Sodium Chloride Reduces Production of Curvacin A, a Bacteriocin Produced by *Lactobacillus curvatus* Strain LTH 1174, Originating from Fermented Sausage

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Lactobacillus curvatus **LTH 1174, a strain originating in fermented sausage, produces the antilisterial bacteriocin curvacin A. Its biokinetics of cell growth and bacteriocin production as a function of various concentrations of salt (sodium chloride) were investigated in vitro during laboratory fermentations using modified MRS medium. A model was set up to describe the effects of different NaCl concentrations on microbial behavior. Both cell growth and bacteriocin activity were affected by changes in the salt concentration. Sodium chloride clearly slowed down the growth of *L. curvatus* LTH 1174, but more importantly, it had a detrimental effect on specific curvacin A production (k_B) and hence on overall bacteriocin activity. Even a low salt concentration (2%, wt/vol) decreased bacteriocin production, while growth was unaffected at this concentration. The inhibitory effect of NaCl was mainly due to its role as an a_w-lowering agent. Further, it was clear that salt interfered with bacteriocin induction. Additionally, when 6% (wt/vol) sodium chloride was added, the minimum biomass concentration necessary to start the production of curvacin A (X_B) was 0.90 g (cell dry mass) per liter. Addition of the cell-free culture supernatant or a protein solution as a source of induction factor resulted in a decrease in X_B, an increase in k_B, and hence an increase in the maximum attainable bacteriocin activity.**

Fermentation of raw meat normally results in stable and safe end products with a long shelf life (40). This outcome is due mainly to the low end pH caused by the production of large amounts of lactic acid by the lactic acid bacteria (LAB) naturally present or intentionally added as starter culture. Other characteristics of raw fermented sausages also help prevent the outgrowth of spoilage and pathogenic bacteria; of these, the two most important are fast acidification during fermentation (usually achieved by addition of fermentable carbohydrates) and a steady decrease in water activity (a_w) during fermentation and ripening of the sausage. For a_w lowering, it is important that a sufficient amount of salt (2.4 to 3.0% [wt/wt] NaCl) be added to the meat batter (40, 45). Salt is used not only as a preventive measure against food spoilage and pathogenic bacteria but also as a flavor compound. The curing agent nitrite is added to the sausage batter to create the typical cured flavor, to produce color, to prevent lipid rancidity, and to inhibit the growth of salmonellae and clostridia (14, 40). These established methods, however, are not very selective and thus not only affect the undesirable microorganisms but also affect the functionality of the added meat starter cultures (42).

At present, consumers are requesting safe, natural food products as well as mild and light foods with a low acid, sugar, salt, or fat content (27). This trend has stimulated research on the additives used in meat processing, such as NaCl and curing agents. For instance, to decrease the high Na^+ content of meat, attempts are being made to replace at least part of the NaCl with other salts such as potassium chloride or calcium chloride, without affecting the fermentation process or the quality of the end product (26). The request for more-natural products has also provided an incentive to search for safe, food-grade preservatives of biological origin (16, 60). Furthermore, some food-borne pathogenic bacteria, such as *Listeria monocytogenes*, can survive in fermented sausage despite the various hurdles they encounter, and they may pose a health risk to consumers (21, 35, 40). The spread of antibiotic resistance genes among listeriae and other pathogens is raising new concerns (61). Moreover, countries such as the United States have adopted a zero-tolerance policy for *Listeria* contamination of processed meat products (59). This policy has serious consequences, in particular for European exporting companies, since the European Union applies a tolerance of less than 100 cells of *L. monocytogenes* in fermented meat products. Hence, there is a need for novel and/or extra preventives in meat processing.

Bacteriocins produced by LAB are antibacterial peptides or proteins active against other gram-positive, mainly closely related bacterial species, including some undesirable spoilage bacteria and food-borne pathogens (17). The only legally approved bacteriocin in many countries is nisin, approved for use as a preservative in a limited number of food products (15). Unfortunately, this bacteriocin is not very efficient in a meat environment, because of its low solubility, uneven distribution, and lack of stability (60). An alternative and interesting approach for the use of bacteriocins in fermented sausages is the use of bacteriocinogenic LAB starter cultures adapted to the specific meat environment (43). Several bacteriocins produced by strains isolated from fermented sausages are highly active against listeriae (13, 29, 33, 48, 55, 66). Moreover, several investigators have already shown the possibility of using LAB strains that produce listericidal bacteriocins to reduce *Listeria* counts in fermented meat products (6, 7, 10, 11, 22, 33, 34, 38,

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58, 66). This is one of the reasons why *Listeria* contamination of fermented sausage is very rare, although *Listeria* contamination of the raw meat materials does occur (56). Although the meat itself may be contaminated, it is also possible that the contamination occurs in the meat plant. Hence, one of the most important objectives remains the prevention of *Listeria* contamination through good manufacturing practice, thereby decreasing the hygiene risk. However, the presence of this organism in the food-processing environment seems inevitable. Although the risk of contamination can be reduced, it seems unlikely that it can be eliminated. In addition, in situ production of bacteriocins enhances the competitiveness of the starter organisms toward fortuitous flora and hence ensures a stable and safe end product (31, 66). Therefore, it is of the utmost importance to know how the growth and bacteriocin production of the functional starter cultures used are affected by various sausage ingredients such as salt.

