^{-1}). The primary inhibitory effect attributable to *L. lactis* was a 2 to 3 log cycle decrease in the maximum population density obtained by *L. monocytogenes*. The extent of the inhibition was decreased at 10 °C, but was only slightly affected by pH in the range of 6.0 to 8.0. *L. monocytogenes* did not grow in the sprout broth at pH 5.0 at any of the incubation temperatures.

Keywords: *Listeria monocytogenes*, competition, pH, alfalfa sprouts

Listeria monocytogenes is a Gram positive bacterium that can cause life threatening food-borne infections. Although listeriosis is relatively rare, *L. monocytogenes* can be isolated readily from a wide range of foods, including various vegetables (FARBER et al., 1989; HEISICK et al., 1989; SIZMUR & WALKER, 1988; BEUCHAT, 1996). Outbreaks of listeriosis have been linked to fresh produce such as raw celery, tomatoes, lettuce and coleslaw (BEUCHAT, 1995). During the past several years, sprouted seeds (i.e., sprouts) of various plants, such as alfalfa and clover, have become prominent as a cause of *Salmonella* and enterohemorrhagic *Escherichia coli* outbreaks associated with fresh produce (PÖNKA et al., 1995; MAHON et al., 1997; CENTERS FOR DISEASE CONTROL AND PREVENTION, 1997; ITOH et al., 1998). A common characteristic of these outbreaks has been an initial low level contamination of the seeds with the food-borne pathogen,

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followed by growth of the pathogen to elevated levels during the germination of the seeds. The production of sprouts typically involves germinating for a period of 4 to 5 days under conditions that are also conducive to microbial growth. Both *Salmonella* and *E. coli* 0157:H7 have been shown to grow during the germination of sprouts (AABO & BAGGESEN, 1997; HARA-KUDO et al., 1997). While no cases of listeriosis have been linked to sprouts, the presence of *L. monocytogenes* in sprouts has resulted in product recalls in the United States. The closely related but non-pathogenic species *Listeria innocua* has been isolated from sprouts (BECKER & HOLZAPFEL, 1997). Furthermore, preliminary studies in our laboratory have indicated that *L. monocytogenes* could multiply during the germination of sprouts (PÁLMAI & BUCHANAN, 2002).

During the germination of sprouts, microbial population increase rapidly. Microbial analyses of commercial sprouts found that they can carry high numbers of microbial flora (10^2–10^6 g^{-1}) (SPLITTSTOESSER et al., 1983; PROPOKOWICH & BLANK, 1991; PATTERSON & WOODBURN, 1980), including both coliforms (10^4 g^{-1}) and fecal coliforms (10^2–10^3 g^{-1}). Past research has suggested that the microflora of a food is an important factor influencing the growth of *L. monocytogenes*, and this has been proposed as a potential means of controlling the pathogen in foods (QUINTO et al. 1996; BUCHANAN & BAGI, 1997, 1999; BREIDT & FLEMING, 1998). It was of interest to determine if a similar approach could be used to control the potential growth of *L. monocytogenes* during the germination of sprouts. Accordingly, one objective of the current study was to isolate lactic acid bacteria (LAB) from the normal microflora of sprouts that have inhibitory activity against *L. monocytogenes*. Since past research (BUCHANAN & BAGI, 1997, 1999) has indicated that the effectiveness of competing bacteria against *L. monocytogenes* is dependent on the growth conditions, a second objective was to characterize the effect of pH and temperature on the ability of the LAB selected for further study to inhibit the growth of *L. monocytogenes*, using alfalfa sprout juice as a model system.

1. Materials and methods

1.1. Isolation of LAB inhibitory to L. monocytogenes

1.1.1. Microorganisms. Listeria monocytogenes F4258, a clinical isolate obtained from CDC, was used throughout the study. The microorganism was maintained in Brain Heart Infusion broth (BHI) stored at 4 °C, and subcultured weekly by incubating freshly inoculated BHI at 30 °C for approximately 18 h. Working cultures were prepared by inoculating 0.2 ml of the stock culture into 10 ml BHI and incubating at 30 °C overnight.

