Growth Response of Salmonella Typhimurium in the Presence of Natural and Synthetic Antimicrobials: Estimation of MICs from Three Different Models

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ABSTRACT

The aim of this study was to determine the MICs of 14 antimicrobials for Salmonella Typhimurium with three methods and to check the influence of experiment duration on the estimation of MICs. The growth of Salmonella Typhimurium in a brain heart infusion medium containing various concentrations of natural aromatic compounds, organic acids, or salts was monitored by absorbance measurements for 24 or 72 h. Three different ways of analyzing optical density (OD) curves were tested for the determination of MICs. Both quantitative methods gave similar MICs for most of the compounds. The semi-quantitative method does not allow estimating the MIC for all compounds. Noticeable differences were found between MICs obtained for 24- or 72-h experiments, whatever the method used. The proposed methods and models can be used for the estimation of MICs from OD data. MICs could be used for a quantitative approach to Salmonella Typhimurium growth.

Addition of preservatives is one of the most commonly used systems to enhance microbiological safety and stability of food products. Among these, natural antimicrobials have great potential because of the consumer’s demand for fresher, additive-free, and more natural-tasting food products. A large number of studies have examined the in vitro antimicrobial activity of naturally occurring compounds (4, 5, 11, 18, 38). Organic acids (e.g., acetic, lactic, citric) have been recognized for many years for their antimicrobial activities against numerous food poisoning bacteria (13, 19, 39, 44). Their efficiency is primarily based on their ability to reduce the pH in the water phase of foods (37). Plant essential oils and their constituents are also widely used as flavoring agents in food, and it is now well established that many of them have great efficiency against foodborne bacteria (9, 16, 30, 33, 34, 40, 41, 43). Generally, the antimicrobial activity of essential oils is primarily attributable to their phenolic compounds (22).

Salmonellosis is an important public health problem having a significant economic impact. Although most infections cause moderate disease, serious infections leading to death do occur (1). In France, it is estimated that between 32,200 and 43,300 nontyphoidal Salmonella infections occur annually (1). These infections lead to 97 to 563 annual deaths.

Thus, food industrials and risk assessors are interested to have quantitative information of the growth of Salmonella with respect to the physicochemical properties of the food products. They are particularly interested to know if the components of the food products are able to prevent its growth. The measurement of absorbance is one of the major techniques used to obtain quantitative data on the effects of the antimicrobial compounds. The inhibitory effect of antimicrobials on microorganisms is generally determined by arbitrarily fixing the exposure period and by determining the antimicrobial’s MIC, which is the lowest concentration of antimicrobial in a tested range leading to the total inhibition of growth (no increasing of absorbance). More recently, other methods based on a mathematical analysis of absorbance measurements were proposed in which MICs are calculated either from the areas under the absorbance curves (6, 25–27) or from the growth rates (2, 7, 10).

The first objective of this article was to determine the MICs for Salmonella enterica serotype Typhimurium of 14 antimicrobials by three different methods: a semiquantitative method and two methods based on the mathematical analysis of absorbance growth curves. The second objective was to evaluate the influence of the duration of the exposure to antimicrobials on the determination of the MICs.

MATERIALS AND METHODS

Antimicrobial agents. Citric acid, pyropolyphosphoric acid, sodium lactate, thymol, geraniol, carvacrol, eugenol, citral, trans-cinnamaldehyde, and α-terpineol were obtained from Sigma-Aldrich Chemicals (St. Louis, Mo.). Acetic acid, sodium, and po-
In this study, we computed the areas under the OD curves by means of trapezoidal integration (TRAPZ subroutine of MATLAB 6.5.1 software).

\[
\text{fa}(c) = \int_{0}^{\infty} \text{OD} \, dc
\]

A Lambert and Pearson (27) proposed a model based on a Gompertz function to fit fractional area data sets. The Lambert-Pearson (LPfa) model was then updated by Lambert and Lambert (26).

