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PREDICTION *LISTERIA MONOCYTOGENES* GROWTH IN MILK – COMPARISON WITH PATHOGEN MODELING PROGRAM 7.0

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Key words: *Listeria monocytogenes*, predictive microbiology, food safety

Securing food safety is one of the priority political tasks of almost every country. Assessment of risk in its microbiological aspect is a systematic process of identification and evaluation of hazards resulting from microbiological contaminations. Predictive microbiology that serves identification and understanding of microorganisms' ecology in food, influence of the technological process, distribution and storage on their survival and inputting that information into devices and systems monitoring the technological process is the tool of risk assessment. The reaction of microorganisms to the environment can be presented in the form of predictive models.

The aim of studies was to construct mathematic models of *Listeria monocytogenes* 38 growth in sterilization milk during storage at 3–15°C and compare them with estimations based on Pathogen Modeling Program 7.0 (PMP 7.0). Microbiological tests were carried out using the impedimetric method (monitoring system Bactometer M64 – Biomerieux). The behaviour of *Listeria monocytogenes* 38 in milk was presented in the form of a regression function with 95% confidence level and a cumulative model (polynomial response surface). Selected results of analyses were compared with predictions obtained from the PMP ver. 7.0 software. It was established that models obtained from tests on the specific product were different from those obtained on the basis of microbiological media. The predictive models that might be useful in creating microbiological quality should be constructed for each type of food individually.

INTRODUCTION

Food consumption poses a continuous hazard as foreign harmful chemical compounds, heavy metals, pathogenic microorganisms and their toxins can occur in it. From the health point of view food containing pathogenic microorganisms can cause serious diseases or food poisoning [Libudzisz, 2000; Kowalik *et al.*, 2003].

The microbiological quality of food, *i.e.* the level of microorganisms' contamination, is then equally dependent on the quality of processed raw products, technological and organizational conditions of production, storage as well as sanitary and hygienic culture and knowledge of the personnel. Generally, food represents rich and convenient growth environment for multiple groups of microorganisms, including the pathogenic ones.

Listeria monocytogenes, a G(+) rod, that causes listeriosis, which is found with the frequency of 10 cases per 1 million populations is one of such microorganisms. Bacteria of all *Listeria* species are widely spread in the environment. They have been isolated from the soil, water, plants, animal fodder, fresh and frozen foods as well as excrements of humans and animals showing no symptoms of disease. The invasive form of the disease is characterised by high mortality rate; it attacks people belonging to high risk groups – pregnant women, the elderly and people with impaired immune system (symptoms: cerebrospinal meningitis, septicemia, abortion, influenza-like symptoms [Osek, 2005; McLauchlin *et al.*, 2004]. It has been established that *Listeria monocytogenes* can be present in a variety of foods, most frequently meat (beef, pork, poultry), fish and milk and dairy products.

Microbiological problems are of major importance to the food industry due to both quality of product manufactured and possible negative economic effects resulting from the activity of undesirable microorganisms.

Systems monitoring technical processes (such as HACCP) were developed to predict the directions of changes, identify hazards and sources of hazards, and to prevent them [Kołożyn-Krajewska *et al.*, 2003].

Tools supporting quality systems by allowing quantitative (and not only qualitative) hazard risk assessment were also developed. Predictive microbiology, a new sub-discipline of food microbiology that deals with development of mathematic models describing the reaction of microorganisms to specific environmental conditions and the rapidness of detecting them in the process of food production and distribution, is one of such tools [Zwietering *et al.*, 1999; Brown & Stringen, 2002].

Complicated microorganisms' growth processes must be presented in a simple model while taking into consideration the specificity of microorganisms and predictive usefulness [Kręgiel & Oberman, 2004; Ross & McMeekin, 1994; Tienungoon *et al.*, 2000].

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Techniques used in microbiological predicting are increasingly complex and precise as a result of progress in technology and computer software (*e.g.* Food Micro Model and Pathogen Modeling Program).