Lactobacillus curvatus LTH 1174, a strain originating in fermented sausage, is the producer of the listericidal bacteriocin curvacin A and has been shown to be a successful starter strain for European sausage fermentations (66). Previously, it was also shown that this strain displays maximum bacteriocin activity levels under the temperature and pH conditions used for these fermentations (47). However, the effects of different sausage ingredients on the behavior of this strain have yet to be determined so as to estimate the bioavailability of bacteriocin in the meat. The present study was undertaken to examine and model the inhibitory effects of different NaCl concentrations in a modified de Man-Rogosa-Sharpe (mMRS) medium on the growth and bacteriocin production of the meat starter culture *L. curvatus* LTH 1174.

MATERIALS AND METHODS

Microorganisms and media. *L. curvatus* LTH 1174 was used as the producer of the antilisterial bacteriocin curvacin A (63). A curvacin A-sensitive indicator organism, *Listeria innocua* LMG 13568, was used to determine bacteriocin activity levels (41). Strains were stored at $-80°C$ in MRS medium and brain heart infusion medium (both from Oxoid, Basingstoke, United Kingdom), respectively, both containing 25% (vol/vol) glycerol as a cryoprotectant. To produce fresh cultures, the strains were propagated twice at 30°C for 12 h before experimental use. Solid medium for enumeration of cells was prepared by adding 1.5% (wt/vol) agar (Oxoid) to the broth. The overlays used for estimation of bacteriocin titers were prepared with 0.7% (wt/vol) agar.

mMRS was used as the fermentation medium for *L. curvatus* LTH 1174. The modification consisted in a doubling of the concentration of the complex nutrient source (bacteriological peptone [Oxoid], Lab Lemco [Oxoid], and yeast extract [VWR International, Darmstadt, Germany]). This was done to avoid a severe growth limitation of *L. curvatus* LTH 1174 due to nutrient depletion in standard MRS medium (44). In addition, calculations of the amino nitrogen content of this medium (9) indicated that this composition more closely simulates the actual sausage environment (14). Furthermore, maximum curvacin A activity was found when the complex nutrient source was doubled, while specific bacteriocin production and the specific apparent bacteriocin inactivation rate were comparable to those with standard MRS (J. Verluyten, W. Messens, F. Leroy, V. Schrijvers, and L. De Vuyst, unpublished data). All media and solutions were sterilized at 121°C for 20 min.

Fermentation experiments. A series of in vitro fermentations were performed using mMRS supplemented with different concentrations of salt (0, 2, 4, and 6% [wt/vol] NaCl) to investigate their effects on both the growth and bacteriocin production of *L. curvatus* LTH 1174. The fermentations without added sodium chloride and those with 4% (wt/vol) NaCl added were performed in triplicate to show the reproducibility of the experiments. Standard deviations were calculated both on the experimental values and on the biokinetic parameters derived from the primary model. To determine whether the effect of the added NaCl was due solely to a reduction in a_w, an additional fermentation was carried out in the

presence of 9.9% (wt/vol) glycerol (sterilized separately) in the absence of added salt. Based on extrapolation of data from previous studies (12, 46), addition of 9.9% (wt/vol) glycerol to the basal growth medium should result in the same decrease in a_w as addition of approximately 4% (wt/vol) NaCl (42).

Fermentations were carried out in a 15-liter laboratory fermentor (BiostatC; B. Braun Biotech International, Melsungen, Germany) with a working volume of 10 liters of mMRS as previously described (41). Briefly, temperature (25°C) and pH (held constant at 5.5) control was performed online (Micro-MFCS for Windows NT; B. Braun Biotech International). For preparation of the inoculum, 10 ml of MRS medium was inoculated with 0.5 ml of a freshly prepared *L. curvatus* LTH 1174 culture and then incubated at 30°C for 12 h. Five milliliters of this preculture was added to 100 ml of MRS medium. After 13 h of growth at 30°C, this culture was used to inoculate the fermentor.

To examine the effect of added induction factor (IF), two additional fermentations were performed using mMRS containing 6% (wt/vol) NaCl at a controlled temperature of 25°C and a constant pH of 5.5. To obtain a source of IF, a fermentation was performed in MRS at a controlled temperature of 22°C and a constant pH of 5.4, a set of conditions optimal for the production of curvacin A (47). After 24 h of growth, cells were removed by centrifugation (at 20,500 × g and 4°C for 30 min), and 50 ml of the cell-free culture supernatant (CFS; curvacin A activity, 1,600 arbitrary units [AU] ml^{-1}) was used as a source of IF for the first fermentation containing 10 liters of mMRS. This fermentation using the CFS was also performed in triplicate, and standard deviations were calculated. As an alternative source of the IF, a precipitate of curvacin A and possibly its IF was prepared as follows. The pH of the CFS was adjusted to 6.5, 300 g of ammonium sulfate per liter (48% saturation) was added, and the suspension was stirred at 4°C for 12 h. After centrifugation (at 20,500 × g and 4°C for 30 min), the pellicle and pellet were harvested and dissolved in 50 mM sodium phosphate buffer at pH 6.5 (5 ml per liter of CFS). The second fermentation was carried out by adding 1.5 ml of this protein solution (ammonium sulfate precipitate; curvacin A activity, 153,600 AU ml^{-1}) to the fermentor (10 liters of mMRS).

