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J Huang, C Lacroix, H Daba, Re Simard. Inhibition of growth of *Listeria* strains by mesenterocin 5 and organic acids. *Le Lait*, 1993, 73 (4), pp.357-370. <hal-00929347>

HAL Id: hal-00929347

<https://hal.science/hal-00929347v1>

Submitted on 11 May 2020

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Inhibition of growth of *Listeria* strains by mesenterocin 5 and organic acids

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(Received 2 December 1992; accepted 15 April 1993)

Summary — The influence of lactic and acetic acid concentration on specific growth rate (μ) of *Listeria* strains in tryptic soy broth (TSB) was investigated. For each strain and acid, minimal and maximal inhibitory concentrations (MIC_{min} and MIC_{max} corresponding respectively to $\mu = \mu_{max}$ and $\mu = 0$) were determined. In the range of MIC_{min} and MIC_{max} ; and for all the *Listeria* strains tested, μ decreased with increasing lactic and acetic acid concentrations in the TSB medium, although it was little affected in buffered (0.15 mol/l phosphate) TSB. For acid concentrations exceeding the MIC_{max} by 0.05% (w/v), viable counts of *Listeria* strains remained nearly constant in unbuffered TSB at $\approx 10^7$ cfu/ml for 24 h. Mesenterocin 5 produced during overnight culture of *Leuconostoc mesenteroides* UL5 in MRS medium at 30°C was used in an inhibition test. Unconcentrated ultrafiltered-diafiltered mesenterocin 5 supernatant (0.1 ml) was added to 9.9 ml of TSB and 0.4 ml of 6 separate *Listeria* suspensions. Mesenterocin 5 exhibited, for the first 2 h at pH 7.0 and 30°C, a bacteriostatic effect on one strain and a bactericidal effect on 5 strains, ie, initial viable counts of 10^7 cfu/ml decreased by 1.0 to 3.0 log. The total biomass measured by optical density (OD) then remained constant for a period of 3 to 16 h before it eventually increased. After this extended lag-phase, mesenterocin 5 concentration (1 x C to 8 x C) affected the specific growth rate of most *Listeria* strains to a much lesser extent in contrast to the influence of organic acid concentration.

organic acid / mesenterocin 5 / bacteriocin / *Listeria* growth

Résumé — Inhibition de la mésentéroïne 5 et des acides organiques sur la croissance de plusieurs souches de *Listeria*. Le taux de croissance (μ) de plusieurs souches de *Listeria* dans un milieu de soja tryptique (TSB) a été étudié en fonction de la concentration en acides lactique et acétique. Pour chaque souche et chaque acide, les concentrations inhibitrices minimales et maximales (MIC_{max} et MIC_{min} , correspondant respectivement à $\mu = \mu_{max}$ et $\mu = 0$) ont été déterminées. Dans l'intervalle MIC_{min} et MIC_{max} le taux de croissance spécifique des *Listeria* a diminué pour des concentrations croissantes en acide dans le milieu TSB, alors qu'il n'a pas varié en milieu TSB tamponné (0,15 mol/l phosphate). Pour des concentrations en acide excédant de 0,05% (p/v) MIC_{max} , la viabilité (environ 10^7 cfu/ml) des *Listeria* a été conservée pendant 24 h dans le milieu non tamponné. Un extrait de mésentéroïne 5 produite lors d'une culture d'une nuit de *Leuconostoc mesenteroides* UL5 dans du milieu MRS à 30°C, a été utilisé pour le test d'inhibition; 0,1 ml de surnageant non concentré, ultrafiltré et diafiltré de la culture, a été ajouté à 9,9 ml de milieu TSB et 0,4 ml d'une culture de

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Listeria. Pour 5 des 6 souches de *Listeria* testées, un effet bactéricide a été observé avec une décroissance lors des 2 premières heures à pH 7,0 et 30°C de 1 à 3 log du nombre initial de 10^7 ctu/ml, alors qu'un effet bactériostatique a été obtenu pour la dernière souche. Par la suite, la biomasse totale évaluée par densité optique est demeurée stable pendant 3 à 16 h, pour croître ensuite. Après cette phase de latence prolongée, l'effet de la concentration en mésentéroline 5 (1 x C à 8 x C) a été peu marqué sur le taux de croissance spécifique de la plupart des souches, contrairement à l'effet des acides organiques.

acide organique / mésentéroline 5 / bactériocine / croissance de *Listeria*

INTRODUCTION

One of the major problems in the food industry, particularly in dairy products, is the occasional presence of pathogens such as *Listeria monocytogenes*, which has been associated with food-borne disease outbreaks (Farber and Peterkin, 1991). It has been reported that *L. monocytogenes* can survive at high temperature, low pH or throughout processing of some products, and in some cases may cause post-pasteurization contamination (Doyle *et al*, 1985, 1987; Parish and Higgins, 1989; Grau and Vanderlinde, 1990). For instance, *L. monocytogenes* was found to survive for > 400 d in aged Cheddar cheese (Ryser and Marth, 1987). Some environmental and processing conditions, such as pH, temperature, oxygen level, carbon dioxide, sodium chloride and nitrite may influence the growth or survival of *L. monocytogenes* in foods (Doyle, 1988; Sorrells *et al*, 1989). Lactic acid bacteria (LAB) generally preserve foods because their growth-associated formation of organic acids and the resulting decrease in pH exert an inhibitory effect on microbial contaminants (Daeschel, 1989). A high acidity is, however, not desirable in some products (Prentice and Neaves, 1988). It therefore seems timely to develop effective preservatives which specifically inhibit *L. monocytogenes*.

