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Development of a Prediction Software for the Growth Kinetics of Pseudomonas spp. in Culture Media using Various Primary Models

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Abstract

Background and Objective: *Pseudomonas* spp. are bacteria with the widest effects on food spoilage. These bacteria can be found in several environments such as soil and water. The major purpose of this study was to develop a software; by which, the growth behaviours of *Pseudomonas* spp. in culture media could be predicted.

Material and Methods: A total number of 509 bacterial data points of Pseudomonas spp. in culture media were collected from the ComBase database. Temperature and pH were used as the major prediction variables for the description of Pseudomonas spp. behaviours in culture media. Modified Gompertz, Baranyi and Huang models, the most commonly used models in predictive food microbiology to predict the count of microorganisms, were used as well. Fitting capability of each model was assessed and compared with other capabilities considering their statistical indices of the root mean square error, RMSE; coefficient of determination, R2; corrected Akaike information criterion, AICc; and Bayesian information criterion, BIC.

Results and Conclusion: Huang model provided better predictions with 0.951 of R^2 and 0.825 of *RMSE*, compared to those of traditionally used models. Prediction capability of the Huang model was assessed considering externally collected data from the ComBase database. Huang model in the validation process provided satisfactory statistical indices (bias factor = 1.027 and accuracy factor = 1.075). These results have revealed that Huang model can be reliably used as a model of describing the growth behaviours of *Pseudomonas* spp. Furthermore, developed software in this study includes significant potentials for predicting *Pseudomonas* counts in culture media.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

During storage and distribution, food decomposition caused by spoilage microorganisms significantly affects food quality and shelf-life [1,2]. Food spoiling is described as a process of changes in a product that renders it unfit for the ingestion [3]. Food spoiling is induced by physical and bio (chemical) interactions, as well as parasite activity. The underlying mechanisms are extremely complicated and difficult to uncover; therefore, they are still mysterious [4]. However, the microbiological proliferation of spoilage microorganisms and effects of their extracellular enzymes such as produced proteases, lipases and carbohydrates are primarily responsible for the loss of desired qualities and considerable product variations. At all phases of production (harvesting, processing and storage), food can be contaminated with a wide range of microbes, although only a small percentage of these microbes can grow into food products, causing major spoilage. Visible yeast or mould colonies, gas production, turbidity of liquids, discolouration and changes in distinctive taste and aroma are signs of microbial deterioration [5]. Microorganisms that contaminate food and cause foodborne diseases are studied in food microbiology. Microbes are present in the foods as foods are seldom sterile. Microbial loads are maintained in foods and their compositions significantly vary. Microorganisms are detected in raw material microflora, as well as animal slaughter and food harvesting, processing, storage and

distribution [6,7]. In a vast majority of cases, food is ingested with no problems or negative health consequences [7].

Food predictive microbiology is a complex scientific area that includes various principles and uses. Technically, predictive microbiology is a scientific area of food microbiology with the aim to statistically characterize microbial activity in food settings for the development of appropriate mathematical models. The mathematical simple implementation of a real system based on its highly significant aspects is described through mathematical equations [8]. Predictive models are useful tools for determining food shelf-life and safety, analysing hazards, establishing crucial control points and developing risk assessment programs. Traditional microbial counting techniques can be used to assess microbial loads in meals; however, these techniques are time-consuming and expensive, needing expert personnel. Furthermore, these techniques provide information only for the conditions; under which, the analysis was carried out. Therefore, use of classic enumerated techniques to assess growth of microorganisms under fluctuating environmental factors is not viable. In contrast, predictive models can forecast development of microbes in foods through processing and storage at actual time [9].