1.1.2. Isolation of LAB strains from alfalfa sprouts. Commercially available alfalfa sprouts were purchased at a local supermarket, and 25 g samples were stomached with 225 ml peptone water. The liquid was surface plated on MRS agar (Difco, Detroit, MI) plates, which were then incubated for 48 h at 30 °C. Colonies were randomly picked, transferred into MRS broth and incubated overnight at 30 °C. Isolates were Gram

stained, and tested for catalase production. The ability of the isolates to grow at 15 °C and 45 °C was determined using MRS broth. The isolates' carbohydrate fermentation patterns were determined using API 50 CH (Biomérieux) strips. Identifications were carried out by comparing the observed fermentation patterns with the identification table supplied with the test kit.

1.1.3. Characterization of LAB isolates for inhibitory activity against L. monocytogenes. Isolates were screened for inhibitory activity against *L. monocytogenes* using the agar spot technique. An overnight culture of an isolate was diluted tenfold in peptone water. A loopful of the diluted LAB culture was spot-inoculated onto the surface of duplicate MRS agar plates, (one plate inoculated with four different isolates - repeated twice) which were then dried under a biological hood for 20 min. A 2 ml portion of an overnight culture of *L. monocytogenes* was mixed with 200 ml BHI of soft agar (10 g agar/l), and 6–8 ml was used to overlay each spot inoculated MRS agar plate. The plates were then incubated for 24 h at 30 °C and examined for zones of inhibition.

Isolates with large inhibition zones were chosen for further examination. Agar spot method was repeated under anaerobic conditions. Method was also repeated on Tryptone Soy Agar with Yeast Extract (TSAYE) without glucose plates to determine if the inhibition was due to acid production (LEWUS et al., 1991).

Those isolates that appeared to be producing a bacteriocin were spot-inoculated onto MRS Agar, dried under hood and incubated for 24 h at 30 °C. Proteinase K enzyme solution (2 μl of a 10 mg ml^{-1} solution) was placed next to the colony, and the plates were held at 30 °C for 2 h to allow the diffusion of the enzyme. A second layer of BHI agar seeded with *L. monocytogenes* was poured on the plate and the plates were incubated at 30 °C for 24 h. This technique was carried out by applying the enzyme both directly to the agar and via an impregnated paper disc (LEWUS et al., 1991). The enzyme was added before and after the formation of the spot-colonies. In addition, the effect of pepsin, trypsin, α-chimotrypsin, proteinase K, and catalase on the ability of the isolates to inhibit *L. monocytogenes* were carried out using the agar well diffusion technique (HARRIS et al., 1989).

1.2. Competition studies

1.2.1. Preliminary determination of relative growth of selected LAB isolates and Listeria monocytogenes *in sprout broth.* Commercially grown alfalfa sprouts were purchased in a supermarket. Sprouts and sterile distilled water in 1:4 weight ratio were placed into filter Stomacher bags and macerated for 1 min. The resultant broth was transferred in 10 ml portions into test tubes, capped, and autoclaved for 15 min at 121 °C.

Preliminary studies were conducted to compare the growth rates of *L. monocytogenes* and four of the LAB isolates. Tubes of autoclaved sprout broth were inoculated with overnight cultures of individual LAB isolates or *Listeria* cultures to a level of 10^3 CFU ml^{-1}. Half of the tubes were incubated at 30 °C, the other half at room temperature (approximately 23 °C). After 0, 3, 6, 24, and 30 h, samples of each isolate from each incubation temperature were surface plated on MRS agar for the LAB

isolates and Oxford Listeria Selective Agar for *L. monocytogenes* using a spiral plater. The plates were incubated at 30 °C for 24 h and then enumerated.

1.2.2. Microorganism. Lactococcus lactis subsp. *lactis* SP26 was selected for further study. The microorganism was maintained in MRS broth at 4 °C, and transferred weekly. Working cultures were prepared by inoculating 0.2 ml of culture into 10 ml broth and incubating at 30 °C overnight.

1.2.3. Experimental design, growth conditions and model system. The study examined the effect of initial pH (6.0, 7.0, and 8.0) and incubation temperature (10, 20, 30 °C) on the growth of *L. monocytogenes* alone and in the presence of *L. lactis* using a complete factorial design. Each pH/temperature combination was examined in duplicate on two separate occasions for a total of four replicates per variable combination. In addition, the growth of *L. monocytogenes* at pH 5.0 as both a mono-culture and as a co-culture was examined separately.