Recently, purification and characterization of bacteriocins produced by LAB that inhibit undesirable organisms have received considerable attention (Tagg *et al*, 1976; Babel, 1977; Barefoot and Klaenhammer, 1984; Benkerroum and Sandine, 1988; Klaenhammer, 1988; Muriana and Klaenhammer, 1991; Piard and Desmazaud, 1992; Piard *et al*, 1992). Nisin, which is generally recognized as safe (GRAS), has received FDA approval in the USA for use in certain processed cheeses (Delves-Broughton, 1990). Today, European countries employ nisin and nisin-producing starters for antimicrobial roles in food processing and fermentations. Although many non-spore-forming bacteria are sensitive to nisin, its industrial use is limited solely to the prevention of spore outgrowth of *Clostridium botulinum* in processed cheese and canned foods (Delves-Broughton, 1990). Therefore, research for new efficient bacteriocins capable of eliminating or inhibiting the growth of *Listeria* strains has become a priority for the food industry. Bacteriocins produced by LAB in particular ensure the safety of foods, and display an inhibitory activity against pathogens when used in food products as food preservatives. Recently, Berry *et al* (1990) have observed that when fully processed meat frankfurters were inoculated with both *L. monocytogenes* and *Pediococcus acidilactici*, a bacteriocin-producing strain, the growth of

L. monocytogenes was efficiently inhibited, although no reduction in cell numbers occurred. The identification of other bacteriocins with antilisterial activity shows great promise for controlling the growth of *L. monocytogenes* in foods (Pucci *et al*, 1988; Spelhaug and Harlander, 1989).

Only a few investigators have reported the characterization of bacteriocins produced by *Leuconostoc* as compared with bacteriocins produced by other species of LAB (Hardings and Shaw, 1990; Daba *et al*, 1991; Hastings *et al*, 1991; Hastings and Stiles, 1991; Lewus *et al*, 1992). Our previous investigation has shown that the bacteriocin mesenterocin 5, secreted by the food-grade microorganism *Leuconostoc mesenteroides*, is proteinaceous and displays an antimicrobial activity against a number of undesirable Gram-positive bacteria, including *Listeria*, in a well diffusion assay (Daba *et al*, 1991). The growth-associated formation of organic acids by *L. mesenteroides* may inhibit the growth of *Listeria* strains to some extent similar to the bacteriocin (Babel, 1977; Barefoot and Klaenhammer, 1984; Daeschel, 1989). The mechanism by which the bacteriocin controls the growth behavior of *Listeria* in suspension has not yet been studied. Therefore, it is important to separately and quantitatively characterize the inhibitory effects of mesenterocin 5 and organic acids on *Listeria* strains in foods.

The main objective of this study was to compare the inhibition pattern of organic acids and mesenterocin 5 produced by *L. mesenteroides* on the growth of various pathogenic *Listeria* strains. We also determined the effects of mesenterocin 5 and organic acids on the growth kinetics of the tested strains.

MATERIALS AND METHODS

Bacterial strains

The microorganism used for bacteriocin production was *Leuconostoc mesenteroides* UL5, isolated from local Cheddar cheese and maintained as a part of our strain collection (Daba *et al*, 1991). The *Listeria* strains used were *L. grayi* 29, *L. innocua* 18, *L. ivanovii* 28, *L. monocytogenes* 1089, *L. monocytogenes* Scott A3, and *L. welshimeri* 89. The origin and characteristics of both *Leuconostoc* and *Listeria* strains have been previously described in detail by Daba *et al* (1991).

Culture media and conditions

L. mesenteroides was grown at 30°C in MRS medium (56 g/l, Institut Rosell, Montreal, Canada), with the addition of 0.1% Tween 80. *Listeria* strains were grown at 30°C in tryptic soy broth (30 g/l, Difco Laboratories, Detroit, MI) with the addition of 6 g yeast extract/l (Difco). In some tests, *Listeria* strains were incubated in buffered (0.15 mol/l phosphate) TSB medium. This allowed the medium pH to be maintained at 6.75 when lactic or acetic acid was added to the medium to reach the maximal inhibitory concentration, while the pH of unbuffered medium decreased from 7.0 to ≈5.0 under these conditions. The buffered medium was also designed to minimize the effect of phosphate ions on the growth of *Listeria* strains. Agar media were prepared by adding 1.5% agar (Difco) to the above liquid media.

Production of mesenterocin 5

Twenty ml MRS medium was inoculated with 0.2 ml freshly-thawed *L. mesenteroides* stock kept frozen at -80°C. For the production of mesenterocin 5, cultures were grown overnight at

30°C in a rotary shaker (100 rpm). Unconcentrated ultrafiltered supernatant obtained from a fresh culture exhibited an activity of 2 048 arbitrary units per ml (AU/ml) against *L. ivanovii* 28 at 30°C for a culture period of 18 h, as described previously (Daba *et al*, 1991). A 10-ml volume of the overnight culture was concentrated by using a YM membrane with 3 kDa cutoff (Amicon Corp, Danvers, MA) in a 10-ml standard cell model (Amicon Corp) to a final vol of 2 ml, or 1 ml for the test shown in table IV. A pressure of 4 kg/cm² was maintained using nitrogen gas. To eliminate the antilisterial effect of organic acids, the concentrated supernatant was diafiltered with 8 or 9 ml of MRS medium (pH 7.0). The 2 ml of concentrated solution was finally adjusted to 10 ml with sterile TSB medium, and the 1 ml of concentrated sample to 1.25, 2.50, 5.00, and 10 ml (corresponding to 8 x C, 4 x C, 2 x C and 1 x C concentrations). These ultrafiltered-diafiltered supernatants were immediately frozen at -80°C overnight. When used, the frozen supernatants were kept at -20 °C for 2 h, then at 0°C for another 2 h, and were finally thawed at room temperature.