The observed fractional areas were modeled as follows:

\[
\frac{c_{\text{fa}(c)}}{A_0} = \exp \left( \frac{c}{B_2} \right)
\]

TABLE 1. Confidence interval (95%) and estimated value of parameters of model fitted to fractional area data of Salmonella Typhimurium for 14 antimicrobials for 24- and 72-h experiments

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>NIC (mM)</th>
<th>MIC (mM)</th>
<th>NIC (mM)</th>
<th>MIC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
<td>24 h</td>
<td>72 h</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>0.55 (0.46; 0.64)</td>
<td>0.99 (0.86; 1.12)</td>
<td>0.59 (0.42; 0.77)</td>
<td>1.03 (0.79; 1.27)</td>
</tr>
<tr>
<td>trans-Cinnamaldehyde</td>
<td>1.04 (0.89; 1.18)</td>
<td>2.64 (2.21; 3.08)</td>
<td>1.40 (1.25; 1.56)</td>
<td>2.51 (2.24; 2.78)</td>
</tr>
<tr>
<td>Eugenol</td>
<td>1.18 (0.85; 1.52)</td>
<td>3.24 (2.56; 3.91)</td>
<td>1.28 (0.97; 1.60)</td>
<td>3.60 (2.90; 4.29)</td>
</tr>
<tr>
<td>Geraniol</td>
<td>0.94 (0.87; 1.02)</td>
<td>2.71 (2.56; 2.87)</td>
<td>0.85 (0.73; 0.98)</td>
<td>3.89 (3.46; 4.32)</td>
</tr>
<tr>
<td>Menthol</td>
<td>0.82 (0.65; 0.99)</td>
<td>2.55 (2.17; 2.92)</td>
<td>0.77 (0.57; 0.97)</td>
<td>4.12 (3.31; 4.93)</td>
</tr>
<tr>
<td>α-Terpinol</td>
<td>2.10 (1.87; 2.33)</td>
<td>4.34 (3.97; 4.70)</td>
<td>1.90 (1.34; 2.46)</td>
<td>5.52 (4.12; 6.92)</td>
</tr>
<tr>
<td>Thymol</td>
<td>0.44 (0.25; 0.63)</td>
<td>1.09 (0.73; 1.46)</td>
<td>0.44 (0.25; 0.63)</td>
<td>1.09 (0.73; 1.46)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>21.99 (20.75; 23.23)</td>
<td>35.61 (34.00; 37.21)</td>
<td>28.92 (27.84; 29.99)</td>
<td>41.61 (39.55; 43.67)</td>
</tr>
<tr>
<td>Citric acid</td>
<td>10.80 (9.55; 12.05)</td>
<td>22.56 (20.59; 24.52)</td>
<td>14.27 (10.95; 17.59)</td>
<td>23.32 (19.07; 27.56)</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>36.36 (30.65; 42.06)</td>
<td>44.72 (36.46; 52.97)</td>
<td>42.41 (37.89; 46.93)</td>
<td>57.27 (51.54; 63.00)</td>
</tr>
<tr>
<td>Pyrophosphoric acid</td>
<td>10.23 (9.17; 11.29)</td>
<td>14.89 (13.47; 16.31)</td>
<td>10.28 (6.16; 14.40)</td>
<td>21.01 (15.26; 26.76)</td>
</tr>
<tr>
<td>Potassium acetate</td>
<td>160.53 (138.66; 182.40)</td>
<td>827.02 (757.59; 896.45)</td>
<td>327.57 (301.24; 353.91)</td>
<td>945.67 (893.07; 998.27)</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>308.39 (272.71; 344.06)</td>
<td>1,057.90 (974.35; 1,141.40)</td>
<td>535.33 (488.24; 582.41)</td>
<td>1,206.10 (1,124.20; 1,288.00)</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>148.83 (125.91; 171.75)</td>
<td>828.99 (750.39; 907.59)</td>
<td>220.06 (173.63; 266.49)</td>
<td>977.81 (844.94; 1,110.70)</td>
</tr>
</tbody>
</table>

Strain and Growth conditions. The strain used in this study was S. enterica subsp. enterica serovar Typhimurium ATCC 13311. Growth was carried out in Brain Heart Infusion Broth (Basingstoke, UK) in the absence or presence of antimicrobials. The bacteria were grown in Brain Heart Infusion Broth (Oxoid, Basingstoke, UK) in the absence or presence of antimicrobials.
where \( c \) is the concentration of inhibitor, and \( p_1 \) and \( p_2 \) are model parameters.

