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28 **Food Microbiology**

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30 # Modelling the growth kinetics of *Listeria monocytogenes* in pasta salads at different storage

31 temperatures and packaging conditions

32

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51 **ABSTRACT**

52 The aim of this study was to model *Listeria monocytogenes* growth kinetics in ready to eat pasta salads,
53 containing fresh and cooked ingredients. With this aim, laboratory prepared salads, representing two
54 formulations of commercial pasta salads, were spiked with *L. monocytogenes* and tested under categorised
55 packaging and storage temperature conditions. *L. monocytogenes* enumeration results collected in 15 different
56 laboratory prepared salad datasets were analysed with primary and secondary models. The models showing the
57 best fit to describe *L. monocytogenes* growth kinetics in the laboratory prepared salads were then validated
58 within commercial pasta salads. Baranyi no-lag was the best primary model fitting datasets collected at 12°C,
59 whereas the exponential model gave the best results for datasets collected at 4°C. The maximum microbial
60 specific growth rate (μ_{max}) mean values obtained at 4 and 12 °C for salads packaged under air packaging
61 conditions were 0.008±0.003 and 0.036±0.006 $\log_{10}$ (cfu/g) h^{-1}, respectively. At the same temperatures, the μ_{max}
62 mean values obtained under modified atmosphere were 0.005±0.005 and 0.026±0.005 $\log_{10}$ (cfu/g) h^{-1},
63 respectively. The Gamma secondary model was able to predict the growth kinetics of *L. monocytogenes* at both
64 temperatures and packaging conditions and the μ_{max} at the optimum temperature and the optimum pH (μ_{opt})
65 estimated by the model corresponded to 0.247±0.009 $\log_{10}$ (cfu/g) h^{-1}. Baranyi model without lag phase was used
66 to generate growth kinetics under different scenarios. In the comparison of the predicted $\log_{10}$ concentrations
67 respect to the observed ones the residues rarely exceeded 1 Log_{10} cfu/g. The selected models can be applied to
68 describe the growth kinetics of *L. monocytogenes* in other pasta salads with comparable pH, shelf life and storage
69 conditions.

70

71 **Key words**: *Listeria monocytogenes*, maximum growth rate, pasta salads, storage temperatures, packaging
72 conditions, primary and secondary models.

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76 **1. Introduction**

77 Over the past years, consumer demand for convenience and fresh foods with minimally preservatives
78 and low thermal processing has resulted in a fast growth of chilled ready-to-eat (RTE) meal market worldwide
79 (Quested et al., 2010). Many refrigerated RTE foods are treated with heat processes, with maximum
80 temperatures reaching 70-95°C, packaged under vacuum or modified atmosphere (usually anaerobic) and then
81 stored refrigerated (Peck, 2006). The combination of heat treatment and refrigerated anaerobic storage is
82 designed to prevent pathogen and spoilage organisms' growth. However, few data are available on impact of
83 ingredient and bacteria interactions in RTE products, like pasta salads, during storage at different conditions on
84 presence and growth of foodborne pathogens. Bovo et al. (2015) showed that *Salmonella enterica* populations
85 in salads consisting of lettuce alone and in mixed ingredient salads, formulated with shredded Cheddar cheese
86 and cooked chicken, remained essentially unchanged during 3 days at 6°C. However, storage of mixed ingredient
87 salads at 14°C led to significant growth of *S. enterica* ($P < 0.05$, approximately 4.0 $\log_{10}$ cfu/g over 3 days).

88 In RTE products, including pasta salads, fresh ingredients, like vegetables and cheese, retain most of their
89 endogenous microflora after minimal processing, but, at the same time, has potential for contamination with
90 *Listeria monocytogenes*, due to extensive handling during processing or cross-contamination from the processing
91 environment. *L. monocytogenes* is of particular concern in RTE products because of its ability to grow in presence
92 or absence of oxygen, as well as at refrigeration temperatures (WHO, 2004). Therefore, it can multiply during
93 RTE product shelf-life, increasing the risk to reach numbers high enough to cause human illnesses. In 2016, the
94 EU notification rate for listeriosis has been 0.47 cases per 100,000 population, corresponding to a 9.3% increase
95 compared with 2015 (EFSA, 2017). Moreover, the EU listeriosis case fatality has been 16.2% among the 1,524
96 confirmed cases with known outcome (EFSA, 2017). There has been a statistically significant increasing trend of
97 confirmed listeriosis cases in the EU/EEA during the overall period 2008–2016, as well as during the period from
98 2012 to 2016.

99 In order to demonstrate that *L. monocytogenes* will not exceed 100 cfu/g during the shelf life, food
100 business operators (FBOs) and regulatory authorities can use predictive models, often included in user-friendly

101 software, like ComBase and Sym'Previus (Tenenhaus-Aziza and Ellouze 2015; Cornu et al., 2011). The application
102 of microbial predictive models in food safety research have been reviewed comprehensively (Pérez-Rodríguez
103 and Valero, 2013; Peleg and Corradini 2011; Li et al., 2007) but few data refer to the application of predictive
104 models to composite foods containing raw and cooked ingredients (Sahu et al., 2017; Lokerse et al., 2016; Hwang
105 and Tamplin, 2005).

106 Microbial models can be judged according to different criteria, such as number of parameters addressed,
107 their biological meaningful as well as model ability to cover different microbial growth phases. According to
108 Whiting and Buchanan (1993), mathematical models used in predictive microbiology could be generally
109 categorised into three types, including primary, secondary, and tertiary models. The primary models are the
110 mathematical models that describe the change in the microbial population as a function of time, under a single
111 set of conditions (Baranyi and Roberts, 1995; Buchanan et al., 1997; Gibson et al., 1987; Huang, 2008; Zwietering
112 et al., 1990). The secondary models are the models describing the responses of one or more parameters of a
113 primary model to one or more environmental conditions (e.g., temperature, pH, etc.) (Buchanan et al., 1989;
114 Huang et al., 2011a, 2011b; Ratkowsky et al., 1983; Rosso et al., 1993). The tertiary models are computer
115 software or expert systems that utilize the primary and secondary models to predict the fate of microorganisms
116 in foods (Whiting and Buchanan, 1993). Predictive models can be included in microbial food safety risk
117 assessments. Growth model parameter estimation for risk assessment purposes should be able to separately
118 evaluate the variability and uncertainty of the estimates. Pouillot et al., 2003 proposed a Bayesian procedure for
119 growth parameter estimation, which makes it possible to separate these two components by means of
120 hyperparameters. In a Bayesian framework, model parameters are random variables, firstly described by a prior
121 distribution (Lee P. M., 2012). These prior distributions are uninformative or vague when information about
122 parameters is absent or poor. However, informative prior distributions may be obtained with expert knowledge
123 or data from previous studies. From a combination of prior distributions and data, posterior distributions over
124 all parameters are computed using Bayes' theorem. In hierarchical Bayesian models, these parameters are
125 themselves defined by distributions that have proper "hyperparameters". In these models hyperprior

126 distributions are assigned to hyperparameters. Through the prior and hyperprior distributions, hierarchical
127 Bayesian models account for both variability and uncertainty of the parameters of interest (Lee P. M., 2012).
128 Since *L. monocytogenes* can be isolated and can grow in RTE mixed products kept at refrigeration
129 temperatures, the aim of this study was to model the growth kinetic of *Listeria monocytogenes* in ready to eat
130 pasta salads during storage under different temperatures (i.e., 4 and 12°C) and packaging conditions (air vs
131 modified atmosphere). With this aim, laboratory prepared salads, representing two different formulations of
132 pasta salads sold in Italy and Norway, were spiked with *L. monocytogenes* and tested under categorised
133 temperature and packaging conditions. The models showing the best fit for the data collected in the laboratory
134 prepared salads were validated using results obtained testing commercial pasta salads, stored at the same
135 temperatures and packaging conditions, as well as at 6°C. Furthermore, lactic acid bacteria were quantified in
136 both laboratory prepared salads and commercial products in order to verify the occurrence of what is described
137 as Jameson effect. This effect relies on the assumption that high concentrations of Lactic Acid Bacteria (LAB)
138 reduce the growth of *L. monocytogenes* in the same way that LAB reduce their own growth when their
139 concentration approaches the maximum population density (Gimenez and Dalgaard, 2004).
140

141 **2. Materials and methods**

142 *2.1 Laboratory prepared salads and commercial pasta salads investigated*

143 Two formulations of full meal pasta salads, with meat, cheese and vegetables were investigated in this
144 study (Table 1). For each formulation, laboratory prepared salads (MS) and commercial pasta salads were used.
145 The laboratory prepared salads reflected commercial salad formulations and packaging conditions but were
146 prepared in the laboratory, using local ingredients or ingredients provided by the local producers of commercial
147 pasta salads. The laboratory prepared salads were used in the trials to select the best models describing the
148 growth kinetics of *L. monoctogenes* under categorized conditions. The commercial pasta salads were real
149 products sold in Italy and Norway and were used in the model validation trials.

150 For the preparation of the laboratory prepared salads, the authors tried to mimic the same operations
151 observed in the investigated commercial companies in both Italy and Norway (Skjerdal et al., 2017). Dried pasta
152 was cooked for 11 minutes and immersed into cold water (3°C) for 10 minutes. Chicken meat was immersed in
153 hot water (80-85°C) until a core temperature of 75 °C was reached. The chicken meat was chopped and the
154 smoked ham cut. Cheeses were grated to diameter maximum 0.5 cm. Radicchio and arugula were washed with
155 running water for 10 minutes, dried on paper and cut in pieces. Bell pepper was cut in pieces of approximately
156 0.5*0.5*3 cm and boiled for 3 minutes before cooling in ice cold water. The sauces, consisting of oil, lemon juice,
157 salt and pepper were mixed separately before mixing carefully with the other ingredients. The laboratory
158 prepared salads were packaged in 25 g portions under air or modified atmosphere (MAP). The applied MAPs
159 reflected the commercial ones and consisted of 50% CO_2 and 50% N_2 (i.e., MAP1) for MS1 and 60% CO_2 and 40%
160 N_2 (i.e., MAP2) for MS2. Samples packaged under air were boxed in stomacher bags, while samples in CO_2
161 enriched atmosphere were placed in plastic trays (Promens art.nr. 5960000011, Ålesund, Norway) sealed with a
162 film of Oriented Polypropylene and Polyvinyl Alchool using a packing machine (Orved VGP, Verona, Italy and
163 Multivac, chamber machine C200, Wohlfertschwenden, Germany). The laboratory prepared MAP salads were
164 packaged in the same plastic trays and sealed with the same films used to pack commercial pasta salads.
165
166 *2.2 L. monocytogenes strains and challenge trials*
167 *2.2.1 Inoculum preparation*
168 Six *L. monocytogenes* strains were selected for the challenge studies (Table 2) and inoculated alone, or
169 in cocktail, to account for variations in pathogen growth and survival. Beside the Scott A reference strain, the
170 other isolates were chosen as they have been found in typical ingredients or production facilities of ready-to-eat
171 foods and because of their rapid growth. Inoculum of *L. monocytogenes* was prepared as described in the
172 European Union Reference Laboratory for *L. monocytogenes* technical document for conducting shelf-life studies
173 on *L. monocytogenes* in ready to eat foods (Beaufort et al., 2014). The same strains were used by Stratakos et
174 al., (2016) for studies in potato salads. Strains for challenge tests were taken from frozen stock cultures and