To test the possible inhibition of hydrogen peroxide, the following procedure, previously described by Piard *et al* (1990) was used: 1 ml unconcentrated ultrafiltered supernatant of *L. mesenteroides* strain and 1 ml filtrate obtained from supernatant ultrafiltration were treated with catalase (Sigma, 7 080 U/mg) diluted in potassium phosphate buffer (0.05 mol/l, pH 7.0) to a final concentration of 100 U/ml. The solutions were incubated at 37°C for 30 min and then assayed for bacteriocin activity using the critical dilution micromethod (Daba *et al*, 1991) and compared to a control sample not treated with catalase.

Measurement of the specific growth rate of *Listeria* strains

To determine the specific growth rate of *Listeria* strains in the presence of mesenterocin 5 or organic acids, the following procedure was used: 9.9 ml TSB medium in test tubes (19 x 150 cm, Hycl Inc, Houston, TX) was first mixed with 0.4 ml *Listeria* culture diluted and standardized to an optical density (OD) of 0.2 (corresponding to $\approx 1 \times 10^8$ cfu/ml) at a wavelength of 600 nm (Spectronic 20, Bausch and Lomb, Rochester,

NY). Lactic acid (85%, w/v) and acetic acid (100%, w/v) were both diluted 10- and 100-fold with sterile TSB medium. An appropriate volume of lactic or acetic acid was withdrawn from the diluted acid solution and adjusted to 0.1 ml with sterile TSB medium. This preparation, or 0.1 ml thawed mesenterocin 5 solution from the ultrafiltered-diafiltered supernatant (8 x C, 4 x C, 2 x C and 1 x C concentrations) stored at -80°C, was added to the above *Listeria* suspension to afford the desired concentration (tables I-IV). The OD₆₀₀ of the mixed solution was measured at 30-min intervals during the exponential phase. The specific growth rate of *Listeria* strains was determined from at least 5 points of the OD₆₀₀ measurement. The maximum specific growth rate (μ_{max}) was obtained by measuring the growth of the corresponding strains in the absence of mesenterocin 5 or organic acids. The experiment was performed in duplicate.

Cell concentration measurement

Total biomass was determined spectrophotometrically by measuring the OD at a wavelength of 600 nm. *Listeria* viable cell numbers were estimated by dilution plating: 0.1-ml samples were withdrawn from a test tube, diluted appropriately and then spread onto TSB agar plates in triplicate. Viable cell numbers of each sample were calculated from the average number of colonies on 3 Petri dishes and expressed as colony forming units (cfu) per ml medium.

Mode of action of mesenterocin 5 on *Listeria* strains

The determination of mesenterocin 5 mode of action on indicator *Listeria* strains was based on the method previously described by Barefoot and Klaenhammer (1984), modified as follows: 0.1 ml ultrafiltered-diafiltered supernatant containing mesenterocin 5 was added to a sterile test tube containing 9.9 ml of TSB medium. A 0.4-ml volume of overnight *Listeria* culture diluted and standardized to an OD₆₀₀ of 0.2 was then added to the test tube to yield a cell concentration of $\approx 1 \times 10^7$ cfu/ml. OD and viable cell concentration (cfu/ml) were measured at 30-min intervals during incubation at 30°C. Bactericidal

or inhibitory effect on *Listeria* strains was determined directly by measuring the viable cell concentration during incubation. Reported data are the average from 2 separate experiments.

RESULTS AND DISCUSSION

The effect of lactic and acetic acid concentration on the specific growth rate of Listeria strains

The relationship between the specific growth rate (μ) of *L. monocytogenes* Scott A3 and organic acid concentration was studied (fig 1). Two important parameters, MIC_{min} and MIC_{max} , corresponding to the minimal and maximal inhibitory concentrations, respectively, could be found for the strain tested. When the acid concentration is lower than MIC_{min} , μ is no longer affected by acid concentration. This characteristic parameter can reflect cell growth capacity in the presence of an acid. If the acid

concentration is higher than MIC_{max} , no cell growth occurs ($\mu = 0$). In the range of MIC_{min} and MIC_{max} , μ decreased from μ_{max} to 0 with increasing acid concentration. Our experiments also showed that other strains tested exhibited the same growth behavior as *L. monocytogenes* Scott A3, and that the effect of lactic and acetic acids on the growth behavior of *Listeria* strains was similar (data not shown).

The MIC_{min} and MIC_{max} of lactic and acetic acids for several *Listeria* strains were determined (table I). Generally, the MIC_{max} for a given strain was 5–17-fold higher than the MIC_{min} for both acids. For the same strain, the MIC_{min} of lactic acid was slightly lower than that of acetic acid, but the MIC_{max} of lactic acid was generally slightly higher than that of acetic acid with the exception of *L. grayi*. This indicates that the range of inhibition for lactic acid was slightly wider than that for acetic acid. In addition, the MIC_{min} and MIC_{max} of lactic and acetic acids were largely dependent upon the *Listeria* strain, showing that

Table I. MIC_{min} * and MIC_{max} * of lactic and acetic acids for various *Listeria* strains. MIC_{min} * et MIC_{max} * des acides lactique et acétique de plusieurs souches de *Listeria*.

