Effect of sodium chloride on growth and bacteriocin production by *Lactobacillus amylovorus* DCE 471

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Received 12 May 2002; received in revised form 13 January 2003; accepted 27 January 2003

**Abstract**

A kinetic investigation of the effect of sodium chloride on cell growth of *Lactobacillus amylovorus* DCE 471 and amylovorin L471 production was carried out through in vitro experiments using a temperature and pH prevailing during sourdough fermentations. Sodium chloride interfered both with cell growth and bacteriocin production. Biomass formation and amylovorin L471 production decreased in the presence of increasing salt concentrations. Maximum bacteriocin activities were observed after the addition of 10 g l\(^{-1}\) of NaCl, while the maximum specific growth rate reached an optimum at 5 g l\(^{-1}\) of NaCl. High salt concentrations (20–40 g l\(^{-1}\)) resulted in biphasic fermentation profiles. Based on these results, incorporation of 5–10 g l\(^{-1}\) of sodium chloride in the water phase of type II sourdough preparations might be beneficial to enhance bacterial growth and amylovorin L471 production, and so contribute to the competitiveness of the strain in a sourdough environment. © 2003 Elsevier Science B.V. All rights reserved.

**Keywords:** *Lactobacillus amylovorus* DCE 471; Bacteriocin production; Salt; Modelling

1. Introduction

In spontaneous food fermentations, lactic acid bacteria (LAB) are very often the dominating microflora, frequently resulting in the inhibition of spoilage bacteria and foodborne pathogens (Motlagh et al., 1991). Their microbial antagonism is due to the production of organic acids, ethanol, diacetyl, hydrogen peroxide, and carbon dioxide, alone or in combinations, and can further result from the production of bacteriocins (De Vuyst and Vandamme, 1994a). Bacteriocins are peptides or proteins with an antibacterial activity usually only against closely related species (De Vuyst and Vandamme, 1994b). Furthermore, production of bacteriocins by starter cultures during food fermentations enhances their competitiveness (Todorov et al., 1999). The practical use of these functional starter cultures will contribute to safer and more uniform end products (De Vuyst, 2000). However, the choice of an appropriate starter culture for controlled fermentation processes is of utmost importance, since strain competitiveness and bacteriocin production is influenced by specific conditions that prevail in the food matrix (Callewaert et al., 2000; Leroy et al., 2002).

The species *Lactobacillus amylovorus* has been isolated frequently from rye-based type II sourdoughs and from Sudanese Kisra fermentations of sorghum
flour (Hamad et al., 1992; Müller et al., 2001; Vogel et al., 1999). From a technological point of view, type II sourdough fermentations require a faster acidification than traditional or type I sourdoughs, without affecting the baking and sensorial properties of the bread. *L. amylovorus* seems suitable as a novel starter culture for type II sourdough and Kisra fermentations since it will, together with the flour amylases, contribute to the release of maltose from the cereal, that serves as an energy source for the sourdough LAB strains. Further, it may enhance the acidification process and play an important inhibitory role due to its competitiveness (Messens et al., 2002). The homofermentative, fast acidifying, amylovorin L471-producing strain, *L. amylovorus* DCE 471, has been well documented (Callewaert et al., 1999; De Vuyst et al., 1996a,b). Earlier reports describe the kinetics of growth and bacteriocin production by *L. amylovorus* DCE 471 in MRS medium (De Vuyst et al., 1996a,b; Lejeune et al., 1998). Further, the strain has been found to exhibit maximum bacteriocin activity levels when cultivated in a sourdough simulation medium (SSM) in the temperature and pH ranges that occur during the production of industrial type II sourdough fermentations (Messens et al., 2002). However, under suboptimal growth or stress conditions, including salt, biphasic fermentation kinetics have been observed (Neysens et al., 2003).

When considering in situ food applications of bacteriocin-producing strains in cereals, the water activity, lowered by the presence of solid substrates, and the interference of mineral compounds such as salt that is present in the cell environment, with cellular growth and bacteriocin production, are important (Leroy and De Vuyst, 2000). Indeed, some papers describe the disadvantageous effect of the addition of salt on bacteriocin production and/or activity by LAB strains (De Vuyst et al., 1996a; Nilsen et al., 1998). In contrast, when it is applied at low concentrations, sodium chloride exerts a beneficial effect on the production of sakacin P and lactacin 481, two bacteriocins produced by *Lactobacillus sakei* and *Lactococcus lactis*, respectively (Gänzle et al., 1996; Uguen et al., 1999). Raw materials and additives used for sourdough production contain variable amounts of salt. On the other hand, several sourdough processes incorporating 20–50 g l⁻¹ of NaCl have been developed recently (Gänzle et al., 1998).