175 streak onto Brain Heart Infusion (BHI) agar plates (Oxoid, Milan, Italy) incubated for 24-48 h at 37°C. At the end
176 of the incubation period, cells from a single colony on BHI agar were inoculated into 10 ml of BHI broth (Oxoid,
177 Milan, Italy) incubated at 37±1°C for 24±2 hours to obtain stationary cells at approximately 1x10^9 cells/ml. A
178 second subculture was prepared by adding 100 µl of the first subculture to 10 ml of fresh BHI broth, incubated
179 at 7±1°C for 7 days to obtain cold adaptation of the bacteria and a cell density of 10^9 cfu/ml. Subcultures were
180 prepared for each strain separately. For preparation of inoculum cocktails, 2 ml of vortexed subculture of each
181 strain were mixed in a tube and diluted in five tenfold successive steps in sterile physiological saline (0.9% w/v
182 sodium chloride) kept at 7°C water to obtain *L. monocytogenes* concentration of 10^4 cfu/g and minimize
183 carryover of growth medium components from the inoculum. For single strain inoculums, the subcultures were
184 diluted accordingly. The concentration of *L. monocytogenes* in the inoculum was confirmed on
185 Agar *Listeria* according to Ottaviani and Agosti (ALOA) (AEB 150072, AES laboratories, Chemunex, Essex, UK)
186 according to ISO 11290-2.
187
188 *2.2.2 Inoculum of laboratory prepared salads and commercial pasta salads*
189 Both laboratory prepared salads and commercial pasta salads were inoculated as described in the
190 technical document mentioned above (Beaufort et al., 2014). Laboratory prepared salads packed under air were
191 inoculated with 0.64% inoculum, mixed well with a sterile spoon and portioned in 25 gr sample units in stomacher
192 bags. Laboratory prepared salads in modified atmosphere were inoculated with the same amount, packaged and
193 then supplemented with the appropriate gas mixture through a septum (Rubber sheet 20mmx200mm; TO125,
194 Toray Engineering Co. Ltd, Japan) in order to avoid contamination of the equipment. *L. monocytogenes* free
195 controls were added with sterilized physiological saline water in the same ratio and in the same way as the
196 inoculated samples. Commercial salads were transported to the laboratory in 70±10 minutes, under refrigeration
197 conditions (i.e., 2±2°C) and inoculated in the laboratory. Commercial pasta salad 1 was packaged in 250 g trays,
198 whereas commercial pasta salad 2 in 2 kg trays. The 250 g trays were inoculated and then re-packaged under air
199 or MAP1. The commercially packed salads in 2 kg trays (approximately 25*30 cm bottom area) were prepared

200 to three inoculated samples and one control sample, using the following procedure: the salad was inoculated
201 through the top plastic foil by penetrating a septum with syringe in three of the four corners. The needle was
202 circulated in order to spread the inoculum within a circle with 7 cm diameter. In the fourth corner of the tray,
203 water was spread in the same way and used as blank sample. At each sampling point, both inoculated sample
204 and blank samples were analysed to check that blank sample was still *Listeria* free and verify that transfer of
205 *Listeria* between samples in the tray was minimal. After inoculation and packaging, the laboratory prepared
206 salads were incubated at 4 and 12 °C for 10-13 days (Table 3), whereas commercial pasta salads at 4, 6 and 12 °C
207 up to the end of the shelf life (i.e., 12 days for commercial pasta salad 1 and 21 days for commercial pasta salad
208 2) (Table 3). Inoculation levels, storage periods and sampling frequencies were chosen to obtain as many relevant
209 data for modelling as possible.
210
211 *2.3 Sample analysis*
212 Inoculated laboratory prepared salads and commercial pasta salads were tested for *L. monocytogenes*
213 enumeration on ALOA agar plates (AEB 150072, AES Laboratories, Chemunex, Essex, UK) according to ISO
214 method 11290-2. Briefly, 10 grams of sample were homogenized in 90 ml of physiological solutions (0.9% NaCl)
215 and then 1 ml spread on three ALOA agar plates to achieve as minimum enumeration level 1 cfu/10 grams. Higher
216 levels were quantified by spreading 100 µl of tenfold dilutions on ALOA agar plates, incubated at 37±1°C for
217 24±2h. Both inoculated and control samples were also analysed for Lactic Acid bacteria (LAB) enumeration on
218 MRS agar (Oxoid, Milan, Italy) using ISO 15214:1998 and pH quantification using ISO 2917:1999.
219 Data for modelling initial growth were collected by sampling every third hour for 150 hours, using 10^3
220 cfu/g as *L. monocytogenes* inoculum level in order to quantify variability associated to inoculation level and
221 analytical method applied. Data for modelling during the entire storage period were collected using 10^2 cfu/g
222 inoculation level and sampling once a day, or every two days, during the first 8 days and then less frequently
223 during later storage. Each dataset was obtained using either duplicate or triplicate samples (Table 3). Lactic acid
224 bacteria and pH were quantified once a day, or every two days.

225

226 *2.4 Data modelling*

227 *2.4.1 Primary models*

228 Baranyi model, with or without lag and stationary phase (Barany and Roberts, 1994) and the exponential 229 growth model, were applied to calculate the μ_{max} (h^{-1}) of *L. monocytogenes* in laboratory prepared salads and 230 commercial pasta salads under categorized conditions. Moreover, lag and stationary phase were determined. 231 According to Baranyi (Baranyi, 1997), the lag phase (h) is determined by its relation with μ_{max} and the 232 physiological state of the cells at time zero, while the transition to the stationary phase is shaped by a linear 233 saturation respect to the maximum population density (log_{10} cfu/g) X_{max}.

234 The differential equation for Baranyi model is the following:

235 $dx(t)/dt = a(t) * \mu_{max} * (1-x(t)/X_{max}) * x(t)$

236 $a(t) = q(t) / (1 + q(t))$

237 $q(t) = q_0 * \exp \{\mu_{max} *t \}$

238 $\lambda = \ln (1 + 1/q_0) / \mu_{max}$

239 where x is the number of cells, μ_{max} is the maximum growth rate, lambda is the lag period, q(t) is the physiological 240 state of the cell. If q(t)=0 there is no growth. The explicit solution of the equation is available in Grijspeerdt and 241 Vanrolleghem, 1999.

242 The Baranyi model without lag phase is obtained from the previous one setting a(t)=1, whereas the 243 Baranyi model without stationary phase is obtained by setting X_{max} to infinity. The exponential model 244 corresponds to the version of the Baranyi model without lag and stationary phase. In this work all observational 245 data are expressed as log_{10} cfu/g. Moreover, maximum growth rate values are expressed in term of log_{10} cfu/g 246 increases per hour to be easily understood in terms of growth potentials; the maximum growth rate (h^{-1}) to 247 include for modelling purposes can be retrieved by multiplying these values by the Ln (10).

248

249 *2.4.2 Secondary models*

250 The secondary models describe the dependence of primary model parameters on environmental
251 conditions. Since the Gamma model of Rosso (Rosso et al., 1995) includes the environmental conditions
252 investigated in this study (i.e., storage temperature and product pH), that model was applied to the datasets.
253 The model introduces the concept of μ_{max} at the optimum temperature and the optimum pH (μ_{opt}) and describes
254 the variation of the μ_{max} in relation to the environemental conditions (i.e., storage temperature and product pH).
255 The dependence over temperature and pH includes minumum, optimum and maximum values of the cardinal
256 parameters allowing pathogen growth:

$$\mu_{max} = \mu_{opt} \cdot \gamma(T)\ \gamma(pH)$$

$$\gamma(T) = CM2(T)$$

$$\gamma(pH) = CM1(pH)$$

$$CM_n(x) = (x-x_{max})*(x-x_{min})^n / (x_{opt} - x_{min})^{n-1}*[(x_{opt}-x_{min})(x-x_{opt})-(x_{opt}-x_{max})((n-1)x_{opt}+x_{min}-nx)]$$

257 In this paper, minimum, optimum and maximum values for the cardinal parameters were taken from Augustin
258 and Carlier, 2000.
259
260 *2.5 Statistical analysis*
261 Primary and secondary models were fitted applying the Python routine "scipy.optimize.curve_fit"
262 (https://www.scipy.org) using the non-linear least squares optimization method. Models are defined as functions
263 and the parameters are determined by the algorithm over the data points coordinates. The fitting procedure
264 returns the optimized parameters of the model and their matrix of covariance. Python is an open source
265 programming language (https://www.python.org/) and many libraries and platforms are freely available for data
266 analysis.
267 Primary models (i.e., Baranyi with lag and stationary phase, Baranyi without lag phase and exponential
268 model) were fitted taking as input the growth kinetics of *L. monocytogenes* as a function of time, to obtain the
269 optimized values for the initial concentration level, the lag phase (if present), the maximum growth rate and the

270 maximum concentration level. The Gamma model was fitted taking as input the maximum growth rate values,
271 estimated in the previous step, as a function of the environmental parameters (i.e., storage temperature and
272 product pH) (Fig S1). The pH values were estimated as the average between the initial and final pH values
273 quantified in each dataset (Table 3).

274 Goodness of fit was assessed considering the coefficient of determination (R^2), the adjusted coefficient
275 of determination (R^2) and the root mean square error (RMSE) between the observed data and the $\log_{10}$ cfu/g
276 predicted by the model. To compare primary models with a different number of degrees of freedom (DFs), the
277 Bayesian information criterion (BIC) parameter was considered as goodness of fit indicator. The model with the
278 lowest BIC is the most suitable to describe the data, as long as it does not over fit the data. The optimized
279 secondary model was used to predict the growth curves under different scenarios and the predictions were
280 compared to real data. In order to validate the models selected, commercial pasta salads were tested at 4 and
281 12°C, as laboratory prepared salads, as well as at 6°C. This temperature was selected for the validation trials
282 because, according to a non-representative study performed in the Italian region where parts of this research
283 were performed, 6°C corresponds to the mean temperature of consumer's refrigerators (De Cesare et al., 2013).
284

285 *2.6 Interaction between L. monocytogenes and Lactic Acid Bacteria growth*

286 Challenged and control samples were analysed for Lactic Acid Bacteria once a day or every two days
287 along the product shelf life. Moreover, in order to test if in our trials *L. monocytogenes* exponential growth phase
288 continued when LAB reached the stationary phase, the growth of *L. monocytogenes* after LAB had reached the
289 stationary phase was estimated, by subtracting the contamination level of *L. monocytogenes* at that point to the
290 maximum contamination level estimated for the same dataset by the primary model.
291

292 ## 3. Results

293 *3.1 pH and Lactic Acid Bacteria evolution during storage of laboratory prepared salads and commercial pasta*
294 *salads.*

295 The initial pH values measured in the laboratory prepared salads investigated in this study ranged
296 between 5.41 and 6.15. In MS1, containing smoked ham, edamer and fresh vegetables, the pH values slightly
297 increased over time in samples packaged under air and decreased in samples packaged under MAP (Table 3). In
298 MS2, containing cooked chicken, mozzarella, norvegia and heat treated vegetables, the pH values were either
299 stable or decreased under air as well as MAP (Table 3). The only exception was observed in dataset 6, in which
300 the pH slightly increased between 5.51 and 5.63 during storage under air for 12 days at 4°C. In commercial pasta
301 salads the initial pH ranged between 6.08 and 6.22. It decreased to values ranging between 4.44 and 5.34 in
302 pasta salads packaged under MAP and between 5.24 and 5.26 in products packaged under air (Table 3).
303 Concerning LAB, their initial values in MS1 ranged between 3.89 and 5.36 log_{10} cfu/g, whereas in MS2 between
304 5.90 and 6.30 log_{10} cfu/g. In both laboratory prepared salads, LAB reached values ranging between 7.92 and 9.18
305 log_{10} cfu/g, as well as 7.39 and 8.87 log_{10} cfu/g, during storage under air at 12 and 4°C, respectively (Table 3).
306 Under MAP, their final concentrations were always higher than 8.80 log_{10} cfu/g at both storage temperatures
307 (Table 3). In commercial pasta salads the initial LAB concentrations ranged between 5.26 and 7.63 log_{10} cfu/g.
308 During storage under air and MAP their values increased 8.94 to 9.14 log_{10} cfu/g and 6.80 to 9.23 log_{10} cfu/g,
309 respectively (Table 3). Overall, the initial and final pH values observed in the two laboratory prepared salads
310 stored at the same temperature (i.e., 12 and 4°C) were not significantly different (Table S1). However, the final
311 pH values detected in laboratory prepared salads packaged under air or MAP during storage at both 12 and 4°C
312 had p values close to the significance level (i.e., $p \leq 0.05$) (Table S1). Moreover, the average pH values in
313 laboratory prepared salads packaged under air or MAP during storage at 4°C were significantly different (Table
314 S1) and the same is true considering only the packaging condition beside storage temperature (Table S2).
315 Concerning LAB counts, they were significantly different in the two formulation of laboratory prepared salads,
316 before and after storage at 12 and 4°C, but they did not show differences in relation to the different packaging
317 conditions at any storage temperature (Table S1 and S2).