| Strains | LA** concentration (% w/v) | | AA** concentration (% w/v) | |
|----------------------------------|----------------------------|-------------|----------------------------|-------------|
| | MIC_{min} | MIC_{max} | MIC_{min} | MIC_{max} |
| <i>L. grayi</i> 29 | 0.055 | 0.30 | 0.048 | 0.25 |
| <i>L. innocua</i> 18 | 0.015 | 0.23 | 0.021 | 0.19 |
| <i>L. ivanovii</i> 28 | 0.015 | 0.26 | 0.017 | 0.19 |
| <i>L. monocytogenes</i> 1089 | 0.028 | 0.23 | 0.036 | 0.32 |
| <i>L. monocytogenes</i> Scott A3 | 0.028 | 0.38 | 0.030 | 0.25 |
| <i>L. welshimeri</i> 89 | 0.032 | 0.28 | 0.038 | 0.28 |

* MIC_{min} and MIC_{max} are respectively the minimal and maximal inhibitory concentrations, expressed as % (w/v). Reported data are the average from 2 separate experiments; SEs for MIC_{min} and MIC_{max} were 0.002 and 0.02%, respectively. ** LA: lactic acid; AA: acetic acid.

* MIC_{min} et MIC_{max} sont respectivement les concentrations inhibitrices minimales et maximales, exprimées en % (p/v). Les résultats sont les moyennes de 2 expérimentations séparées. Les erreurs standard pour MIC_{min} et MIC_{max} sont respectivement de 0,002 et 0,02%. ** LA : acide lactique; AA : acide acétique.

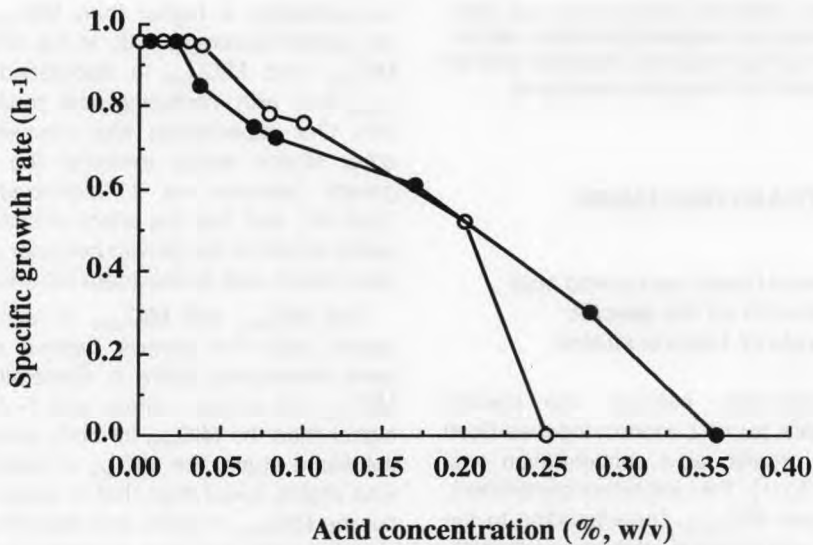


Fig 1. Relationship between lactic (●) and acetic (○) acid concentrations and the specific growth rate of *Listeria monocytogenes* Scott A3. The strain was cultured in unbuffered TSB at 30°C. The specific growth rate was obtained by measuring OD₆₀₀ during the exponential phase.

Relation entre les concentrations en acides lactique (●) et acétique (○) et le taux de croissance spécifique de la souche de Listeria monocytogenes Scott A3. La souche a été incubée en milieu TSB non tamponné à 30°C. Le taux de croissance spécifique a été mesuré par la DO₆₀₀ en phase exponentielle.

MIC_{min} and MIC_{max} are characteristic for each strain. *L. innocua* and *L. ivanovii* appeared to be the most sensitive strains to lactic and acetic acids while *L. grayi* and *L. welshimeri* were the most resistant.

In the range of MIC_{min} and MIC_{max}, the specific growth rate of the 6 *Listeria* strains tested generally decreased with increasing lactic or acetic acid concentration (table II). The organic acids caused only a decrease in the specific growth rate of a strain without significantly affecting the lag-phase (data not shown). As well, increased acid concentration in the TSB medium resulted in a pH decrease from 7.0 to < 5.0 due to the low buffering capacity of this medium. Therefore, the effect of organic acids on the specific growth rate of *Listeria* may

originate from both pH decrease and presence of acids.

The role of lactic and acetic acids in inhibiting the growth of *Listeria* strains

The inhibition of *Listeria* growth by lactic and acetic acids was examined using phosphate-buffered medium. The pH of the medium was reduced from 7.0 to 6.75 when 0.30% of lactic acid or 0.26% acetic acid was added. In unbuffered medium, however, these additions reduced the pH to ≈ 5.0. Comparison of growth rates in buffered medium (table III) to those in unbuffered medium (table II) shows that the maximum specific growth rate (μ_{max}) was

Table II. Specific growth rate (μ , h^{-1})^{*} of *Listeria* strains cultured in unbuffered TSB containing different concentrations of lactic and acetic acids.