A single large batch of sprout broth was prepared to ensure that the composition of the broth was consistent over the course of the multiple experiment study. The whole amount was prepared at once in smaller portions. 240 ml sterile distilled water was added to 60 g sprouts in sterile Stomacher filter bags. Contents of bags were blended in a Stomacher for 15 s. Stomached sprout broth was prefiltered through cheesecloth and then centrifuged at 10,000 x *g* for 25 min. The supernatant was gently collected and then stored in a freezer (–20 °C) until use. The day before an experiment, the volume of sprout broth required was thawed. The pH was adjusted with HCl or NaOH to achieve the target pH and the broth transferred in 100 ml portions to 250 ml screw-cap bottles. Bottles were capped and autoclaved for 15 min at 121 °C.

The bottles of sprout broth that had been adjusted to the desired incubation temperature were inoculated with 1.0 ml of an appropriate dilution of an overnight culture of *L. monocytogenes* to achieve an initial cell concentration of approximately 10^3 CFU ml^{-1}. The bottle designated to be co-cultures also received 1.0 ml of an overnight culture of *L. lactis* to achieve an initial cell concentration of approximately 10^4 CFU ml^{-1}.

In order to facilitate timed sampling of the 20 and 30 °C cultures duplicate cultures were inoculated in a staggered manner. Bottles of sprout broth were inoculated at a specified time in the morning, and then refrigerated at 2 °C. That evening, a designated number of bottles were transferred to a 20° or 30 °C incubator. The next morning both the bottles that had been incubated overnight and the bottles still in refrigerated storage were sampled. After sampling, the refrigerated bottles were transferred to the designated incubator. This allowed samples from both the early stationary phase (14 h) and the lag phase (0 h) to be followed simultaneously. Though the length of the cold storage period was different for the two groups of cultures (8 h vs. 22 h), preliminary studies indicated that it did not significantly affect the determination of growth kinetics values derived by the data. The data from the staggered inoculum cultures were combined to yield a single growth curve.

At designated times, 4-ml samples were aseptically removed from each bottle. One ml was appropriately diluted and plated in duplicate using the Automated Spiral

Plater. The *L. monocytogenes* + *L. lactis* co-culture samples were plated on MRS agar (Oxoid) (LAB count), Oxford Listeria Selective agar (Oxoid) (*L. monocytogenes* count) and BHI agar plates (Oxoid) (total count). The samples from the *L. monocytogenes* mono-culture were plated on Oxford Listeria Selective agar and BHI agar. All plates were incubated for 24 h at 30 °C, and then enumerated using an automated Spiral Counter.

The remainder of the sample (3 ml) was used to determine the pH of the sprout broth (VWR Benchtop pH/ISE Meter, Model No 8005).

1.2.4. Curve fitting. Growth curves were generated by fitting the observed data to two mathematical models, the Gompertz equation (GIBSON et al., 1988; BUCHANAN et al., 1989) and the three-phase-linear model (BUCHANAN et al., 1997) using curve-fitting software (Damert, 1994). The model parameters where then used to calculate exponential growth rates (μ), lag phase durations (λ) and maximum population densities (MPD).

2. Results and discussion

2.1. Isolation of LAB with activity against L. monocytogenes

Of the 40 isolates from alfalfa sprouts that grew on MRS agar plates, 38 were Gram positive and catalase negative. When screened using an agar spot test, *Listeria monocytogenes* F4258 was inhibited by 37 of the 38 isolates. Two types of zones were observed; one with sharply defined (Type #1), and another with an undefined, diffuse edge (Type #2). Sixteen of the isolates that had large zones of inhibition were examined further using agar spot assays with the plates being incubated anaerobically. All 16 produced inhibition zones under anaerobic conditions, though the size of the zone was smaller than those observed when the plates were incubated aerobically. Again, two types of inhibition zones were observed.

The 16 isolates were agar spot tested on TSAYE agar which did not contain glucose to determine which of the strains inhibited *L. monocytogenes* primarily due to acid production. The 6 strains that produced Type #2 inhibition zones on MRS plates did not inhibit *L. monocytogenes* on TSAYE without glucose plates. This indicates that Type #2 inhibition is due to acid production associated with catabolism of glucose.