Assays. At regular time intervals, samples were withdrawn aseptically from the fermentor to determine cell counts (CFU), biomass (cell dry mass [CDM]), levels of soluble bacteriocin activity in CFS, lactic acid concentrations, and residual glucose concentrations. Briefly, CDM was determined gravimetrically after membrane filtration (41); the amount of lactic acid produced and the residual glucose concentration were determined by high-performance liquid chromatography (18); and the level of bacteriocin activity was determined by a modified critical dilution method using *L. innocua* LMG 13568 as the indicator strain (18). The standard deviations for the CDM, glucose, and lactic acid measurements were 0.11, 0.04, and 0.02 g liter^{-1}, respectively.

Primary and secondary modeling. Primary modeling of cell growth, glucose consumption, lactic acid production, and bacteriocin production and inactivation was performed both to fit the data and to estimate the values of the biokinetic parameters representative of growth and curvacin A production. Abbreviations for biokinetic parameters are explained, and the equations used are given, in Table 1. These equations are the same as those reported by Messens et al. (47), except that bacteriocin production was made dependent on X_B, defined as the minimum biomass concentration required for the onset of bacteriocin produc-

TABLE 1. Equations used for primary model development

Model	Equation[a]
Cell growth	$dX/dt = \{\mu_{max} [1 - (X/X_{max})]^n - \alpha\}X$ when $t > \lambda$
Sugar consumption (glucose)	$dS/dt = -[(1/Y_{X/S})(dX/dt)] - m_S X$
Lactic acid production	$dL/dt = -(Y_{L/S})(dS/dt)$
Bacteriocin production	$dB/dt = [(k_B)(dX/dt)] - [(k_{inact})(XB)]$ when $X > X_B$

[a] Abbreviations: X, biomass concentration (in grams [CDM] per liter); t, time (in hours); λ, duration of the lag phase (in hours); μ_{max}, maximum specific growth rate (in h^{-1}); X_{max}, maximum attainable biomass concentration (in grams [CDM] per liter); n, inhibition exponent; α, specific death rate (in h^{-1}); S, residual glucose concentration (in grams of glucose per liter); $Y_{X/S}$, cell yield coefficient (in grams [CDM] per gram of glucose); m_s, maintenance coefficient (in grams of glucose per gram of CDM per hour); L, lactic acid production (in grams of lactic acid per liter); $Y_{L/S}$, yield coefficient for the conversion of glucose into lactic acid (in grams of lactic acid per gram of glucose); B, bacteriocin activity in the CFS (in AU per liter); k_B, specific bacteriocin production (in AU per gram of CDM); k_{inact}, apparent rate of bacteriocin inactivation (in liters per gram of CDM per hour); X_B, minimum biomass concentration for the onset of bacteriocin production (in grams [CDM] per liter).
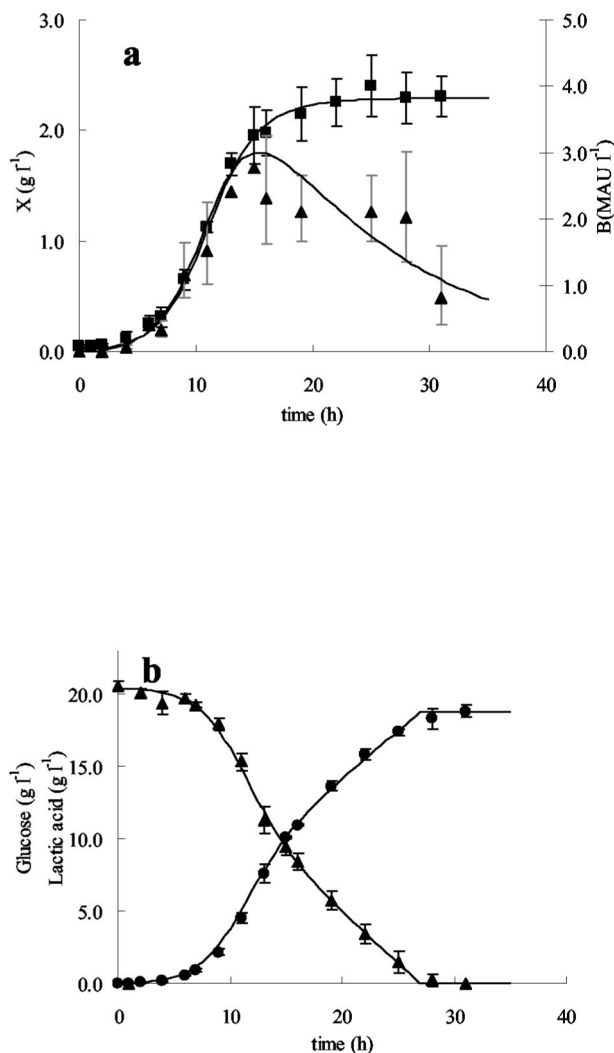
FIG. 1. Modeling of biokinetic parameters for *L. curvatus* LTH 1174 in mMRS at a controlled temperature of 25°C and a constant pH of 5.5. (a) Biomass (in grams [CDM] per liter) (■) and bacteriocin production (in MAU per liter) (▲). (b) Residual glucose concentration (▲) and lactic acid formation (●) (both in grams per liter). Symbols represent experimental values; lines are drawn according to the model. Experimental values are averages from three fermentations. Error bars, standard deviations.