The aim of these studies was to investigate the behavior of *Listeria monocytogenes* 38 (isolated from raw milk) in milk during its storage at temperatures ranging from 3 to 15°C for 21 days and to create a model of *Listeria* growth taking into consideration estimates of possible deviations in technical processes, influence of specific infection on product's quality and comparing that model with the model obtained on microbiological medium according to Pathogen Modeling Program 7.0 software.

MATERIAL AND METHODS

Sterile, regenerated skim milk with 10% dry mass content was used as the material for the investigation. The strain of *Listeria monocytogenes* 38 was obtained from the Chair of Industrial and Food Microbiology, Faculty of Food Sciences, University of Warmia and Mazury, Olsztyn, Poland. Following regeneration in heat-resistant bottles, milk was sterilised in an autoclave and, after cooling, inoculated with *Listeria monocytogenes* 38 in quantities assuring concentration in milk at a level of 10^3 cells/mL. During the tests, the strain was stored at 6°C and activated at 37°C on selective medium LEB (24h) (MERCK, Poland).

The infected milk samples were maintained in precise incubators (Memmert) at 3, 6, 9, 12 and 15°C. Each temperature cycle included determination on day 0, immediately after inoculation and after day 4, 7, 11, 14, 17 and 21 of storage. The number of *Listeria monocytogenes* 38 was determined in the impedimetric monitoring system Bactometer M64 (Biomerieux). The Bactometer was calibrated according to the reference plate method as recommended by the manufacturer. Sterile bactometric modules were used as carriers of the special medium with the required electric properties. The number of *Listeria* was determined in module wells at 37°C during the time dependent on the number of microorganisms. That method is a quantitative analysis of the number of microorganisms faster than the traditional plate method. Incubation of the infected milk sample was carried out in 3 replications for each temperature (3–15°C). The determination of the number of *Listeria* in definite days was made in 3 repetitions (inoculation into 3 wells of bactometric module).

The estimation of bacterial growth was done using the following software: Excel 2000 (MS Office 2000 PL), Statistica 6.0 (StatSoft Polska Sp. z o.o, Kraków, Poland) and Pathogen Modeling Program 7.0 (USDA). Computer simulation of *Listeria* growth was carried out under conditions in milk taking into account such factors as temperature, pH (for milk 6.6) and water activity (for milk 0.97).

On the basis of the results obtained during tests, the graph of scatter with the line of regression and its 95% confidence level was created in Statistica software.

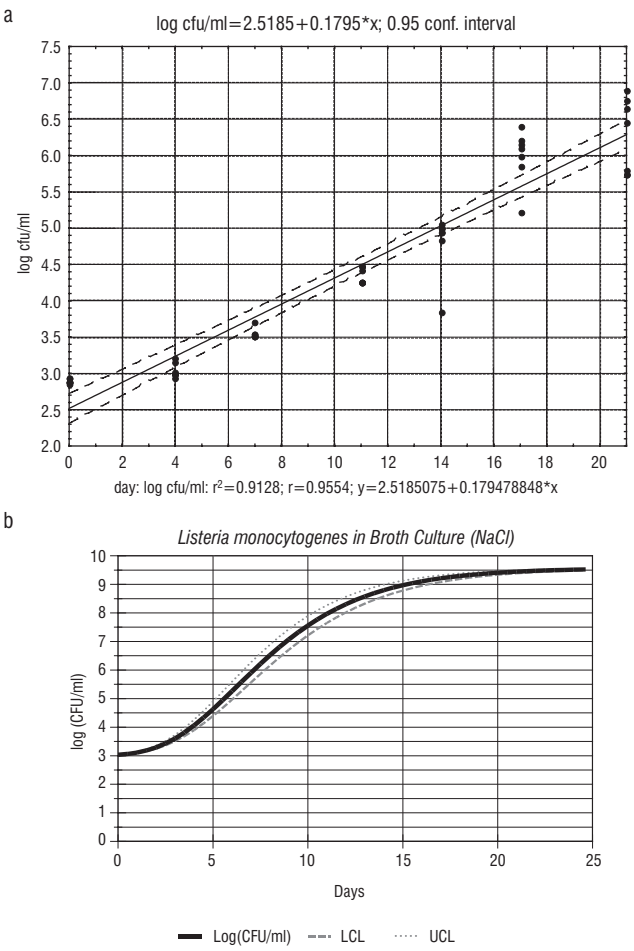
Pathogen growth curves obtained under experimental conditions (linear regression analysis) were compared with the curves obtained from computer simulation (Pathogen Modeling Program 7.0).