The 10 isolates that produced Type #1 inhibition on MRS agar also inhibited *L. monocytogenes* on TSAYE agar. These strains were tested for bacteriocin by performing agar spot tests or agar well diffusion assays with various proteolytic enzymes (i.e., pepsin, trypsin, α-chymotrypsin and proteinase K). None of the enzymes affected the zones of inhibition. While this cannot rule out that the type #2 zones of inhibition was not the result of bacteriocin activity, the results do indicate that if present, the bacteriocin is unusual in relation to the range of proteolytic enzymes to which it is resistant. The Type #1 zones of inhibition were also unaffected by the presence of catalase, indicating that the activity of the 10 *L. lactis* isolates was not due to the production of peroxides.

The identity of the 16 isolates was determined using API 50 CH strips. Fourteen of the isolates were identified as *Lactococcus lactis* subsp. *lactis*; four with very good identification and ten with acceptable identification. The latter group fermented melibiose and D-raffinose in addition to the sugars fermented by the other four *L. lactis* isolates. The remaining two were identified as *Lactobacillus brevis*. *Lactococcus lactis* subsp. *lactis* is well known for its activity and has been isolated from a number of different foods (CAI et al., 1997; KELLY et al., 1998).

Lactococcus lactis has a homolactic fermentation i.e. produce mainly lactic acid as an endproduct in its metabolism. In addition to decreasing the pH of the environment, lactic acid has additional antimicrobial activity associated with its ability as a weak lipophilic acid to pass across the cell membrane in its undissociated form, dissociate within the cell and acidify the cell interior (SHELEF, 1994). It is likely that the inhibitory activity of *L. lactis* is associated with its fermentation of sugars present in the sprout broth to lactic acid. The sprout broth contained approximately 1 g per liter total sugars in glucose equivalents; a level that support microbial growth and significant pH depression.

The randomly selected four isolates (SP03, SP26, SP36, later all identified as *L. lactis* subsp. *lactis*, and SP06: *Lactobacillus brevis*) grew well in the alfalfa broth reaching about 5×10^8 CFU ml^{-1} MPD (Table 1). The *L. monocytogenes* strain grew slower than the isolates, especially at room temperature, where the growth curves showed a somewhat longer lag time than the other strains. It reached an MPD of about 10^8 CFU ml^{-1}.

Lactococcus lactis subsp. *lactis* SP26 strain was chosen for further study on the basis of its ability to grow rapidly and its inhibitory activity was restricted to acid production. We decided not to use a Type #1 isolate for the subsequent competition study, since the mechanism of action could not be conclusively demonstrated. A strain that produced acid only was selected based on the assumption that even if a bacteriocin-producing isolate was later identified, significant inhibition by acid production would be an important safeguard against the acquisition of resistance to the bacteriocin by *L. monocytogenes* isolates.

2.2. Competition studies

The rate and extent of growth of *L. monocytogenes* were determined in the presence or absence of *Lactococcus lactis* in alfalfa sprout broth adjusted for different combinations of initial pH (5, 6, 7, or 8) and temperatures (10, 20, or 30 °C). The use of autoclaved sprout broth was used as model system, since it better approximated the commodity of interest, but still allowed the effects of co-culturing and initial pH to be studied under highly controlled conditions. Four growth curves for *L. monocytogenes* were obtained for each growth condition, the data were fitted with the Gompertz and the three-phase linear growth models to estimate MPD, μ, and λ.

Table 1. Preliminary study in alfalfa sprout broth: growth of *L. monocytogenes* F4258 and four selected lactic acid bacteria strains (SP03, SP36 - Type #1 and SP06, SP26 - Type #2 inhibition)

Strain	Time (h)	30 °C $\log_{10}$ CFU ml^{-1}	Room temp.(appr. 23 °C) $\log_{10}$ CFU ml^{-1}
Listeria monocytogenes F4258	0	2.90	3.24
	3	3.38	NT[a]
	6	4.34	3.31
	24	8.09	6.29
	30	8.08	7.42
Strain SP03	0	3.33	3.33
	3	3.97	NT
	6	4.96	4.58
	24	8.40	8.33
	30	8.37	8.41
Strain SP06	0	3.05	3.05
	3	4.35	NT
	6	5.43	5.07
	24	6.83	6.71
	30	6.39	6.96
Strain SP26	0	3.38	3.38
	3	4.09	NT
	6	5.24	4.91
	24	8.39	8.49
	30	8.18	8.30
Strain SP36	0	3.33	3.33
	3	4.26	NT
	6	5.44	5.12
	24	8.50	8.58
	30	8.09	8.52