tion due to induction (19, 44). For fermentations performed in triplicate, standard deviations were calculated on all biokinetic parameters estimated via primary modeling.

The equations were integrated by the Euler integration technique in Microsoft Excel 97 (version 8.0a). All parameters needed for the modeling were estimated by manual adjustment until the best fit was obtained. This procedure has been followed previously and led to reliable results (41, 47). It has the advantage of avoiding the unrealistic fitting solutions that are sometimes generated by computational solving.

By use of secondary modeling, all biokinetic parameters (μ_{max}, X_{max}, n, $Y_{X/S}$, m_s, $Y_{L/S}$, k_B, and k_{inact}) derived from the primary model were expressed as functions of the salt concentration. For this purpose, empirical models were used. Standard deviations were calculated where appropriate.

RESULTS

Fermentation profiles. Figure 1 represents both the mean experimental and the mean modeled values of biomass con-

centrations and bacteriocin activities in the supernatant (Fig. 1a) and of residual glucose concentrations and lactic acid production levels (Fig. 1b) for a control fermentation carried out in mMRS at a controlled temperature of 25°C and a constant pH of 5.5 without added NaCl. The biokinetic parameters derived from the primary model are listed in Table 2. The profiles of the fermentations carried out in the presence of NaCl were similar. Empirical models were set up to show the relationship between the biokinetic parameters derived from the primary model and the salt concentration (Table 2 and Fig. 2). Details are described below.

Effects of sodium chloride. Addition of 2% (wt/vol) NaCl had no effect on μ_{max}, while at higher salt concentrations, μ_{max} decreased linearly with increasing salt concentrations (Fig. 2a). Addition of sodium chloride did not affect X_{max} much. A slightly higher X_{max} was found at high salt concentrations (4 and 6% [wt/vol] NaCl) (Fig. 3a), a finding that can be ascribed to the formation of cell aggregates as observed microscopically, probably caused by stress due to these high salt concentrations. Neither the cell yield coefficient ($Y_{X/S}$) nor the maintenance coefficient (m_s) was changed by the addition of salt (Table 2 and Fig. 2b).

Modeling of the bacteriocin activity in the CFS for the various salt concentrations tested is represented in Fig. 3b. Note that for the fermentation without added sodium chloride, the error bars on the bacteriocin data are larger due to the increased uncertainty at high bacteriocin activities, as a result of the twofold dilution assay used. Bacteriocin activity was strongly affected by added salt. A severe drop in B_{max} was observed even at a concentration of 2% (wt/vol) NaCl, while growth remained unaffected (Fig. 2c). At higher salt concentrations, a further decrease in bacteriocin activity was seen, and with 6% (wt/vol) added NaCl, B_{max} reached only 12% of the activity observed without added salt (Fig. 2c and 3b). This was due to a linear decrease in k_B with increasing NaCl concentrations (Fig. 2d). In addition, the minimum biomass concentration required for curvacin A production to start (X_B) increased when 6% (wt/vol) NaCl was present in the medium. Up to an NaCl concentration of 4% (wt/vol), X_B was nil, indicating that curvacin A production started from the beginning of fermentation. However, when 6% (wt/vol) NaCl was present, bacteriocin production started only at a biomass concentration of 0.90 g (CDM) liter^{-1} (Fig. 3). Apparently, a critical NaCl

TABLE 2. Values of biokinetic parameters and empirical-model equations showing the relationship between biokinetic parameters and sodium chloride concentrations[a]

Biokinetic parameters (primary model)[b]	Empirical-model equations (secondary model)
$\mu_{max} = 0.52 \pm 0.02$	$\mu_{max} = 0.51 - 0.01 (\% \text{ NaCl})^2$
$X_{max} = 2.30 \pm 0.17$	$X_{max} = 2.39 \pm 0.21$
$n = 1.17 \pm 0.06$	$n = 1.20 - 0.06 (\% \text{ NaCl})$
$Y_{X/S} = 0.24 \pm 0.01$	$Y_{X/S} = 0.23 \pm 0.01$
$m_S = 0.30 \pm 0.03$	$m_S = 0.32 \pm 0.03$
$k_B = 1.90 \pm 0.10$	$k_B = 1.84 - 0.24 (\% \text{ NaCl})$
$k_{inact} = 0.035 \pm 0.005$	$k_{inact} = 0.041 \pm 0.004$

