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Short Communication

Application of Artificial Neural Networks on growth prediction of *Staphylococcus aureus* in milk

[1*]Orawan C., [1]Panwadee, S. and [2]Bandit, S.

[1]Division of Microbiology, Department of Science, Faculty of Liberal Arts and Science, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand
[2]Department of Teacher Training in Mechanical Engineering, Faculty of Technical Education, King Mongkut's University of Technology North Bangkok, Bangsue, Bangkok 10800, Thailand

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Abstract

Staphylococcus aureus is the most frequently occurring major pathogen of cow mastitis and also predominant species in Staphylococcal food poisoning outbreak. Prediction of *S. aureus* growth in milk by using artificial neural network (ANN) was investigated. Input parameters consisting of temperature (25-40°C), pH (5-8), shaking speed (50-120 rpm) and initial cell concentration (10^1 to 10^4 CFU/ml) were randomly combined to obtain culture conditions. Thirty data sets were used for training and optimization of program learning and 10 data sets were used for ANN prediction. The results exhibited that growth prediction had a relative error at 8.99%. Validation was carried out and the relative error was obtained at 10.95%. Thus, the use of ANN modeling technic can be used to predict bacterial growth in the complex effects of environmental variable conditions in liquid food.

Introduction

Staphylococcus aureus is the predominant species involved in staphylococcal food poisoning outbreaks and also considered the most frequently occurring major pathogen of cow mastitis (Leitner *et al*., 2003). Enterotoxin producing *S. aureus* are an important cause of foodborne illness. Enterotoxins have been associated with foodborne diseases, with *Staphylococcus* enterotoxin A and D being the most frequently associated (Oliveira *et al*., 2011). Commonly, food containing the pre-formed enterotoxin is often normal in odors, appearance and taste. It has been reported that population of *S. aureus* usually need to reach 5-6 log CFU/g either at the end of exponential phase or during stationary phase of growth before detectable amount of enterotoxins would be produced (Fujikawa and Morozumi, 2006). Exposure of raw milk to abuse temperatures during transportation and storage may have been underlying contributing factor that permitted *S. aureus* growth and subsequent staphylococcal enterotoxin production in the contaminated milk.

Growth predictive model are currently accepted as informative tools that assist in rapid and cost-effective assessment of microbial growth for risk assessment and education purposes. More recently, predictive microbiology has been used to forecast the growth of spoilage microorganisms in order to study the shelf life of a food product. Improvement of food quality and safety required the development of appropriate tools to predict microbial growth. Thus mathematical models that can successfully predict microbial growth are needed to adequately describe the changing conditions generally associated with the manufacture and storage of foods. Artificial neural networks (ANNs) are information processing systems that consist of many interconnected processors, called neurons, resembling the elementary principles of the nervous system (Ramos-Nino *et al*., 1997). Artificial neural networks have been employed in recent years as an alternative to conventional regression models, due to their ability to describe highly complex and non-linear problems in many fields of science (Hajmeer *et al*., 1997). ANNs normally has no restriction on the type of relationship between the growth parameters (input patterns) and the desired output, whereas regression-based models require the order of the model to be stated before hands. In contrast, ANNs directly explored the knowledge contained input-output patterns by adjusting the parameters of the non-linear ANN topology, as the input output patterns are repeatedly presented to the network (Ham and Kostanic, 2001). When the system is supervised using an appropriate training data set, it can then be used to predict growth kinetic values

*Corresponding author.
Email: *faasowc@ku.ac.th*
Tel: +66-34-281-105 ext 7668; Fax: +66-34-351-402

for different growth conditions within the initial experimental range. Thus prediction of enterotoxin A producing *S. aureus* growth in milk by using artificial neural network (ANNs) was investigated.

Materials and Methods

Microorganism and medium

Staphylococcus aureus TISTR029, an enterotoxin A producing strain, was purchased from culture collection of Thailand Institute of Scientific and Technological Research. The strain was cultured in Nutrient broth at 37°C with shaking at 120 rpm.

Inoculum preparation

For the preparation of *S. aureus*, the strain was inoculated in flask with 100 ml of Nutrient broth, incubated at 30°C for 18 h. After that cell suspension was collected by centrifugation at 10,000 rpm, 4°C for 15 min. Cells were washed twice with 0.01 M sterile phosphate buffer, pH 7.5. Then cells were re-suspended by using 0.85% normal saline in order to prepare cell suspension at standard McFarland no. 0.5 with approximate cell density at 1.5×10^8 cell/ml.