The objective of this study was to investigate the effect of both different concentrations of added sodium chloride and water activity on both cell growth and amylovorin L471 production by *L. amylovorus* DCE 471 using sourdough fermentation conditions. A model has been set up to describe the influence of salt on microbial behaviour.

## 2. Materials and methods

### 2.1. Microorganisms and media

*L. amylovorus* DCE 471 was used as producer of the bacteriocin amylovorin L471 throughout this study (De Vuyst et al., 1996b). The amylovorin-sensitive *Lactobacillus delbrueckii* subsp. *bulgaricus* LMG 6901ᵀ strain was used as indicator organism to determine bacteriocin activity levels (De Vuyst et al., 1996b). Both strains were maintained as described previously (Callewaert et al., 1999). Fermentations were carried out in a sourdough simulation medium (SSM) that was characterized by the presence of fructose (10 g l⁻¹) and maltose (10 g l⁻¹), peptides and amino acids (as trypton (Oxoid, Basingstoke, Hampshire, UK), 10 g l⁻¹), growth factors and vitamins (yeast extract (VWR International, Darmstadt, Germany), 12 g l⁻¹), salts and minerals (cysteine–HCl, 0.5 g l⁻¹; KH₂PO₄, 2 g l⁻¹; MgSO₄·7H₂O, 0.2 g l⁻¹; MnSO₄·H₂O, 0.05 g l⁻¹), and Tween 80 (1 ml l⁻¹) (Messens et al., 2002). Lab-Lemco (Oxoid), a rich nitrogen source consisting of peptides and amino acids, was added to avoid biphasic growth kinetics. Solid media to determine cell counts were prepared by the addition of 15 g l⁻¹ of granulated agar (Oxoid) to the broth. Media were autoclaved at 121 °C for 20 min. Sugars were autoclaved separately and aseptically added to the broth.

### 2.2. Inoculum preparation

The inoculum was build up in two steps. First, 10 ml of SSM was inoculated with 0.1 ml of a freshly prepared *L. amylovorus* DCE 471 culture and incubated at 37 °C for 12 h. Then this preculture was used to inoculate 90 ml of SSM. After incubation at 37 °C for 12 h, this second preculture was used to inoculate the
fermentor. The \( L. \) \textit{delbrueckii} subsp. \textit{bulgaricus} LMG 6901\(^T \) culture, needed for the preparation of the overlays, was propagated twice in MRS broth before use.

### 2.3. Fermentation experiments

All fermentations were carried out at a constant temperature of 37 °C and a constant pH of 5.4. The pH was controlled by automatic addition of 10 N NaOH. Preparation of the fermentor and on-line control of the fermentation process (temperature, pH, agitation) were performed as previously described (Messens \textit{et al.}, 2002). Fermentation experiments were carried out in a 15-l laboratory fermentor (Bio Stat\textregistered C; B. Braun Biotech International, Melsungen, Germany) with a working volume of 10 l. To investigate the influence of different amounts of added salt on both cell growth and production of amylovorin L471 by \( L. \) \textit{amylovorus} DCE 471, a series of in vitro fermentations was performed making use of fermentation liquors with added NaCl (2.5, 5, 10, 20, 30 and 40 g l\(^{-1} \)). The corresponding \( a_w \) values were approximately 0.995, 0.993, 0.990, 0.985, 0.978 and 0.972, respectively. An additional fermentation without salt was performed in triplicate; the standard error of the mean (S.E.M.) is given below. SSM contained approximately 0.93 g of NaCl per liter due to the presence of the complex nutrients trypton, yeast extract and Lab Lemco. The amount of Na\(^+\) and the corresponding \( \frac{m}{S} \) were always immediately cooled on ice. Determination of biomass concentration (\( X \)), colony forming units (CFU), total lactic acid concentration (\( L \)), residual maltose (\( M \)) and residual fructose (\( F \)) concentrations, and bacteriocin activity levels (\( B \)) were carried out as described elsewhere (De Vuyst \textit{et al.}, 1996a,b; Lejeune \textit{et al.}, 1998; Leroy and De Vuyst, 1999a). Summarizing, biomass (as cell dry mass, CDM) was determined by gravimetry after membrane filtration and bacteriocin activity was determined by a twofold critical dilution method. Activity was expressed in arbitrary units (AU) per ml or mega arbitrary units (MAU) per liter. To be sure that the inhibition zones could be attributed to amylovorin L471 activity alone, it was first checked that the presence of salt in the supernatant did not prevent growth of the indicator organism. The lactic acid concentration and the residual fructose and maltose concentrations were determined by high-pressure liquid chromatography (HPLC) using a Waters chromatograph (Waters, Milford, MA). The standard deviations for the maltose, fructose, lactic acid and CDM measurements were 0.040, 0.035, 0.025 and 0.11 g l\(^{-1} \), respectively.