The polynomial collective time and temperature model (response surface) for growth and survival of *Listeria monocytogenes* in the stored milk covering the experimental data range was presented.

RESULTS AND DISCUSSION

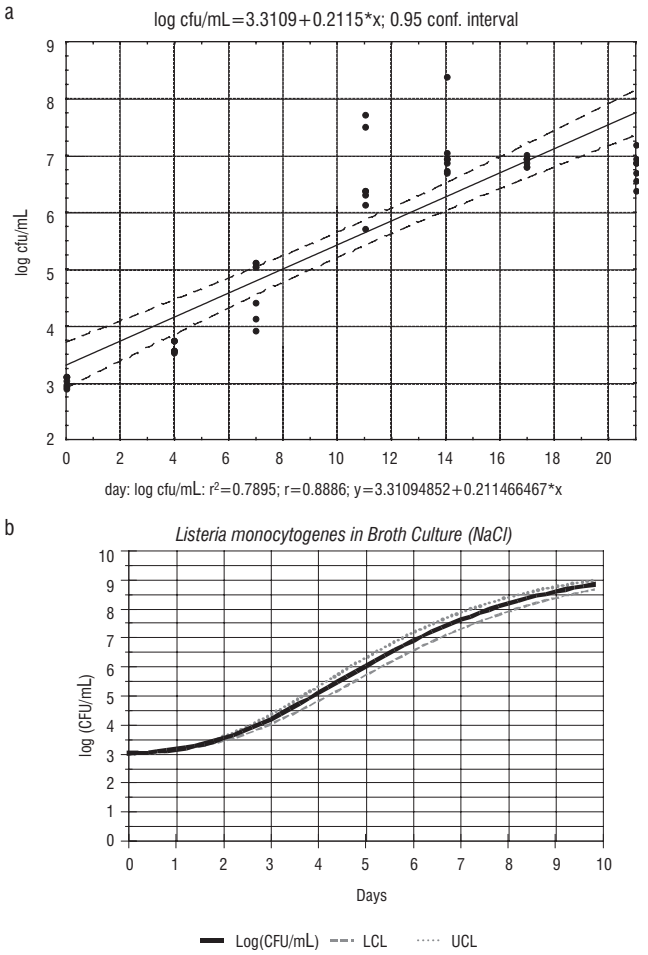
Linear models (linear regression analysis) presenting behavior of *Listeria monocytogenes* 38 during storage at 3, 6, 9, 12 and 15°C for 21 days were created on the basis of the results obtained. The models allow determining the temperature at which the most favorable conditions for *Listeria monocytogenes* 38 development existed.

Rapid frequency of cells division was observed at 9°C, 12°C and 15°C (Graphs 3a, 4a, 5a). A long adaptation process was observed at 3°C (Graph 1a), where sudden growth occurred after day 14 of storage while after 21 days it exceeded 10^6 cfu/mL.