Duration to achieve a defined goal level under various temperature circumstances was the most critical parameter in predictive models used to characterize growth behaviours of spoilage bacteria. Modelling growth behaviours of *Pseudomonas* spp., one of the most common bacterial genera isolated from a variety of foods, is critical for addressing the best storage conditions for food products and predicting their shelf-lives. *Pseudomonas* spp., one of the most common microbes extensively identified from various foods, have already been investigated and simulated using certain prediction models [10]. No studies have been published in the literature comparing prediction capabilities of the predictive models by illustrating them in software. Because *Pseudomonas* spp. are bacteria that include the widest effects on food spoilage, behaviours of *Pseudomonas* spp. were studied using temperature and pH, which are important factors affecting microorganism behaviours. It is a big gap in the literature that software development to compare prediction capabilities of the models for the description of microorganism behaviours has not been carried out. Therefore, the major purpose of this article was to develop a software; by which, growth behaviours of *Pseudomonas* spp. in culture media could be predicted.

2. Materials and Methods

In this study, four major steps were carried out (Fig. 1), including i) growth data were collected, ii) primary and secondary modelling processes were carried out, iii) comparison of the performances of the models was carried out and iv) validation step and software development were completed. Detailed information on these four major steps were provided in the following subsections:

2.1. Data gathering

ComBase database (www.combase.cc) provides approximately 60000 bacterial data from research organisations and research papers. In this database, bacterial data are available with their specific characteristics and conditions, including food category, food name, temperature, pH, water activity, conditions and time, enabling us to classify microbial factors and responses. To describe growth behaviours of *Pseudomonas* spp. in culture media, 509 bacterial data points were collected from the ComBase database with their specific and individual information (time, temperature and pH) in Excel format.

2.2. Modelling

2.2.1. Primary models

Three various primary models of modified Gompertz [11], Baranyi [12] and Huang [13] models were selected using one-step modelling approach [14,15] for fitting of the growth data points from ComBase database as well as Eq. (1), Eq. (2) and Eq. (3), respectively:

$$y(t) = y_0 + (y_{max} - y_0).exp\left\{-exp\left[\frac{\mu_{max}.e}{(y_{max} - y_0)}.(\lambda - t) + 1\right]\right\} \quad \text{Eq.1}$$

$$y(t) = y_0 + \mu_{max}F(t) - ln\left(1 + \frac{e^{\mu_{max}F(t)} - 1}{e^{(y_{max} - y_0)}}\right) \quad \text{Eq.2}$$

$$y(t) = y_0 + y_{max} - ln(e^{y_0} + [e^{y_{max}} - e^{y_0}].e^{-\mu_{max}B(t)}) \quad \text{Eq.3}$$

F (t) and B(t) were the adjustment functions respectively described by Baranyi and Roberts [12] and Huang [13] in Eq. (4) and Eq. (5):

$$F(t) = t + \frac{1}{v}ln\left(e^{-vt} + e^{-\mu_{max}\lambda} - e^{(-vt-\mu_{max}\lambda)}\right) \quad \text{Eq.4}$$

$$B(t) = t + \frac{1}{4}ln\left(\frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda}}\right) \quad \text{Eq.5}$$

Where, t was the time (h), y(t) was the concentration of bacterial populations (log CFU·ml^{-1}) at time t, y_0 was the initial concentration of bacterial populations (log CFU·ml^{-1}), y_{max} was the maximum concentration of bacterial populations (log CFU·ml^{-1}), μ_{max} was the maximum specific bacterial growth rate (h^{-1}), λ was the duration of lag phase (h) and v was the increase rate of limiting substrate, assumed equal to μ_{max}.