### 2.5. Primary model development

Growth, acidification and bacteriocin production by \( L. \) \textit{amylovorus} DCE 471 were modeled using the following set of equations:

\[
\frac{dX}{dt} = \mu_{\text{max}} \times (1 - \frac{X}{X_{\text{max}}})^n - a \times X
\]

for \( t \geq \lambda \). \hfill (1)

\[
\frac{dM}{dt} = -1 \times \frac{Y_{X/M} \times dX}{dt} - m_M \times X
\]

\[
\frac{dF}{dt} = -1 \times \frac{Y_{X/F} \times dX}{dt} - m_F \times X
\]

\[
\frac{dL}{dt} = -1 \times \frac{Y_{L/S} \times (dM/dt + dF/dt)}{dt}
\]

\[
\frac{dB}{dt} = k_B \times \frac{dX/dt - k_{\text{mact}} \times X \times B}{X > X'}
\]

where \( X \) is the biomass concentration (in g CDM l\(^{-1} \)), \( X_{\text{max}} \) is the maximum attainable biomass concentration (in g CDM l\(^{-1} \)), \( \mu_{\text{max}} \) is the maximum specific growth rate (in h\(^{-1} \)), \( t \) is the time (in h), \( \lambda \) is the duration of the lag phase (in h), \( n \) is the inhibition
exponent, $z$ is the specific rate of death (in h\(^{-1}\)), $M$ is the residual maltose concentration (in g maltose \(1^{-1}\)), $F$ is the residual fructose concentration (in g fructose \(1^{-1}\)), $m_M$ is the maintenance coefficient on maltose (in g maltose (g CDM\(^{-1}\)) \( h^{-1}\)), $m_F$ is the maintenance coefficient on fructose (in g fructose (g CDM\(^{-1}\)) \( h^{-1}\)), $Y_{X/M}$ is the cell yield coefficient based on maltose (in g CDM (g maltose\(^{-1}\)), $Y_{X/F}$ is the cell yield coefficient based on fructose (in g CDM (g fructose\(^{-1}\)), $L$ is the amount of lactic acid produced (in g lactic acid \(1^{-1}\)), $Y_L/S$ is the yield coefficient for lactic acid production based on fructose and maltose (in g lactic acid (g fructose + g maltose\(^{-1}\)), $B$ is the soluble bacteriocin activity (in AU g CDM\(^{-1}\)) \( h^{-1}\)), $k_B$ is the specific bacteriocin production (in AU g CDM\(^{-1}\)), $k_{inact}$ is the apparent rate of bacteriocin inactivation (in liter (g CDM\(^{-1}\)) \( h^{-1}\)), $X'$ is the biomass concentration which is needed to produce detectable amounts of bacteriocin (in g CDM \(1^{-1}\)). The equations were integrated with the Euler integration technique in Microsoft Excel 2000. To avoid unrealistic fitting solutions without physiological relevance, and to exclude computational solving problems (e.g. convergence problems), all parameters needed for the modeling were estimated by manual adjustment until a good visual fit of the curves was obtained.

2.6. Secondary modeling

During the secondary modeling all biokinetic parameters derived from the primary model were expressed as a function of the salt concentration. For $\mu_{\text{max}}$, the experimental model as proposed by Gänzle et al. (1998) was used. For the other biokinetic parameters ($X_{\text{max}}$, $Y_{X/M}$, $m_M$, $Y_{X/F}$, $m_F$, $B_{\text{max}}$, $k_B$, and $k_{\text{inact}}$) empirical models were set up. Where appropriate, the quadratic correlation coefficient ($r^2$) is given.

