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THE INFLUENCE OF PREDICTIVE MICROBIOLOGICAL MODELS ON SAFETY AND QUALITY OF FOOD PRODUCTS – REVIEW

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Abstract: The huge health consciousness of Polish society causes citizens to look for food products of only very high microbiological quality. Such a demand has resulted in the systematic introduction and improvement of food safety assurance systems. The present study evaluates tools of predictive microbiology with particular regard to the USDA Agricultural Research Service Pathogen Modeling Program (PMP70) which is a very useful software package available at *www.arserrc.gov/mfs/pahogen.htm* as well as ComBase Predictor available at *www.modelling.combase.cc*. The application of predictive microbiological tools enables scientists to quantitatively assess the microbiological risk of the appearance of food borne pathogens in food products. The program operation is illustrated by the example of *Escherichia coli* O157:H7 cultivated in broth medium. It predicts the growth and behavior of the pathogen under some specific environmental parameters introduced as the input to the program. Such a tool constituting a software package allows for the prediction of growth of different microorganisms and guarantees the operation of the food safety assurance systems in food processing.

Key words: predictive microbiology, ARS Pathogen Modeling Program, ComBase Predictor, food safety, *Escherichia coli* O157:H7.

Introduction

Predictive microbiology belongs to the field of food microbiology. It has enjoyed immense popularity for the last two decades. That term covers some sort of quantitative science allowing for the estimation of the influence of processing, distribution and storage procedures on the preserving the microbiological safety and quality of foods [1, 2]. Moreover, predictive microbiology deals with responses of microorganisms to different food conditions with the application of prognostic programs of bacterial growth [3]. The aim of this study is to indicate how it is possible to predict the growth of particular microorganisms being exposed to different factors such as temperature, pH and water activity by the usage of prognostic programs [4]. The study particularly focuses on predicting growth and decreasing the number of *Escherichia coli* O157:H7 cultivated in broth medium in order to assess a potential health risk. The process of modeling growth and decreasing the number of cells depending on exposure to different temperatures during meat processing, its storage and distribution creates a lot of problems [5]. It is commonly believed that there is a different range of temperature, pH and water activity which leads to the best growth of microorganisms including food pathogens. The knowledge of their values facilitates the prevention of growth of unwanted organisms in food [6, 7].

Due to the fact that there is a huge variety of spoilage microorganisms, it is very useful to work out the fastest and most efficient prognostic models which give the information on the combination of different environmental factors and is the most beneficial for the growth of such organisms [8]. Having such knowledge enables the prevention of their multiplication in food. There are also other factors which are significant for the optimal growth of microorganisms. They include the composition of the atmosphere in which the food is stored, preservatives as well as food structure [9].

The application of prognostic programs makes it possible to assess to what extent an amount of water contained in food, pH value and storage temperature of storage are responsible for the growth of microorganisms. Such programs are designed to measure the response of microflora to environmental factors [10]. Furthermore, they are able to indicate the relationship between the growth and those three factors. In a case where there is a big difference between the calculated and observed responses, it can be stated that there were also other factors which might have had an influence on the growth of microorganisms. Such factors should also be taken into consideration [11].

To sum up, it can be stated that the application of validated prognostic programs for predicting growth of microorganisms has obtained its confirmation with reliable but time-consuming microbiological results from the scientific literature. In such a way their application enables scientists to obtain reliable data on microorganisms growth within a relatively short period of time. It is particularly useful in the development of safe products to avoid any spoilage microorganisms which might have a huge influence on the decrease in shelf-life of such products [12].

Predictive Microbiology is a term which involves the scientific discipline whose aim is to predict growth and decrease the number of bacteria cells which are a function of different environmental factors [13]. It can be stated that growth is described by the features such as the „lag" time (characterized as the amount of time from an initial equilibrium state after exposure to an environmental factor such as temperature and lasting to the moment when growth starts) as well as generation time (characterized as the amount of time needed for a population to double in size) [14]. On the other hand, a decrease in the number of cells is described as a value called the „D-valu", which is the period of time needed to reach a 1 (one) common logarithm decline of the population at a specific temperature.