The MIC and the noninhibitory concentration (NIC), which is the concentration below which the inhibitors have no effect on the growth of the microorganism, can be retrieved from the \( p_1 \) and \( p_2 \) values of the LP\(_{fa}\) model (26):

\[
\text{MIC} = p_1 \cdot \exp \left( \frac{1}{p_2} \right) \quad \text{and} \quad \text{NIC} = p_1 \cdot \exp \left( \frac{1 - e}{p_2} \right)
\]

where the value of \( e \) is the exponential of 1.

We propose a new parameterization of the LP\(_{fa}\) model in order to have a direct estimation of confidence intervals on fitted values of MIC and NIC. Thus, we replace \( p_1 \) and \( p_2 \) model parameters (equation 2) by NIC and MIC parameters by equations 3 and 4. Then, we obtain

\[
fa(c) = \exp \left\{ \frac{-c}{\text{MIC}/\exp \left[ \ln(\text{NIC}/\text{MIC}) \right]} \right\}^{e/[\ln(\text{NIC}/\text{MIC})]}
\]

**Dose-response curve modeling and MIC determination: estimation based on growth rate model.** The maximum specific growth rates (\( \mu_{\text{max}} \)) were estimated from OD growth kinetics by fitting the modified Gompertz model (2, 10). The logarithmic transformation of OD was done to stabilize the variance (2).

\[
\ln(\text{OD}(t)) = \ln(\text{OD}_0) + A \cdot \exp \left\{ -\exp \left[ \frac{\mu_{\text{max}} \cdot c}{A} \cdot (\text{lag} - t) + 1 \right] \right\}
\]

where \( t \) is the time, \( \text{OD}_0 \) is the OD value at time \( t = 0 \), \( A \) is equal to \( \ln(\text{OD}_{\text{max}}) - \ln(\text{OD}_0) \), \( \text{OD}_{\text{max}} \) is the maximum OD, \( \text{lag} \) is the duration of the lag phase, \( \mu_{\text{max}} \) is the growth rate, and \( e \) is the exponential of 1. \( \text{OD}_0, \text{OD}_{\text{max}}, \text{lag}, \) and \( \mu_{\text{max}} \) are model parameters.

We tested two models to assess the antimicrobial concentration (\( c \)) dependence of \( \mu_{\text{max}} \). First, we fitted a square-root model (3, 7, 12, 15) with a shape parameter (\( \beta \)). The model (SR\(_{\beta}\)) was as follows:

\[
\sqrt{\mu_{\text{max}}(c)} = \sqrt{\mu_{\text{max}}(c = 0)} \cdot f(c), \quad \text{with}
\]

\[
f(c) = \begin{cases} 
1 - \frac{c}{\text{MIC}}, & c < \text{MIC} \\
0, & c \geq \text{MIC}
\end{cases}
\]

where the model parameters are MIC, the MIC of the antimicrobial; \( \mu_{\text{max}}(c = 0) \), the growth rate in the absence of the antimicrobial (\( c = 0 \)); and \( \beta \), the shape parameter. \( \beta \) represents the sensitiveness of the microorganism to an antimicrobial (7).

Second, we proposed to model \( \mu_{\text{max}} \) and to determine the MIC and NIC of the inhibitors by a model based on the LP\(_{fa}\) model (LP\(_{\n}\)):

\[
\sqrt{\mu_{\text{max}}(c)} = \sqrt{\mu_{\text{max}}(c = 0)} \cdot g(c), \quad \text{where}
\]

\[
g(c) = \exp \left\{ \frac{-c}{\text{MIC}/\exp \left[ \ln(\text{NIC}/\text{MIC}) \right]} \right\}
\]

where the model parameters are MIC, NIC, and \( \mu_{\text{max}}(c = 0) \).