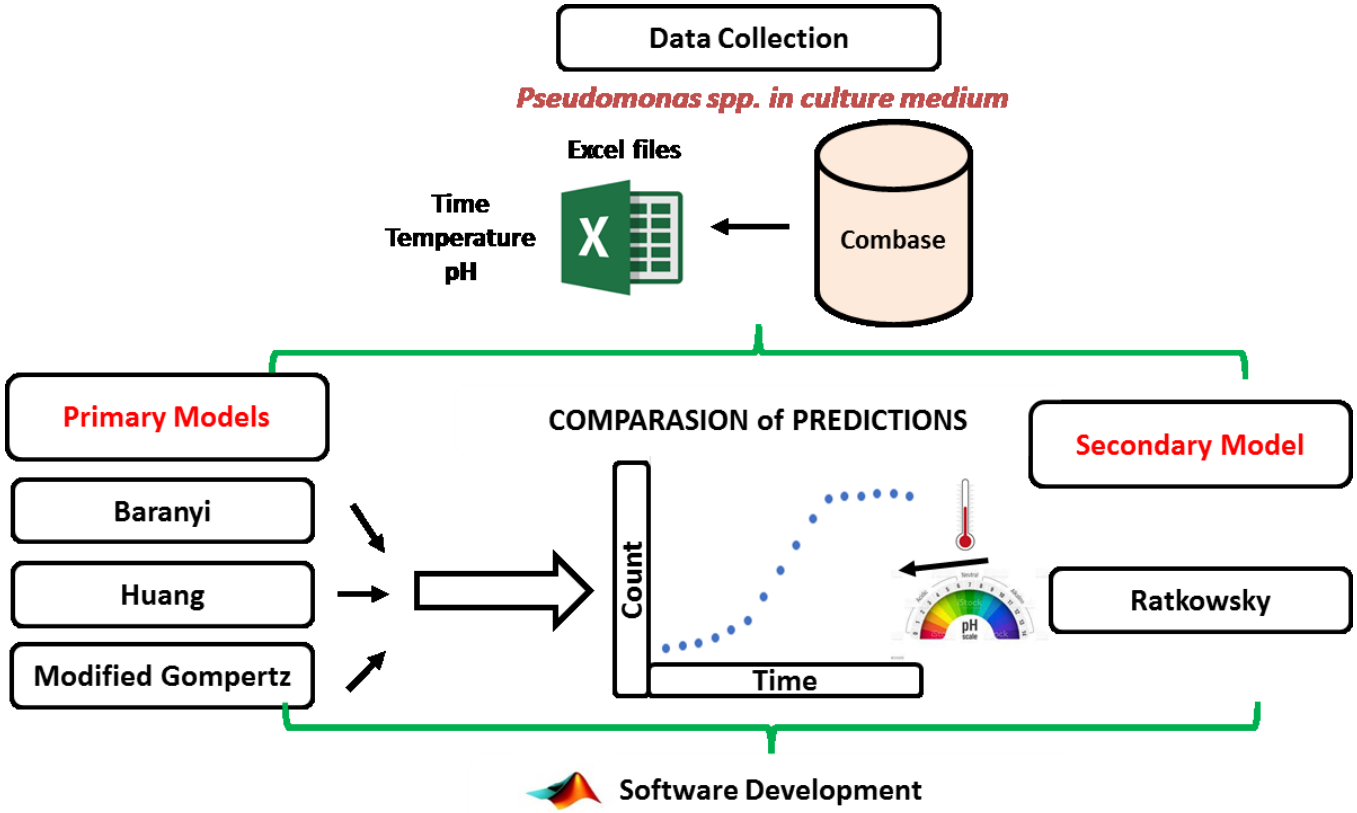

Figure 1. Steps for the development of prediction software to describe growth behaviours of *Pseudomonas* spp. in culture media

2.2.2. Secondary Models

Ratkowsky model [16] was used to assess effects of storage temperature and pH on μ_{max} as follows [Eq. (6)]:

$$\mu_{max} = b_1(\mathrm{T} - T_0)^2 \times (pH - pH_{\min}) \qquad \text{(Eq.6)}$$

Where, T was the storage temperature (°C), T_0 was the theoretical lowest bacterial growth temperature (°C), pH was the acidity of food product, pH_{min} was the theoretical lowest bacterial growth acidity, μ_{max} was the maximum specific bacterial growth rate (h^{-1}) and b_1 was the regression coefficient. Additionally, λ (lag phase duration) was described as a function of μ_{max} with respect to temperature using Eq. (7) [17]:

$$\lambda = \frac{b_2}{\mu_{max}(T,pH)} \qquad \text{Eq.7}$$

Where, b_2 was the regression coefficient, $\mu_{max}(T, pH)$ was the function of temperature and pH, leading to λ as a function of storage temperature and pH.

