Modelling the Growth of *Listeria monocytogenes* on Fresh-Cut Cucumbers at Various Storage Temperatures

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Abstract: The primary objective of this study was to investigate the behavior of *Listeria monocytogenes* (*L. monocytogenes*) on fresh-cut cucumbers. Fresh-cut cucumber samples were inoculated with a mixture of six strains of *L. monocytogenes*. The inoculated samples were stored at 5, 10, 15, 20, 25, 30, and 35 °C. The results demonstrated that *L. monocytogenes* was able to grow on fresh-cut cucumbers at all the evaluated temperatures, although its growth decreased but was not inhibited at 5 °C. An extreme storage temperature of 35 °C considerably reduced the lag time. *L. monocytogenes* growth on fresh-cut cucumbers was controlled for several days by storage at a low temperature, mainly at 5 °C. Thus, this product should only be stored at low temperatures. The growth process was fitted by the Baranyi model, with the specific growth rates equally well-fitted to the Ratkowsky square-root model. The R-square and mean square error values for the corresponding Ratkowsky square-root models were 0.97 (R² > 0.95) and 0.02, respectively. The Baranyi and Ratkowsky square-root models exhibited good relevancy. The predictive models developed in this study can be used to estimate the risk assessment of *L. monocytogenes* on fresh-cut cucumber.

Keywords: *Listeria monocytogenes*; predictive microbiology; growth modelling; risk assessment

1. Introduction

The Cucurbitaceae plant family consists of several members, including squash, pumpkins, melons, and cucumbers, which are all economically important agricultural products [1]. Among these plants, cucumber is an important vegetable crop that is widely cultivated and has a good flavour and low caloric content [2]. In 2020, the total cucumber production was approximately 72.83 million tons in China and account for 80% of the global output [3]. Fresh-cut cucumbers are one of the major types of food used in ready-to-eat food, salad mixes, cold dishes, and cooked vegetable mixtures [4,5]. The safety of fresh-cut cucumber is becoming a serious problem, as fresh-cut vegetables generally contain vitamins and other nutrients for pathogen growth, and food poisoning can occur because of careless treatment during processing, storage, and distribution [6]. Outbreaks of foodborne illnesses linked to fruits and vegetables have resulted in increased concern over the safety of ready-to-eat vegetables [7]. As ready-to-eat vegetables have become increasingly popular, it has been suspected that fresh-cut fruits and vegetables may be vehicles for pathogenic bacteria.

*Listeria monocytogenes* is a Gram-positive, nonspore-forming bacillus that has been the cause of many listeriosis outbreaks with high fatality rates. It is a psychrotrophic microorganism that can grow at refrigeration temperatures (4–15 °C). This pathogen is widely present in the environment, particularly in soil and decaying vegetation. *L. monocytogenes* was isolated from a variety of raw vegetables and fruits. Reports have shown that a number of outbreaks are associated with the consumption of lettuce contaminated with *L. monocytogenes* [8]. In 2010, diced celery, used as an ingredient in chicken salad in a
hospital, was the cause of a listeriosis outbreak in Texas that led to ten confirmed cases and five deaths in immunocompromised, hospitalised patients [9]. In 2011, a listeriosis outbreak caused by contaminated cantaloupe emphasised the need to understand how *L. monocytogenes* grows on fruits and vegetables. According to a survey, cucumbers infected with *Escherichia coli* O157:H7 have also caused the deaths of at least ten people, leading to the removal of cucumbers from markets in Germany, Austria, and the Czech Republic by some retailers [10]. Recent studies have also demonstrated the possible growth of *L. monocytogenes* on fresh-cut cantaloupe, watermelon, papaya, pineapple, lettuce, carrots, beetroot, and mini spinach, although the results were highly dependent on the produce type and the storage temperature [11–13]. A study revealed that *L. monocytogenes* could grow in fresh-cut melon at 8 °C [14]. In addition, *L. monocytogenes* can also grow in fresh-cut red cabbage and turnips at different storage temperatures [15,16]. Therefore, this pathogen should be considered a potential threat to the safety of fresh-cut vegetables.