318

319 3. 2 *Estimation of L. monocytogenes maximum growth rates and primary model selection*

320 The maximum growth rate (μ_{max}) of *L. monocytogenes* in each dataset was estimated minimizing a
321 nonlinear least square through the routine in Python "scipy.optimize.curve_fit" (Fig. S2). This routine provides
322 the optimized parameters and their matrix of covariance, from which the standard deviation (μ_{max} sd) over each
323 estimated parameter can be determined by applying the square root over the diagonal of the matrix. The most
324 suitable models fitting the different datasets collected in this research are Baranyi without the lag phase and the
325 exponential model, both compatible with the absence of a lag phase (Table 4, Table S3). In particular, Baranyi
326 no-lag (DF=3) was the most suitable model to describe all datasets, except for those labelled as 4, 5, 6, 7 and 16,
327 collected at 4°C, in which the stationary phase was not reached (Table 4, Table S3). The exponential model was
328 the best primary model to describe the behavior of *L. monocytogenes* in the laboratory prepared salads 1
329 packaged under modified atmosphere and stored at 4°C for 12 days and laboratory prepared salads 2 stored
330 under the same modified atmosphere as well as air at the same temperature for the same period (Table 3, Table
331 4). The maximum growth rates calculated for the laboratory prepared salads (i.e., MS1 and MS2) were compared
332 and they did not show significant variations (Table S1). Therefore, both formulations of laboratory prepared
333 salads were treated as the same product to estimate the optimum growth rate. However, it should be stressed
334 that, in general, each ingredient formulation deeply affects *L. monocytogenes* growth kinetics that must be then
335 estimated case by case.

336 The growth curves for datasets 12 and 13 showed a multi-phase behavior (Fig. S2). This trend might be
337 due to the presence of preservatives in the meat added in MS2 in these two datasets, which was indeed verified
338 by the authors. Therefore, for these datasets, a multi-phases intermediate stationary phase was used to
339 determine the μ_{max} in each exponential growth phase. In particular, Baranyi model without lag phase was used
340 to determine the parameter of the first growth step; Baranyi model with lag phase was used to determine the
341 parameters of the second growth step; finally, the transition point between the two steps was chosen to
342 maximize the fit coefficient of determination (Fig. S2).

343 The μ_{max} values obtained at 4°C (i.e., datasets 4, 5, 6, 7, 10, 11, 14, 15) were lower than those calculated
344 at 12°C (i.e., datasets 1, 2, 3, 8, 9, 12, 13) (Table 4). In particular, the μ_{max} mean values obtained at 4 and 12°C
345 were 0.007±0.004 and 0.033±0.008 log_{10} (cfu/g) h^{-1}, respectively. Overall, the μ_{max} values obtained in the
346 laboratory prepared salads stored at 12 and 4°C were not significantly different in the two laboratory prepared
347 salads (Table S1). The same result was observed for the μ_{max} values obtained under modified atmosphere and
348 under air during storage at 12°C. However, μ_{max} values obtained in the laboratory prepared salads packaged
349 under MAP and air and stored at 4°C showed significant variability (Table S1). This result might be due to higher
350 variability of the growth rate at 12°C (Table 4).
351 Packaging under MAP (i.e., datasets 3, 5 and 7) reduced μ_{max} in comparison to packaging under air (i.e.,
352 datasets 2, 4 and 6) (i.e., μ_{max} mean values 0.012±0.012 *vs* 0.018±0.015 log_{10} (cfu/g) h^{-1}). In datasets 2 and 3, in
353 which the stationary phase was reached, the maximum concentration of *L. monocytogenes* at the end of the
354 shelf life was higher under air in comparison to MAP (Table 4). Furthermore, significant differences between X_m
355 values were observed in different laboratory prepared salads (Tables S1 and S2) possibly related to differences
356 in the ingredients composition. Most of the datasets showed very good coefficient of determination and
357 goodness, meaning adjusted R^2 values close to one and low RMSE values (Table 4). The observed lower scores
358 were related to specific dataset features (i.e., high variability in the enumeration results) more than a lack of the
359 model (Fig. S2). In order to reduce the uncertainty and better estimate the variability in microbial counts in the
360 laboratory prepared salads, a higher number of repetitions at each time point should be considered, finding a
361 balance between an appropriate number of repetitions and sampling time points.
362
363 *3.3 Calculation of L. monocytogenes optimum growth rates by using calculated maximum growth rates*
364 All the calculated maximum growth rates at 4 and 12°C detailed in Table 4 were used to determine the
365 Gamma model parameters in order to predict *L. monocytogenes* growth at different temperature and pH
366 conditions. Pasta salads stored under air and MAP were modeled separately but the estimated optimum growth
367 rates were almost the same. For this reason, data were used all together to get a unique value of optimum

368 growth rate (Table 5). Since there was no difference in the estimated optimum growth rates for salads packaged
369 under air and MAP, differences in the maximum growth rates might be linked to product pH. This hypothesis is
370 supported by the results showing that significant differences in product pH values were observed in laboratory
371 prepared salads packaged under air and MAP (Tables S1 and S2).

372 Baranyi model without lag phase was used to generate *L. monocytogenes* growth kinetics under different
373 scenarios using the following parameters: (1) maximum growth rate predicted by the Gamma model; (2)
374 estimated initial contamination level for each scenario; (3) highest maximum contamination level (X_m) estimated
375 in each scenario in order to be more restrictive. Comparison of the predicted $\log_{10}$ concentrations respect to the
376 observed ones are shown in Fig. 1A and the residues, rarely exceeded 1 Log_{10} cfu/g (Fig. 1B). The growth kinetics
377 for each dataset are available in Fig. S3 and the coefficients of determination as well as RMSE in Table S4.

378 Fig. 2 a and b shows the comparison between the experimental μ_{max} values showed in Table 4 and the
379 μ_{max} values predicted using the Gamma model with the parameters reported in Table 5. Fig. 2 a shows that in 8.3
380 and 25% of the cases considering confidence intervals of three and one standard deviations, respectively, μ_{max}
381 values fall out of the prediction areas. These cases refer to dataset 10 and 11, which were underestimated by
382 the prediction of the secondary model (Fig. S3). The results of datasets 10 and 11 were probably due to the
383 variability in microbial composition associated to the ingredients used for those laboratory prepared salads. In
384 fact, in the samples tested in datasets 10 and 11 the final concentrations of lactic acid bacteria were lower in
385 comparison to other datasets collected at 4°C and, therefore, the decrease in pH values was not observed.

386

387 *3.4 Interaction between L. monocytogenes and Lactic Acid Bacteria growth*

388 Lactic acid bacteria (LAB) were enumerated in all datasets along the product shelf life. Initial and final
389 concentrations for each dataset are summarized in Table 3. LAB initial concentrations were different among
390 different datasets, because of their different concentration in the ingredients used in formulation of both
391 laboratory prepared salads and commercial products. Therefore, LAB reached the stationary phase at different

392 times along the product shelf life (Table 6). The *L. monocytogenes* growth potential showed that the pathogen
393 was able to grow even when LAB reached their stationary phase in laboratory prepared salads and commercial
394 pasta salads packaged under air and stored at all tested temperatures (i.e., 4, 6 and 12°C) (datasets 8, 9, 12-15,
395 20 and 21). However, in datasets 17 and 22-24 growth of *L. monocytogenes* was not significant after LAB reached
396 the stationary phase. Therefore, the inhibitory effect of LAB versus *L. monocytogenes* growth, described as
397 Jameson effect, was possibly observed (Table 6).
398
399 **4. Discussion**

400 The use of mathematical models to predict the growth kinetics of *L. monocytogenes* in ready-to-eat foods
401 can be used to support assessment and management of the consumer health risk due to *L. monocytogenes* in
402 those products. According to Gimenez and Dalgaard (2004) a comparisons between model prediction and data
403 from challenge tests and naturally contaminated products can help to determine the approximate range of
404 applicability of selected models. Models to predict the combined effect of product characteristics and storage
405 conditions on growth and growth boundary of *L. monocytogenes* hold great practical interest for the industry
406 and for food inspection authorities (Mejlholm et al., 2010). Numerous models to predict the combined effect of
407 product characteristics and storage conditions on growth and growth boundary of *L. monocytogenes* are
408 available in the scientific literature (Mejlholm et al., 2010) but few concern composite foods containing raw and
409 cooked ingredients (Sahu et al., 2017; Lokerse et al., 2016; Hwang and Tamplin, 2005). Therefore, in this study
410 we modelled the growth kinetic of *Listeria monocytogenes* in different recipes of ready to eat pasta salads during
411 storage under different temperatures (i.e., 4 and 12°C) and packaging conditions (air vs modified atmosphere).
412 The results of the present study indicated that ready to eat full meal pasta salads support the growth of
413 *L. monocytogenes.* Baranyi no lag was the most suitable primary model to estimate the pathogen μ_{max} for
414 datasets collected at 12°C, whereas the exponential model provided the best results fitting datasets collected at
415 4°C, in which the stationary phase was not reached. Two different compositions of pasta salads were used in this
416 study, with ingredients from two European countries characterised by different climate and therefore different

417 availability of raw ingredients during the year. Since the results collected for both product formulations were
418 comparable, the models suggested in this paper should be valid for similar products, meaning commercial
419 products with similar pH and ingredient composition. To evaluate the goodness of fit, the adjusted R^2 and RMSE
420 parameters were calculated. Moreover, the BIC parameter was used to select the correct description. In few
421 cases, since the estimated q_0 parameter presented high uncertainty, the Baranyi no lag model was selected. The
422 results of this study were cross sectional data; therefore, poor values of fit goodness do not always mean that
423 the model used was not appropriate. In fact, those values might be due to the biological fluctuations in the data
424 sets (Fig. S2). The μ_{max} assessment is generally more precise when experimental measures are closer in time and
425 the transition to the stationary phase can be identified more accurately. However, to determine the maximum
426 contamination level, having sampling points in a longer period (t >150 h) is helpful. Therefore, in the validation
427 trials, corresponding to datasets 16 to 24, samplings for longer periods were considered.

428 In some datasets, referred to both laboratory prepared salads as well as commercial pasta salads, *L.*
429 *monocytogenes* growth continued even when lactic acid bacteria (LAB) reached the stationary phase (Table 6).
430 This observation indicates that the Jameson effect (Cornu et al., 2011; Jameson, 1962), referred to the
431 simultaneous deceleration of both microbial populations as result of competition, does not apply to all scenarios
432 investigated in our research. It is important to consider that multi ingredient products, like pasta salads, are
433 systems characterized by different microenvironments, where the growth of *L. monocytogenes* may occur in
434 some compartments, even if the average LAB and pH levels in the product should not allow that. This hypothesis
435 has been stated also by Mejlholm and Dalgaard (2015), who reported higher maximum population densities of
436 *L. monocytogenes* in mayonnaise based seafood salads than predicted, when the pH in the salad was below 6.0.
437 They suggested the potential occurrence of microenvironments with higher pH as a possible reason for this
438 discrepancy. The pH of pasta salads studied in the present paper was in the same range as in the salads studied
439 by Mejlhom and Dalgaard (2015) and we did observe different pH in the ingredients during the experiment period
440 (data not shown). However, the models indicated in this paper cover the sum of models for the different
441 compartments in the salads, where various pH values and LAB concentrations occur.

