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Research/Technical Note

Exploring a Multilayer Perceptron Model on Dual-Species Biofilm Growth Data

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Abstract

Salmonella Typhimurium in ready-to-eat leafy salads is a well-known threat in food production. The aim of this study was to design an approximation multi-layer perceptron model (MLP) in order to predict the final population of *S.* Typhimurium in dual-species biofilms. Previously collected data (n=48; log CFU/cm^2) of various isolates from either rocket or spinach salads, which grew together with *S.* Typhimurium to form biofilms, were used as attributes for the development of the two models. The target (output) variable was the final population of *S.* Typhimurium in both models of the rocket and spinach datasets. For the rocket isolates, the highest efficiency (learning epoch=1000, learning rate=0.001, and momentum value=0.1) was achieved with a shallow MLP with one hidden layer of 3 neurons, and a correlation coefficient 75% (RMSE<0.23). For the spinach isolates, similar results were found (correlation coefficient 76%), although with higher error (RMSE <0.35), and a hidden layer with 4 neurons, which might indicate more complex microbial interactions in this leafy vegetable. Further research is needed to train a model with a large data set of dual-species biofilms along with more input variables. Such neural networks could be helpful in efforts to control *Salmonella* diseases linked to the consumption of contaminated fresh salads.

Keywords

Rocket; Spinach; *Salmonella* Typhimurium; Dual-Species Biofilm; Neural Network; Model

1. Introduction

The ability of *Salmonella* Typhimurium (ST) to attach to rocket and spinach leaves has been well-documented for over a decade now [1-3]. ST can form biofilms along with the native biota of leafy vegetables, enhancing its survival through the processing of leafy greens, and making it hard to eradicate. Mixed culture biofilms have been shown to be more resistant to common disinfectants [4-7], thus posing a food safety hazard, especially in the ready-to-eat (RTE) food category.

Biofilm data are usually composed of a mix of continuous (log CFU/cm^2 - gr) data, such as those of microbial growth of strains, or discrete (data for the number of biological repeats, type of substrate used in the study, etc. These kinds of datasets are usually analyzed using ANOVA (analysis of variance) to identify significant differences in the continuous variables, followed by a series of pair-wise comparisons (i.e., Tukey's test) to identify the specific groups of data where the significant differences are found. This type of analysis requires that the dataset be normally distributed.

Neural networks used in food microbiology were found to perform reasonably well and be suitable for practical applications, such as predicting growth kinetic parameters of food-

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borne pathogens [8]. To develop a useful formula for predictions, once the artificial neural network (ANN) is trained for a particular dataset to find optimal weights, by estimating several input and output parameters. Furthermore, ANNs can be constructed without any assumptions about the relationship between the input and output. Unlike analytical approaches, ANNs require no explicit mathematical equation and no limiting assumptions of normality or linearity of data [9].

Calculating a specific microorganism's population in a dual-species biofilm is a daunting procedure and experimentally challenging. The exploratory process described here, used a neural network to predict the final population of *S.* Typhimurium in dual-species biofilms. Data consisted of various isolates recovered from either rocket or spinach salads and cultured together with *S.* Typhimurium to form dual-species biofilms on stainless steel coupons at 20°C, immersed in rocket or spinach extract. These data were used to train a simplified multi-layer perceptron model with the software package Neural Designer v.7 [10].

2. Methods

Among the available workflows in Neural Designer, approximation modeling was selected, as the objective was to estimate the value of y given the value of x—that is, to address a regression problem. The neural network learns from the patterns represented in the given dataset, consisting of instances (different rocket and spinach isolates) of input variables.

The input layer represents the independent input variables (predictors) of the approximation model. In this analysis, 5 inputs were used - the inoculum population of native isolate (in - log CFU/ml), type of extract (r - rocket / s - spinach), final population of native biota (t) and *S.* Typhimurium (s) in dual-species broth biofilms (b_fn_t / b_fn_s - log CFU/ cm^2), and final population of native biota in dual-species biofilms formed on stainless steel immerged in rocket or spinach extract (r_fn_t or s_fn_t - log CFU/cm^2), respectively. The hidden layer is where the relationships between the input and output layers are mathematically explored and eventually optimized for a specific model. The output layer represents the dependent target variable, the final ST population in either rocket or spinach extract biofilms (r_fn_s or s_fn_s - log CFU/cm^2). A separate model was built for each broth extract, considering the final ST population in dual-species biofilms as the response variable to the same input variables (n=48). The raw data used to build the model were retrieved from Doulgeraki et al. 2014 [11]. Table 1 shows the descriptive statistics of all input and target variables of this analysis.

Supervised learning with the implementation of the backpropagation algorithm was applied since a target variable was used, and this is one of the most common MLP training methods [12]. A complete dataset of 48 values was randomly split to generate the training (62.5%), the selection (19%), and testing (19%) subsets. The training occurs in two stages: the forward and the backward. In the forward stage, the predictive weights of the model input variables are calculated and transmitted through the layers until they reach the output layer.

In the backward stage, an error signal is calculated after comparing the output of the model values to the expected ones. The learning rate of 0.001 (the change in the value of weights in each iteration) and the momentum of 0.1 (speed of the learning process) were used by default, in 1000 learning epochs. The training phase of the model is an iterative process, each time optimizing the previously calculated weights, minimizing the error until a level of precision is accomplished.