3. Results

3.1. Fermentation runs

Fig. 1 displays the experimental and modeled values of both cell growth and amylovorin L471 activity of L. amylovorus DCE 471 during fermentations performed at a controlled temperature of 37 °C and a constant pH of 5.4 in the presence of various amounts of added sodium chloride. No initial lag phases ($\lambda$) were observed upon the addition of up to 10 g \(1^{-1}\) of NaCl. Increasing the added amount of NaCl led to initial lag phases of maximum 1 h. The consumption of maltose and fructose and the production of lactic acid were modeled using Eqs. (2), (3) and (4), respectively (data not shown). The inhibition exponent was kept constant ($n=1$) for all experiments. To model the observed cell death during the stationary growth phases, the specific rate of death ($z$) varied in the range between 0.005 and 0.039 h\(^{-1}\). The simultaneous consumption of maltose and fructose resulted in the production of lactic acid. No acetic acid nor mannitol were produced (data not shown). It was clear that the production of amylovorin L471 displayed primary metabolite kinetics as the bacteriocin was produced during the exponential growth phase. Highest bacteriocin activities occurred at the end of the active growth phase when reaching maximal cell densities. The beginning of the stationary phase was characterized by the cessation of bacteriocin production and a sharp decrease in bacteriocin activity.

It was observed that growth under suboptimal conditions, i.e. added amounts of 20 g \(1^{-1}\) of NaCl and more, resulted in biphasic fermentation profiles. This has previously been observed for L. amylovorus DCE 471, cultivated under suboptimal growth conditions of temperature and pH (Neysens et al., 2003). In all these cases, each of these profiles were characterized by two exponential and two stationary phases (Fig. 1). When comparing both growth phases of the same fermentation experiment, the maximum biomass was much lower during the first growth phase than during the second one. Furthermore, with exception of the experiment in the presence of 40 g \(1^{-1}\) of added NaCl, the higher the amount of added salt, the longer it took before the second growth phase started. In the presence of 20 g \(1^{-1}\) of added NaCl an intermediate lag phase of 18 h was observed compared with 36 h in the presence of 30 g \(1^{-1}\) of added NaCl. Both growth and bacteriocin production phases were modeled separately (see below). However, the experimental bacteriocin activities which were obtained during second growth phase were not fitted well with the model. This is because the nature of the method used for their determination requires that experimental data
Fig. 1. Modeling of cell growth (in grams of CDM per liter) (a, b) and bacteriocin activity (in MAU per liter) (c, d) of L. amylovorus DCE 471 grown in a sourdough simulation medium in the presence of 0.0 g l\(^{-1}\) (○); 2.5 g l\(^{-1}\) (■); 5.0 g l\(^{-1}\) (▲); 10.0 g l\(^{-1}\) (□); 20.0 g l\(^{-1}\) (◇); 30.0 g l\(^{-1}\) (●) and 40.0 g l\(^{-1}\) (△) of added sodium chloride. Symbols represent the experimental values; full lines are drawn according to the model.
obtained during the early production phase should be fitted more stringent than those obtained in the late production phase. Hence, the higher the bacteriocin activity, the higher the dilution and consequently the higher the error because of the twofold dilution procedure.

3.2. Influence of added sodium chloride

Table 1 shows the effect of added NaCl on the biokinetic parameters of *L. amylovorus* DCE 471. When the added amount of salt was higher than 5 g l\(^{-1}\), cells grew more slowly and biomass production became less efficient. This could be observed during both the first and second growth phase (Table 1). Comparing the fermentations in the presence of added NaCl, it was observed that during the first growth phase the maximum specific growth rate (\(\mu_{\text{max}}\)) was higher when the quantity of added salt was below or equal to 5 g l\(^{-1}\) of added salt. The maximum attainable biomass concentration (\(X_{\text{max}}\)) was lower when the addition of salt was increased (Table 1). Increasing the added amounts of NaCl from 0 to 30 g l\(^{-1}\) resulted in a loss of biomass of 1.68 g of CDM per liter, compared to a loss of 1.44 g of CDM per liter when the strain was cultivated on small scale in MRS (De Vuyst et al., 1996a). The \(\mu_{\text{max}}\) values of the first and second growth phase were not similar. The latter phase was characterized by a higher \(\mu_{\text{max}}\) and a lower \(X_{\text{max}}\) for concentrations up to 30 g l\(^{-1}\) of NaCl (Table 1). When the added amount of salt to the medium was 40 g l\(^{-1}\) of NaCl, \(X_{\text{max}}\) and \(\mu_{\text{max}}\) were considerably lower compared with the values obtained during the first growth phase in the presence of less NaCl. However, the highest \(\mu_{\text{max}}\) and lowest \(X_{\text{max}}\) values were found during the second growth phase of the fermentation where 40 g l\(^{-1}\) of NaCl was added.