A quantitative risk assessment can provide estimates of how remedial actions can lower the listeriosis risk [17]. Risk analysis is a technique that has been considered in relation to foodborne illnesses [18]. Presently, the majority of studies investigating food poisoning and foodborne diseases have concentrated on the quantitative risk assessment, with mathematical tools of predictive microbiology being applied for risk assessment [19]. Predictive models can be used to estimate variations in pathogen density at any given time and temperature in processing operations during storage and distribution. Estimating changes in microbial numbers in a production chain can be useful in the decision-making of critical control points (CCPs) in the HACCP and in risk assessment. Growth models for *L. monocytogenes* were first described by Buchanan and Phillips (1990) [20]. Survival models for *Listeria* spp. in aerobic and anaerobic environments were subsequently developed for the Pathogen Modelling Program [21]. These models include the Gompertz model, logistic model, and Baranyi model. Predictive modelling was performed using a Baranyi function to estimate pathogen growth kinetic parameters (growth rate, lag phase duration, and maximum population). In this study, *L. monocytogenes* growth on fresh green coconut was successfully modelled using the Baranyi model [22], and, then, a secondary model based on the Ratkowsky model was derived to predict growth parameters (maximum growth rate, lag time, and maximum population) as a function of temperature (5–35 °C) [23]. The growth of *Listeria monocytogenes* on fresh-cut mixed salad at different pre-growth temperatures (4, 21, and 37 °C) was fit using the Baranyi–Roberts mode [24]. The growth kinetics of *Salmonella* on three types of mangoes (‘Ataulfo’, ‘Kent’, and ‘Tommy Atkins’) was also evaluated with Linear and Baranyi models at 12, 20, and 30 °C [25]. Research continues to improve the scope, precision, and applicability of these models, which can be used to predict the effects of combinations of preservative factors on growth in many foods and can be particularly useful in ensuring the safety of new or modified foods.

Cucumbers are common, widely consumed vegetables that are regularly purchased from supermarkets and farmers’ markets. This study was performed under similar storage conditions to evaluate the survival of *L. monocytogenes* in fresh-cut cucumbers, which are widely consumed by people worldwide. The objectives of this research were to investigate and develop a growth model of *L. monocytogenes* in fresh-cut cucumbers and to describe its behavior via predictive mathematical growth models. The results obtained during the course of this study will be helpful for regulatory agencies conducting risk analyses of fresh-cut cucumbers contaminated with *L. monocytogenes*.

2. Materials and Methods
2.1. Bacterial Culture and Inoculation Preparation

*L. monocytogenes* CICC 21633, *L. monocytogenes* CICC 21634, *L. monocytogenes* CICC 21635, *L. monocytogenes* CICC 21662, and *L. monocytogenes* CICC 21583 were obtained from the China Center of Industrial Culture Collection (Beijing, China). *L. monocytogenes* GIM 1.229 were obtained from the Guangdong Microbiology Culture Center (Guangzhou, China). The six strains of *L. monocytogenes* were individually cultivated on trypticase soy-
yeast extract broth (TSB-YE, Hopebio, Qingdao, China) at 37 °C for 24 h. The bacterial suspensions were individually inoculated on trypticase soy-yeast extract agar (TSA-YE, Hopebio, Qingdao, China) for recovery. The plates were incubated at 37 °C for 24 h. A single colony from the TSA-YE was inoculated in a test tube with 10 mL of TSB-YE. After incubation for 12 h at 37 °C, the cell suspensions were transferred to another test tube with 90 mL of TSB-YE and stored at 37 °C for 24 h without agitation. The cell was washed twice by removing the supernatant and suspending the cell pellet in 10 mL of 0.1% peptone. The six strains of *L. monocytogenes* were mixed and combined in equal volumes (5 mL of each strain). The cocktails containing all strains were enumerated on TSA-YE to confirm the cell concentrations. The concentrations of the inoculums were diluted with 0.1% peptone water to approximately 3–4 log CFU/mL.