[a]NT: Not tested

The three-phase linear model gave better estimates of the growth kinetics (presented in Table 2) than the Gompertz model, which tended to overestimate the μ of *L. monocytogenes* grown in co-culture (data not shown). This reflected the fact that when the increase in cell numbers was relatively small as was the case when *L. monocytogenes* was grown in co-culture, the curve fitting program had a difficult time fitting the limited number of data points available to the Gompertz model.

Table 2. Growth kinetics of *Listeria monocytogenes* F4258 in co-culture with *L. lactis* SP26, and in single culture as control grown in alfalfa sprout juice (values were derived from parameters estimated by the Three-Phase Linear model)

		L. monocytogenes mono-culture					*L. monocytogenes* co-culture				
Temp. (°C)	Initial pH	IPD ($\log_{10}$ (CFU ml^{-1}))	Lag Phase Duration (h)	MPD ($\log_{10}$ (CFU ml^{-1}))	μ ($\log_{10}$ (CFU ml^{-1}) h^{-1})	Final pH[a]	IPD ($\log_{10}$ (CFU ml^{-1}))	Lag Phase Duration (h)	MPD ($\log_{10}$ (CFU ml^{-1}))	μ ($\log_{10}$ (CFU ml^{-1}) h^{-1})	Final pH[a]
30	8.0	3.4 ± 0.1	2.9 ± 0.3	8.7 ± 0.1	0.42 ± 0.01	4.4	3.4 ± 0.1	2.7 ± 0.3	5.8 ± 0.0	0.36 ± 0.05	3.6
30	7.0	3.2 ± 0.3	3.1 ± 0.4	8.4 ± 0.1	0.45 ± 0.02	4.5	3.2 ± 0.3	2.8 ± 0.5	5.3 ± 0.3	0.39 ± 0.04	3.4
30	6.0	3.4 ± 0.0	3.9 ± 0.2	7.9 ± 0.1	0.39 ± 0.05	4.8	3.3 ± 0.05	3.7 ± 0.05	4.7 ± 0.3	0.36 ± 0.13	3.4
30	5.0	3.4			No growth	5.0	3.40		No growth		3.5
20	8.0	3.4 ± 0.0	2.8 ± 0.1	8.7 ± 0.0	0.20 ± 0.00	4.8	3.3 ± 0.05	1.7 ± 0.4	5.8 ± 0.05	0.17 ± 0.01	3.7
20	7.0	3.6 ± 0.1	2.5 ± 0.3	8.6 ± 0.0	0.20 ± 0.02	4.3	3.5 ± 0.1	2.2 ± 0.9	5.5 ± 0.1	0.19 ± 0.02	3.6
20	6.0	3.4 ± 0.1	3.3 ± 2.1	8.3 ± 0.1	0.17 ± 0.01	4.5	3.4 ± 0.0	4.0 ± 0.6	5.1 ± 0.0	0.19 ± 0.05	3.4
20	5.0	Not tested					Not tested				
10	8.0	3.5 ± 0.0	9.2 ± 4.9	8.9 ± 0.0	0.055 ± 0.004	4.6	3.4 ± 0.0	5.9 ± 0.7	6.6 ± 0.0	0.055 ± 0.003	3.9
10	7.0	3.5 ± 0.1	8.7 ± 3.7	8.8 ± 0.1	0.057 ± 0.001	4.7	3.4 ± 0.1	5.8 ± 0.9	6.4 ± 0.1	0.056 ± 0.003	4.0
10	6.0	3.5 ± 0.1	14.5 ± 2.3	8.5 ± 0.2	0.049 ± 0.003	4.9	3.5 ± 0.1	11.0 ± 0.9	5.6 ± 0.2	0.041 ± 0.001	3.8
10	5.0	2.3			No growth	5.0	2.3			No growth	5.1