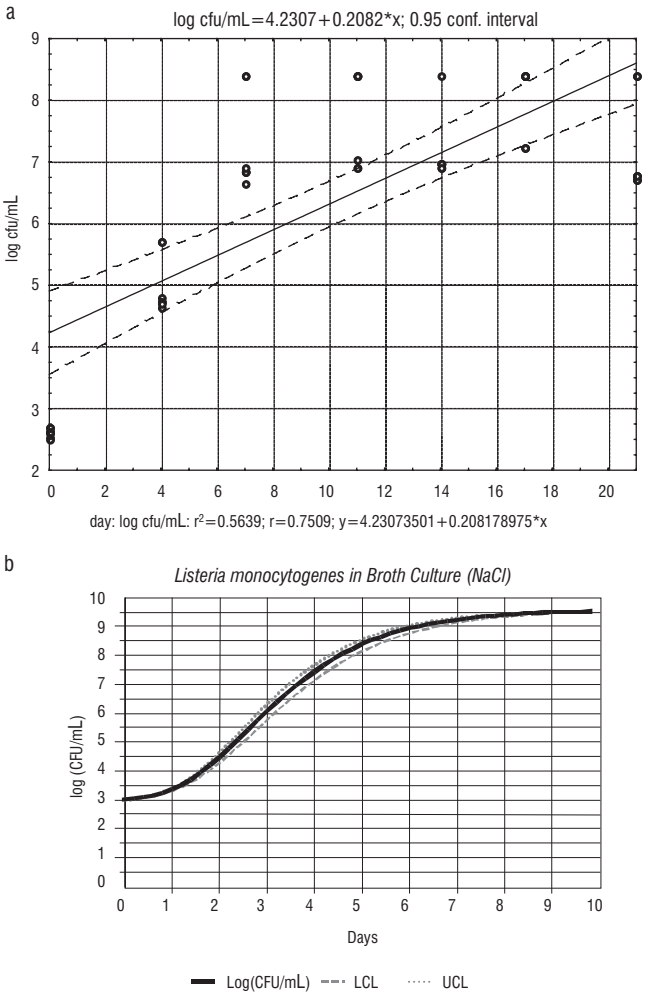


GRAPH 1. *Listeria monocytogenes* 38 growth model in milk stored at a temperature of 3°C a) own research; b) simulation in PMP 7.0.

At 6°C (Graph 2a), bacteria in the test sample grew proportionally to storage time to the ultimate level of 10^7 cfu/mL. The period of most intensive growth occurred between days 7 and 11 of incubation. With the increase of temperature the adaptation time was shorter and increase in the number of cells more rapid. At 9°C (Graph 3a) the bacteria grew gradually bypassing the adaptation stage and reached the ultimate level of 10^7 cfu/mL. The optimum



GRAPH 2. *Listeria monocytogenes* 38 growth model in milk stored at a temperature of 6°C a) own research; b) simulation in PMP 7.0.



GRAPH 3. *Listeria monocytogenes* 38 growth model in milk stored at a temperature of 9°C a) own research; b) simulation in PMP 7.0.

growth temperature was 12°C (Graph 4a) where a high level of growth (exceeding 10^7 cfu/mL) was achieved quickly and then it was maintained until day 21 of storage. At 15°C (Graph 5a) the stage of logarithmic growth of bacteria started with milk inoculation and continued until day 4. Then the stage of dying out was observed followed by gradual growth between day 7 and 14 up to the level exceeding 10^8 cfu/mL.