2.3. Comparison of the goodness of fit

Comparison of the performances of models was carried out using root mean square error (*RMSE*), coefficient of determination (R^2), corrected Akaike information criterion (*AICc*) and Bayesian information criterion (*BIC*) using Eqs. (8), (9), (10) and (11), respectively:

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(x_{obs} - x_{fit})^2}{n - s}} \qquad \text{Eq. 8}$$

$$R^2 = 1 - \left(\frac{SSE}{SST}\right) \qquad \text{Eq. 9}$$

$$AIC_c = (n)\ln\left(\frac{SSE}{n}\right) + 2(s+1) + \frac{2(s+1)(s+2)}{n-s-2} \qquad \text{Eq. 10}$$

$$BIC = n\ln\left(\frac{SSE}{n}\right) + s\ln(n) \qquad \text{Eq. 11}$$

Where, x_{obs} was the experimental bacterial growth, x_{fit} was the fitted value, n was the number of experiments, s was the number of parameters of the model, SSE was the sum of squares of errors and SST was the total sum of squares.

2.4. Validation and software development

Prediction capability of the best primary model was assessed with the independent bacterial growth data of *Pseudomonas* spp. in culture media collected from ComBase database. Comparison was carried out for each method corresponding the bias (B_f) and accuracy (A_f) factors in Eqs. (13) and (14), respectively:

$$B_f = 10^{\frac{\sum_{i=1}^{n}\log(x_{pred}/x_{obs})}{n}} \qquad \text{(Eq. 13)}$$

$$A_f = 10^{\frac{\sum_{i=1}^{n}\left|\log(x_{pred}/x_{obs})\right|}{n}} \qquad \text{(Eq. 14)}$$

Where, x_{pred} referred to the predicted count of microorganisms (log CFU·ml^{-1}), x_{obs} referred to the experimental count of microorganisms (log CFU ml^{-1}) and n referred to the number of experimental data points. The acceptable prediction zone (*APZ*) procedure might be used to assess the overall validated performances of all types of the predictive models [18]. A prediction was considered as desirable within the *APZ* technique when the residual (observed-predicted) was in *APZ* values from -1 log CFU·ml^{-1} (fail-safe) to 0.5 log CFU·ml^{-1} (fail-dangerous). To show validation results visually, prediction software was developed in this study using traditionally used models (modified Gompertz, Branyi and Huang models). All the processes were carried out using Matlab 9.10.0.1710957 (R2021a) Software (MathWorks, Natick, MA, USA).

3. Results and Discussion

Record ID, temperature (°C), pH and time (h) for *Pseudomonas* spp. in culture media were collected from ComBase database and the histograms of data are present in Fig. 2. Totally, 509 bacterial data points of *Pseudomonas* spp. in culture media were used. Technically, the maximum specific growth rate (μ_{max}), which is one of the most important growth kinetic parameters, can be modelled with respect to environmental factors such as temperature and pH. Temperature plays key roles in affecting microbial growth behaviours in foods [19]. In this study, temperature variable ranged 0-25 °C. Other important factor that is directly affecting the growth behaviours of microorganisms is pH. In this study, pH ranged 4.01-7.40 (Fig. 2).