Predictive microbiology is used to work out predictive models of death and growth curves for different pathogens which might constitute a huge health risk. There are two steps which should be estimated to analyze the behavior of microorganisms in food. The first step includes the observation of pathogen in a constant environment and the establishment of its growth/death model. The second step is to assess how the parameters of the first step can be influenced by some environmental factors. There are many significant environmental factors which influence the growth. They include temperature, pH, water activity, the presence of preservatives and antimicrobials as well as the composition of the atmosphere. It can be stated that temperature is one of the few parameters which is analyzed and controlled during food storage. The other parameters might change during some period of time as a result of bacteria multiplication, and they can influence each other [15].

The application of such a program facilitates the prediction of interaction between bacteria and environmental factors. It answers how the actual physiological state of the bacteria looks. It also gives information on the Quantitative Microbial Risk Assessment. Predictive microbiology remains dependent on molecular microbiology and together give the information on how genes are affected by the environmental factors, which is bacterial variability and stress-tolerance. It enables the prediction of growth and death properties of significant and potentially dangerous microorganisms in food. Such a program allows scientists to predict the response of pathogens and spoilage microorganisms to different environmental factors. They include temperature, pH and salt concentration. It also provides an opportunity to observe the reaction of foodborne pathogens to other factors which involve the concentration of carbon dioxide and organic acids. It is commonly regarded that raw beef meat constitutes an excellent medium for bacteria growth. There are some specific bacteria which dominate beef. They involve lactic acid bacteria, *Brochothrix thermosphacta* under anaerobic conditions, *Pseudomonas, Acinetobacter* and *Moraxella* under aerobic conditions [16]. They are considered to be in the majority in raw meat. Aerobic plate counts are usually regarded as an indicator of spoilage microflora [17].

Apart from aerobic bacteria there are some other sources of microflora appearing in meat [18,19]. It might contain some fecal material, soil, hooves, hair and gastrointestinal contents as well as microorganisms coming from the slaughter environment such as equipment surfaces, aerosols, and handling [20]. Unfortunately, even the preservation of the best production and slaughter conditions does not guarantee that raw meat is free from microorganisms [21]. It is known that the growth of different pathogens depends not only on its initial number but also on a number of competing flora in meat [8,22]. The microflora inherently associated with meat is very important for assessing the kinetics of growth of pathogens [23].

Case studies

The research was carried out to analyze the kinetics of microbial growth in rich broth culture media at significantly high initial densities (10^1 and 10^3 CFU per ml). Such initial number of *E. coli* O157:H7 cells were used to model its growth with the application of a commercial software program ARS Pathogen Modeling Program (PMP) and ComBase Predictor Program. The research was carried out with the application of different temperatures, pH, salt and nitrite conditions in pure culture broths. Nowadays, predictive microbiology models are able to predict the growth of microorganisms independently on their initial density per gram or per unit surface area [24,25].

Predictive microbiology has become very popular with the development of first validated and commercialised programme package, Food MicroModel^{TM}, which was used to determine the growth and death of bacterial pathogens after exposure to different environmental factors. In 2003 UK Food Standards Agency (FSA) used Food MicroModel^{TM} to design Growth Predictor program. Nowadays, it is available at *www.ifr.ac.uk/Safety/GrowthPredictor*. There is its US counterpart, which is called PMP (Pathogen Modelling Programme) available at *www.arserrc.gov/mfs/pahogen.html*. The valuable developments of UK and US scientists were joined and led to the creation of ComBase, which is the Combined Database of Microbial Responses to Food Environments available at *www.combase.cc* [26,27].

Results and discussion

The behavior of *E. coli* O157:H7 exposed to some environmental factors was observed by applying these two prognostic programs. There are some initial parameters intro-

duced as the input for operating these programs. They include: temperature, pH, salt content, nitrite content, aerobic or anaerobic growth, as well as initial population density [28, 29]. After the introduction of these data into the programs, the outputs are received, and they include the expected value, the minimum and maximum values for lag phase duration as well as generation time [30–32]. They enable the estimation of growth of a specific pathogen under a given condition [33–35]. However, they do not show how the growth of a pathogen looks under the changing conditions of temperature on the same chart. In order to analyze its growth during temperature variations another chart should be created with the prior introduction of a specific temperature [15].