For both models, the square-root transformation of the growth rate was performed to homogenize its variance (35, 36, 46).

**Fit of models.** The ordinary least-squares criterion was used to fit models to the data set. Estimation of parameters was carried out by nonlinear regression by minimizing the sum of the squared residuals (SSR). The SSR is defined as follows:

\[
\text{SSR} = \sum_{i=1}^{n} (V_{\text{obs}} - V_{\text{fit}})^2
\]

where \( n \) is the number of data points, \( V_{\text{obs}} \) is the observed value, and \( V_{\text{fit}} \) is the estimated value. The minimum SSR was computed with the NLINFIT function of MATLAB 6.5.1 software (MathWorks Inc., Natick, Mass.). The NLPARCI function of MATLAB was used to determine the confidence interval at 95% of the nonlinear parameter estimate.

**RESULTS**

**Dose-response curve modeling and comparison of MICs and NICs.** Estimated values of parameters of the LP\(_{fa}\) model fitted to fractional area data from 24- and 72-h experiments are shown in Table 1. By classifying the 14 antimicrobials by the MICs, three groups of antimicrobials can be retrieved: plant natural extracts, organic acids, and acid salts, with small differences among the MICs within each group.

The parameter values of the two models applied on growth rates determined from the 24-h experiments are shown in Table 2. The MICs obtained from both fitted models are very similar. Both models adequately described the 14 different data sets. By analyzing the SSR of both models, it can be said that their performances were close, whatever the nature of the antimicrobials. We reached the same conclusions on the performance and best-fit MICs for SR\(_{\beta}\) and LP\(_{\n}\) models with 72-h experiments (Table 3).

For both experiment durations, the best-fit MICs (Tables 2 and 3) estimated from growth rate data (either with the SR\(_{\beta}\) model or LP\(_{\n}\) model) and the best-fit values estimated from fa data (Table 1) are approximately the same. This similarity was also observed for the best-fit NICs estimated from growth rate data sets and the best-fit NICs estimated from the fa data sets (Table 1).

In Table 4, the lower and upper possible MICs based on the semiquantitative method are presented. These pairs of values define the range of the MIC. By comparing these ranges of MICs with the confidence intervals of the parameters determined by the two other methods, it can be observed that the confidence limit at 95% can be either larger or narrower than the ranges. This can be explained by un-
TABLE 2. Confidence interval (95%) and estimated value of parameters of models fitted to growth rate data of Salmonella Typhimurium for 14 antimicrobial compounds for 24-h experiments.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (mM)</th>
<th>MIC (mM)</th>
<th>MIC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trans-cinnamaldehyde</strong></td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td><strong>Carvacrol</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td><strong>Cinnamaldehyde</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td><strong>-Terpineol</strong></td>
<td><strong>0.04</strong></td>
<td><strong>0.04</strong></td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td><strong>Acetic acid</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td><strong>Citric acid</strong></td>
<td><strong>0.06</strong></td>
<td><strong>0.06</strong></td>
<td><strong>0.06</strong></td>
</tr>
<tr>
<td><strong>Pyrophosphoric acid</strong></td>
<td><strong>0.07</strong></td>
<td><strong>0.07</strong></td>
<td><strong>0.07</strong></td>
</tr>
<tr>
<td><strong>Potassium acetate</strong></td>
<td><strong>0.08</strong></td>
<td><strong>0.08</strong></td>
<td><strong>0.08</strong></td>
</tr>
<tr>
<td><strong>Sodium lactate</strong></td>
<td><strong>0.09</strong></td>
<td><strong>0.09</strong></td>
<td><strong>0.09</strong></td>
</tr>
<tr>
<td><strong>Sodium acetate</strong></td>
<td><strong>0.10</strong></td>
<td><strong>0.10</strong></td>
<td><strong>0.10</strong></td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the present study, we determined the MIC of 14 antimicrobial compounds for Salmonella Typhimurium by OD measurements. Modeling fractional areas and growth certainty linked to the calculation of fa and μ_max and by the number of concentrations of antimicrobial tested.