[a] pH values were not obtained by curve fitting

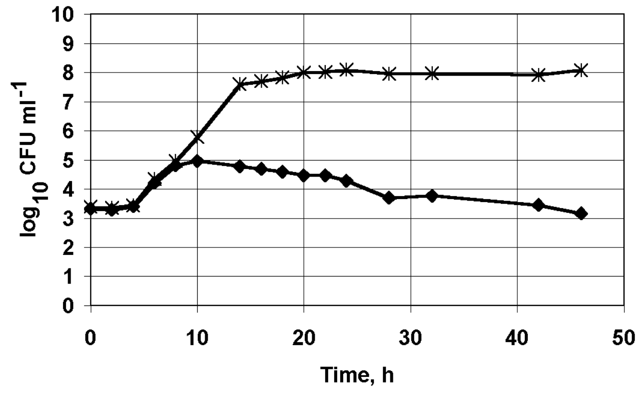
IPD: Initial population density ($\log_{10}$ CFU ml^{-1}); MPD: Maximum population density ($\log_{10}$ CFU ml^{-1}); μ: specific growth rate (1 h^{-1}). Values are averages of four replicates.

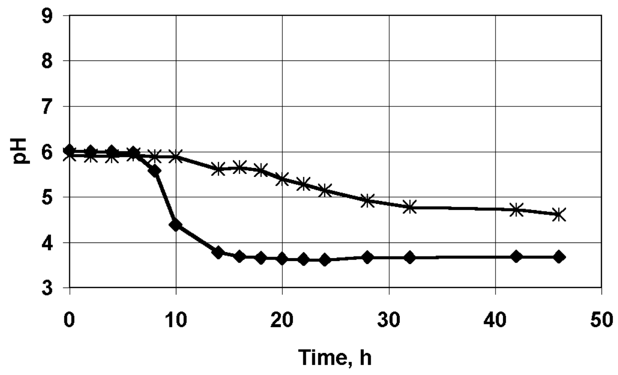
Listeria monocytogenes, in the presence or absence of *L. lactis*, did not grow at pH 5.0 at 10 and 30 °C. At the other pH values, the microorganism grew well in the absence of *L. lactis* at each of the incubation temperatures (Table 2), reaching MPD values of about 5×10^8 CFU ml^{-1} in each case. The MPDs reached by *L. monocytogenes* monocultures at pH 6 were slightly less than those of the corresponding cultures at pH 7 and 8. At a given incubation temperature, initial pH values between 6 and 8 had little effect on the μ and λ of *Listeria monocytogenes* monocultures. As expected, μ decreased with decreasing temperatures.

The presence of *L. lactis* SP26 appeared to have little effect on the μ and λ of *L. monocytogenes* (Table 2). However, the lactic acid bacterium had a profound effect on the MPD reached by *L. monocytogene*s F4258 (Table 2). This is similar to the studies of BUCHANAN and BAGI (1997, 1999), who examined the effect of initial pH and temperature on the interaction of *L. monocytogenes* with *Carnobacterium piscicola* and *Pseudomonas fluorescens*. In both cases, the effect of the competing microorganism on *L. monocytogenes* was largely limited to a suppression of the MPD reached by the pathogen. The combination of temperature and initial pH that favoured that inhibition largely reflected at what combination of the two parameters the growth of the competing microorganism is favoured over *L. monocytogenes*. The same appears to be the case in the current instance.

The MPD of *L. monocytogenes* in the presence of *L. lactis* SP26 was depressed by 2 to 3 log cycles. The extent of MPD depression was affected by both initial pH and incubation temperature. Three examples comparing the growth of *L. monocytogenes* in the presence and absence of *L. lactis* are depicted in Fig. 1. Lowering the initial pH enhanced the effect of the competitive culture. Conversely, increasing incubation temperature enhanced the inhibitory effect of the *L. lactis* culture. Thus, the greatest suppression of *L. monocytogenes* growth was achieved at an initial pH of 6 and an incubation temperature of 30 °C. Higher temperatures (20–30 °C) supported the metabolic activity, thus the acid producing capacity of *Lactococcus lactis.* The differential between the MPDs of *L. monocytogenes* monocultures and co-cultures under the different pH and temperature conditions is presented graphically in Fig. 2. The inhibitory activity observed in the co-culture appears to be attributable to the production of lactic acid by *L. lactis*. The final pH of the co-cultures was approximately 1 pH unit below the corresponding monoculture (Table 2), and the pH decline corresponded temporally with the cessation of *L. monocytogenes* growth. This also suggests that increasing the initial level of *L. lactis* in relation to *L. monocytogenes* would enhance the inhibition effect. This was the case in a preliminary study (data not shown), where the initial level of *L. lactis* was increased to 10^6 CFU ml^{-1} and that for *L. monocytogenes* was reduced to $<10^2$ CFU ml^{-1}. In this instance, *L. monocytogenes* did not grow at all.