2.2. Fresh-Cut Cucumber Preparation

Fresh cucumbers were purchased from a local supermarket (Newmart, Dalian, China). Cucumbers with visible damage and physical defects were discarded. Cucumbers with commercial ripening stage (90% green colouration degree) were chosen to be transferred to the laboratory. The cucumbers were first washed until clean using running tap water, and then washed again with sterile distilled water. Each cucumber plant was scrubbed with sterile cotton again before being cut. The cucumbers were then cut into 0.5 cm × 0.5 cm × 0.5 cm cubed pieces using a sterile knife and cutting board in a sterile room.

2.3. Physical and Chemical Analyses

The fresh-cut cucumbers were subjected to the following chemical and physicochemical analyses. The samples were homogenised, and the pH was measured using a calibrated pH meter (Mettler Toledo FE20, Zurich, Switzerland). Brix (%Brix) was determined using a Digital Hand-held “Pocket” Refractometer (Pocket PAL-1, ATAGO Co., Tokyo, Japan). The titratable acidity was determined using the method described in GB/T 12456-2008 [26]. Water activity (Aw) was measured using an Aqualab Pawkit meter (Aqualab Pawkit, Decagon Inc., Pullman, WA, USA). All analyses were performed in triplicate, and the average values are reported.

2.4. Inoculation and Incubation

Fresh-cut cucumber samples (10 g each) were inoculated with 1 mL of the *L. monocytogenes* cocktail containing six strains at a final concentration of 3–4 log CFU/mL, and uninoculated fresh-cut cucumbers were cultured as the control samples. The fresh-cut cucumber samples (inoculated and uninoculated) were air-dried within a biosafety cabinet (Haier, Qingdao, China) for 1 h. After air-drying, each sample was separately transferred to a 400 mL sterile stomacher bag (Interscience, Saint Nom la Bretèche, France) and stored at different temperatures, including 5, 10, 15, 20, 25, 30, and 35 °C, until the bacterial population reached the stationary phase. Samples were usually analysed at 12–24 h intervals at 5, 10, and 15 °C. Shorter time intervals (3, 6, and 9 h) were selected for temperatures above 15 °C. Three of the inoculated samples were removed and analysed in triplicate at each interval at all temperatures.

2.5. Determination of *L. monocytogenes*

The removed samples were combined with 90 mL of 0.1% peptone water and homogenised in a stomacher at high speed for 1 min. Serial dilutions of each sample were made in 0.1% peptone water, and the samples were surface-plated (0.1 mL) in triplicate onto PALCAM agar media (Hopebio, Qingdao, China). Control samples were plated onto TSA supplemented with PALCAM agar to determine the background microbiology. After incubation at 37 °C, colonies were counted using a colony counter (Acolyte, London, UK). The bacterial populations are expressed as the log CFU/g. The *L. monocytogenes* colonies present black aureole on the plate.
2.6. Model Validation

The Baranyi model equation was used to fit the experimentally obtained data [22]. The reparameterised models are described by Equations (1)–(5). It is calculated based on estimates of three kinetic parameters, including the lag time (lag, expressed in hours in this paper), maximum growth rate (Gr\(_{\text{max}}\), expressed in log CFU/h), and maximum cell number (\(y_{\text{max}}\)). A fitting method for repeated measures was applied to take into account the results from the different replicates analysed at each time interval.

\[
Y_t = y_0 + \mu_{\text{max}} A(t) - \ln 1 + e^{\mu_{\text{max}} A(t)} - 1 \quad (1)
\]

\[
A(t) = t + \frac{1}{\mu_{\text{max}}} \ln \left( e^{-\mu_{\text{max}} t} + e^{-h_0 - e^{-\mu_{\text{max}} t}} \right) \quad (2)
\]

In Equations (1) and (2), \(y_0\) and \(y_{\text{max}}\) are the initial and maximum cell concentrations in ln (CFU/g) units, respectively. \(\mu_{\text{max}}\) is the maximum growth rate. \(h_0\) is a dimensionless parameter quantifying the initial physiological state of the cells. From that, the lag time \(\lambda(h)\) can be calculated as \(h_0/\mu_{\text{max}}\). The \(\mu_{\text{max}}\), which was obtained from the primary model, was fitted by linear regression to the square-root model (3) of the Ratkowsky model (1982) [27].

\[
\sqrt{\mu_{\text{max}}} = b(T - T_{\text{min}}) \quad (3)
\]