[a] Calculated values that are negative are set to zero. Standard deviations are reported where appropriate.
[b] Values for the fermentation carried out at a controlled temperature of 25°C and a constant pH of 5.5 without added sodium chloride. Abbreviations and dimensions are explained in the footnote to Table 1.
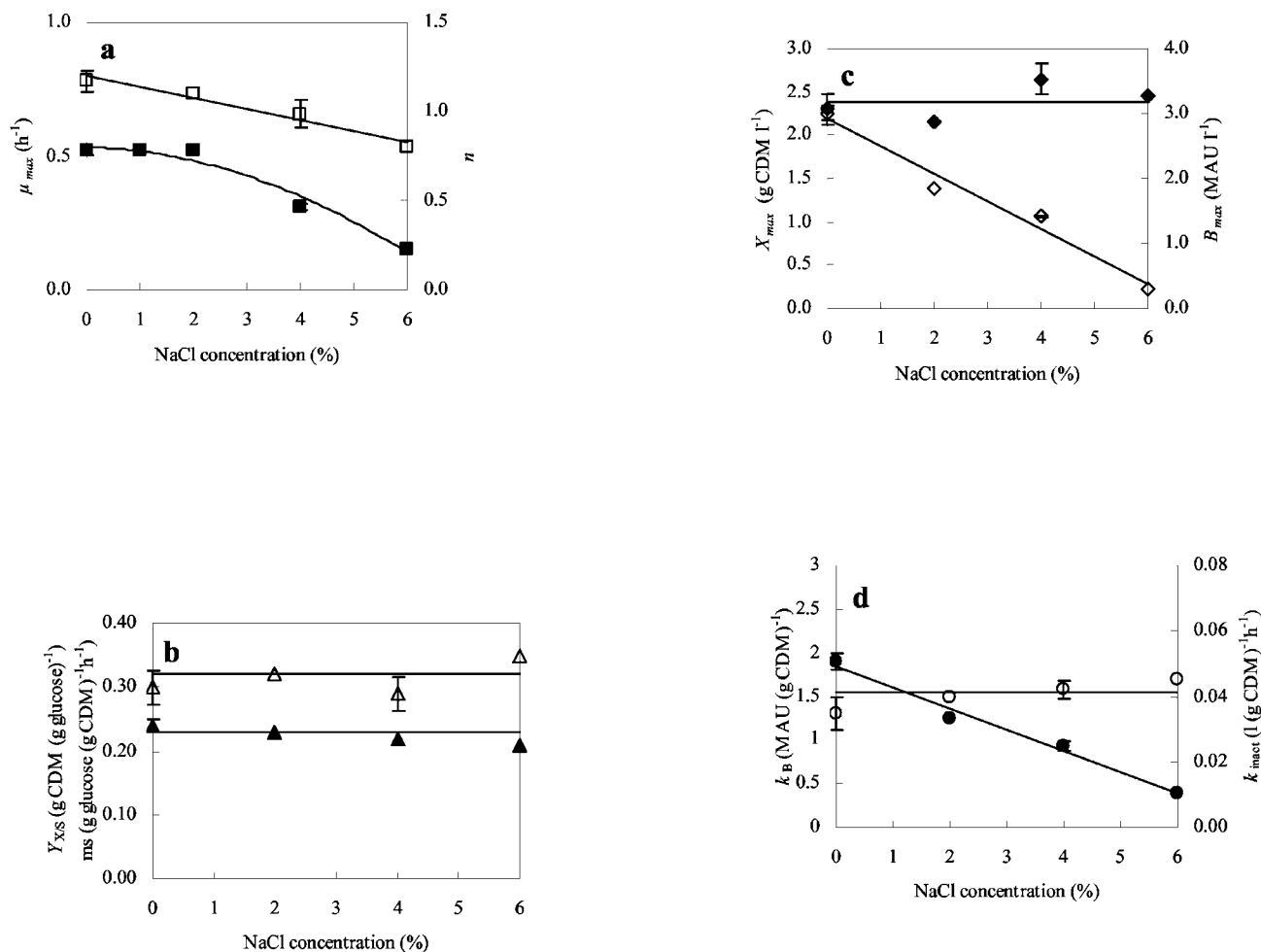
FIG. 2. Secondary modeling of the biokinetic parameters derived from the primary model. Shown are the effects of different NaCl concentrations (expressed in percentages [wt/vol]) added to mMRS on the different parameters for *L. curvatus* LTH 1174 growing at a controlled temperature of 25°C and a constant pH of 5.5. (a) Bacterial growth (μ_{max}) (■) per hour and inhibition exponent (*n*) (□). (b) Sugar metabolism ($Y_{X/S}$) (▲), expressed in grams of CDM per gram of glucose, and m_S (△), expressed in grams of glucose per gram of CDM per hour. (c) Maximum biomass production (X_{max}) (◆), expressed in grams of CDM per liter, and maximum bacteriocin activity (B_{max}) (◇), expressed in MAU per liter. (d) Bacteriocin production (k_B) (●), expressed in MAU per gram of CDM, and apparent rate of bacteriocin inactivation (k_{inact}) (○), expressed in liters per gram of CDM per hour. For 0 and 4% added NaCl, biokinetic parameters are means from three fermentations; error bars, standard deviations. Lines are drawn according to the model.