**Effects of sub-MIC levels on the growth of Salmonella Typhimurium.** The analysis of OD curves by measurement of fractional areas or growth rate allowed us to have a quantitative approach to the influence of subinhibitory concentrations of antimicrobials. Two different kinds of influence of the inhibitors on the growth rate of Salmonella Typhimurium were observed. This classification is based on the shape of the growth rate versus the antimicrobial concentration curve. The first group was a standard sigmoid curve. A typical example of a sigmoid-shaped curve is represented with α-terpineol in Figure 1. For the second group, the inhibitors have an effect on growth rate even for the smaller concentrations. An example is shown in Figure 2 with potassium acetate. These two categories are separated by a threshold value of approximately 2 for β and 5 for MIC:NIC ratios. In the first category, we retrieved all the natural extracts of plants and the organic acids. In the second category, only the three salts were found.

At the opposite, the curves of the plot of the concentration of antimicrobials with the fractional areas were all sigmoid shaped (Figs. 1 and 2). Indeed, the MIC:NIC ratios were inferior to 5 for the 14 inhibitors. Then, both types of analysis of OD curves led to a different appreciation of the influence of subinhibitory concentrations for the three salts.

**Comparison of MIC and NIC 24- and 72-h experiments.** For both aromatic compounds and organic acids, according to best-fit values and their confidence intervals, the NICs determined from 24- or 72-h experiments were not different (Tables 2 and 3). For the three salts of acid, the NICs were also equivalent when growth rates were used. But with fa data, the NICs of the three salts obtained from 72-h experiments were higher than 24-h experiments (Table 1 and Fig. 3). In other terms, for the three salts, the lack of differences between the control and the small concentrations was hidden by the importance of the area defined by the OD curve.

The results of MICs obtained from 24- and 72-h experiments are graphically represented in Figure 4. This figure shows that the MICs estimated from 72-h experiments were equal to or superior than the MICs obtained from 24-h experiments, whatever the method or model used. For most of the natural antimicrobials, the estimation was little affected by the duration of the experiment. Yet, for acid compounds and salts, we observed a noticeable difference between 24- and 72-h experiments (Fig. 4).

The difference between the two durations of experiment was also observed for the range of MICs determined by the semiquantitative method for trans-cinnamaldehyde, citric acid, pyrophosphoric acid, and the three salt of acids (Table 4).
TABLE 3. Confidence interval (95%) and estimated value of parameters of models fitted to growth rate data of Salmonella Typhimurium for 14 antimicrobials for 72-h experiments