A





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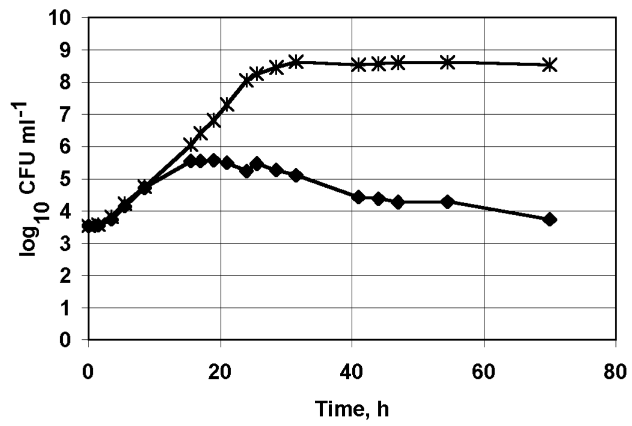

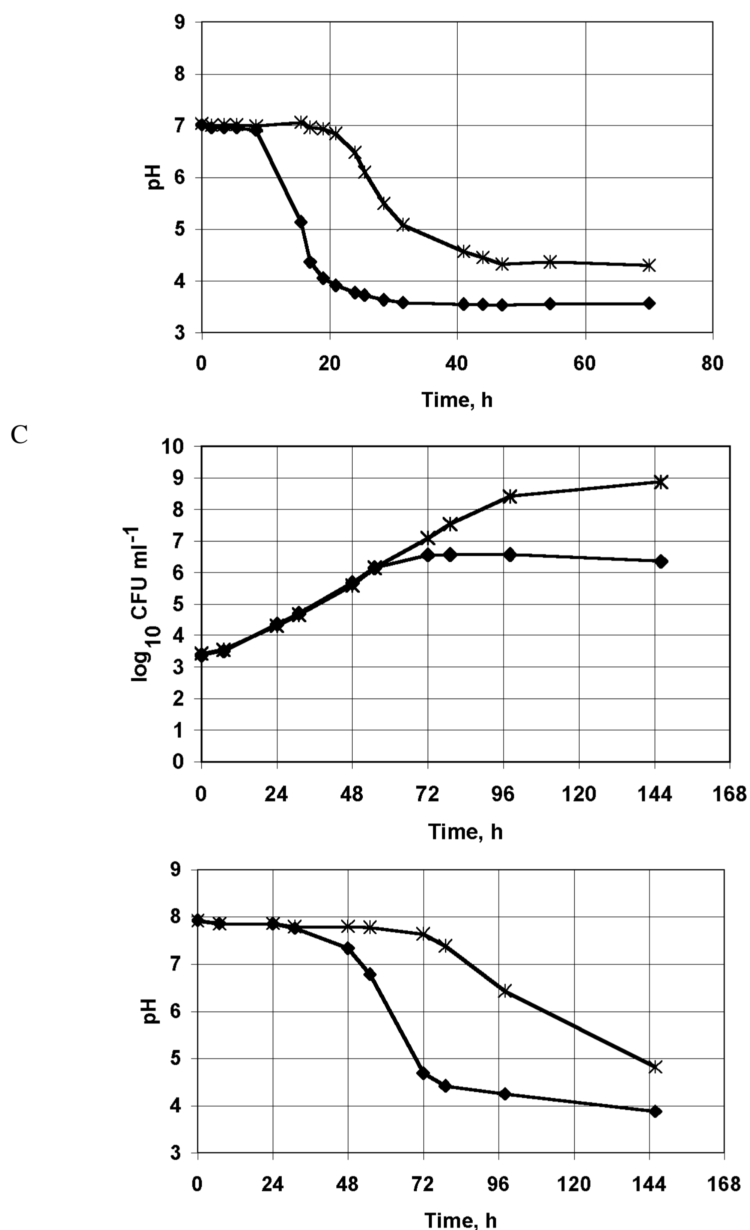

Fig. 1. Examples of the growth kinetics and pH changes in *Listeria monocytogenes* F4258 mono- and co-cultures with *Lactococcus lactis* SP26 in alfalfa sprout broth. A: Initial pH = 6.0, incubation temperature = 30 °C; B: initial pH = 7.0, incubation temperature = 20 °C; C: initial pH = 8.0, incubation temperature = 10 °C. *: Monoculture; ♦ co-culture