In this model, \(\sqrt{\mu_{\text{max}}}\) is the square root of the maximum growth rate, where \(b\) is the slope of the regression line, \(T\) is the temperature (°C), and \(T_{\text{min}}\) is the conceptual minimum growth temperature (°C). The goodness of fit was evaluated by applying the coefficient of determination (R\(^2\)) and standard error (SE). For validation purposes, model predictions were compared with growth data from the literature using the bias factor (\(B_f\)) and accuracy factor (\(A_f\)) described by Ross (1996), which can be calculated using the following equations [28]:

\[
B_f = 10^{\Sigma \log(G_{\text{observed}}/G_{\text{predicted}})/n} \quad (4)
\]

\[
A_f = 10^{\Sigma \log(G_{\text{observed}}/G_{\text{predicted}})/n} \quad (5)
\]

where \(G_{\text{observed}}\) and \(G_{\text{predicted}}\) indicate the \(\mu_{\text{max}}\) values taken from the literature and the \(\mu_{\text{max}}\) values predicted by the model, respectively, and \(n\) is the number of data points.

2.7. Statistical Analysis

All experiments were conducted in triplicate. The experimental factors were simultaneously compared for the pathogen, where the mean log CFU/g was compared at different storage temperatures and storage times. The data for the fresh-cut cucumbers at each time interval were evaluated for significant differences (\(p \leq 0.05\)) using Duncan’s multiple-range test in SPSS software (SPSS 12.0, Chicago, IL, USA).

3. Results

3.1. Physicochemical Characteristics of the Fresh-Cut Cucumbers

The physicochemical characteristics of the fresh-cut cucumbers used in this study are reported in Table 1. The pH of the sample was 5.7, and the Aw was 0.995. The physical and chemical results indicated that cucumbers are an adequate substrate for the growth of micro-organisms, with an average pH above the minimal growth conditions for pathogens (pH > 4.2), and cannot be considered inhibitory. The water activity measurements indicate that fresh-cut cucumbers are vegetables that maintain high water activity.
Table 1. Physicochemical characteristics of fresh-cut cucumbers.

<table>
<thead>
<tr>
<th>Physicochemical Parameters</th>
<th>Value ± S.D. ±</th>
<th>±</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.7 ± 0.02 ±</td>
<td>±</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.995 ± 0.03 ±</td>
<td>±</td>
</tr>
<tr>
<td>Solid soluble (°Brix)</td>
<td>2.1 ± 0.5 ±</td>
<td>±</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>0.58 ± 0.02 ±</td>
<td>±</td>
</tr>
</tbody>
</table>

± standard deviation (SD) (n = 3).

3.2. Primary Model of *L. monocytogenes* Infection on Fresh-Cut Cucumbers

As shown in Figure 1, *L. monocytogenes* was able to grow on fresh-cut cucumbers at different temperatures (5, 10, 15, 20, 25, 30, and 35 °C). The growth of *L. monocytogenes* was observed in terms of population changes at various temperatures. The population of *L. monocytogenes* began to grow from the lag phase and exponential phase to the stationary phase at 5–35 °C. The initial population density was between 3.04 and 3.09 log CFU/g, showing that it was not significantly different. The maximum population density was between 5.4 and 6.4 log CFU/g among the stationary phases at 5–35 °C. The population of *L. monocytogenes* can be well-described using Baranyi models. The growth parameters of *L. monocytogenes* are shown in Table 2. The determination coefficient (R²) values for the models under different storage temperatures ranged from 0.96–0.99, indicating a very good fit of the model to the data. Increasing the storage temperature of fresh-cut cucumbers increased the *L. monocytogenes* growth rate approximately 24-fold from 5 °C to 35 °C. The lag time of *L. monocytogenes* was 82 h at 5 °C, 45.81 h at 10 °C, and 4.72 h at 15 °C. At high temperatures, the pathogen reached its stationary phase at an earlier time. No lag time was observed at 20 °C, 25 °C, 30 °C, and 35 °C. Temperatures below 15 °C appeared to significantly impact the lag time. This indicates that low temperatures effectively control the growth of *L. monocytogenes* in fresh-cut cucumbers. In the stationary phase, the maximum populations of *L. monocytogenes* were 5.43 at 5 °C, 5.94 at 10 °C, 6.29 at 15 °C, 6.13 at 20 °C, 6.22 at 25 °C, 6.10 at 30 °C, and 6.38 log CFU/g at 35 °C.