During the first growth phase, the cell yield coefficients for fructose (\(Y_{X\text{F}}\)) and maltose (\(Y_{X\text{M}}\)) were negatively influenced by higher salt concentrations (Table 1), representing a similar trend as was observed for \(X_{\text{max}}\). The maintenance coefficient for fructose (\(m_{F}\)) followed the same trend as \(Y_{X\text{F}}\). Although the maintenance coefficient for maltose (\(m_{M}\)) did not experience any effect by NaCl, it
gained importance, compared to \( m_F \), when higher amounts of salt were added. The cell yield coefficients as well as the maintenance coefficients for both maltose and fructose were slightly affected by higher amounts of added NaCl only during the second growth phase (Table 1).

A maximum amyllovorin L471 activity (\( B_{\text{max}} \) of 25.6 MAU L\(^{-1}\)) was found when adding 10 g L\(^{-1}\) of NaCl. Further elevation of the amount of salt resulted in a lower \( B_{\text{max}} \) while the specific bacteriocin production (\( k_B \)) leveled off from 20 g L\(^{-1}\) of NaCl and beyond. For this growth phase, the \( k_B \), \( B_{\text{max}} \), and \( k_{\text{inact}} \) values for the fermentation experiment in the presence of 40 g L\(^{-1}\) of added NaCl could not be determined experimentally, due to the very low growth rate and biomass formation. Because there was a low amount of biomass available (\( X_{\text{max}} \) of 0.15 g of CDM L\(^{-1}\)), the production of detectable amounts of amyllovorin L471 was hampered. The trends of the parameters describing the bacteriocin production during the second growth phase were different from the ones obtained during the first growth phase (Table 1). A clear increase in \( k_{\text{inact}} \) was observed from the first to the second growth phase. A similar trend was found for \( k_B \) in the presence of 20 g L\(^{-1}\) of added NaCl, whereas \( k_B \) was unaffected after the addition of 30 g L\(^{-1}\) of NaCl. A decrease of \( B_{\text{max}} \) with the biomass concentration was observed upon elevation of the added amount of NaCl from 20 g L\(^{-1}\) to 30 g L\(^{-1}\). When grown in the presence of 40 g L\(^{-1}\) of added NaCl, \( X_{\text{max}} \) reached only 43% of the concentration that was obtained when no salt was added, at the end of the second growth phase. This corresponded with a \( B_{\text{max}} \) value of 8.44 MAU L\(^{-1}\). The responses of the biokinetic parameters to salt in the range of 0–40 g L\(^{-1}\) of added NaCl during the first growth phase were described with the mathematical relationships displayed in Table 2. The model parameters in the absence of added salt are shown as well.

### 3.3. Influence of \( a_w \)

To investigate whether the inhibitory effect of high amounts of added NaCl on cell growth and bacteriocin production by \( L. \) amylovorus DCE 471 could be ascribed to a decrease in \( a_w \) or to other (ionic) effects, two additional fermentation experiments were performed with glycerol as an \( a_w \)-lowering agent (Table 3). Since a second growth phase could not be observed in the presence of glycerol, the values of the biokinetic parameters were compared with the corresponding values obtained during first growth phases for the addition of NaCl. It appears that \( \mu_{\text{max}} \) was not affected by lowering the \( a_w \) of the growth medium with glycerol: 0.66 h\(^{-1}\) (no salt, Table 1) versus 0.68–0.69 h\(^{-1}\) (with glycerol, Table 3). The \( \mu_{\text{max}} \) and \( X_{\text{max}} \) values of the fermentations in the presence of 20 and 30 g L\(^{-1}\) of added NaCl were compared with the corresponding values obtained after the addition of 50 and 83 g L\(^{-1}\) of glycerol, respectively. Both \( \mu_{\text{max}} \) and \( X_{\text{max}} \) were lower when NaCl was used as \( a_w \)-lowering agent instead of