The figures below were obtained from the data taken from the ARS Pathogen Modeling Program for *E. coli* O157:H7 which was cultivated in broth in aerobic and anaerobic conditions [36]. Broth medium was inoculated with *E. coli* O157:H7 at the initial level of 3.0 log (CFU/ml) which meant 1000 CFU/ml. The temperature was $37°C$, pH 6.5, sodium chloride 0.5 (%[g/dL]), sodium nitrite 10 (ppm), water activity 0,997. Figure 1 presents specific prediction for *E. coli* O157:H7 growth in broth medium in aerobic condition and Figure 2 in anaerobic condition [37, 38].

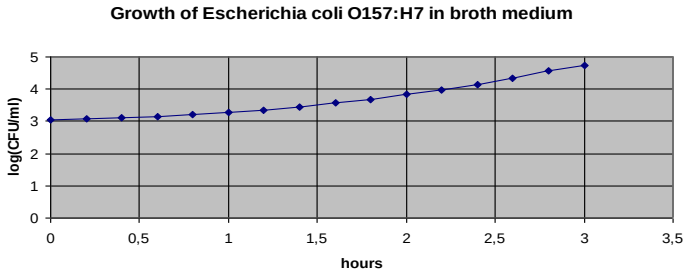

Fig. 1: Prediction of *E. coli* O157:H7 growth in broth medium in aerobic condition. US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, Pennsylvania, USA. [PMP70 version] *www.arserrc.gov/mfs/pathogen.html*.

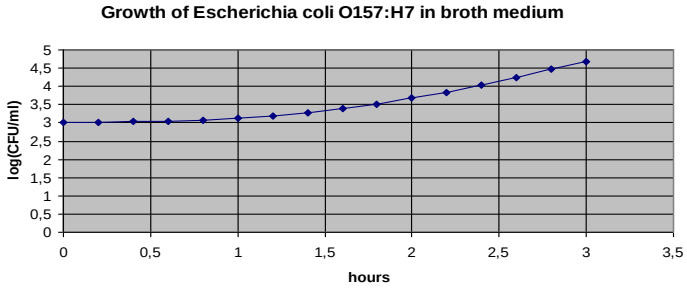

Fig. 2: Prediction of *E. coli* O157:H7 growth in broth medium in anaerobic condition US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, Pennsylvania, USA. [PMP70 version] *www.arserrc.gov/mfs/pathogen.html*.

From the data obtained from the ARS Pathogen Modeling Program it can be observed that *E. coli* O157:H7 cultivated in broth medium multiplies faster in aerobic conditions in comparison to anaerobic ones. After 0.40 hours of incubation time at $37°C$, pH 6.5, sodium chloride 0.5 (%[g/dL]), sodium nitrite 10 (ppm), water activity 0.997, there is 3.10 log(CFU/ml) in aerobic conditions, while for the same input parameters there is 3.03 log(CFU/ml) in anaerobic conditions.

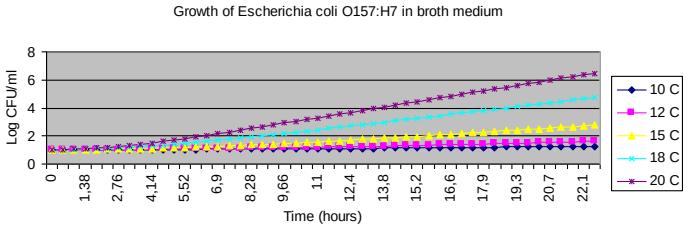

Fig. 3: Prediction of E. coli O157:H7 growth in broth medium at temperature of $10°C, 12°C, 15°C, 18°C$ and $20°C$ by the application of ComBase Predictor Program available at *http://modelling.combase.cc*.