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (mM)</th>
<th>95% CI (mM)</th>
<th>MIC (mM)</th>
<th>95% CI (mM)</th>
<th>MIC (mM)</th>
<th>95% CI (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvacrol</td>
<td>0.90</td>
<td>0.80-1.00</td>
<td>1.00</td>
<td>0.80-1.00</td>
<td>1.00</td>
<td>0.80-1.00</td>
</tr>
<tr>
<td>trans-Cinnamaldehyde</td>
<td>1.60</td>
<td>2.40-3.00</td>
<td>2.40</td>
<td>2.40-3.00</td>
<td>2.40</td>
<td>2.40-3.00</td>
</tr>
<tr>
<td>Eugenol</td>
<td>2.80</td>
<td>3.00-3.20</td>
<td>3.20</td>
<td>3.20-3.80</td>
<td>3.20</td>
<td>3.20-3.80</td>
</tr>
<tr>
<td>Geraniol</td>
<td>3.00</td>
<td>3.20-3.40</td>
<td>3.20</td>
<td>3.20-3.40</td>
<td>3.20</td>
<td>3.20-3.40</td>
</tr>
<tr>
<td>Menthol</td>
<td>3.20</td>
<td>3.80-4.20</td>
<td>3.80</td>
<td>3.80-4.20</td>
<td>3.80</td>
<td>3.80-4.20</td>
</tr>
<tr>
<td>α-Terpinol</td>
<td>4.00</td>
<td>4.50-5.00</td>
<td>4.50</td>
<td>4.50-5.00</td>
<td>4.50</td>
<td>4.50-5.00</td>
</tr>
<tr>
<td>Thymol</td>
<td>0.80</td>
<td>0.80-1.00</td>
<td>1.00</td>
<td>0.80-1.00</td>
<td>1.00</td>
<td>0.80-1.00</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>35.00</td>
<td>50.00-50.00</td>
<td>50.00</td>
<td>50.00-50.00</td>
<td>50.00</td>
<td>50.00-50.00</td>
</tr>
<tr>
<td>Citric acid</td>
<td>25.00</td>
<td>30.00-30.00</td>
<td>30.00</td>
<td>30.00-30.00</td>
<td>30.00</td>
<td>30.00-30.00</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>50.00</td>
<td>60.00-60.00</td>
<td>60.00</td>
<td>60.00-60.00</td>
<td>60.00</td>
<td>60.00-60.00</td>
</tr>
<tr>
<td>Pyrophosphoric acid</td>
<td>16.67</td>
<td>18.76-20.00</td>
<td>18.76</td>
<td>18.76-20.00</td>
<td>18.76</td>
<td>18.76-20.00</td>
</tr>
<tr>
<td>Potassium acetate</td>
<td>815.00</td>
<td>917.00-1,000.00</td>
<td>917.00</td>
<td>917.00-1,000.00</td>
<td>917.00</td>
<td>917.00-1,000.00</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>1,100.00</td>
<td>1,200.00-1,247.80</td>
<td>1,200.00</td>
<td>1,200.00-1,247.80</td>
<td>1,200.00</td>
<td>1,200.00-1,247.80</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>853.00</td>
<td>975.10-1,097.00</td>
<td>975.10</td>
<td>975.10-1,097.00</td>
<td>975.10</td>
<td>975.10-1,097.00</td>
</tr>
</tbody>
</table>

Both methods gave equivalent results. The two different models fitted to growth rates gave equivalent adjustment performances. The modeling approach allowed determining a MIC, even if none of the tested concentration led to total inhibition of growth. Another advantage of the use of modeling to determine MICs is that a confidence is given with the value found. This confidence given to a MIC is, in opposition to the semiquantitative method, dependent on all the tested concentrations. Indeed, the uncertainty in the estimate of fractional areas or growth rate has an influence on the fitted value of MIC. With the semiquantitative method, the range of possible MICs will only depend on the size of the interval between the tested concentrations. Thus, the confidence intervals determined with the modeling approach can be larger or narrower than the range of concentrations of the semiquantitative method, depending on the uncertainty of data sets and the experimental plan.

Whatever the model, the lowest MICs at 24 or 48 h are obtained with thymol and carvacrol. These components with phenolic structures are known to be highly active on foodborne pathogens (14, 22, 28). In the literature, the MICs of thymol and carvacrol are quite the same as in the present study when the assays are done with the dilution method (1 mM for both thymol and carvacrol in L broth at 37°C at 16 h (33); 1 mM for thymol in Luria-Bertani broth at 37°C at 20 h (17); and 1.5 and 1.6 mM for carvacrol, respectively, in nutrient broth at 37°C at 24 h and in tryptone soya broth at 35°C at 36 h (8, 22). It is more difficult to compare the results of the MICs obtained with the diffusion method: MICs can respectively vary for thymol from 1.2 mM (20) to 3 mM (21) and, for carvacrol, from 0.66 mM (23) to 1.5 mM (20). The MICs of trans-cinnamaldehyde, eugenol, geraniol, menthol, and α-terpineol come after. In the literature, the MICs are close to what we found: 3 mM for trans-cinnamaldehyde (17), 3 mM for eugenol (22, 33), 3.2 mM for geraniol (22), 3.2 mM for menthol (21), and 6.5 mM for α-terpineol (22).
acid efficiency, the MIC of pyropolyphosphoric acid is the lowest before citric acid, acetic acid, and lactic acid. In the study of Subramanian and Marth (44), the growth of Salmonella Typhimurium was also more inhibited by citric acid than by lactic acid. The MICs of citric, acetic, and lactic acids for Salmonella Enteritidis have been reported to be close to our values: 35 mM for citric and acetic acids and 57.5 mM for lactic acid (42).