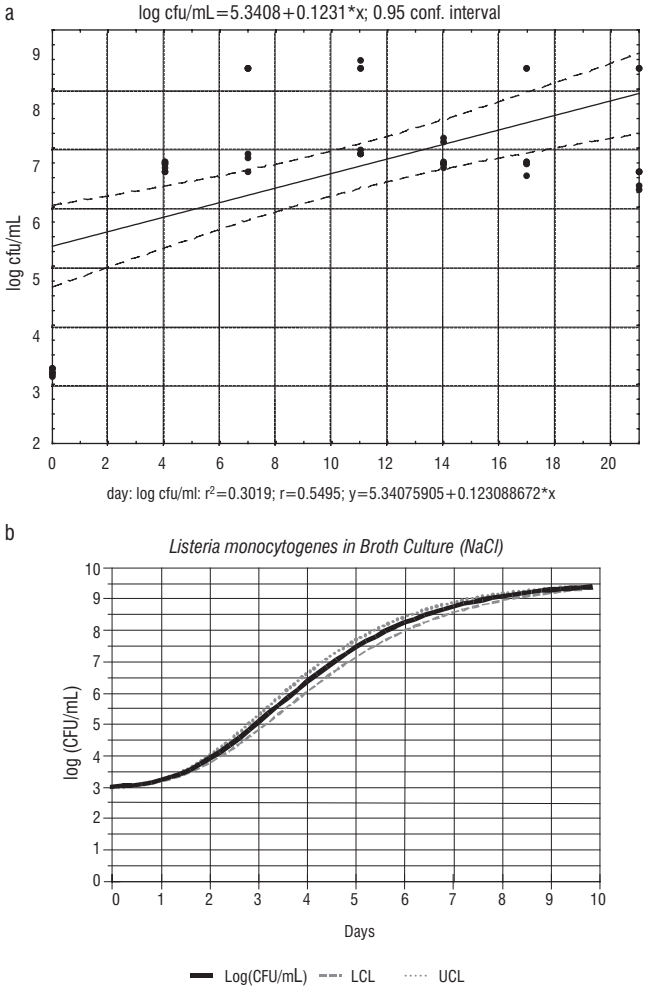
Graphs 1a, b – 5 a, b present the comparison of *Listeria monocytogenes* 38 growth under simulated conditions of PMP 7.0 computer software and during own investigations. The environment parameters (temperature, inoculum value, pH) were input according to those in the milk tested and the curves of *Listeria monocytogenes* 38 logarithmic growth under the described conditions were obtained.

On the basis of comparison between graphs obtained from own tests and graphs presented by PMP 7.0 software it was established that the higher the storage temperature of the contaminated milk, the more similar the growth curves to those created by the computer software. At 6°C, we found out that the intensity of bacterial cells growth is similar in both growth models, however, in the experimental model the ultimate level is not achieved as in the case of growth curve constructed by PMP 7.0 software. At 9°C *Listeria monocytogenes* 38 grows until day 7 (in excess of 10^7 cfu/mL) and maintains that level during all the storage period of the test sample. The growth curve is similar to that of the computer software model (Graphs 3 a, b). Growth of *Listeria monocytogenes* 38 at 12°C and 15°C was also compatible to that presented by PMP 7.0 software. While comparing the models obtained during own studies with those obtained with PMP 7.0 software, the number of *Listeria* cells did not reach such high levels as in the case of PMP 7.0 growth curves where the models were created for microbiological media (Graphs 4 a, b – 5 a, b).

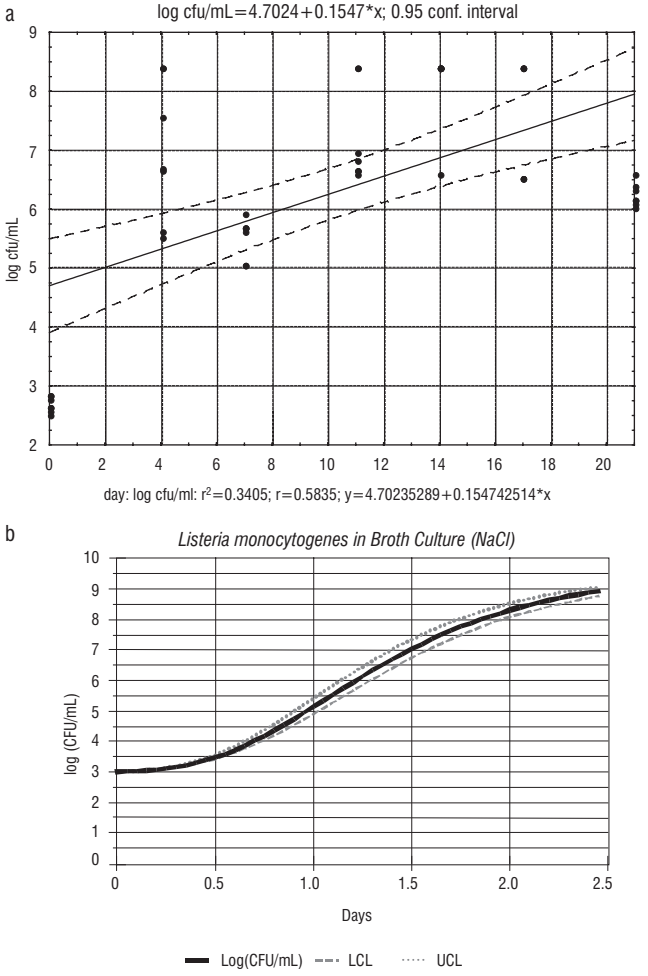
A response surface model (polynomial of second degree, correlation rate 0.78) was also constructed. In case of such a model it is possible to determine the correlation between the variables of time and temperature and estimate the number of microorganisms within the temperature range of 3–15°C (Graph 6).