Experimental design

Lag times (λ) as affected by temperature, pH, shaking speed and initial cell concentration obtained from 30 data sets were used for ANNs training. Optimization of prediction was performed by adjusting momentum value and learning rate in order to obtain the minimum error. Other ten separated data sets were used for lag time prediction. For validation, 10 speculated culture conditions were input to ANN model then the predicted lag times were obtained. After that, the experiments were done according to the input culture conditions and the experimental lag times were obtained.

Experimental procedure

The ANN model was developed by Visual Basic Program. The network consists of three layers, i.e. the input layer, the hidden layers and the output layers as shown in Fig. 1. The input layer consists of temperature (25-40ºC), pH (5-8), initial cell concentration (10^1 - 10^4 cell/ml) and shaking speed (50-120 rpm). The hidden layers are used to adjust the optimum computation weight of each node connected between the input layer and the output layer. The output layer is the estimated lag time (λ) of *S. aureus*. Combination among all inputs was completely randomized resulting in 100 culture conditions. After that, thirty conditions from 100 culture conditions were randomly selected for growth monitoring and

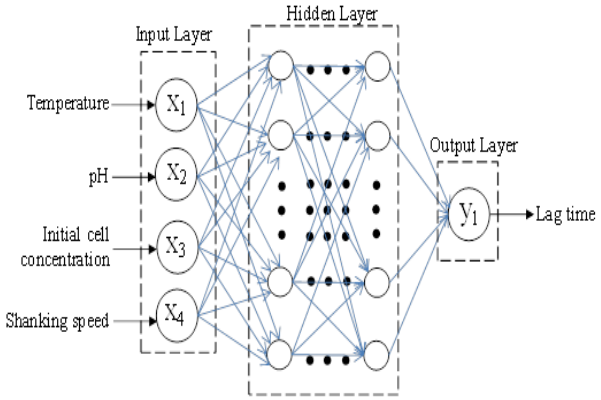

Fig. 1. A schematic of neural network with four parameters of the input layer, the hidden layer and the output layer

ANN training. Other 10 culture conditions were selected for ANN prediction. After inoculation of *S. aureus* into sterilized cow milk, samples were taken every 2 h. Then standard plate count was carried out and the plates were incubated at 30°C for 24-48 h. The cell numbers were plotted versus incubation time and the lag time (λ) was determine by graphical methods (Swinnen, 2004).

Results and Discussion

Training of ANN model

Table. 1 showed data sets of experimental lag time used for ANN training. Learning parameter (0.1-1.0) and momentum (0.1-1.0) were optimized in order to obtain the minimum error. The result exhibited that learning parameter at 0.9 and momentum at 0.5 gave the minimum error after running program for 500 cycles (data not shown).

Prediction of lag time by ANN

After training the model, other 10 data sets obtained from the experiments were used for lag time prediction. The result revealed that the prediction has relative error at 8.99% (Table. 2). The prediction of the lag time poses more problems for the models than other parameters since it depends on several factors, such as the physiological stage and size of the inoculum (Ross *et al*., 2000). The values obtained in this study are within the range described by other authors. In several publications, ANN models have been chosen over others, despite the fact that these models are more complex, because they produce lower error of prediction values (Hajmeer *et al*., 1997; Garcia-Gimeno *et al*., 2002, 2003).

Validation of ANN prediction of S. aureus *lag time*

Ten speculated conditions for *S. aureus* cultivation were input to ANN model to predict lag time. After