The preservation of food is generally achieved by a combination of hurdles. The hurdle technology concept combines several mild preservation factors (29). In this case, it seems important to check if the level of a factor is sufficiently high to have an effect on the considered microorganism. Thus, in a quantitative approach to hurdle technology, knowledge of MICs is essential. Moreover, NICs are of great importance, as we showed that the difference between the NICs and MICs of Salmonella Typhimurium for several antimicrobials (carvacrol, thymol, and lactic acid) was very small. For these three antimicrobials, a small error in the adjustment of a concentration of a food product can lead to the wrong side of the growth–no-growth limit. With the semiquantitative method, the NIC cannot be estimated, and thus, the LP_{fa} or LP_{fa} model presents a clear advantage. For the SR_{fa} model, the NIC is not available, but it provides information on the closeness of MIC and NIC. A value of $\beta$ close to zero indicates that the antimicrobial

FIGURE 1. (A) Optical density growth curve of Salmonella Typhimurium in brain heart infusion broth at 37°C for concentrations of α-terpineol consisted of between 0 and 4.5 mmol·L⁻¹. (B) LP_{fa} model (equation 5) fitted to fractional area data. (C) SR_{fa} model (equation 7) fitted to growth rate data. (D) LP_{fa} model (equation 8) fitted to growth rate data.

FIGURE 2. (A) Optical density growth curves of Salmonella Typhimurium in brain heart infusion broth at 37°C for concentrations of potassium acetate consisted of between 0 and 1.1 mol·L⁻¹. (B) LP_{fa} model (equation 5) fitted to fractional area data. (C) SR_{fa} model (equation 7) fitted to growth rate data. (D) LP_{fa} model (equation 8) fitted to growth rate data.
either does not have any effect on the microorganism or completely inhibits its growth (all-or-nothing effect).

The MIC is usually defined as the lowest concentration that completely inhibits bacterial growth, but experiment duration varies considerably: 16 h (33), 20 h (17), 36 h (22), 48 h (31, 45), or 72 h (32). We compared MICs at 24 and 72 h, and we observed for 72-h experiments that MICs were significantly higher than for 24-h experiments for organic acids, salts, and some aromatic compounds, whatever the modeling approach. This is in agreement with the results of Lambert and Pearson (27). They observed for Staphylococcus aureus an increase in the MIC with the duration of the experiment. In this manner, a too-short duration of experiment could lead to an underestimated MIC. An extended duration could lead to higher values. A drawback of longer durations appeared in this study with fa data sets, as NICs were affected. Indeed, the areas under OD curves for 72-h experiments hid the small effects of antimicrobials for subinhibitory concentrations. Contrary to fa, growth rates are by definition not affected by the length of the stationary phase, and then growth rates should be preferred when the objective is to estimate NICs.

Several authors have shown that the concentration or the level of an environmental factor necessary to prevent the growth of a microorganism decreases with the size of the inoculum (24, 25). As the size of the inoculum in this study is rather high compared with the levels encountered in food products, the estimated MICs provide conservative predictions in food. Nevertheless, before their use for simulating Salmonella Typhimurium growth or growth boundaries in foods, the MICs have to be validated with data obtained from food. Indeed, it appears essential to study the performance of predictive models in food in order to specify to the users the confidence they can have in the predictions.

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