442 The effect of LAB was not included in the secondary model because few data points were collected for
443 LAB and due to the high variability of their enumeration results (Table 3), reflecting the non-homogenous nature
444 of the product investigated and their different concentrations in the ingredients used in the formulations of both
445 laboratory prepared salads and commercial pasta salads. In particular, for specific ingredients, like cheese and
446 fresh vegetables, different storage times and different suppliers resulted in large variability in LAB enumeration
447 results. Pasta salads stored under air and MAP were modeled separately but the estimated optimum growth
448 rates obtained with the Gamma model were almost the same (i.e., 0.247±0.009 and 0.244±0.025 log_{10} (cfu/g) h^{-1}
449 , respectively) (Table 5). Therefore, data were used all together to get a unique value of optimum growth rate
450 (Table 5). That value 0.247±0.009 log_{10} (cfu/g) h^{-1} is compatible with that obtained by Crépet et al., (2009) (i.e.,
451 μ_{opt} = 0.33 ±0.16 log_{10} (cfu/g) h^{-1}) and this result supports the conclusion that the selected model might be applied
452 to describe the growth kinetics of *L. monocytogenes* in other composite foods based on pasta salads, with
453 comparable pH and storage conditions. This seems true even if in this study the Gamma model was not always
454 able to account for μ_{max} variations between several samples, some of which representing extreme cases that fall
455 out the prediction areas (Fig. 2). However, these extreme cases refer to specific datasets (i.e., datasets 10 and
456 11) underestimated by the prediction of the secondary model due to lower initial and final concentrations of
457 lactic acid bacteria in comparison to the majority of the other datasets collected at 4°C.
458 According to the results presented in this paper, the models selected cover the sum of models for
459 different compartments in the tested products, even if they were characterized by different pH values and LAB
460 concentrations. The approach followed in this study represents a further development of the frequentist
461 approach presented by Augustin and Carlier, 2000 and it might be useful to predict the behavior of *L.*
462 *monocytogenes* in composite food matrices. As further implementation, in the context of models' applications
463 in risk assessment studies, the Bayesian approach might be exploited.
464
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471

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562

563 **Figure captions**

564

565 **Fig 1.**

566 Comparison of the observed *L. monocytogenes* counts with those predicted by the model proposed in all the
567 datasets (A) and plot of residues *vs* predicted values (B). The model proposed is a combination of the Gamma
568 model to predict *L. monocytogenes* maximum growth rates and the Baranyi model.

569

570 **Fig 2.**

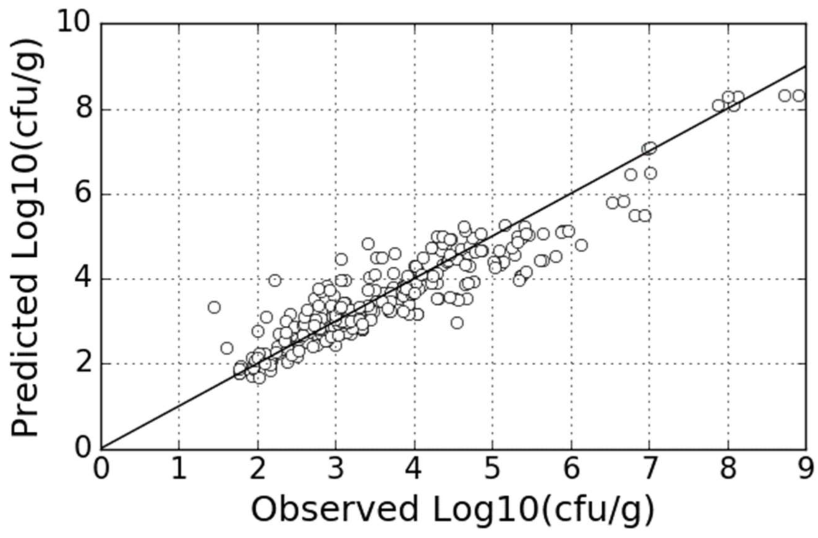
571 Prediction of maximum growth rate by using the Gamma secondary model in comparison with the experimental
572 values (A) and comparison between the maximum growth rate fit estimates and the predictions of the gamma
573 model under different scenarios (B). The optimal growth rate parameter used in the model was calculated using
574 maximum growth rate estimated values at 12 and 4°C and minimizing a non linear least squares. Data for model
575 validation collected at 6°C were compared with the values predicted by the model (A). The dark and light shadows
576 represent the confidence intervals of one and three standard deviations around the estimated maximum growth
577 rate, respectively (A).

578

579 Fig. 1

580 (A)

581



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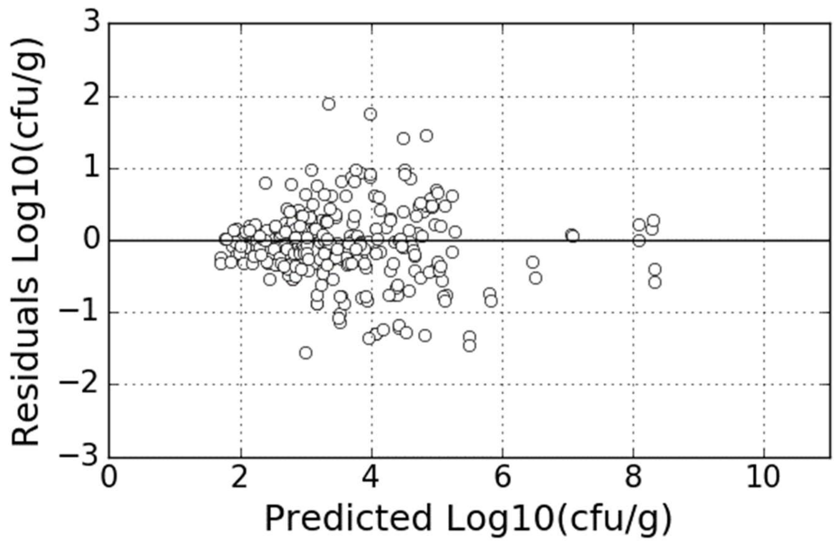
584 (B)

585



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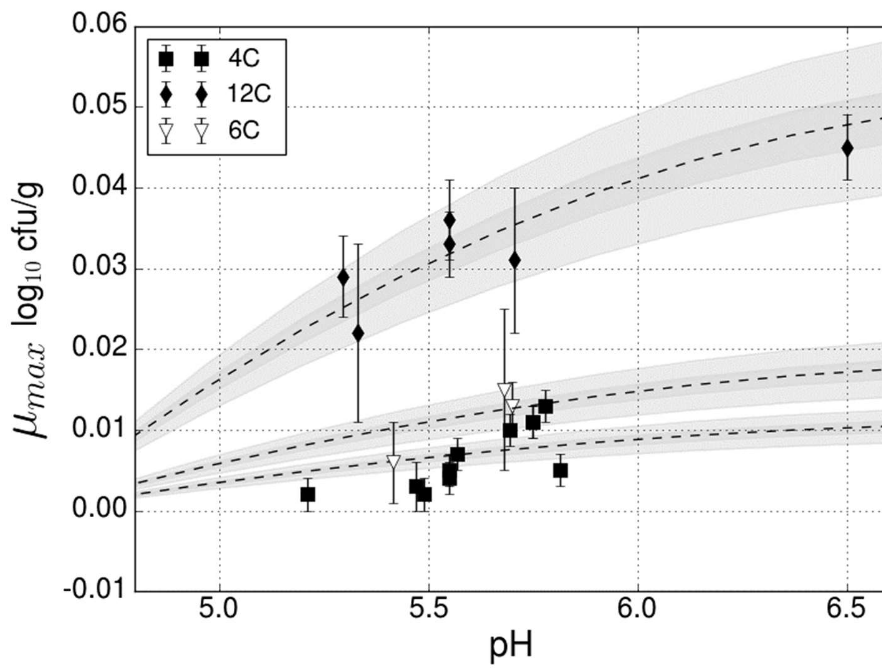
592 Fig. 2

593 (A)

594



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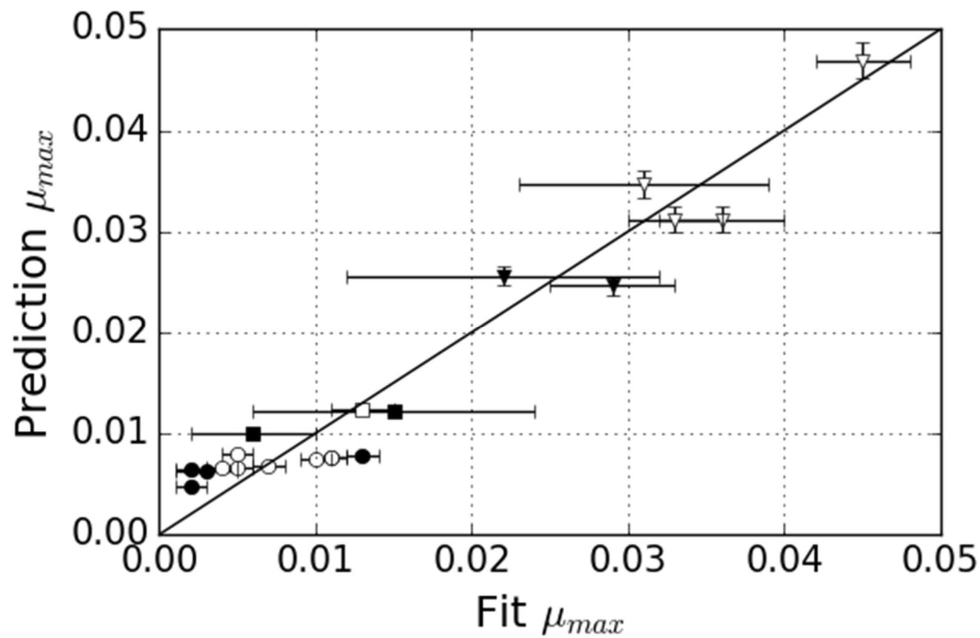
597 (B)



598

599

600 **Supplementary Figures**

601

602 **Fig S1.**

603 Overall modelling approach. The main steps of the modelling approach, fit and prediction are shown in the

604 diagram.

605

606 **Fig S2**

607 Fit (black line) of the primary models of the observed kinetics (white dots) used to calibrate the secondary model.

608 Dataset labels are shown in figure's title. Dataset 18 is missing because few data points were collected.

609

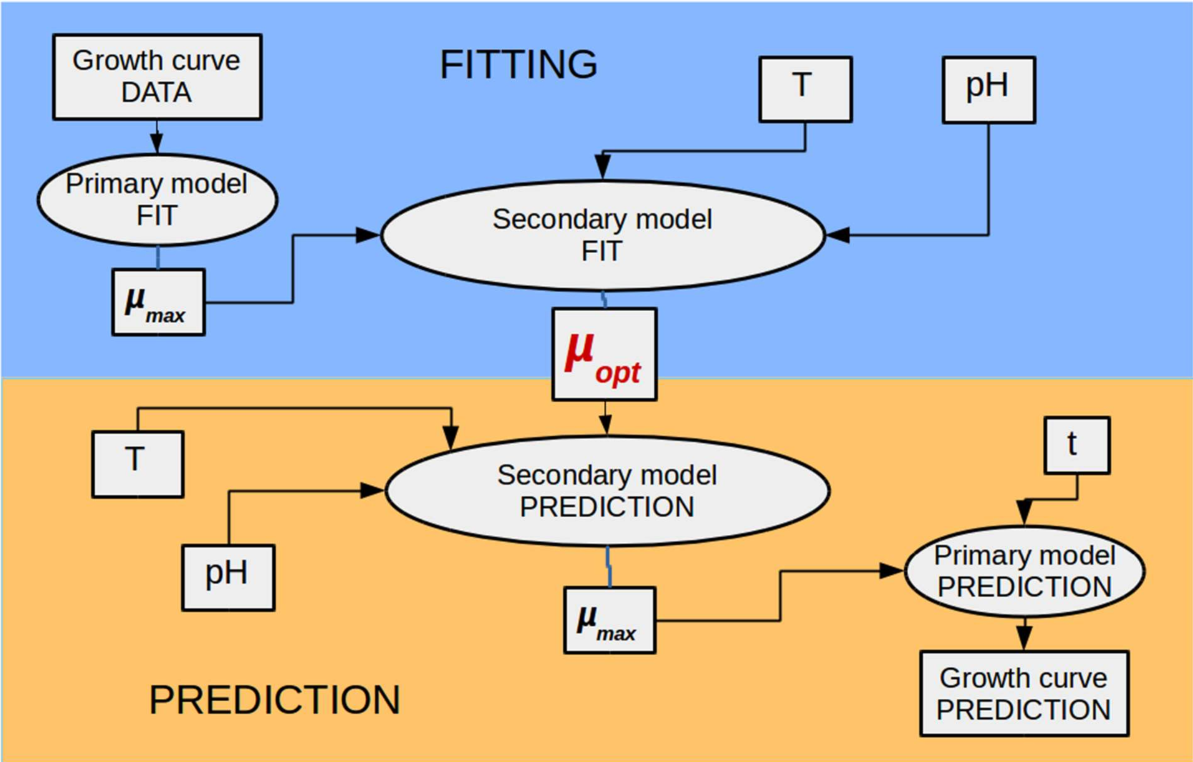
610 **Fig S3**

611 Predicted kinetics by the use of the calibrated Gamma model (black line) are compared to observed kinetics

612 (white dots). Dataset labels are shown in figure's title.