The predictive models constructed in PMP 7.0 do not reflect precisely the real conditions occurring in food products. The main reason for that situation is, *e.g.* absence of accompanying microflora. Food production and distribution conditions are specific and as a consequence of this the models of microorganisms' behavior should be constructed for specific products.

The results obtained can be compared to studies by other authors that tried to estimate the hazard of bacterial growth in food products. A study carried out at the Chair of Food Hygiene of SGGW was aimed at comparing the behavior of *Listeria monocytogenes* in selected food prod-



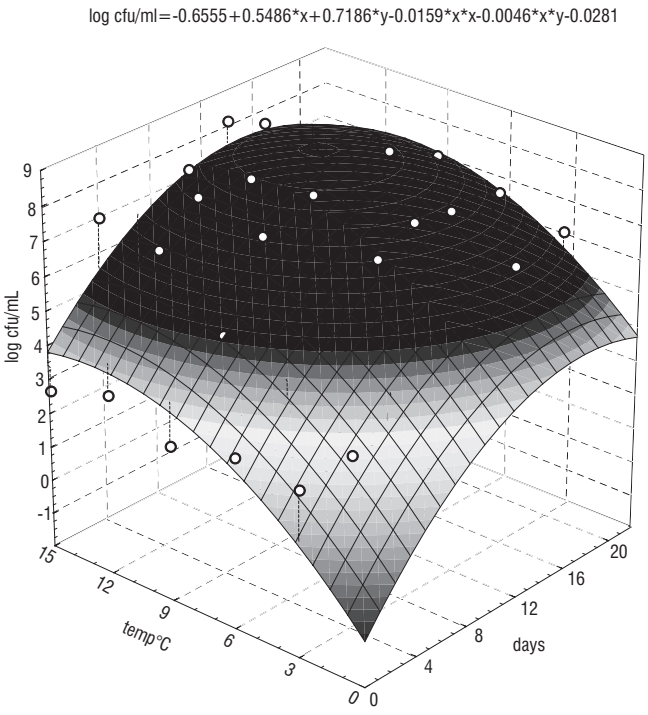
GRAPH 4. *Listeria monocytogenes* 38 growth model in milk stored at a temperature of 12°C a) own research; b) simulation in PMP 7.0.



GRAPH 5. *Listeria monocytogenes* 38 growth model in milk stored at a temperature of 15°C a) own research; b) simulation in PMP 7.0.

ucts (kefir, yogurt, raw milk, thermally processed cottage cheese and seasoned ham) with the results estimated using Pathogen Modeling Program 4.0. Faster than expected by the software, the inactivation of Listeria in dairy products (probably caused by the influence of lactic acid bacteria) and slower than projected growth in seasoned ham were observed [Szczawiński, 1996].