The parameters derived from the primary and secondary models for the growth behaviours of *Pseudomonas* spp. in culture media are shown in Table 1. The goodness-of-fit of the traditional models (modified Gompertz, Baranyi and Huang models) for the estimation of the bacterial data points of *Pseudomonas* spp. in culture media was assessed via analysing their statistical indices (R^2, *RMSE, AICc*, *BIC* and *pAPZ*) (Table 2). The R^2 values from each of the conventional models ranged 0.934-0.951 and the *RMSE* values ranged 0.825-0.841 for the description of data points of *Pseudomonas* spp. in culture media. These results showed that the Huang model provided a better fitting performance than that the modified Gompertz and Baranyi models did. In general, -29.1 < *AICc* < -15.6 and -25.2 < *BIC* < -8.8 were achieved for the modified Gompertz and Baranyi models, respectively, while *AICc* = -33.4 *and BIC* = -29.5 were achieved for the Huang model, demonstrating that the Huang model yielded excellent prediction capability.

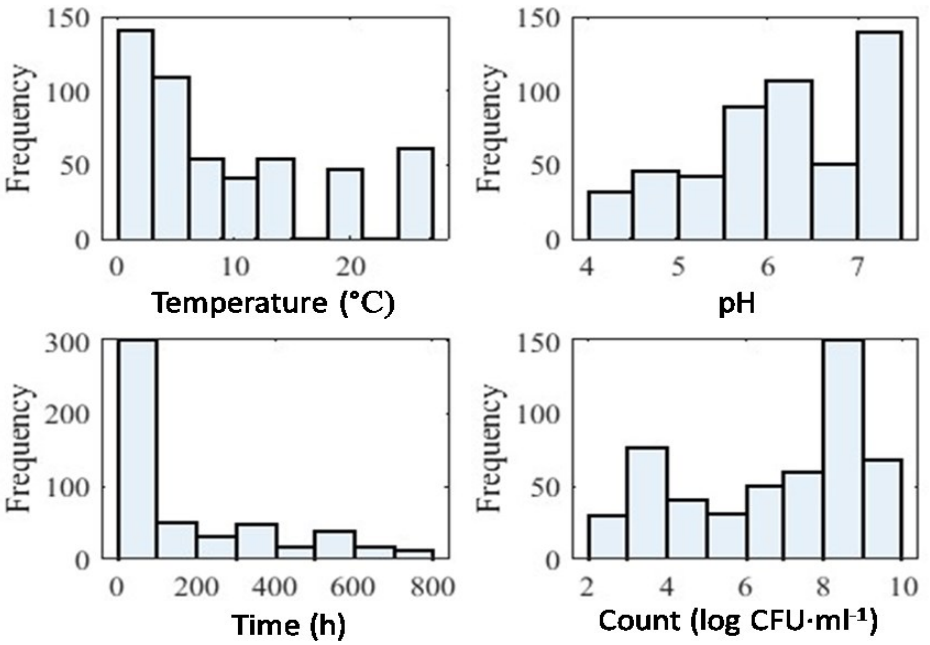

Figure 2. Histograms of the variables for a) temperature (°C), b) pH, c) time (h) and d) initial microbial count (log CFU ml^{-1})

Table 1. Parameters derived from the primary and secondary models

Models	y_0 (log CFU·ml^{-1})	y_{max} (log CFU·ml^{-1})	T_0 (°C)	b_1	b_2	pH_{min}
Baranyi	3.26 ± 0.013	8.72 ± 0.07	-5.38 ± 0.20	0.020 ± 0.001	0.66 ± 0.54	3.32 ± 0.07
Huang	3.34 ± 0.12	8.78 ± 0.07	-5.35 ± 0.21	0.010 ± 0.001	0.49 ± 0.44	3.37 ± 0.07
Modified Gompertz	2.84 ± 0.29	8.90 ± 0.08	-5.30 ± 0.22	0.020 ± 0.001	0.22 ± 0.92	3.43 ± 0.06

Table 2. Comparison of the performances of the models (modified Gompertz, Baranyi and Huang model) for the fitting data

Models	R^2	*RMSE*	*AICc*	*BIC*
Baranyi	0.942	0.835	-29.1	-25.2
Huang	0.951	0.825	-33.4	-29.5
Modified Gompertz	0.934	0.841	-15.6	-8.8