Table 1. Experimental lag time *S. aureus* for ANN model development

Temp. (°C)	Shaking speed (rpm)	Initial cell (cell/ml)	pH	lag time (min)
27	102	7.0×10^{1}	5	72
27	82	2.08×10^{3}	5.9	78
28	90	2.19×10^{4}	6.1	84
28	80	3.2×10^{3}	5.5	96
28	80	3.9×10^{3}	6.5	108
29	111	2.12×10^{3}	6.8	60
30	50	2.2×10^{2}	7.5	84
30	60	1.50×10^{2}	7.5	102
30	82	6.10×10^{2}	5.7	60
30	96	1.58×10^{3}	7.3	62
30	100	1.0×10^{2}	7	77
30	120	8.3×10^{2}	6.4	60
31	85	1.36×10^{3}	6	48
32	58	9.3×10^{2}	5.9	51
32	70	1.29×10^{3}	6.2	58
33	85	1.7×10^{2}	7.9	53
33	97	1.28×10^{3}	7.2	58
34	100	1.0×10^{2}	7	51
34	112	8.0×10^{2}	6.9	48
35	90	7.0×10^{2}	7.5	58
35	117	1.93×10^{3}	6.2	53
36	81	2.26×10^{3}	6.9	36
37	52	1.17×10^{3}	5.8	42
37	82	1.23×10^{3}	7.9	45
37	94	5.6×10^{2}	6.7	42
37	103	1.33×10^{3}	5.8	46
37	120	2.1×10^{2}	8	42
38	94	8.7×10^{2}	6.2	48
38	96	1.17×10^{3}	5.3	41
38	102	2.14×10^{4}	7.9	36

Table 2. Estimation errors for lag time prediction by Artificial Neural Network model

Input parameter				Prediction Lag time (min)	Experimental Lag time (min)	Relative error (%)
Temp (°C)	pH	Initial Cell (cell/ml)	Shaking speed (rpm)			
38	6.2	8.70×10^{2}	94	43.44	48	9.50
37	7.9	1.23×10^{3}	82	45.15	45	0.32
37	5.8	1.17×10^{3}	52	44.10	42	5.01
31	6.0	1.36×10^{3}	85	53.27	48	10.98
30	6.4	8.30×10^{2}	120	52.18	60	13.03
36	6.9	2.26×10^{3}	81	44.94	36	24.84
30	5.7	6.10×10^{2}	82	58.91	60	1.81
27	5.9	2.08×10^{3}	82	85.37	78	9.45
29	6.8	2.12×10^{3}	111	63.89	60	6.48
32	6.2	1.29×10^{3}	70	53.06	58	8.52
					Average	8.99
					S.D.	6.84

that the experiments were carried out according to the input culture conditions then the experimental lag times were obtained. The validation result exhibited that the prediction had an average of relative error at 10.95% (Table. 3). Several authors indicated that ANN models produce better estimations of kinetic parameters than other models such as response surface model (Hajmeer *et al.*, 1997). In the studies conducted by García-Gimeno *et al.* (2002, 2003) into *L. plantarum* and *E. coli* O157:H7, respectively, the ANN models were chosen instead of response surface model because of its lower standard error of prediction, despite the fact that the ANN models had a greater degree of complexity. Hajmeer *et al.* (1997) reported that an ANN for prediction of *Shigella flexneri* had low error values (4%-12% mean absolute relative error) but with considerable degree of complexity (142 parameters).

The possibility of finding out the development of foodborne pathogens in foods by predictive microbiology, and resulting the safety and spoilage of product to a certain level of microorganism would allow us to estimate risk of toxin production and the shelf life of different products. However, the model should include microorganism behavior data to estimate realistic growth and shelf life duration. Furthermore, if the model is embedded into an electronic device such as a time-temperature

Table 3. Estimation errors for validation of lag time prediction by Artificial Neural Network

Input parameter				Prediction Lag time (min)	Experimental Lag time (min)	Relative error (%)
Temp (°C)	pH	Initial Cell (cell/ml)	Shaking speed (rpm)			
27	5.9	2.08×10^3	82	85.37	94	9.18
28	5.5	1.78×10^3	66	82.44	84	1.86
29	8.0	1.14×10^3	85	85.86	90	4.59
30	6.3	1.00×10^2	75	68.40	72	4.99
32	6.5	1.14×10^3	70	54.94	48	14.45
34	6.8	1.13×10^3	120	45.48	54	15.78
35	7.5	6.70×10^2	80	47.05	60	21.59
36	7.5	1.40×10^2	98	45.16	36	25.44
37	5.0	1.45×10^3	80	43.39	41	5.83
38	6.2	1.47×10^3	113	43.33	46	5.81
					Average	10.95
					SD	7.98

integrator and dissolve oxygen detection probe, it might predict microbial growth and its metabolite production from the temperature or dissolved oxygen history of a liquid food.

Conclusion

All of this demonstrates that the artificial neural network has good generalization ability because of its accuracy in estimation of the lag time of S. aureus in bovine milk.

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