Table 1. *Descriptive statistics of independent (input) and dependent (target) variables.*

Parameter ID	Mean	Deviation	Minimum	Maximum
in_native	5.87	0.54	4.38	6.83
in_ST	NA	NA	5.83	5.89
*b_fn_t	6.15	0.51	5.0	7.22
*b_fn_s	4.60	0.52	2.69	5.10
r_fn_t	6.25	0.57	5.02	7.40
s_fn_t	7.07	0.39	5.61	7.55
r_fn_s	5.77	0.68	4.13	7.04
s_fn_s	6.71	0.74	4.19	7.55

*Variables not selected for the final model configuration

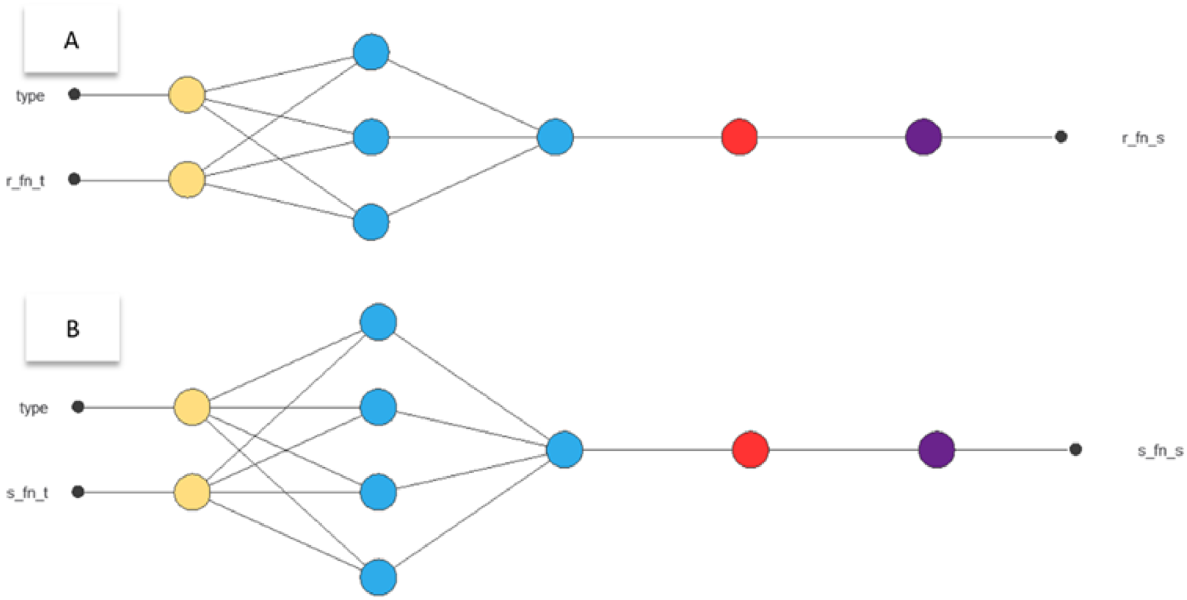

Figure 1. *Neural network models generated with rocket (A) and spinach (B) dual-species biofilm growth data.*

3. Results and Discussion

The optimized models (Figure 1) generated an R^2 coefficient of predicted versus real data, equal to 0.75 and 0.76, for rocket and spinach data, respectively, which are within the expected, though lower limits (≥ 75%) of ANN structure [13]. The model's mean squared errors ranged from 0.10 to 0.23, and from 0.11 to 0.35 for rocket and spinach, respectively.

In the initial data collection, there were more rocket isolates tested in biofilm growth, thus more data points for the ANN model, as compared to the spinach extract, and this could partially explain the lower training errors obtained in the rocket model. Overall, the growth of rocket isolates was strongly correlated with ST growth ($R^2 = 0.79$) in spinach and rocket extract ($R^2 = 0.92$), whereas the spinach isolates' growth showed poor correlation with ST growth in both extracts ($R^2 < 0.2$).

In both MLP models, the two most important inputs were the final populations of native biota in dual-species biofilms and the type of extract. These variables were included in the final model design, in contrast to native or ST populations in biofilms formed in broth, which were not found to be important for the regression analysis. This finding is consistent with microbiological principles, since a nutrient broth environment is considered to be very different than a food-derived broth extract [2]. The influence of specific elements found in spinach, such as zinc, on biofilm formation has been shown earlier [14].

4. Conclusions

Overall, this exploratory analysis of biofilm growth data and simple MLP models seems promising for predicting the *Salmonella* Typhimurium population when knowing the total microbiota population in a mixed culture biofilm. An approximation model performed adequately well and solved the prediction of a pathogen's growth as a regression-like problem. It is important to mention that the inoculum level of both isolates and ST was about 5 log cfu/cm^2, which sets the operational limits of this MLP model.

Further analysis is needed with larger datasets to configure the current model's parameters. Although we assumed linear relationships between input and output, this model is not representative of the implicit relationships among native and pathogenic biota. A larger dataset could dramatically change the number of hidden layers and the neurons in each layer, leading to deeper models, but at the same time, would minimize the risk of underfitting the data. As a result, capturing the problem's complexity with a higher number of input variables would help us develop better predictive models that would support food safety researchers and producers.

Abbreviations

ST	*Salmonella* Typhimurium
RTE	Ready to Eat
ANN	Artificial Neural Network

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Author Contributions

Kyriaki Chatzikyriakidou: Data Curation, Methodology, Software, Visualization, Writing – original draft

Christina Kamarinou: Investigation**,** Writing – review & editing

Agapi Doulgeraki: Conceptualization, Funding acquisition, Investigation**,** Resources, Supervision, Writing – review & editing

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Conflicts of Interest

The authors declare no conflicts of interest.

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