In lag phase, organoleptic characteristics did not change because the number of microorganisms was relatively constant [1]. In fact, temperature and pH are important variables affecting lag phase duration. Figure 3a shows effects of temperature and pH on the lag phase. There are inverse relationships between the two variables (temperature and pH) and the lag phase. In other words, increasing temperature from 0 to 25 °C and pH from 5.0 to 7.0 decreased duration of the lag phase. In contrast, a reverse trend was reported for the specific growth rate, meaning that direct relationships occurred between the two variables (temperature and pH) and specific growth rate (Fig. 3b). Additionally, B_f = 1.027 and A_f = 1.075 belonged to the Huang model. A B_f factor of 1 revealed no structural deviation of the model. The B_f factor of 1.027 indicated that the model overestimated a maximum 2.7%, whereas an A_f factor of 1.075 showed that the predicted value was maximally 7.5% different (either smaller or larger) from the value. These results demonstrated that the Huang model could safely be used because the error rates were relatively small.

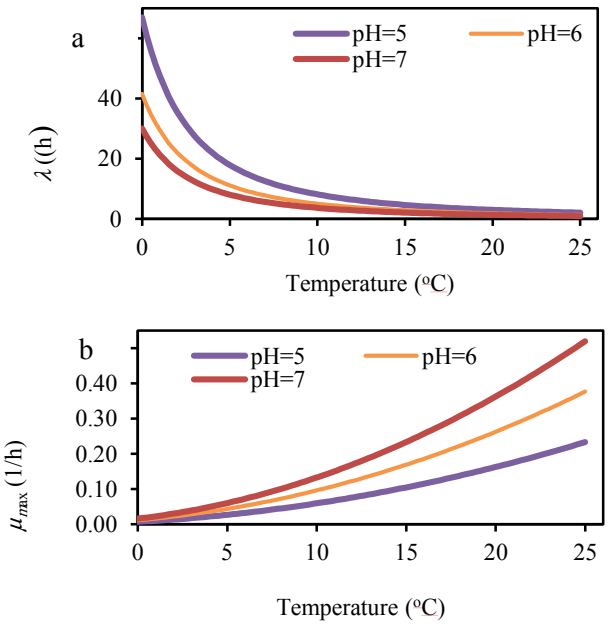

Figure 3. Effects of temperature and pH on a) specific maximum growth rate and b) lag phase duration

Acceptable prediction zone (*APZ*) procedure may be used to assess the overall validation performance of all types of prediction models [18]. A prediction is reported as desirable within the *APZ* technique while the residual (observed-predicted) is included in *APZ* from-1 log CFU·ml^{-1} (fail-safe) to 0.5 log CFU·ml^{-1} (fail-dangerous). No standards are available in predictive microbiology for the classification of model performances. In the US education system, an established performance criterion is described as a test score of 70% correct answers includes the minimum value for the classification of acceptable performances. This established criterion is used in *APZ* method. Thus, when the proportion of residuals in *APZ* (*pAPZ*) is 0.7, the model is classified as a provider of acceptable predictions. In the present study, *pAPZ* for the Huang model was 0.82, simply meaning that the Huang model included an acceptable prediction performance (82%) (Fig. 4).

Fitting and validation processes indicate that the Huang model can be used to describe growth behaviours of *Pseudomonas* spp. in culture media as a function of temperature and pH. In the current study, a software was developed for the prediction of microbial behaviours of *Pseudomonas* spp. in culture media. A schematic of the developed software and its compartments is shown in Fig. 5. Detailed information on this software and a short video about how to use the software are provided in GitHub platform with "Microbial-behaviour-of-*Pseudomonas*-spp.-in-culture-media" repository, accessible at https://github.com/ftarlak/Microbial-behaviour-of-Pseudomonas-spp.-in-culture-medium