613 Fig S1

614



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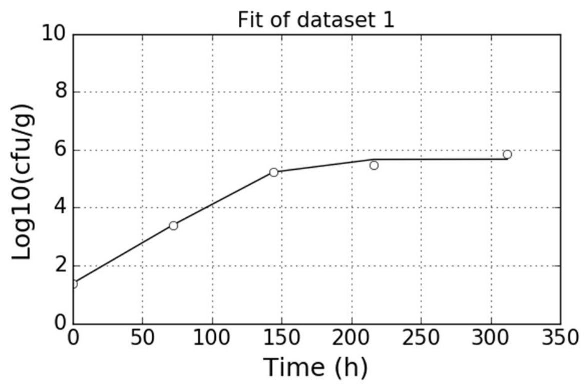
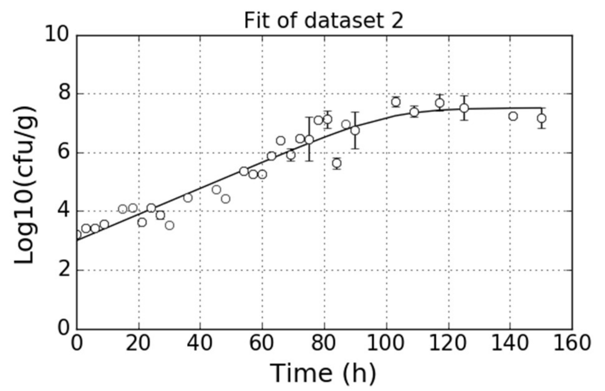
616

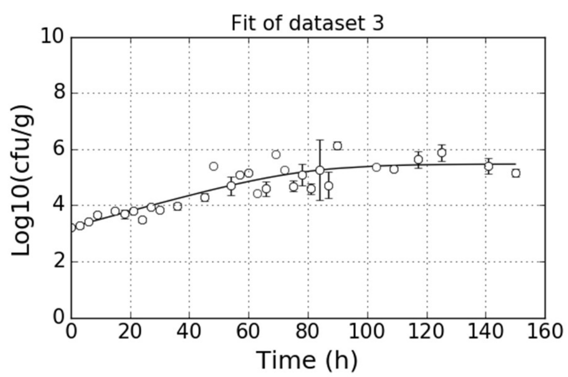
617

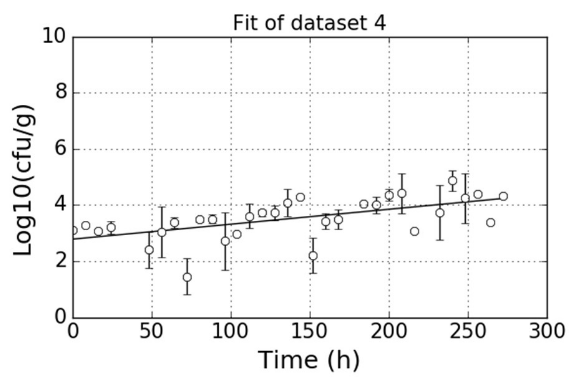
618 Fig S2

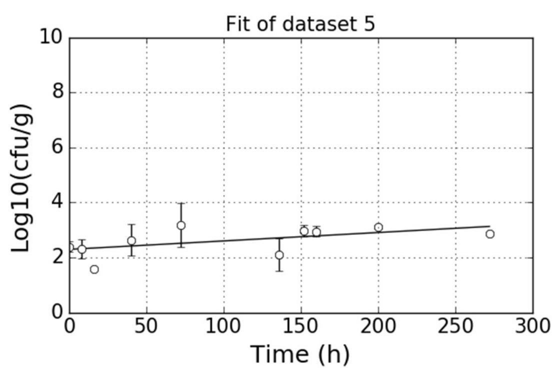
619

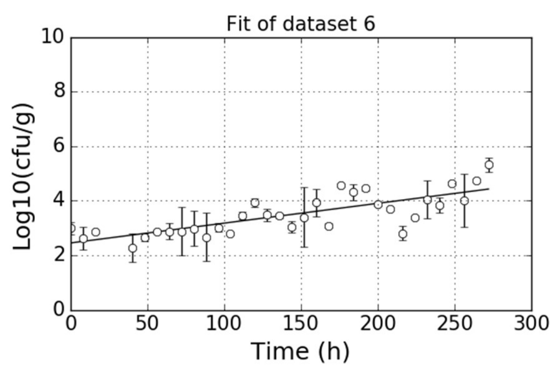


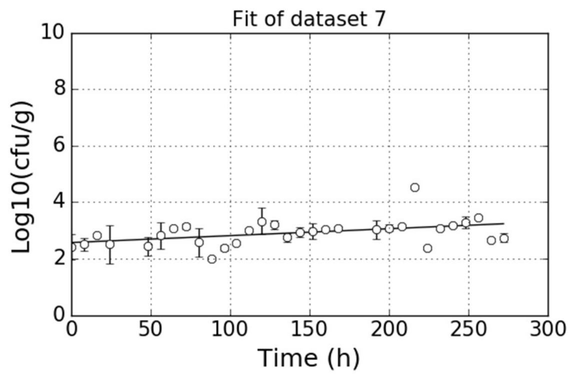


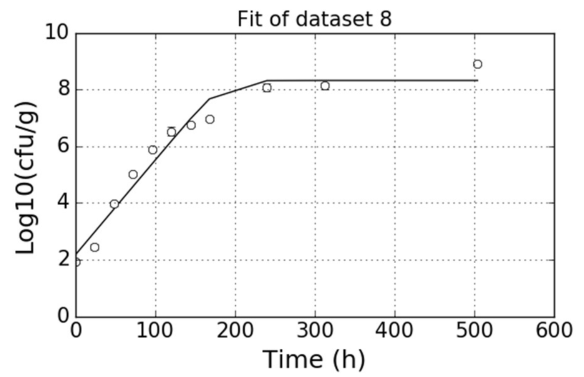


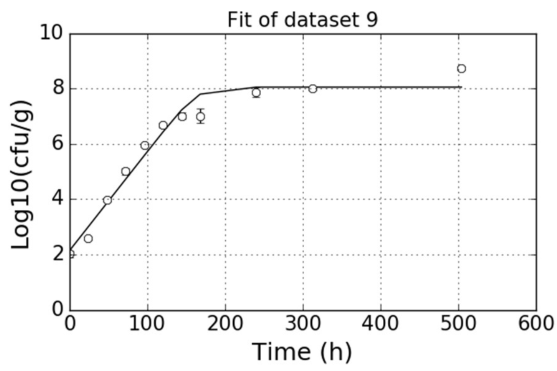


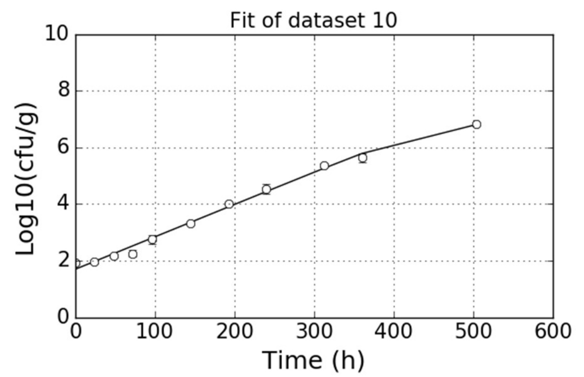


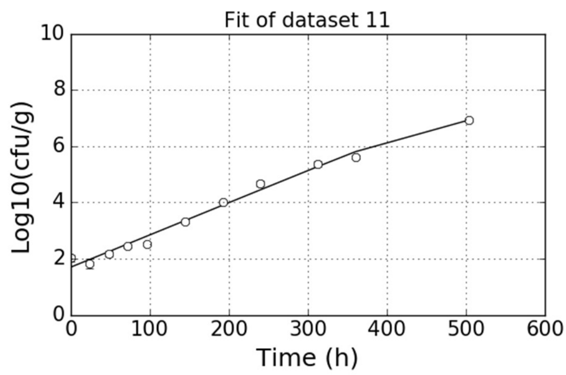


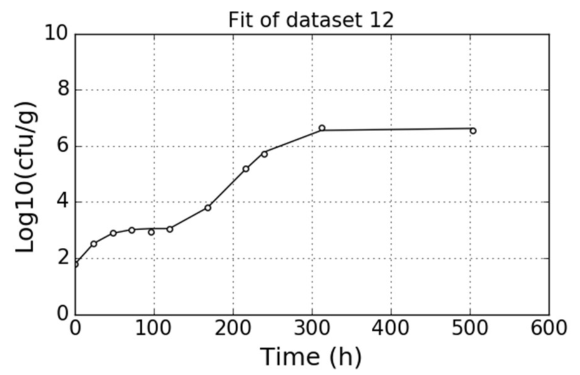

Fit of dataset 7
Log10(cfu/g)
Time (h)

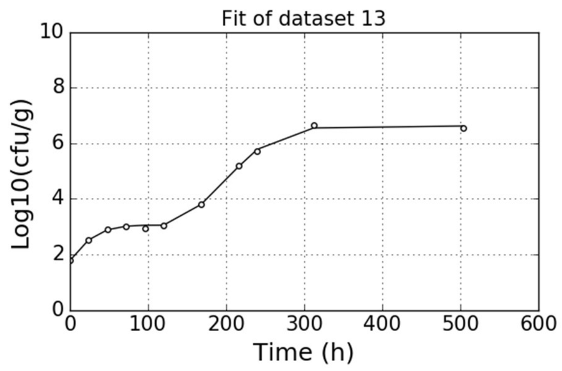

Fit of dataset 8
Log10(cfu/g)
Time (h)

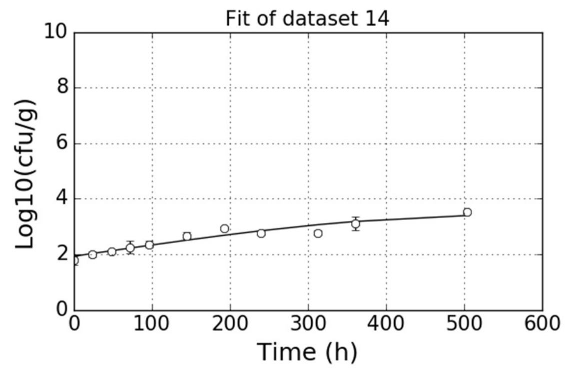

Fit of dataset 9
Log10(cfu/g)
Time (h)

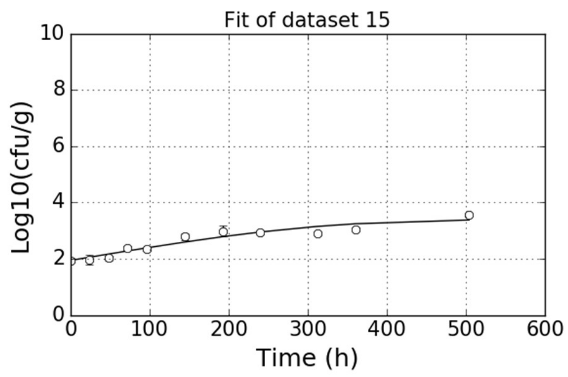

Fit of dataset 10
Log10(cfu/g)
Time (h)