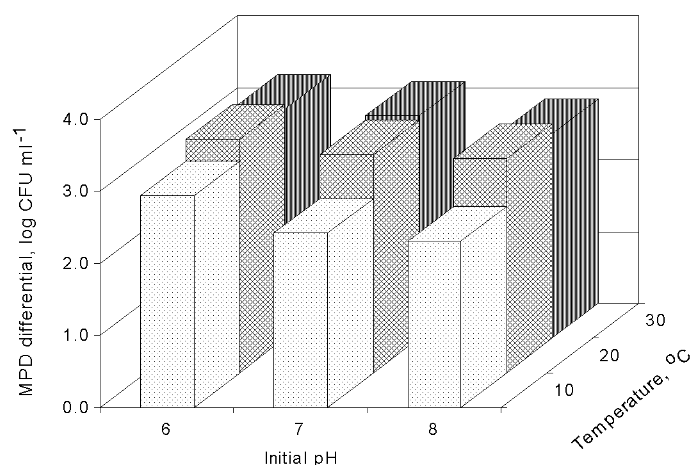

Fig. 2. Differential of maximum population density (MPD) of *Listeria monocytogenes* F4258 in monoculture and as a co-culture with *Lactococcus lactis* in alfalfa sprout broth at different pH and temperature conditions

The results of the current study are similar to those of BREIDT and FLEMING (1998), who developed a model for competitive growth of *Lactococcus lactis* and *L. monocytogenes* in cucumber juice medium. In their model, bacterial cell growth was limited by the accumulation of protonated lactic acid and decreasing pH. Though the antimicrobial activity of the lactate anion was considered, they concluded that pH was the primary factor limiting the growth of *L. monocytogenes.*

3. Conclusion

Lactic acid bacteria have been studied widely for their ability to affect the growth of other foodborne bacteria including pathogenic species (HOLZAPFEL et al., 1995). Their ubiquity and activity against Gram-positive pathogens such as *L. monocytogenes* prompted us to isolate from commercially produced alfalfa sprouts lactic acid bacteria with activity against *L. monocytogenes* F4258. Numerous isolates that produced large inhibition zones against *L. monocytogenes* were isolated readily. It is likely that some of the inhibitory activity observed were associated with bacteriocin production but that could not be confirmed using the relatively simple screening techniques used. Most of the active isolates were identified as *Lactococcus lactis* subsp. *lactis*.

One of the *L. lactis* subsp *lactis* strains, SP26, that inhibited *L. monocytogenes* by acid production only was chosen to evaluate its potential to inhibit *L. monocytogenes* in a model sprout broth system.

The most striking feature in the interaction between *L. monocytogenes* and *L. lactis* was that the inhibitory activity of the lactic acid bacterium was virtually limited to a suppression of the MPD. The relative growth rates of *L. lactis*, a highly acid resistant, mesophilic microorganism, was most favoured over *L. monocytogenes* at an initial pH of 6 and an incubation temperature of 30 °C.

The temporal relationship between the rapid decline in pH and the time when *L. monocytogenes* growth was suppressed suggests that the decline in pH is the primary mechanism responsible for the inhibitory activity of *L. lactis*. This also suggests that factors that lead to earlier or more extensive lactic acid production would enhance the lactic acid bacterium activity. Thus, the use of larger inocula or addition of additional fermentable carbohydrate maybe a means for optimizing the inhibitory activity of *L. lactis*. However, whether lactic acid bacteria such as *L. lactis* can be used successfully to enhance the microbiological safety of sprouts awaits additional studies with intact sprouted seeds under conditions similar to those that would be encountered during the production, distribution and marketing of this product group.

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