*Taux de croissance spécifique (μ , h^{-1}) * de plusieurs souches de Listeria cultivées en milieu TSB non tamponné à différentes concentrations en acides lactique et acétique.*

| Strain | μ_{max} | LA** concentration (% w/v) | | | | AA** concentration (% w/v) | | | |
|----------------------------------|-------------|----------------------------|------|------|------|----------------------------|------|------|------|
| | | 0.03 | 0.07 | 0.09 | 0.17 | 0.04 | 0.08 | 0.10 | 0.20 |
| <i>L. grayi</i> 29 | 1.05 | 1.05 | 0.95 | 0.83 | 0.70 | 1.05 | 0.96 | 0.80 | 0.50 |
| <i>L. innocua</i> 18 | 1.01 | 0.92 | 0.85 | 0.80 | 0.70 | 0.97 | 0.89 | 0.71 | 0.59 |
| <i>L. ivanovii</i> 28 | 1.06 | 0.92 | 0.87 | 0.83 | 0.66 | 0.91 | 0.89 | 0.56 | 0.43 |
| <i>L. monocytogenes</i> 1089 | 0.93 | 0.88 | 0.73 | 0.67 | 0.52 | 0.92 | 0.90 | 0.75 | 0.54 |
| <i>L. monocytogenes</i> Scott A3 | 0.96 | 0.86 | 0.75 | 0.72 | 0.59 | 0.96 | 0.79 | 0.71 | 0.50 |
| <i>L. welshimeri</i> 89 | 1.04 | 1.04 | 0.85 | 0.67 | 0.49 | 0.90 | 0.82 | 0.38 | 0.28 |

* The results are average data from 2 experiments; SE of μ was 0.04 (h^{-1}). ** LA: lactic acid; AA: acetic acid.

* Les résultats sont les moyennes obtenues de 2 expérimentations. L'erreur type sur μ est de 0,04 (h^{-1}). ** LA : acide lactique; AA : acide acétique.

reduced by only \approx 10%, indicating that the phosphate buffer itself had little influence on the specific growth rate. In the phosphate-buffered medium (table III), the specific growth rate for most strains was only slightly affected by increasing the lactic or acetic acid concentration (0.05 to 0.10%), and it decreased by only 2 to 27% even at concentrations as high as 0.26% for lactic acid and 0.30% for acetic acid, which are close or superior to the corresponding MIC_{max} determined in unbuffered medium. The data show that acetic and lactic acid concentrations that were bacteriostatic in unbuffered medium were not bacteriostatic in buffered medium. These results suggest that the inhibitory activities of lactic and acetic acids were mainly due to the pH effect in the range of MIC_{min} and MIC_{max} . However, a significant decrease in the specific growth rate of some strains was also observed in the buffered medium at concentrations of 0.45% for lactic and 0.50% for acetic acid, which largely exceeded the MIC_{max} of all strains tested.

Our observation shows that the growth of *Listeria* strains may be only slightly affected by organic acids in buffered medium when pH is not changed. This may suggest that production of organic acids by lactic acid bacteria in relatively well buffered medium may not provide good protection against the growth of *Listeria*. Moreover, Doyle *et al.* (1987) have shown the survival of *L. monocytogenes* in milk during high-temperature, short-time pasteurization. The survival of *Listeria* even under extreme conditions clearly demonstrates that the investigation of natural and more efficient food preservatives such as bacteriocins is needed.

The bacteriostatic effect of lactic and acetic acid on *Listeria* strains

To further determine the inhibitory activities of organic acids, the *Listeria* strains were incubated in TSB medium containing either acetic or lactic acid at concentra-

Table III. Specific growth rate (μ , h^{-1})^{*} of *Listeria* strains cultured in buffered (0.15 mol/l phosphate) TSB containing different concentrations of lactic and acetic acids.
Taux de croissance spécifique (μ , h^{-1})^{} de plusieurs souches de *Listeria* cultivées en milieu TSB tamponné (0,15 mol/l de phosphate) à différentes concentrations en acides lactique et acétique.*

| Strain | μ_{max} | LA** concentration (% w/v) | | | AA** concentration (% w/v) | | |
|----------------------------------|-------------|----------------------------|------|------|----------------------------|------|------|
| | | 0.05 | 0.09 | 0.26 | 0.06 | 0.10 | 0.30 |
| <i>L. grayi</i> 29 | 0.97 | 0.94 | 0.94 | 0.92 | 0.95 | 0.96 | 0.96 |
| <i>L. innocua</i> 18 | 1.07 | 0.99 | 1.00 | 0.95 | 1.05 | 1.01 | 0.95 |
| <i>L. ivanovii</i> 28 | 1.10 | 1.03 | 0.97 | 0.91 | 1.10 | 1.01 | 0.80 |
| <i>L. monocytogenes</i> 1089 | 1.06 | 0.90 | 0.90 | 0.85 | 0.96 | 0.95 | 0.87 |
| <i>L. monocytogenes</i> Scott A3 | 0.98 | 0.97 | 0.90 | 0.85 | 0.96 | 0.95 | 0.86 |
| <i>L. welshimeri</i> 89 | 1.08 | 1.02 | 1.02 | 1.00 | 1.08 | 1.05 | 0.97 |

^{*} The results are the average data from 2 experiments; SE of μ was 0.04 (h^{-1}). ** LA: lactic acid; AA: acetic acid.

^{*} Les résultats sont les moyennes obtenues de 2 expérimentations. L'erreur type sur μ est de 0,04 (h^{-1}). ** LA : acide lactique; AA : acide acétique.

tions exceeding the MIC_{max} by 0.05%. The pH varied from 5.6 to 4.6 depending on the MIC_{max} for the individual strains. Viable cell concentrations were monitored for 24 or 28 h. Figure 2 shows that the viable cell concentrations of all strains decreased very little after 12 h culture and remained at $\approx 10^7$ cfu/ml. The data confirm that lactic and acetic acids can strongly inhibit the growth of *Listeria* strains, but that the inhibition by the acids is caused by a bacteriostatic rather than a bactericidal effect. Our results are also in agreement with those previously observed by Parish and Higgins (1989), in which *Listeria* strains could survive at low pH. In fact, many investigations have revealed that environmental conditions, such as acidulants, pH, temperature, sodium chloride and nitrite can influence growth profiles or behavior of *Listeria* strains in foods (Conner *et al*, 1986; Doyle *et al*, 1987; Doyle, 1988; George *et al*, 1988; Daeschel, 1989; Farber *et al*, 1989). Our study further indi-

cates that the use of organic acids which display a bacteriostatic effect on *Listeria* may not be sufficient to prevent the growth of *Listeria* after an adaptation period, leading to a potential *Listeria* contamination in foods.