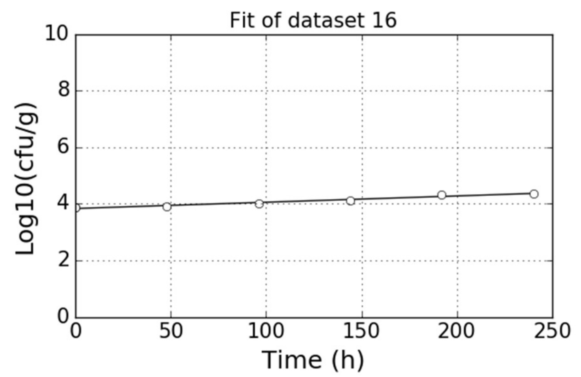

Fit of dataset 11
Log10(cfu/g)
Time (h)

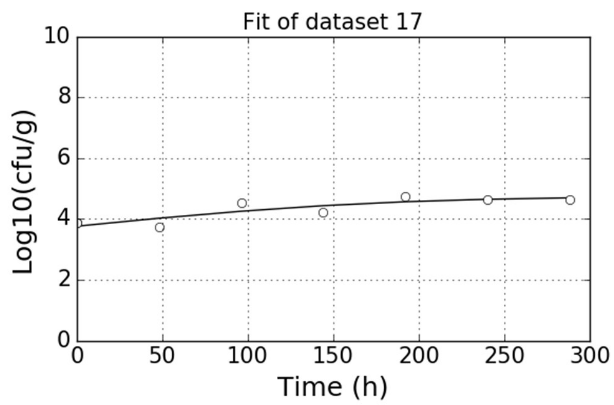

Fit of dataset 12
Log10(cfu/g)
Time (h)

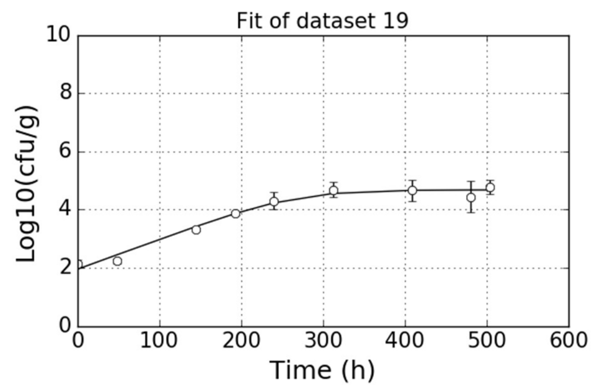

Fit of dataset 13
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
100
200
300
400
500
600

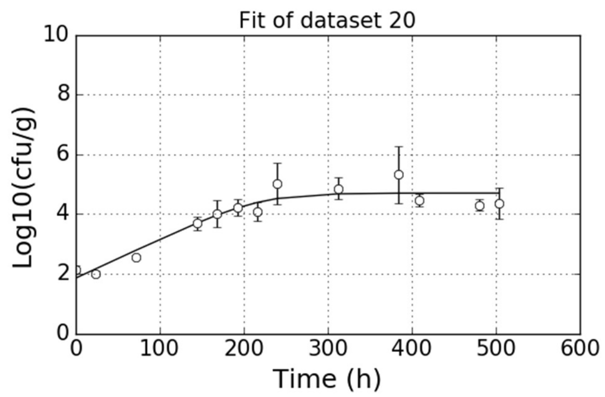

Fit of dataset 14
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
100
200
300
400
500
600

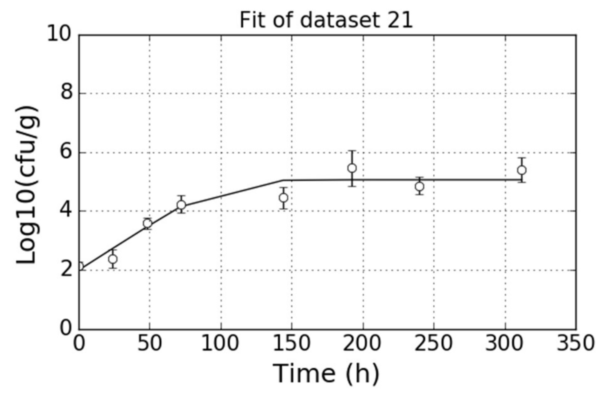

Fit of dataset 15
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
100
200
300
400
500
600

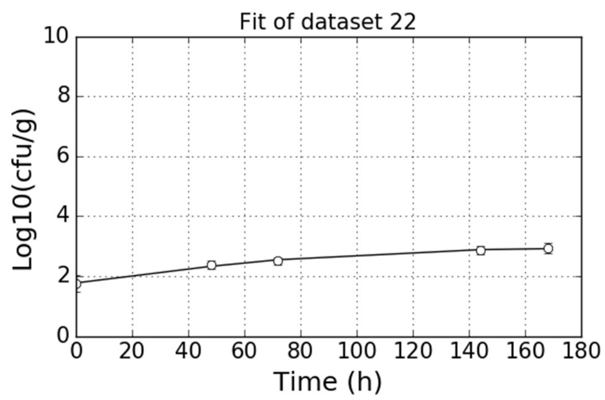

Fit of dataset 16
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
50
100
150
200
250

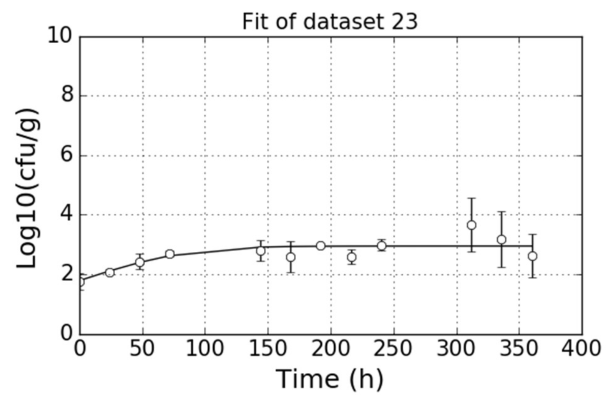

Fit of dataset 17
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
50
100
150
200
250
300

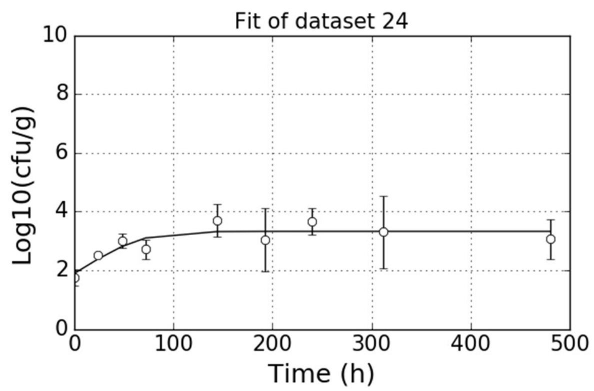

Fit of dataset 19
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
100
200
300
400
500
600











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621 Fig. S3

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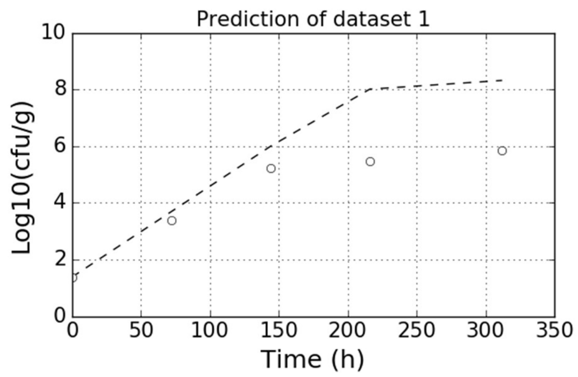
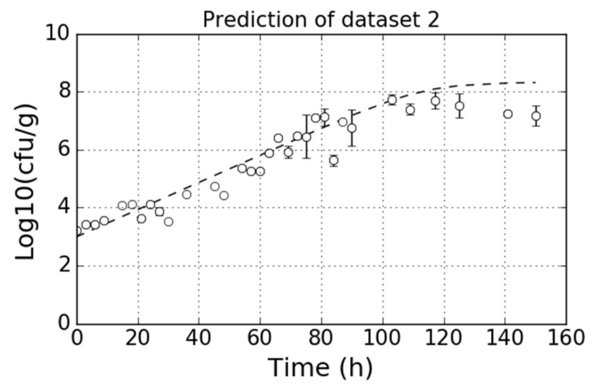
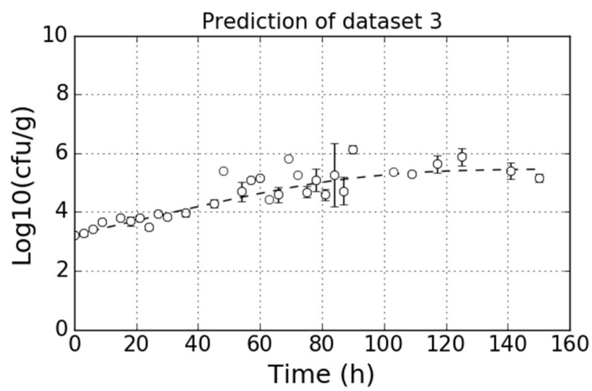
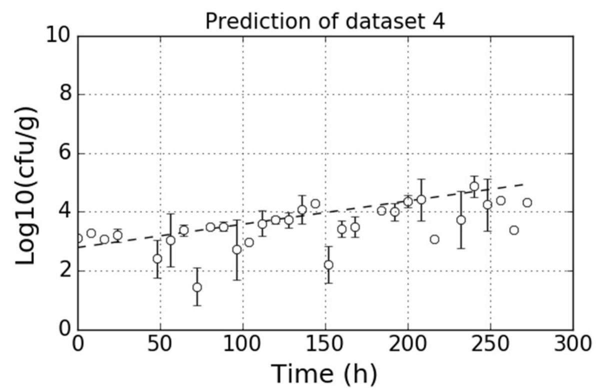
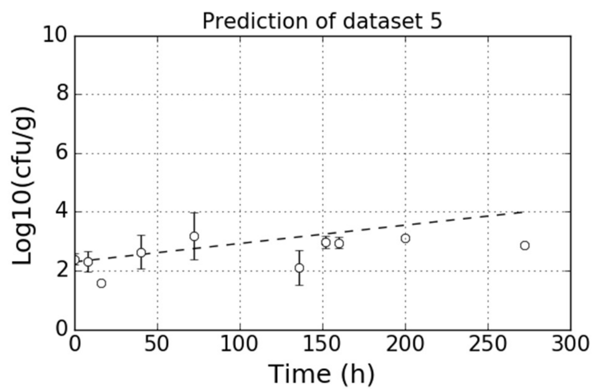
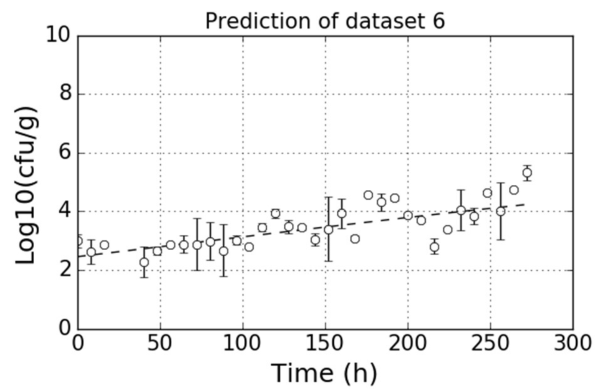

Prediction of dataset 1
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
0
50
100
150
200
250
300
350

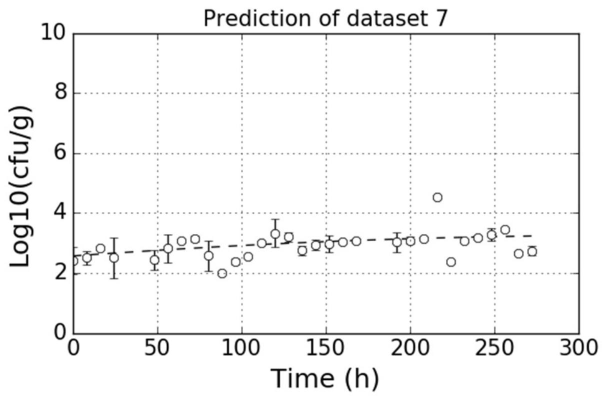

Prediction of dataset 2
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
0
20
40
60
80
100
120
140
160