### Table 2

Mathematical relationships describing the response of the biokinetic parameters (\( \mu_{\text{max}}, Y_{\text{XM}}, Y_{\text{XF}}, m_M, m_F, X_{\text{max}}, k_B, k_{\text{inact}} \) and \( B_{\text{max}} \)) to added NaCl during the first growth phase of \( L. \) amylovorus DCE 471 grown in a sourdough simulation medium at 37 °C and constant pH 5.4.

<table>
<thead>
<tr>
<th>Biokinetic parameter</th>
<th>Mathematical relationship</th>
<th>( r^2 )</th>
<th>Value for [NaCl] = 0 g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{\text{max}} ) (h(^{-1}))</td>
<td>( 0.1414 \times \left( \frac{([\text{NaCl}]+0.10) \times ([\text{NaCl}]-6.92)}{([\text{NaCl}]-6.29)-([\text{NaCl}]-0.30)^2} \right) \times (\mu_{\text{max}})_{\text{NaCl}=0} ) ( \text{g} \text{l}^{-1} )</td>
<td>0.932</td>
<td>0.666</td>
</tr>
<tr>
<td>( X_{\text{max}} ) (g of CDM L(^{-1}))</td>
<td>( (1-0.2446 \times [\text{NaCl}] \times (X_{\text{max}})_{\text{NaCl}=0} \text{g} \text{l}^{-1}) )</td>
<td>0.989</td>
<td>2.034</td>
</tr>
<tr>
<td>( Y_{\text{XM}} ) (g of CDM (g of maltose)-(^{\text{-1}}))</td>
<td>( ([\text{NaCl}]^3-7.7442 \times [\text{NaCl}]+26.2262) \times (Y_{\text{XM}})_{\text{NaCl}=0} \text{g} \text{l}^{-1} )</td>
<td>0.996</td>
<td>0.0305</td>
</tr>
<tr>
<td>( Y_{\text{XF}} ) (g of CDM (g of fructose)-(^{\text{-1}}))</td>
<td>( ([\text{NaCl}]^3-5.2295 \times [\text{NaCl}]^2-0.7540 \times [\text{NaCl}]+55.0163) \times (Y_{\text{XF}})_{\text{NaCl}=0} \text{g} \text{l}^{-1} )</td>
<td>0.997</td>
<td>0.0061</td>
</tr>
<tr>
<td>( m_M ) (g of maltose (g of CDM)-(^{\text{-1}}) h(^{-1}))</td>
<td>( (m_M)_{\text{NaCl}=0} \text{g} \text{l}^{-1} )</td>
<td>1.000</td>
<td>0.31</td>
</tr>
<tr>
<td>( m_F ) (g of fructose (g of CDM)-(^{\text{-1}}) h(^{-1}))</td>
<td>( ([\text{NaCl}]^3-5.5714 \times [\text{NaCl}]^2+2.7276 \times [\text{NaCl}]+18.7723) \times (m_F)_{\text{NaCl}=0} \text{g} \text{l}^{-1} )</td>
<td>0.991</td>
<td>0.0224</td>
</tr>
<tr>
<td>( k_B ) (MAU (g of CDM)-(^{\text{-1}}))</td>
<td>( (-[\text{NaCl}]^4+4.7103 \times [\text{NaCl}]+0.76777) \times (k_B)_{\text{NaCl}=0} \text{g} \text{l}^{-1} )</td>
<td>0.989</td>
<td>8.332</td>
</tr>
<tr>
<td>( k_{\text{inact}} ) (l (g of CDM)-(^{\text{-1}}) h(^{-1}))</td>
<td>( (0.3743 \times [\text{NaCl}]) \times (k_{\text{inact}})_{\text{NaCl}=0} \text{g} \text{l}^{-1} )</td>
<td>0.993</td>
<td>0.084</td>
</tr>
<tr>
<td>( B_{\text{max}} ) (MAU L(^{-1}))</td>
<td>( (-[\text{NaCl}]^2+2.9761 \times [\text{NaCl}]+0.7718) \times (B_{\text{max}})_{\text{NaCl}=0} \text{g} \text{l}^{-1} )</td>
<td>0.953</td>
<td>7.999</td>
</tr>
</tbody>
</table>
glycerol (Table 3). Furthermore, $Y_{X/M}$ was higher in the presence of glycerol whereas $m_M$ was not affected. Both $Y_{X/F}$ and $m_F$ experienced little effect upon glycerol addition. Also, $k_B$ was only slightly affected upon increasing the glycerol concentration from 50 to 83 g l$^{-1}$ and averaged 20 MAU (g CDM)$^{-1}$, but when compared with the corresponding values obtained in the presence of added salt, a lower $k_B$ was observed. This was mainly due to the lower attainable biomass concentration (38% lower at 83 g l$^{-1}$ of glycerol compared with the control) and the higher amylovorin L471 activity (threefold at 83 g l$^{-1}$ of glycerol compared with the control). A decrease in $k_{inact}$ was found when ramping up the glycerol concentration. This phenomenon could not be observed after increasing the amount of added NaCl.