Table 2. Growth parameters of *Listeria monocytogenes* at different temperatures based on the Baranyi model.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>R²</th>
<th>Maximum Growth Rate (log CFU/g)/h</th>
<th>Lag Time (h)</th>
<th>Maximum Population (log CFU/g)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.9957</td>
<td>0.0065</td>
<td>82.0073</td>
<td>5.4310</td>
<td>0.0668</td>
</tr>
<tr>
<td>10</td>
<td>0.9861</td>
<td>0.0300</td>
<td>45.8138</td>
<td>5.9386</td>
<td>0.1303</td>
</tr>
<tr>
<td>15</td>
<td>0.9919</td>
<td>0.0525</td>
<td>4.7227</td>
<td>6.2910</td>
<td>0.1075</td>
</tr>
<tr>
<td>20</td>
<td>0.9872</td>
<td>0.0771</td>
<td>Na</td>
<td>6.1303</td>
<td>0.1447</td>
</tr>
<tr>
<td>25</td>
<td>0.9613</td>
<td>0.1040</td>
<td>Na</td>
<td>6.2184</td>
<td>0.1853</td>
</tr>
<tr>
<td>30</td>
<td>0.9879</td>
<td>0.1212</td>
<td>Na</td>
<td>6.0974</td>
<td>0.1304</td>
</tr>
<tr>
<td>35</td>
<td>0.9872</td>
<td>0.1571</td>
<td>Na</td>
<td>6.3773</td>
<td>0.1450</td>
</tr>
</tbody>
</table>

Na = no result.
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(A) (B) (C) (D) (E) (F) (G)

Figure 1. Growth of L. monocytogenes on fresh-cut cucumbers at different temperatures: (A) 5 °C, (B) 10 °C, (C) 15 °C, (D) 20 °C, (E) 25 °C, (F) 30 °C, and (G) 35 °C. The black solid rhombuses represent the observed data points; the red thin solid curves were obtained using the Baranyi model.
3.3. The Secondary Models of L. monocytogenes on Fresh-Cut Cucumbers

As demonstrated in Figure 2, the secondary models were suitable for describing the effect of temperature on the specific growth rates of L. monocytogenes on fresh-cut cucumbers. The parameters of the primary model could be applied to the second model under fluctuating temperature conditions. The parameters of the secondary models were SSE (sum of squares for error) = 0.0020, $R^2 = 0.9709$, adjusted $R^2 = 0.9651$, and RMSE (root mean square error) = 0.0200. The Ratkowsky square-root model yielded good results ($R^2 = 0.97$). Regression lines of the secondary models shown were drawn based on the mean growth kinetics parameters of L. monocytogenes. The obtained $B_f$ and $A_f$ were 1.02 and 1.19, respectively. These values confirmed that the developed model could accurately predict L. monocytogenes growth in the range of 5–35 °C, even though the $B_f$ value (>1) indicated that the model slightly overestimated growth based on the growth studies used.

![Figure 2. The relationship between the Ratkowsky square root of the growth rate and temperature of L. monocytogenes.](image)