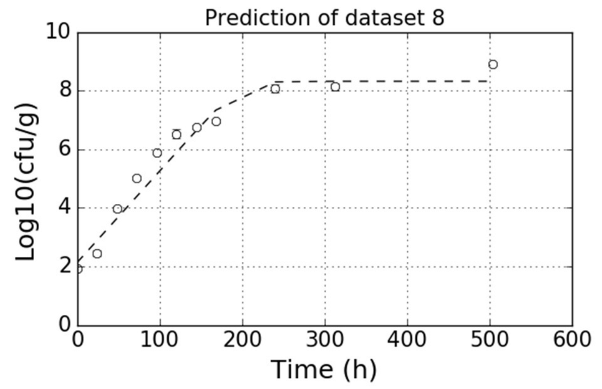

Prediction of dataset 3
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
0
20
40
60
80
100
120
140
160

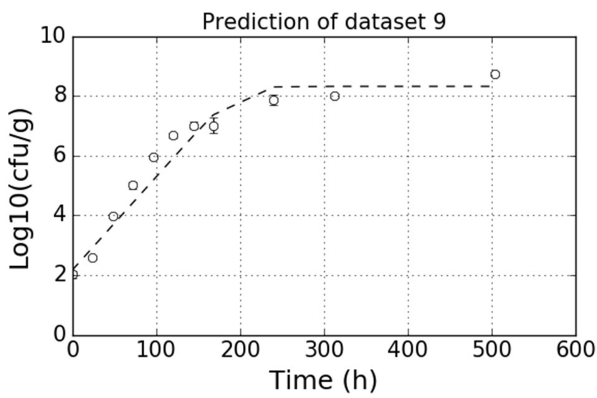

Prediction of dataset 4
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
0
50
100
150
200
250
300

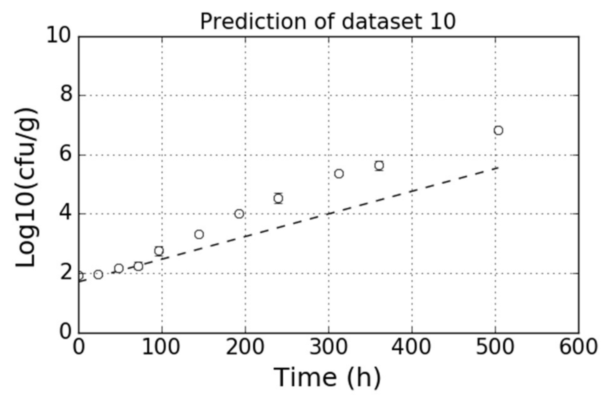

Prediction of dataset 5
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
0
50
100
150
200
250
300

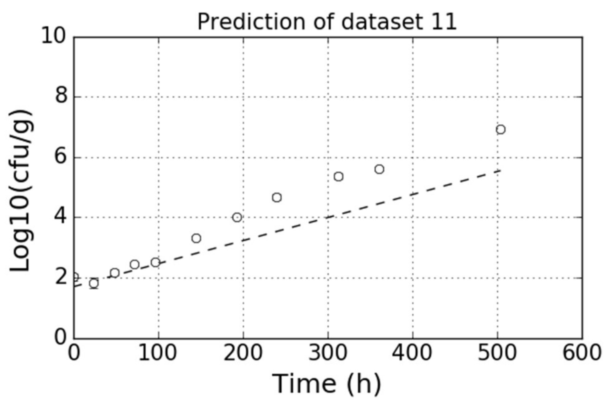

Prediction of dataset 6
Log10(cfu/g)
Time (h)
0
2
4
6
8
10
0
50
100
150
200
250
300

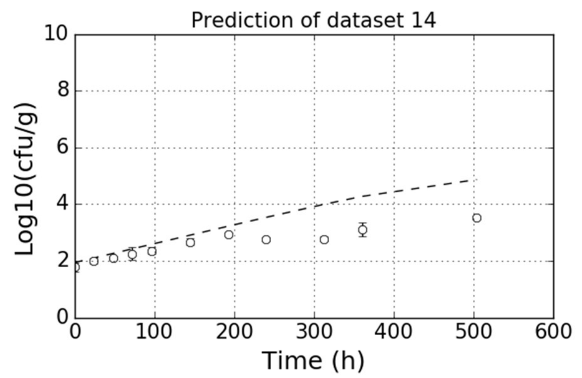

Prediction of dataset 7
Log10(cfu/g)
Time (h)

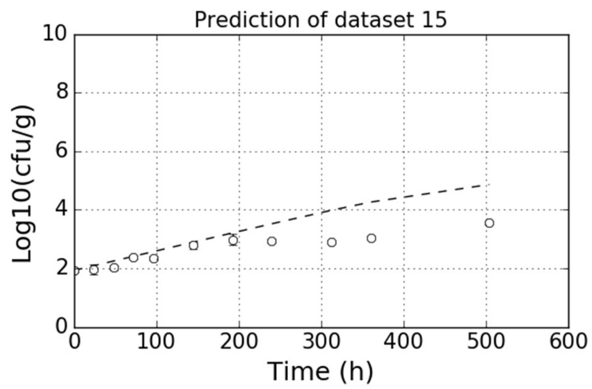

Prediction of dataset 8
Log10(cfu/g)
Time (h)

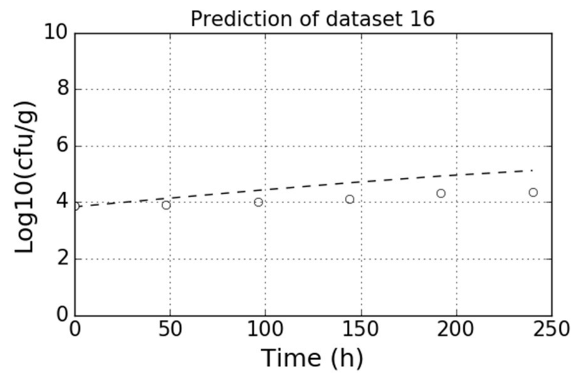

Prediction of dataset 9
Log10(cfu/g)
Time (h)

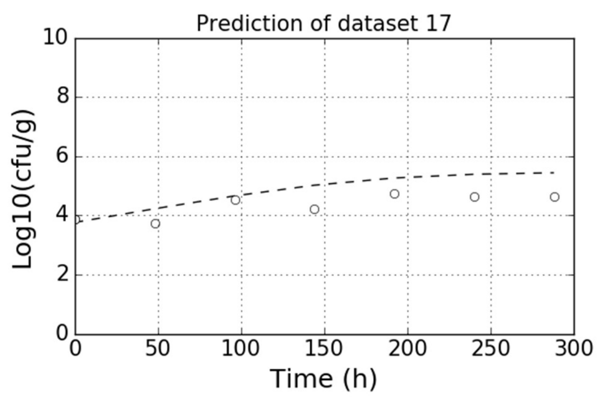

Prediction of dataset 10
Log10(cfu/g)
Time (h)

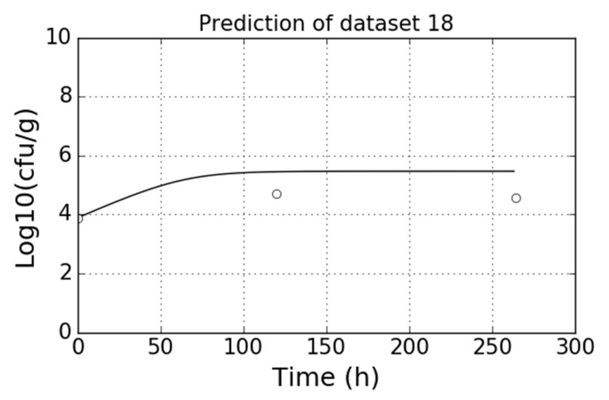

Prediction of dataset 11
Log10(cfu/g)
Time (h)

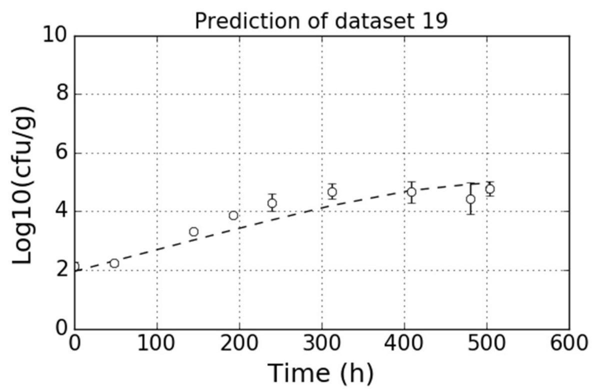

Prediction of dataset 14
Log10(cfu/g)
Time (h)

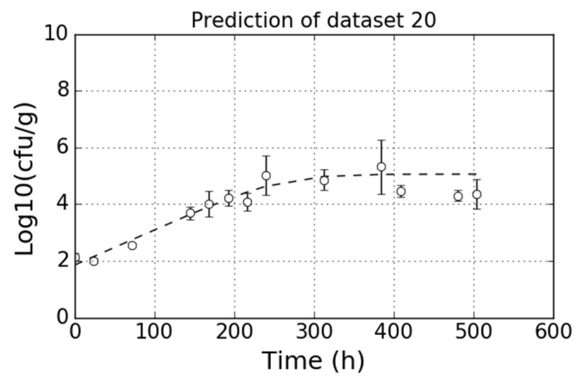

Prediction of dataset 15
Log10(cfu/g)
Time (h)

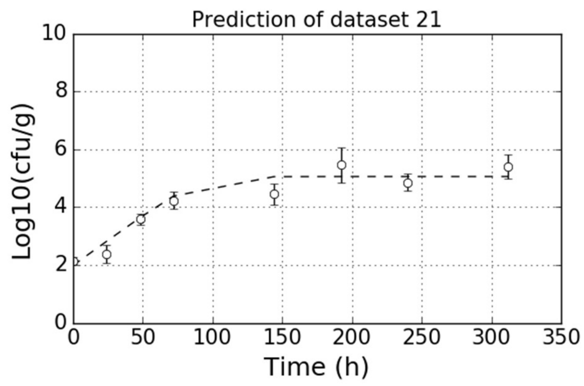

Prediction of dataset 16
Log10(cfu/g)
Time (h)

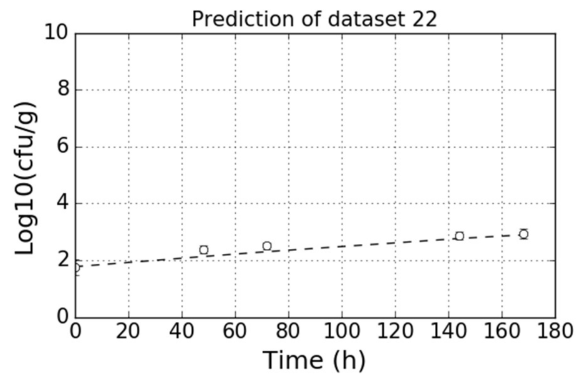

Prediction of dataset 17
Log10(cfu/g)
Time (h)

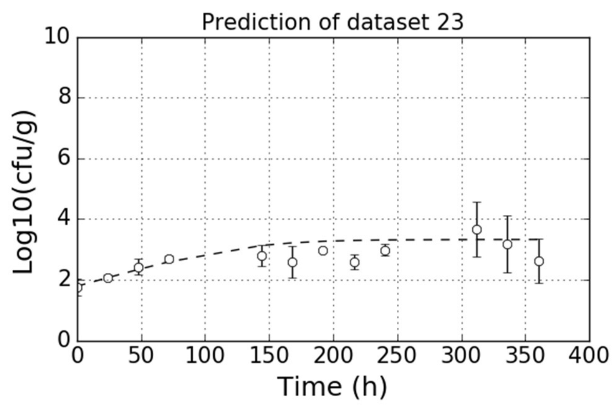

Prediction of dataset 18
Log10(cfu/g)
Time (h)

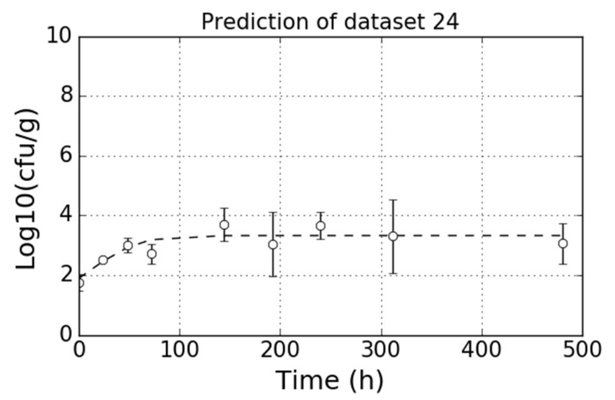

Prediction of dataset 19
Log10(cfu/g)
Time (h)


Prediction of dataset 20
Log10(cfu/g)
Time (h)









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626 **Table 1**. Ingredients of full meal commercial pasta salads and corresponding laboratory prepared salads.
627 Percentages of each ingredient correspond to the ratio between ingredient weight and total product weight.