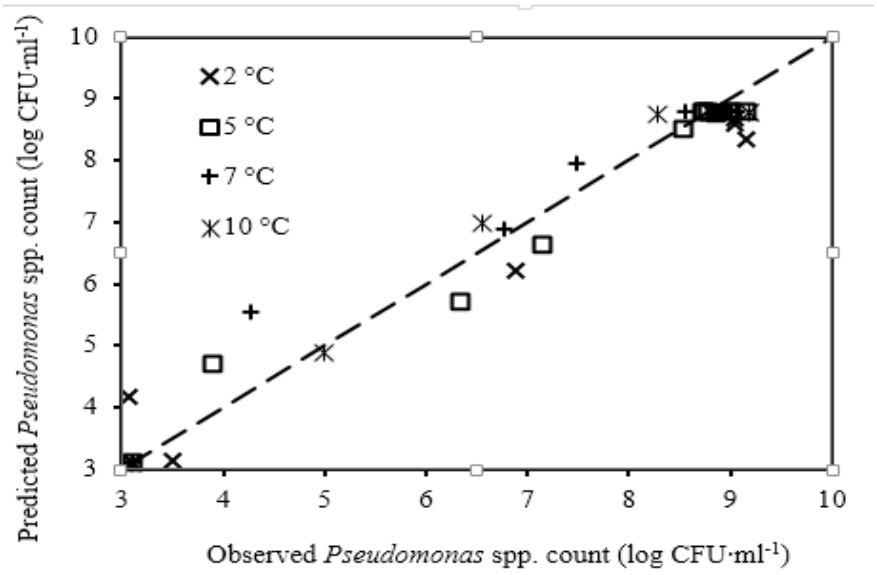

Figure 4. Observed and predicted counts of *Pseudomonas* spp. in culture media using Huang model for external validation