The effects of mesenterocin 5 on the growth kinetics of *Listeria* strains

The viability loss of *Listeria* strains in the presence of mesenterocin 5 was studied (fig 3). Mesenterocin 5 exerted a bactericidal effect on 5 of the 6 strains tested during the first 2 h of culture in unbuffered TSB at pH 7.0, the exception being *L. monocytogenes* Scott A3. As a result, the bactericidal effect led to a significant decrease in viable cell concentration by a factor of 10 to 1 000, even for an initial viable cell concentration as high as 10^7 cfu/ml. The cell concentration remained constant before it eventually increased (fig 4). The

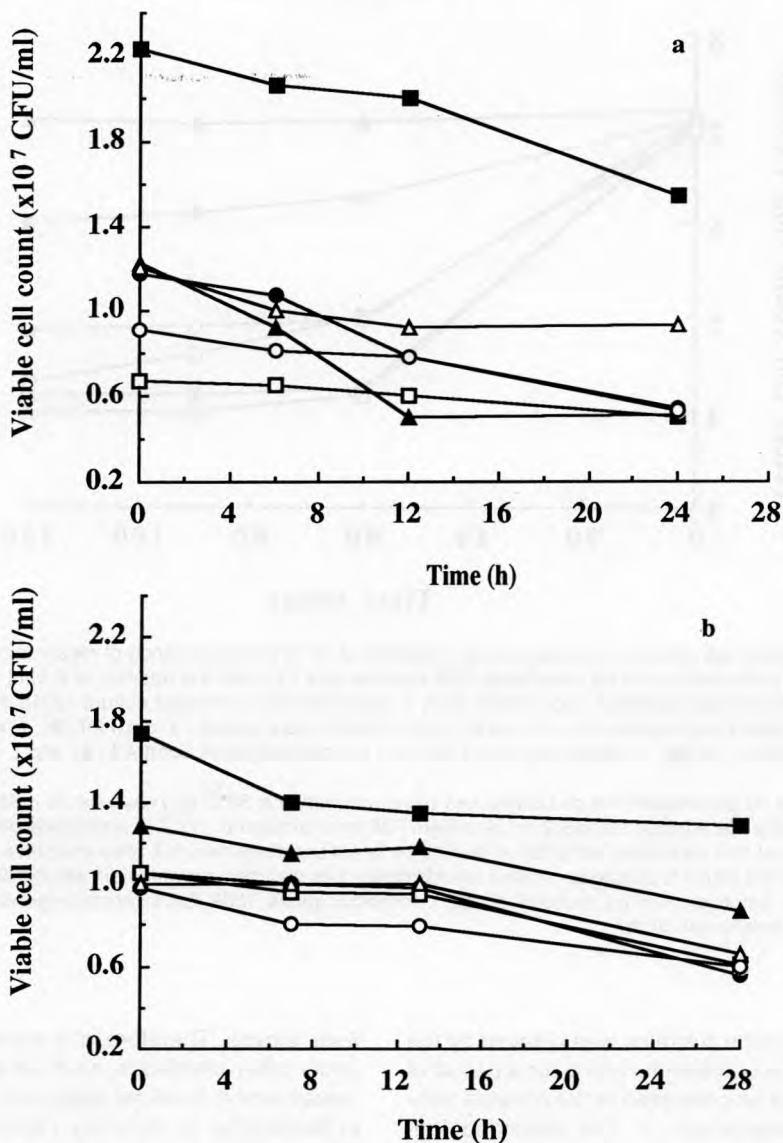


Fig 2. Evolution of viable cell counts of *Listeria* strains grown at 30°C in non-buffered TSB medium (pH 7.0) and in the presence of lactic (a) and acetic (b) acids. Both acid concentrations in the culture medium exceeded the MIC_{max} of the corresponding strain by 0.05% (w/v). The *Listeria* strains used were: *L. grayi* 29 (●), *L. innocua* 18 (○), *L. ivanovii* 28 (■), *L. monocytogenes* 1089 (□), *L. monocytogenes* Scott A3 (▲) and *L. welshimeri* 89 (Δ).

Évolution du dénombrement de *Listeria* lors d'une incubation à 30°C en milieu non tamponné (pH 7,0) et en présence d'acides lactique (a) et acétique (b). Les concentrations des 2 acides excèdent la MIC_{max} de chaque souche correspondante de 0,05% (p/v). Les souches de *Listeria* utilisées ont été : *L. grayi* 29 (●), *L. innocua* 18 (○), *L. ivanovii* 28 (■), *L. monocytogenes* 1089 (□), *L. monocytogenes* Scott A3 (▲) et *L. welshimeri* 89 (Δ).