concentration exists above which NaCl clearly interferes with the induction of bacteriocin production. Probably the IF that was added to the fermentor with the inoculum was sufficient to initiate bacteriocin production from the beginning up to an NaCl concentration of 4% (wt/vol).

Glucose was consumed more slowly at higher concentrations of NaCl, resulting in retarded production of lactic acid (Fig. 4).

Effect of a_w. To determine whether a reduction in a_w was solely responsible for the inhibitory effect of NaCl on bacterial growth and curvacin A production with *L. curvatus* LTH 1174 or if there were other (ionic) effects responsible, one additional fermentation was performed using glycerol as an a_w-lowering agent.

When the different biokinetic parameters used for the primary model were compared, the values obtained for fermentation in the presence of glycerol (9.9%, wt/vol) were comparable to those obtained for fermentation with 4% (wt/vol) added NaCl. Only the value of X_{max} (2.06 versus 2.65 ± 0.18 g

[CDM] liter^{-1}) was somewhat lower. The difference in X_{max} may be ascribed to the formation of cell aggregates, resulting in a nonhomogenous fermentation liquor.

Effect of added IF. To determine the effect of added IF on curvacin A production, fermentations in the presence of 6% (wt/vol) NaCl were performed by adding either CFS or protein solution (ASP) as a source of IF. These fermentations were compared with a fermentation in which no CFS or ASP was added. Bacteriocin activity was determined immediately after inoculation and addition of CFS or ASP. No inhibition of the indicator strain was observed, indicating that the amount of bacteriocin that was added in this way did not contribute to the observed inhibition during these experiments. Growth was comparable in the three cases, although less formation of aggregates was observed during the fermentations with added IF. This resulted in an X_{max} of 2.02 ± 0.08 or 2.10 g (CDM) liter^{-1} with added CFS or ASP, respectively.

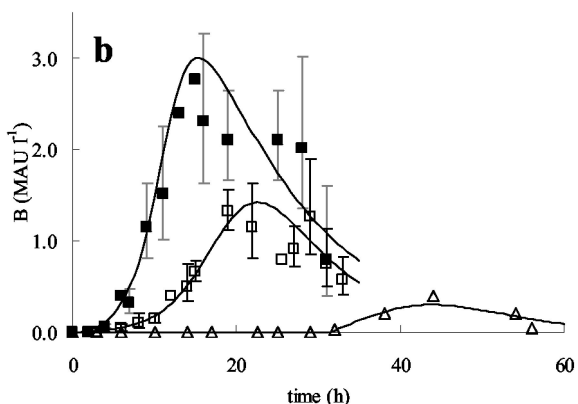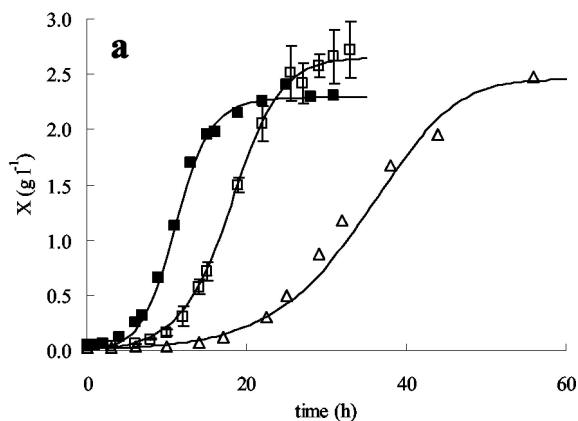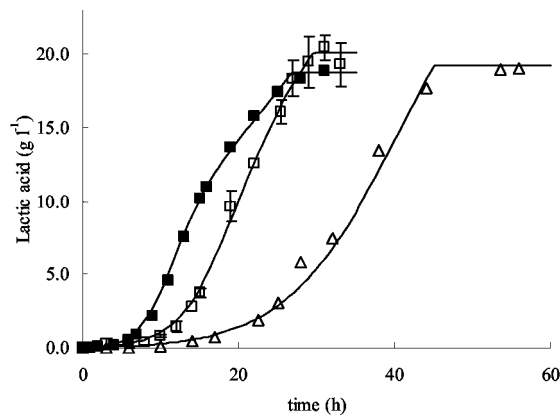Modeling of bacteriocin activity for fermentations with and

FIG. 4. Effects of different concentrations of sodium chloride (expressed in percentages [wt/vol]) added to mMRS on lactic acid production. Symbols: ■, 0% NaCl; □, 4% NaCl; △, 6% NaCl. Symbols represent experimental values; lines are drawn according to the model. For 0 and 4% added NaCl, the experimental values and the model derived are means from three fermentations; error bars (shown only for 4% added NaCl), standard deviations.