Figure 3 illustrates the growth of *E. coli* O157:H7 in broth medium at different temperatures. The data were generated on the basis of the growth modeling program called ComBase Predictor Program. The initial concentration of the strain was 1 log CFU/ml of broth medium. The acidity expressed in pH value amounted to 6.2 while the concentration of NaCl was 0.5%. The changeable factor was the temperature at which *E. coli* O157:H7 was incubated, and it amounted to $10°C, 12°C, 15°C, 18°C$ and $20°C$. The temperatures values were chosen in such a way to create conditions similar to the ones at which for example meat contaminated with *E. coli* O157:H7 can be stored on cool shelves, where the cooling chain can be discontinued. With regard to the temperature significant differences in the pathogen growth were observed. The temperature $10°C$ occurred to be safe and did not cause significant growth in a number of *E. coli* O157:H7 cells. After 22.54 hours a number of *E. coli* O157:H7 incubated at $10°C$ increased from 1 log CFU/ml as an initial value to 1.28 log CFU/ml, while at $12°C$ increased to 1.63 log CFU/ml, at $15°C$ increased to 2.78 log CFU/ml, at $18°C$ increased to 4.73 log CFU/ml and at $20°C$ increased to 6.47 log CFU/ml. It means that the growth in the storage temperature causes a significant increase in a number of live cells of the examined foodborne pathogen. This information proves that the proper storage conditions should be maintained in order to prevent the pathogen multiplication in food products.

Figure 4 shows the of *E. coli* O157:H7 in broth medium containing different amount of NaCl. The data, as in previous case, were also generated from the growth modeling program called ComBase Predictor Program. The initial concentration of the strain also amounted to 1 log CFU/ml of broth medium. The temperature at which the strain was inoculated was $10°C$. The acidity expressed in pH value amounted to 6.2 while the concentration of NaCl was chan-

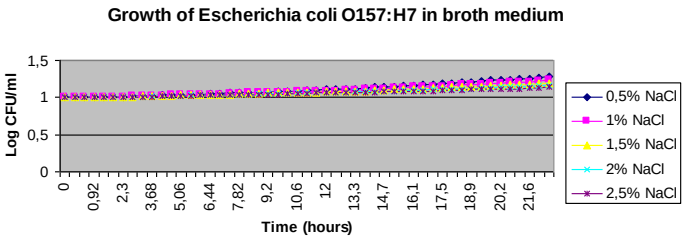

Fig. 4: Prediction of *E. coli* O157:H7 growth in broth medium with NaCl concentration of 0.5%, 1%, 1.5%, 2% and 2.5% by the application of ComBase Predictor Program available at *http://modelling.combase.cc*.

geable and amounted 0.5%, 1%, 1.5%, 2% and 2.5%. It can be observed that the concentration of salt does not have a significant influence on the pathogen growth. After 22.54 hours a number of *E. coli* O157:H7 incubated at broth medium containing 0.5% of NaCl increased from 1 log CFU/ml as an initial value to 1.28 log CFU/ml, while at 1% increased to only 1.23 log CFU/ml, at 1.5% increased to 1.19 log CFU/ml, at 2% increased to 1.16 log CFU/ml, and at 2.5% increased to 1.14 log CFU/ml. Such data indicate that *E. coli* O157:H7 shows a huge resistance towards the increasing concentration of NaCl, and the salt concentration does not belong to factors which cause the growth inhibition of pathogen in growth media.

Future trends

1. Predictive microbiology is regarded to be a promising and rapidly developing area of food microbiology. It uses mathematical models to analyze microbial evolution in laboratory media as well as foods which is a function of environmental conditions. It combines existing microbiological knowledge (microbial behaviour and physiology) with mathematical predictive models.
2. Predictive microbiology enables scientists to predict changes in microbial populations in a product during food processing, to assess the shelf life of the food during storage as well as estimates a risk of appearing a specific pathogen in food under various conditions.
3. The progress in introduction of microbial predictive models is impressive and such models are applied as a standard research tool in assessing and designing food processes.
4. However, it is not possible to count exclusively on predictive models to assess the safety of foods and process systems.
5. There is still a need for laboratory testing to unequivocally confirm the ability of a pathogen to grow or survive in the food product.

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