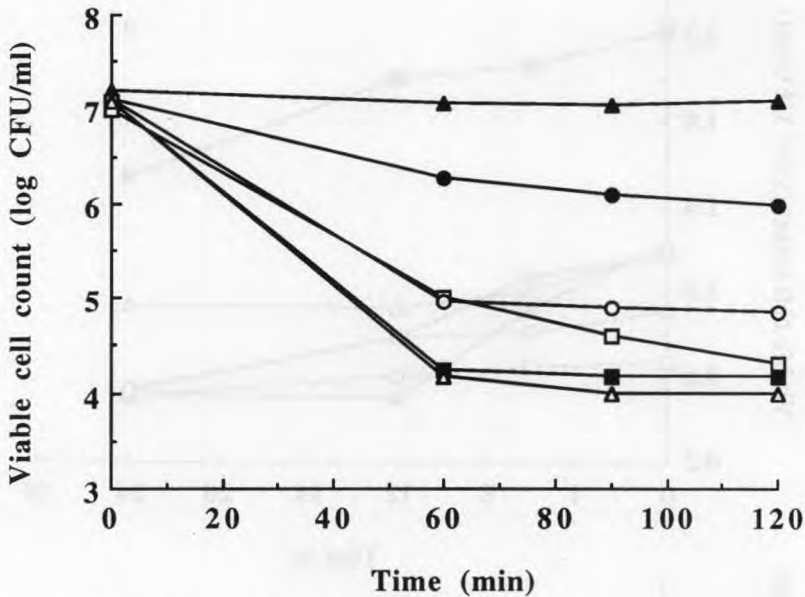


Fig 3. Viable cell counts of *Listeria* strains incubated at 30°C in the presence of mesenterocin 5. The test was performed in 9.9 ml unbuffered TSB medium (pH 7.0) with the addition of 0.1 ml unconcentrated ultrafiltered-diafiltered supernatant from *L. mesenteroides* overnight culture (2048 AU/ml) and 0.4 ml *Listeria* suspension. The following *Listeria* strains were tested: *L. grayi* 29 (●), *L. innocua* 18 (○), *L. ivanovii* 28 (■), *L. monocytogenes* 1089 (□), *L. monocytogenes* Scott A3 (▲) and *L. welshimeri* 89 (Δ).

Évolution du dénombrement de *Listeria* lors d'une incubation à 30°C en présence de mésentérocin 5. Le test a été effectué dans 9,9 ml de milieu TSB non tamponné (pH 7,0) additionné de 0,1 ml de surnageant non concentré, ultrafiltré et diafiltré de la culture d'une nuit de *L. mesenteroides* (2048 AU/ml) et de 0,4 ml de la culture de *Listeria* standardisée. Les souches suivantes ont été testées: *L. grayi* 29 (●), *L. innocua* 18 (○), *L. ivanovii* 28 (■), *L. monocytogenes* 1089 (□), *L. monocytogenes* Scott A3 (▲) et *L. welshimeri* 89 (Δ).

growth of the 5 strains was delayed by the presence of mesenterocin 5 for a period of 2 to 14 h as compared to the controls without mesenterocin 5. The extended lag-phase can be partly explained by the bactericidal effect of mesenterocin 5 and the resulting sharp decrease in viable cell numbers in the medium, and by the longer adaptation to the new environment required for the affected *Listeria* strains. This effect of mesenterocin 5 was observed in cell suspensions at pH 7.0 and 30°C, conditions very favorable to the growth of *Lis-*

teria strains. Therefore, it is expected that under other conditions, such as at pH 5.0, mesenterocin 5 will be even more efficient in eliminating or inhibiting *Listeria* strains since both the growth rate of *Listeria* strains is reduced and mesenterocin 5 activity is improved at this reduced pH (Daba *et al*, 1992).

The mode of action of mesenterocin 5 on *L. monocytogenes* Scott A3 is inhibitory and bacteriostatic under the test conditions used, similar to the effect of organic acids in the range of MIC_{min} and MIC_{max}.

This bacteriostatic rather than bactericidal effect of mesenterocin-5 on *L monocytogenes* Scott A3 may be explained by the relatively low ratio of mesenterocin 5 concentration to *L monocytogenes* Scott A3 concentration. Mesenterocin 5 could, however, display a strong bactericidal effect on *L monocytogenes* Scott A3, largely depending on mesenterocin 5 concentration, indicator strain concentration and culture conditions in either TSB or milk (unpublished data).

Table IV shows the relationship between the specific growth rate of *Listeria* strains determined after the lag-phase and mesenterocin 5 concentration. *L monocytogenes* 1089 and *L monocytogenes* Scott A3 showed sensitivity to mesenterocin 5 concentration during the exponential phase, *ie*, their specific growth rates decreased with an increase in mesenterocin 5 concentration. This characteristic made it

possible to estimate mesenterocin 5 activity in culture medium by determining the decrease in the specific growth rate of the indicator strain *L monocytogenes* Scott A3 during exponential phase in a standardized test (unpublished results). *L grayi* 29 and *L welshimeri* 89 were much less affected by mesenterocin 5 concentration. Interestingly, the extended lag-phase of 5 *Listeria* strains was little affected by the mesenterocin 5 concentrations (1 x C to 8 x C) used. Therefore, for each strain and medium, other mesenterocin 5 concentrations < 1 x C should be studied in order to determine the minimum mesenterocin 5 concentration without significantly affecting the extended lag-phase under the test conditions.

According to our results, it is unlikely that the acid concentration in the ultrafiltered-diafiltered mesenterocin 5 supernatant was sufficient to cause the extended

Table IV. Specific growth rate (μ , h^{-1})* of *Listeria* strains grown in unbuffered TSB containing different mesenterocin 5 concentrations.