4. Discussion

Bacterial metabolism is sensitive to salt, because salt exhibits specific ionic and water binding properties (Korkeala et al., 1992). The latter effect is of utmost importance because the addition of salt to the fermentation liquor leads to a decrease in $a_w$. Decreases in $a_w$ below the optimum values for growth often result in a linear decrease of the growth rate (McMeekin et al., 1987). On the other hand, homofermentative LAB are in general less salt sensitive than heterofermentative LAB (Korkeala et al., 1992). It appears that cultivation of LAB in environments with a lot of salt (NaCl concentrations higher than 30 g l$^{-1}$) hampers bacterial growth, while lower amounts from 10 to 20 g l$^{-1}$ can exhibit a positive effect (Gänzle et al., 1998; Korkeala et al., 1992; Passos et al., 1993). During the first growth phase of *L. amylovorus* DCE 471, increasing the quantity of added salt gave rise to a linear decrease of the growth rate after reaching a maximum in the presence of 5 g l$^{-1}$ of NaCl. Also, an enhanced growth of some sourdough LAB present in an aqueous flour suspension occurs after its supplementation with a salt concentration of 5 g l$^{-1}$ (Kline and Sugihara, 1971). When increasing the amount of added salt, *L. amylovorus* DCE 471 is no longer able to consume the available carbon and nitrogen source in an efficient way (De Vuyst et al., 1996a). Furthermore, cells appeared to use the available energy for cell growth and multi-

<table>
<thead>
<tr>
<th>Glycerol (g l$^{-1}$)</th>
<th>$l_{max}$ (h l$^{-1}$)</th>
<th>$Y_{X/M}$ (g CDM (g maltose)$^{-1}$)</th>
<th>$Y_{X/F}$ (g CDM (g fructose)$^{-1}$)</th>
<th>$m_M$ (g CDM h$^{-1}$)</th>
<th>$m_F$ (g CDM h$^{-1}$)</th>
<th>$X_{max}$ (g CDM l$^{-1}$)</th>
<th>$k_B$ (MAU (g CDM)$^{-1}$)</th>
<th>$k_{inact}$ (l (g CDM)$^{-1}$ h$^{-1}$)</th>
<th>$B_{max}$ (MAU l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 (0.997)</td>
<td>0.66 ± 0.05</td>
<td>0.78 ± 0.04</td>
<td>0.35 ± 0.04</td>
<td>0.46 ± 0.06</td>
<td>0.35 ± 0.04</td>
<td>0.46 ± 0.06</td>
<td>0.35 ± 0.04</td>
<td>0.46 ± 0.06</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>50.0 (0.985)</td>
<td>0.70</td>
<td>0.32</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>83.0 (0.983)</td>
<td>0.66</td>
<td>0.78</td>
<td>0.32</td>
<td>0.25</td>
<td>0.25</td>
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* The fermentation was done in triplicate.
plication rather than for maintenance. For biomass formation, maltose was preferred above fructose. However, when increasing the added quantity of salt up to 10 g l\(^{-1}\), it was observed that fructose was the preferred energy source for maintenance. As the increase in added salt gave rise to lower \(\mu_{\text{max}}\) values, slow growing cells needed less energy derived from fructose. Moreover, in the presence of more than 20 g l\(^{-1}\) of added NaCl, two distinct growth and bacteriocin production phases were observed.