FIG. 3. Effects of sodium chloride (concentrations expressed in percentages [wt/vol]) added to mMRS on the production of biomass (expressed in grams [CDM] per liter) (a) and bacteriocin production (expressed in MAU per liter) (b) as a function of time. Symbols: ■, 0% NaCl; □, 4% NaCl; △, 6% NaCl. Symbols represent experimental values; lines are drawn according to the model. For 0 and 4% added NaCl, the experimental values and the model derived are means from three fermentations; error bars, standard deviations.

cases. Addition of IF also affected the onset of curvacin A production, as reflected in the X_B. When either CFS or ASP was added, bacteriocin production started from the beginning of the fermentation and X_B was reduced to only 0.03 ± 0.03 g (CDM) liter^{-1}, compared with 0.90 g (CDM) liter^{-1} when no IF was added. Finally, sugar metabolism was not affected by the addition of IF, resulting in comparable values for $Y_{X/S}$ and m_S.

DISCUSSION

In the past few years, suppression of food-borne pathogens such as *L. monocytogenes* in meat has become a major issue, and much work has been devoted to the selection of starter strains that are antagonistic to food pathogens (45). However,

without the addition of IF (CFS or ASP) is represented in Fig. 5. Addition of IF resulted in an increase in B_{max} from 0.30 mega arbitrary unit (MAU) liter^{-1} (no IF added) to 0.79 ± 0.10 or 0.52 MAU liter^{-1} when CFS or ASP was added, respectively. These bacteriocin activities were the result of specific bacteriocin production levels of 0.40 MAU (g of CDM)$^{-1}$ (no IF added) and 0.62 ± 0.06 or 0.40 MAU (g of CDM)$^{-1}$ when CFS or ASP was added, respectively, indicating that CFS was a better source of IF than ASP. Possibly the precipitation of the IF was not very successful, or the IF may form inactive complexes in a more isolated form. Another possibility is that other compounds present in CFS stimulate bacteriocin production. The values for k_{inact} were comparable in the three
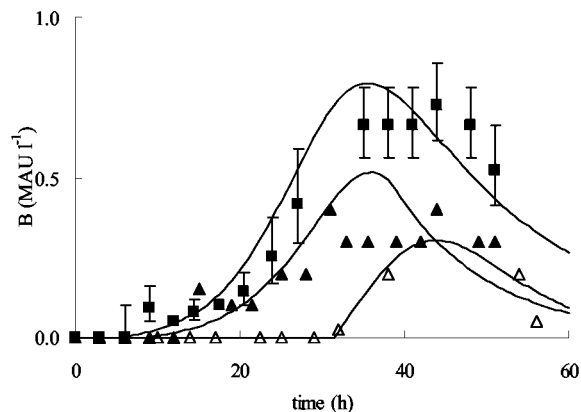

FIG. 5. Modeling of bacteriocin production (in MAU per liter) by *L. curvatus* LTH 1174 either with no IF added (△) or with the addition of CFS (■) or ASP (▲) in mMRS with 6% (wt/vol) added sodium chloride at a controlled temperature of 25°C and a constant pH of 5.5. Symbols represent experimental values; lines are drawn according to the model. For the fermentation with CFS added, the experimental values and the model derived are means from three fermentations; error bars, standard deviations.

the production of a bacteriocin under laboratory conditions does not necessarily mean a sufficient and effective bacteriocin production and bioavailability in the food ecosystem. Severe limiting factors include, for instance, restricted nutrient availability for the bacteriocin-producing cells, adsorption of the bacteriocin onto meat particles, fats, and proteins, and/or the presence of endogenous proteases degrading the bacteriocin (60). In addition, meat processing technology may interfere with the bacteriocin production capacity of the starter cultures or cocultures used.

Salt affects the growth and bacteriocin production of LAB. In the case of *L. curvatus* LTH 1174, a low concentration of NaCl (2%, wt/vol) showed no effect on bacterial growth, while an inhibition that increased linearly was evident with higher salt concentrations. The strong negative effect of high salt concentrations on the growth of LAB has been reported previously (18, 54, 64). However, low concentrations of salt (1 to 2%, wt/vol) can sometimes enhance bacterial growth (23, 36, 64, 65).