*Taux de croissance (μ , h^{-1}) * de plusieurs souches de Listeria cultivées en milieu TSB non tamponné à différentes concentrations en mésentéroïcine 5.*

| Strain | μ_{max} | Bacteriocin concentration | | | |
|---------------------------------|-------------|---------------------------|-------|-------|-------|
| | | 1 x C ** | 2 x C | 4 x C | 8 x C |
| <i>L grayi</i> 29 | 1.05 | 0.89 | 0.87 | 0.86 | 0.83 |
| <i>L innocua</i> 18 | 1.01 | 0.72 | 0.65 | 0.55 | 0.53 |
| <i>L ivanovii</i> 28 | 1.06 | 0.72 | 0.71 | 0.70 | 0.63 |
| <i>L monocytogenes</i> 1089 | 0.93 | 0.66 | 0.61 | 0.39 | 0.31 |
| <i>L monocytogenes</i> Scott A3 | 0.96 | 0.79 | 0.67 | 0.63 | 0.56 |
| <i>L welshimeri</i> 89 | 1.04 | 0.76 | 0.83 | 0.77 | 0.78 |

* The reported results are average data from 2 experiments, SE of μ was 0.04 (h^{-1}). ** C correspond to mesenterocin 5 concentration in a mixed solution which contained 0.1 ml unconcentrated ultrafiltered-diafiltered supernatant of *L mesenteroides* overnight culture (2 048 AU/ml) and 9.9 ml TSB medium with the addition of 0.4 ml *Listeria* culture diluted and standardized to an OD_{600} of 0.2.

* Les résultats présentés sont la moyenne de 2 expérimentations. L'erreur type sur μ est de 0.04 (h^{-1}). C correspond à la concentration en mésentéroïcine 5 dans une solution contenant 0,1 ml de surnageant non concentré, ultrafiltré et diafiltré de la culture d'une nuit de *L mesenteroides* (2048AU/ml), 9,9 ml de milieu TSB et 0,4 ml d'une culture de *Listeria* diluée et standardisée à une DO_{600} de 0,2.

lag-phase or strong inhibition when *Listeria* strains were tested. In fact, we found that *L. mesenteroides* produced 0.77% of lactic acid and 0.11% of acetic acid after overnight culture in unbuffered MRS medium at 30°C. Consequently, even when 0.1 ml of mesenterocin 5 untreated supernatant was added directly to 10.3 ml TSB-*Listeria* suspension, lactic and acetic acid concentrations during the test should have been only $\approx 0.007\%$ and 0.001% , respectively. These acid concentrations are generally much lower than the MIC_{min} under the test conditions used. The effect of hydrogen peroxide on inhibitory activity can be excluded by comparing the activities of samples treated or not with catalase using the critical-dilution micromethod. The activity of hydrogen peroxide in the filtrate from ultrafiltered supernatant was only 8 AU/ml. Moreover, bacteriocin activi-

ty in the unconcentrated ultrafiltered supernatant was not affected by catalase treatment. Thus, a filtered supernatant from the *L. mesenteroides* culture may be directly used to determine the effect of mesenterocin 5 on *Listeria* strains without interference from organic acids when using minimum or higher mesenterocin 5 concentration. To significantly extend the lag-phase of 10.3 ml of *Listeria* suspension with initial numbers of 10^7 cfu/ml, only 0.1 ml unconcentrated ultrafiltered-diafiltered supernatant from an overnight culture of *L. mesenteroides* was required. This report indicates that the amount of mesenterocin 5 produced is sufficient to result in a significant inhibition in the *Listeria* strains, and that it is possible to produce mesenterocin 5 by a simple fermentation process with the selected *L. mesenteroides* strain.

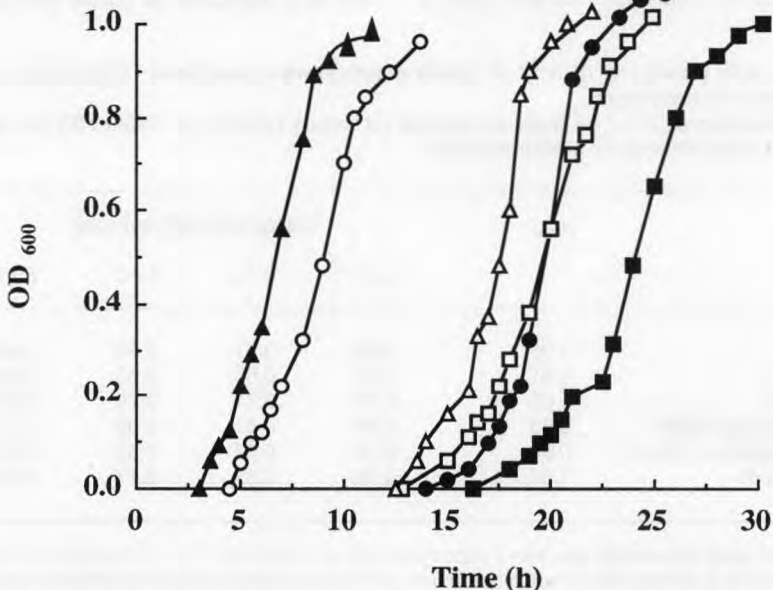


Fig 4. Growth profiles of *Listeria* strains grown at 30°C in the presence of mesenterocin 5 during the exponential phase. OD_{600} at $t = 0$ h was zero. The test conditions were the same as in the figure 3. *Profil de croissance en phase exponentielle des souches de Listeria incubées à 30°C en présence de mésentérocin 5. La DO_{600} à $t = 0$ h était de zéro. Les conditions de test sont identiques à celles indiquées sur la figure 3.*

ACKNOWLEDGMENTS

The authors wish to thank C Paquin for her technical assistance, the FCAR (Fonds pour la Formation des Chercheurs et l'Aide à la Recherche) and the MESS (Ministère de l'Enseignement Supérieur et de la Science Québec) for financial support.

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