628

Ingredient	Commercial pasta salad 1 Lab prepared salad 1 (MS1)	Commercial pasta salad 2 Lab prepared salad 2 (MS2)
Cooked pasta (Fusilli)	51%	38%
Meat	8% (smoked ham)	23% (chicken)
Cheeses	19% (edamer)	10% (mozzarella) and 3% (norvegia)
Vegetables	13% (radicchio) and 3% (arugula)	15% (bell pepper, heat treated)
Salt	0.57%	0.57%
Black pepper	0.05%	0.05%
Oil	5% (sunflower oil)	10% (olive oil)
Lemon juice	0.38%	0.38%

629

630 **Table 2**. *Listeria monocytogenes* strains used in the challenge studies.

Strain label	Name	Serotype	Genoserogroup	Remarks
1	LR102	1/2a	IIa	Human isolate from the listeriosis outbreak with *Camembert* cheese in Norway, 2007
2	VI 51028	-	IIb	From the environment in fish slaughter house
3	0227-359	-	IIa	Isolated from meatballs
4	0113-131	-	IIa	Ready-to-eat chicken meat for use in salads
5	VI 51010	4b	-	Scott A J. Reference strain
6	12MOB 045 LM		II	Recommended (Beaufort et al., 2004) for challenge studies in products with meat, both for chilled storage (8°C) acidic conditions (pH 5), and low water activity (a_w 0.95).

631

632

633 **Table 3**. Description of the datasets used for models' selection and validation: *Listeria monocytogenes* (LM) and Lactic Acid Bacteria (LAB) were
634 enumerated in different lab prepared salads (MS), labelled as 1 and 2, as well as commercial pasta salads, labelled as 1 and 2, packaged under air or
635 under different modified atmospheres (MAP) (i.e., MAP1=50%CO_2+50%N_2; MAP2=60%CO_2+40%N_2).

Dataset label	Number replicates	Sample label	Packaging condition	T (°C)	Time (days)	Ingredient origin	LM strain(s) challenged in the samples	LM initial count (Log_{10} cfu/g)	Initial pH (mean)	Final pH (mean)	LAB initial count (Log_{10} cfu/g)	LAB final count (Log_{10} cfu/g)
Datasets for model's selection												
1	2	MS 1	Air	12	13	Italy	1-5	1.39	5.41	5.76	3.89	9.18
2	2	MS 1	Air	12	10	Italy	1-5	3.23	6.15	6.85	4.69	7.92
3	2	MS 1	MAP1	12	10	Italy	1-5	3.23	6.15	4.44	4.69	8.90
4	2	MS 1	Air	4	12	Italy	1-5	2.89	5.78	5.85	5.36	8.79
5	2	MS 1	MAP1	4	12	Italy	1-5	2.89	5.60	5.34	5.36	8.90
6	2	MS 2	Air	4	12	Italy	1-5	2.89	5.51	5.63	5.90	8.78
7	2	MS 2	MAP1	4	12	Italy	1-5	2.89	5.66	4.76	5.90	8.81
8	3	MS 1	Air	12	13*	Norway	4	1.84	5.60	5.50	4.44	8.30
9	3	MS 1	Air	12	13*	Norway	6	2.08	5.60	5.50	4.44	8.30
10	3	MS 1	Air	4	13*	Norway	4	1.84	5.60	5.90	4.44	7.39
11	3	MS 1	Air	4	13*	Norway	6	2.08	5.60	5.90	4.44	7.39
12	3	MS 2	Air	12	13*	Norway	4	1.77	6.00	5.10	6.30	8.87
13	3	MS 2	Air	12	13*	Norway	6	1.95	6.00	5.10	6.30	8.87
14	3	MS 2	Air	4	13*	Norway	4	1.77	6.00	5.10	6.30	8.87
15	3	MS 2	Air	4	13*	Norway	6	1.84	6.00	5.10	6.30	8.87
Datasets for model's validation												
16	2	Pasta salad 1	MAP1	4	12	Italy	1-5	3.41	6.08	4.90	5.26	6.80
17	2	Pasta salad 1	MAP1	6	12	Italy	1-5	3.41	6.08	4.75	5.26	7.82
18	2	Pasta salad 1	MAP1	12	12	Italy	1-5	3.41	6.08	4.52	5.26	9.01
19	3	Pasta salad 2	Air	4	21	Norway	1-5	2.11	6.15	5.24	6.60	8.94
20	3	Pasta salad 2	Air	6	21	Norway	1-5	2.11	6.15	5.25	6.60	8.93
21	3	Pasta salad 2	Air	12	21	Norway	1-5	2.11	6.15	5.26	6.60	9.14
22	3	Pasta salad 2	MAP2	4	21	Norway	1-5	1.60	6.22	5.34	7.63	8.85
23	3	Pasta salad 2	MAP2	6	21	Norway	1-5	1.60	6.22	5.14	7.63	8.99
24	3	Pasta salad 2	MAP2	12	21	Norway	1-5	1.60	6.22	4.44	7.63	9.23

636 *Samples were stored further up to day 21 to ensure the stationary phase.

637 **Table 4**. Optimized parameters of the most suitable primary model calculated for each dataset. For each dataset storage temperature (T), initial and
638 final pH are reported as well as *L. monocytogenes* growth parameters (i.e., maximum growth rate (μ_{max}) and corresponding standard deviation (μ_{max}
639 sd); initial concentration (X_0) and corresponding standard deviation (X_0 sd); maximum concentration at the end of the product shelf life (X_m) and
640 corresponding standard deviation (X_m sd). Goodness of fit is shown by values of adjusted coefficient of determination (i.e., adjusted R2) and root mean
641 square error (RMSE) between the observed data and the $\log_{10}$ (cfu/g) h^{-1} predicted by the model.
642

Dataset label	T	Initial pH	Final pH	μ_{max}	μ_{max} sd	X_0	X_0 sd	X_m	X_m sd	Adjusted R^2	RMSE	Model selected
1	12	5.41	5.76	0.028	0.002	1.386	0.167	5.670	0.127	0.98	0.11	Baranyi no lag
2	12	6.15	6.85	0.045	0.003	2.991	0.158	7.510	0.206	0.92	0.39	Baranyi no lag
3	12	6.15	4.44	0.029	0.004	3.215	0.163	5.468	0.166	0.77	0.37	Baranyi no lag
4	4	5.78	5.85	0.005	0.001	2.784	0.214	-	-	0.31	0.58	Exponential
5	4	5.60	5.34	0.003	0.002	2.296	0.222	-	-	0.11	0.40	Exponential
6	4	5.51	5.63	0.007	0.001	2.454	0.168	-	-	0.59	0.45	Exponential
7	4	5.66	4.76	0.002	0.001	2.570	0.149	-	-	0.13	0.40	Exponential
8	12	5.60	5.50	0.036	0.004	2.139	0.308	8.051	0.252	0.95	0.39	Baranyi no lag
9	12	5.60	5.50	0.033	0.003	2.173	0.319	8.319	0.280	0.95	0.41	Baranyi no lag
10	4	5.60	5.90	0.011	0.001	1.700	0.103	7.079	0.296	0.98	0.17	Baranyi no lag
11	4	5.60	5.90	0.011	0.000	1.698	0.080	6.917	0.208	0.99	0.13	Baranyi no lag
12 Phase1 (t<168h) phase 2 (t<168h)	12	6.00	5.10	**0.054** **0.040**	**0.001** **0.004**	**1.777** **2.839**	**0.009** **0.168**	**3.069** **6.620**	**0.013** **0.070**	0.99	0.04	Multi phases Intermediate lag phase ~ 3 day
13 Phase1 (t<168h)	12	6.00	5.10	**0.053** **0.031**	**0.018** **0.007**	**1.931** **3.005**	**0.133** **0.367**	**3.262** **7.023**	**0.188** **0.234**	0.98	0.12	Multi phases Intermediate lag phase ~ 3 day

Dataset label	T	Initial pH	Final pH	μ_{max}	μ_{max} sd	X_0	X_0 sd	X_m	X_m sd	Adjusted R^2	RMSE	Model selected
phase 2 (t<168h)												
14	4	6.00	5.10	0.005	0.001	1.942	0.106	3.419	0.197	0.87	0.15	Baranyi no lag
15	4	6.00	5.10	0.004	0.001	1.930	0.099	3.495	0.268	0.88	0.15	Baranyi no lag
16	4	6.08	4.90	0.002	0.001	3.835	0.041	-	-	0.88	0.15	Exponential
17	6	6.08	4.75	0.006	0.004	3.762	0.227	4.746	0.329	0.46	0.19	Baranyi no lag
18	12	6.08	4.52	**0.025**	-	**3.890**	-	**5.468**	-	0.95	0.68	Baranyi no lag
19	4	6.15	5.24	0.010	0.001	1.952	0.135	4.676	0.096	0.97	0.14	Baranyi no lag
20	6	6.15	5.25	0.013	0.002	1.855	0.245	4.702	0.157	0.88	0.31	Baranyi no lag
21	12	6.15	5.26	0.031	0.008	1.986	0.350	5.059	0.210	0.87	0.33	Baranyi no lag
22	4	6.22	5.34	0.013	0.001	1.775	0.049	2.953	0.050	0.98	0.03	Baranyi no lag
23	6	6.22	5.14	0.015	0.009	1.786	0.282	2.949	0.121	0.52	0.28	Baranyi no lag
24	12	6.22	4.44	0.022	0.010	1.892	0.290	3.327	0.145	0.65	0.27	Baranyi no lag

643 *values in bold were predicted using the Gamma model and not calculated with the primary model

644

645 **Table 5**. Optimized parameters of the secondary models calculated taking into account the uncertainty over the μ_{max} values at 4 and 12°C.

Model	μ_opt log$_{10}$ (cfu/g) h^{-1}	Parameters (Augustin and Carlier, 2000)	Value
Gamma model Air	0.247±0.009	T_min	-2.7°C
		T_opt	37°C
Gamma model MAP	0.244±0.025	T_max	45.5°C
		pH_min	4.55
Gamma model MAP+Air	0.247±0.009	pH_opt	7.1
		pH_max	9.61

646

647

648 **Table 6**. Results showing the growth or not growth of *L. monocytogenes* (LM) after the achievement of the stationary phase by lactic acid bacteria

649 (LAB). Datasets 12 and 13 were not considered because LM showed a multi-phase growth.

Dataset label	Storage time when LAB reached stationary phase (days)	LAB concentration in the stationary phase (log_{10} cfu/g)	Growth of LM after LAB reached the stationary phase (δ log_{10} cfu/g)
1	9	8.63	<0.5*
2	5	7.92	*
3	6	8.90	*
4	7	8.79	-
5	9	8.90	-
6	9	8.78	-
7	8	8.81	-
8	>13	8.30	+0.7
9	>13	8.30	+0.7
10	>13	-	-
11	>13	-	-
14	13	8.90	+0.7
15	13	8.90	+0.7
16	8	6.80	-
17	8	7.82	-0.168
18	6	9.01	-
19	14	8.80	-
20	6	8.90	+1.0
21	3	9.00	+1.0
22	6	8.80	<0.3
23	6	8.90	<0.3*
24	3	8.80	<0.5*

650 *= large variations between replicates were observed; -= not determined because the stationary phase was not reached