Although a strong negative effect of salt was observed on the maximum specific growth rate (μ_{max}) of *L. curvatus* LTH 1174, the maximum attainable biomass concentration (X_{max}) did not decrease. This indicates that the a_w constraints caused by NaCl addition may not impose large energetic burdens (37). In addition, replacing the sodium chloride with glycerol as an a_w-lowering agent resulted in comparable inhibition; hence, the effect exerted by NaCl was probably due to its effect on a_w. Although an ionic effect cannot be excluded, such an effect is not likely, because of the natural salt resistance of the LAB present in fermented meat and vegetable products (5, 36). However, *Lactobacillus sakei* CTC 494 shows a decreasing trend of X_{max} with increasing NaCl concentrations (42). This may indicate that adding NaCl to the growth medium interferes with the efficiency of the substrate conversion into biomass for the latter strain. A linear decrease in the growth rate with a_w values below the optimum is usually observed (46, 53).

Even at a concentration of 2% (wt/vol) NaCl, which did not affect the bacterial growth of *L. curvatus* LTH 1174, the maximum attainable bacteriocin activity (B_{max}) decreased by almost 40% from that in the fermentation without added salt. This can be explained by the decrease in specific bacteriocin production (k_B), since X_{max} values were comparable. At higher NaCl concentrations, k_B decreased further. In contrast, Uguen et al. (64) reported an increased lacticin 481 production when the osmolarity of the growth medium increased due to added NaCl. Also, for plantaricin S, the highest production is observed at a sodium chloride concentration of 2.5% (wt/vol) (39). On the other hand, the production of sakacin K by *L. sakei* CTC 494 is negatively affected by added NaCl (42), as is the case for the antilisterial carnobacteriocin B2 produced by *Carnobacterium piscicola* A9b (30). Although enterococci are more salt resistant, production of the enterocins A and B by *Enterococcus faecium* CTC 492 is also inhibited in the presence of NaCl (3). However, enterocin production can be increased in the meat matrix by the addition of a small amount of the bacteriocin, which acts as an inducer (4).

A possible explanation for the inhibition of bacteriocin production by NaCl is interference with the binding of the IF to its receptor (50). The IF is excreted by the producing strain, and when it reaches a critical concentration, the binding to its receptor initiates bacteriocin production. For *C. piscicola* A9b, the induction capacities of both acetate and carnobacteriocin B2 itself are negatively affected by the addition of NaCl (51). Moreover, for *E. faecium* CTC 492, addition of IF can overcome the inhibition of enterocin production (3, 50). Even at NaCl concentrations that do not affect growth, the induction of bacteriocin production decreases, indicating that higher concentrations of the inducer are necessary to sustain bacteriocin production (50). Likewise, curvacin A production by *L. curvatus* LTH 1174 is abolished at elevated temperatures (35°C) due to reduced synthesis of the cationic pheromone peptide Sap-Ph (19, 47). Possibly, addition of NaCl to the growth medium interferes with pheromone stability or binding to its receptor and/or reduces pheromone synthesis. Indeed, addition of CFS or ASP as a source of IF increased B_{max}. In contrast, addition of IF could not reverse the negative effect of NaCl on sakacin P production (49), which was explained by the fact that sakacin P production is an "all or nothing process" (20).

Alternatively, in the actual sausage environment, the lower specific bacteriocin production may very well be counterbalanced by an increased relative bacteriocin activity, due to a higher susceptibility of pathogens in the presence of certain sausage ingredients such as salt. For instance, the addition of NaCl acts synergistically on the activity of curvacin A against *Escherichia coli* O157:H7 (24). Other strains of *E. coli* tested are also sensitive to nisin and curvacin A at a pH of <5.5 and in the presence of >3% (wt/vol) NaCl (25). For the enterotoxin-producing organism *Bacillus cereus*, a similar higher susceptibility to bacteriocin AS-48 is observed at an NaCl concentration of 5% (wt/vol) (1). For *L. monocytogenes*, a higher susceptibility to curvaticin 13 or nisin is also observed at higher NaCl concentrations (8, 62). Fewer bacteriocin molecules seem to be necessary to exert the same lethal effect. Moreover, during fermentation, a pH drop occurs, which will render certain species more susceptible to the bacteriocin that is produced in situ.

L. sakei and *L. curvatus* are the dominant LAB in European sausage starters (28). However, their adaptive responses to the meat environment differ in that *L. curvatus* is slightly more sensitive to salt than *L. sakei* (36), as is also shown in papers on *L. curvatus* LTH 1174 and *L. sakei* CTC 494 (42; this study). This difference may at least partially explain the dominance of *L. sakei* over *L. curvatus* in fermented sausages, as is often observed upon isolation of these strains from such products (2, 32, 52, 57). The use of bacteriocin-producing meat starter cultures, such as *L. curvatus* and *L. sakei*, for sausage fermentation enhances their competitiveness and contributes to a safer and more uniform end product. It is essential to understand the effects of different sausage-related environmental factors on the induction of curvacin A production in order to better understand the potential for application of this strain in the production of fermented sausages. The potential of this strain can be considerably broadened by the addition of CFS containing IF to achieve a higher level of bacteriocin production under unfavorable conditions. The addition of fermentation liquor or lyophilized whole-culture medium is an avenue to be investigated.

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