It has been shown that amyllovorin L471 production displays primary metabolite kinetics (Callewaert et al., 1999; De Vuyst et al., 1996a; Lejeune et al., 1998). During the first growth phase, however, amyllovorin L471 production was negatively affected by NaCl, because the amount of biomass formed was lower, as well as the apparent rate of bacteriocin inactivation. As bacteriocins are proteins and glycerol is a protective and stabilizing agent, this component might prevent the adsorption and/or degradation of the bacteriocin and protect it from inactivation. Concerning bacteriocin production, it was shown that addition of 10 g l\(^{-1}\) of NaCl does not influence bavaricin A production at 10 °C (Larsen et al., 1993). When the NaCl concentration is increased to 30 g l\(^{-1}\), the production of active bavaricin A is lowered. At 50 g l\(^{-1}\) of NaCl, bavaricin A activity is no longer detected, although the bacteria are still growing. As bacteriocin production by \(L. \) amylovorus DCE 471 best fits the temperature and pH conditions that prevail during the preparation of type II sourdoughs (Messens et al., 2002), the incorporation of low amounts of NaCl might be an additional benefit for its competitiveness and the production of amyllovorin L471, because of the simulation of growth in SSM by addition of a small amount of NaCl (5–10 g l\(^{-1}\)). Moreover, the addition of up to 15 g l\(^{-1}\) of NaCl will not interfere with yeast activity, since a decrease in the specific growth rate and an increase in the lag phase is only observed during fermentations with \(\text{Saccharomyces cerevisiae}\) for NaCl concentrations above 30 g l\(^{-1}\) in the water phase (Carvalheiro et al., 1999). On the other hand, for up to 15 g l\(^{-1}\) of added NaCl, no inhibitory effect on growth is observed.

Because the use of glycerol instead of NaCl as an \(a_w\)-lowering agent did not appear to have the same effect on the biokinetic parameters of \(L. \) amylovorus DCE 471, the water binding effect of the NaCl molecules cannot be the main reason for the decreased amyllovorin L471 production. When compared with the corresponding experiments in the presence of added NaCl, the production of biomass as well as maintenance based on the consumption of fructose were found to be less efficient. Again, maltose was the preferred energy source for biomass formation. Opposite observations were made with \(L. \) sakei CTC 494 (Leroy and De Vuyst, 1999b). These authors observed no significant changes upon the replacement of NaCl by glycerol. On the other hand, a more pronounced growth inhibition of lactobacilli in sausage occurred in the presence of salt (Doßmann et al., 1996). Furthermore, since the addition of glycerol did not lead to biphasic fermentation profiles, it is clear that the inhibitory effect of NaCl, leading to a sharp decrease in \(\mu_{\text{max}}\) and a pronounced increase in \(k_{\text{IB}}\), is more important than the water activity.

In this paper, we examined the effect of salt on growth and bacteriocin production by \(L. \) amylovorus DCE 471, a potential starter culture for use in type II sourdough preparations. A mathematical model was set up to describe growth and amyllovorin L471 production characteristics under both optimal and stress conditions. When compared to the addition of glycerol, NaCl had a more pronounced effect on cell growth resulting in biphasic growth kinetics for high added values. The incorporation of low amounts of NaCl (5–10 g l\(^{-1}\)) in the water phase of type II sourdough preparations might have a positive effect on both cell growth and bacteriocin production, thereby resulting in a faster acidification of the raw material and an enhanced competitiveness of \(L. \) amylovorus DCE 471 in a cereal environment.

**Acknowledgements**

The authors acknowledge the financial support from the Institute for the Encouragement of Innovation through Science and Technology in Flanders (IWT), in particular the STWW project ‘Functionality of Novel Starter Cultures in Traditional Fermentation Processes’. Also, the financial support from the Research Council of the Vrije Universiteit Brussel, the Fund for Scientific Research-Flanders, and from different food companies, is greatly appreciated.
Vincent Schrijvers is acknowledged for his part in the practical work during this study.

References


Leroy, F., De Vuyst, L., 1999b. The presence of salt and a curing agent reduces bacteriocin production by Lactobacillus sakei CTC 494, a potential starter culture for sausage fermentation. Applied and Environmental Microbiology 65, 5350–5356.