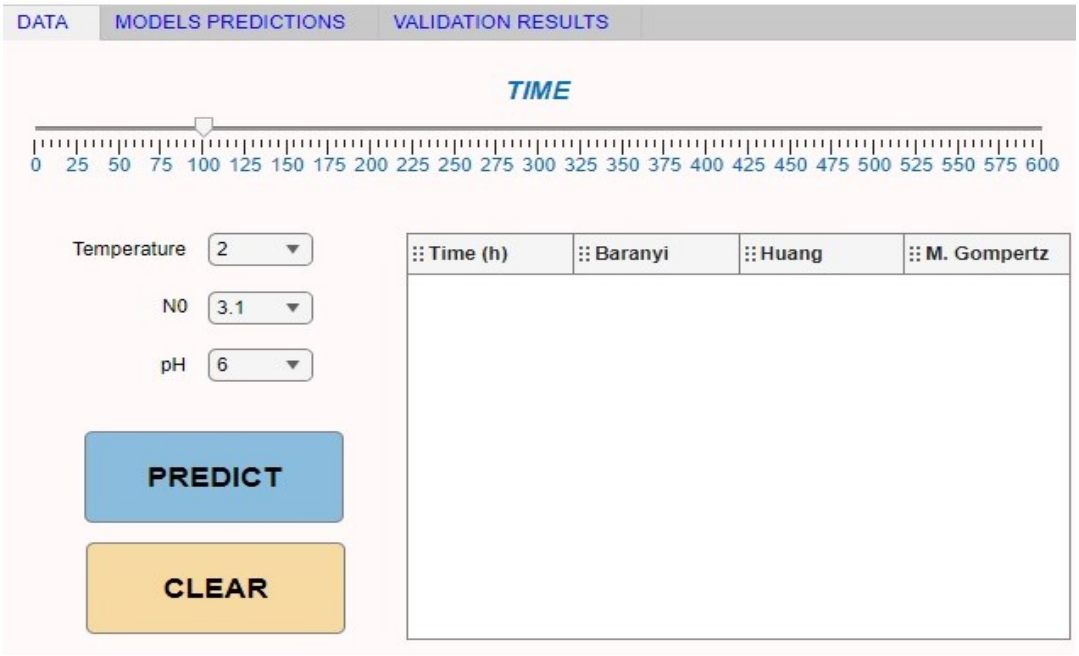

Figure 5. Illustration of the developed software and its compartments

4. Conclusion

In this study, prediction abilities of the modified Gompertz, Baranyi and Huang models as frequently used models in predictive food microbiology to predict the count of microorganisms were assessed and compared for quantitative description of *Pseudomonas* spp. in culture media. Temperature and pH were used as the major prediction variables considering one-step modelling approach for the estimation of *Pseudomonas* spp. behaviours in culture media. Each of the models provided satisfactory prediction ability; however, Huang model provided the best statistical indices (R^2 = 0.951, *RMSE* = 0.825, *AICc* = -33.4, *BIC* = -29.5). Results revealed that the Huang model could reliably be used to assess growth behaviours of *Pseudomonas* spp. in culture media. Furthermore, developed software in this study included significant potentials for predicting *Pseudomonas* counts in culture media.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

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توسعه نرم‌افزار پیش بینی سینتیک رشد گونه‌های *سودوموناس* در محیط‌های کشت با استفاده از مدل‌های اولیه گوناگون

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واژگان کلیدی
- محیط‌های کشت
- سینتیک رشد
- میکروبشناسی پیشگویانه
- مواد غذایی

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چکیده

سابقه و هدف: گونه‌های *سودوموناس* باکتری‌هایی هستند که بیشترین اثر را در فساد مواد غذایی دارند. این باکتری‌ها در محیط‌های بسیاری مانند خاک وآب یافت می‌شوند. هدف اصلی این مطالعه توسعه نرم افزاری است که با آن رفتارهای رشد گونه‌های سودوموناس در محیط‌های کشت پیش بینی شود.

مواد و روش ها: در مجموع ۵۰۹ داده باکتریایی گونه‌های *سودوموناس* در محیط‌های کشت از پایگاه داده ComBase گردآوری شد. درجه حرارت و pH متغیرهای عمده پیش بینی رفتارهای گونه‌های *سودوموناس* در محیط‌های کشت بودند. مدل‌های اصلاح شده Gompertz، Gompertz و Huang متداول‌ترین مدل‌های مورد استفاده در پیش بینی میکروبیولوژی مواد غذایی برای پیش بینی تعداد میکروارگانیسم‌ها نیز مورد استفاده قرار گرفتند. قابلیت برازش هر مدل ارزیابی و با توجه به سایر شاخص‌های آماری خطای ریشه میانگین توان‌های دوم ($RMSE$[1])؛ ضریب تعیین (R^2)؛ معیار اصلاح شده اطلاعات آکائیکه ($AICc$) و معیار اطلاعات بیزی (BIC) مقایسه شد.

یافته‌ها و نتیجه‌گیری: در مقایسه با سایر مدل‌های معمول مورد استفاده، مدل Huang پیش بینی بهتری با R^2 برابر ۰/۹۵۱ و $RMSE$ برابر ۰/۸۲۵ را به‌دست داد. با در نظر گرفتن داده‌های گردآوری شده خارجی از پایگاه داده ComBase توانایی پیش بینی مدل Huang مورد بررسی قرار گرفت. در فرایند اعتبار سنجی، مدل Huang شاخص‌های آماری رضایت‌بخشی را به‌دست داد (فاکتور اریبی[2] برابر ۱/۰۲۷ و فاکتور دقت[3] معادل ۱/۰۷۵). ابن نتایج نشان داد که مدل Huang را می‌توان به‌طور قابل اعتمادی به‌عنوان مدلی برای توصیف رفتارهای رشد گونه‌های *سودوموناس* در محیط‌های کشت استفاده کرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1] Root-mean-square deviation
[2] bias factor
[3] accuracy factor