4. Discussion

Sugar and soluble solids provide carbohydrates for microbial growth, and, together with these characteristics, they make fresh-cut cucumbers highly prone to the survival and growth of pathogens. Many researchers have reported that L. monocytogenes can grow at low temperatures at high pH values and with high concentrations of salts; hence, it can survive and multiply in a great variety of food products [29]. The growth of L. monocytogenes on fresh-cut cucumbers stored at 5 °C did not occur until 82 h, and at 10 °C for 45.8 h in this study. Another report showed that the lag time was 45.5 h at 5 °C and 39.1 h at 10 °C for lettuce [30]. The maximum L. monocytogenes cell concentration was 5.4 log CFU/g after 700 h at 5 °C, 5.9 log CFU/g after 250 h at 10 °C, and 6.22 log CFU/g after 240 h 25 °C in this study. Some reports have also shown a similar result in terms of the maximum population on vegetables. In particular, the population of L. monocytogenes and Salmonella can reach 5.6 CFU/g and 3.9 CFU/g on fresh-cut cucumber at 23 °C after 4 days [31]. The L. monocytogenes population increased from approximately 3.5 log CFU/g to 5.7 log CFU/g on conventional lettuce stored at 10 °C [32]. At 7 °C, the relative increase in the number of L. monocytogenes ranged from 2.4 in pepper to 3.2 log CFU/g in courgette. The calculated maximum increase exceeded 3.4 log CFU/g in Galia melon, potato, and pasta products [33]. The lag time decreased from 82 h to 4.72 h as the temperature increased from 5–15 °C in this study. These results suggest that the storage of fresh-cut cucumbers at temperatures lower than 10 °C reduces the risk of pathogenic infections during cucumber shelf life. Therefore, these data emphasise the importance of a low temperature during manufacturing processing steps for microbiological safety, hygienic handling, and distribution process management [34]. However, at supermarkets
and restaurants, improper storage temperatures or difficulty maintaining the product below 10 °C for 48 h may considerably increase the potential hazards of fresh-cut cucumbers. On the other hand, although the effect of temperatures on the behavior of *L. monocytogenes* in cucumber was evaluated, the quality of the cucumber will decrease if it is stored for a few days at temperatures lower than 10 °C, due to the physiological disorder cold damage or chilling injury [35].

Temperature has been shown to be a major environmental factor affecting microbial growth kinetics in foods [36]. Some reports have shown that the growth of the pathogen can be well-described by primary models, such as the Baranyi model, the Modified Gompertz model, and the logistic model [37]. The determination coefficient (R^2) values for the Baranyi models under different storage temperatures ranged from 0.96–0.99, indicating a very good fit of the model to the data. The Baranyi model predicted that the growth rate would increase gradually and the lag time would decrease gradually as the storage temperature increased (10, 15, 20, 25, 30, and 35 °C). Other reports have also found an increasing growth rate of *L. monocytogenes* on fresh-cut cantaloupe, watermelon, and honeydew as the storage temperature increased from 4 to 25 °C [38,39]. Some reports have shown similar results where the growth rate was greater when using the Baranyi model [40–42]. The value of the maximum growth rate was successfully determined using a linear relationship between the square root of the parameter and temperature, as described by Ratkowsky et al. [27]. The RMSE is a measure of the precision of a predictive model and accounts for the differences between the predicted and observed values. The lower the RMSE value for a model is, the more precisely the data are described [28]. Moreover, the RMSE values (0.02) obtained in this study are very close to those found in models reported in the literature, which indicates that the models built here are generally suitable for modelling the growth of *L. monocytogenes* on fresh-cut cucumbers. Therefore, the models developed in this study will provide a reliable prediction of *L. monocytogenes* on fresh-cut cucumbers, which will allow the prediction of bacterial growth on fresh-cut cucumbers during distribution under real temperature conditions that are likely to fluctuate over time.

5. Conclusions

Fresh-cut cucumbers possess intrinsic characteristics (pH, Aw, Brix, etc.) that permit the growth of micro-organisms, and storage at refrigeration temperatures below 10 °C can act as risk factors for the growth of micro-organisms such as *L. monocytogenes*. Consequently, action should be taken to control the cucumber storage temperature since this is the only factor among those studied that has been shown to significantly influence the growth of *L. monocytogenes* in this vegetable. The observations made in this work recommend the conservation of fresh-cut cucumbers at temperatures below 5 °C to guarantee product safety. In addition, predictive modelling appears to be a useful tool for determining *L. monocytogenes* growth rates in these products.

**Author Contributions:** Conceptualisation, K.F. and W.H.; methodology, K.F.; software, S.; validation, K.F.; investigation, K.F.; resources, W.H.; data curation, S.; writing—original draft preparation K.F.; writing—review and editing, S. and J.M.; project administration, K.F. and W.H.; funding acquisition, S. and K.F. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors on request.

**Conflicts of Interest:** The authors declare no conflicts of interest.
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