Studies using model ready to consume meat products were also carried out at the Division of Gastronomic Technology and Food Hygiene of SGGW. The results were compared with those obtained from Pathogen Modeling Program 5.1 software. On the basis of those studies it was established that despite large differences in the numbers of pathogenic bacteria as compared to the microflora accompanying them, the growth of selected pathogenic microorganisms was much slower than that estimated according to PMP software. For the data collected, summary models of growth, survival and inactivation of pathogenic microorganisms (*Listeria monocytogenes*, *Salmonella enteritidis*) in the model food product were constructed as well. The studies confirm, among others, the belonging of *Listeria monocytogenes* to psychrotrophic microorganisms as very clear growth at 5°C was confirmed. The results obtained also confirmed the correctness of recommendations concerning storage of food products at temperatures below 4°C to pre-



GRAPH 6. Surface graph for *Listeria monocytogenes* 38 growth in milk stored within the temperature range of 3–15°C.

vent growth of *Listeria* spp. Particular attention should be drawn to large differences between pathogenic bacteria growth rates found in own studies and those estimated on the basis of Pathogen Modeling Program software. The results presented confirm that bacterial growth on selected microbiological media differs from the growth of the same bacteria on a food product as the medium. Many factors involved in food, such as, *e.g.* availability of nutrients, antimicrobiological factors or influence of accompanying microflora, are not taken into account in models designed on the basis of experiments on microbiological media. This, in turn, results in the need for designing models for specific food products [Jałosińska-Pieńkowska *et al.*, 1999]

The comparison of growth *Listeria monocytogenes* 38 in milk obtained from PMP7.0 shows many discrepancies. It was found that the same number of bacterium, which was determined after several days of storage in own investigations, is estimated considerably earlier by computer programme. Together with the increase of storage temperature the time difference are much greater.

We should find that the growth *Listeria* in own studies was much more slow and not as steady in comparison to the growth estimated on the basis of PMP 7.0. The best solution would be to create the model product for each type of food, which could be the proper representative for study of specific microbiological hazards.

Predictive microbiology allows better understanding of pathogenic microorganisms ecology leading to a better assessment of, *e.g.* the raw material, compares information with control criteria and includes the information obtained in microbiological contaminations monitoring systems. It is a very important tool supporting food safety assurance systems [Kręgiel & Oberman, 2004].

Thanks to predictive microbiology the database concerning hazards in the production of different groups of foods is developed that is also available on the Internet (www.combase.cc). That database is updated with the latest research achievements.

That means that exchange of information in that field is becoming necessary.

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PROGNOZOWANIE WZROSTU *LISTERIA MONOCYTOGENES* W MLEKU – PORÓWNANIE Z PROGRAMEM PATHOGEN MODELING 7.0

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Narzędziem oceny ryzyka jest mikrobiologia prognostyczna służąca do identyfikacji i zrozumienia ekologii drobnoustrojów w żywności, wpływu procesu technologicznego, dystrybucji i przechowywania na ich żywotność oraz wprowadzeniu tych informacji do urządzeń i systemów monitorujących proces technologiczny. Reakcję drobnoustrojów na środowisko można przedstawić w postaci modeli prognostycznych.

Celem badań było opracowanie modeli matematycznych wzrostu *Listeria monocytogenes* 38 w mleku podczas przechowywania w zakresie temperatur 3–15°C. Badania mikrobiologiczne wykonano z wykorzystaniem metody impedymetrycznej (system monitorujący – Bactometer M64 – Biomerieux). Zachowanie się *Listeria monocytogenes* 38 w mleku przedstawiono w postaci funkcji regresji z 95% przedziałem ufności oraz modelu zbiorczego (wielomianowa powierzchnia odpowiedzi) (Wykresy 1a – 5a i 6). Wybrane wyniki analiz porównano z prognozami otrzymanymi z programu komputerowego Pathogen Modeling Program ver.7.0. Wzrost *Listeria monocytogenes* 38 w temperaturze 12°C i 15°C był porównywalny z modelem w programie PMP 7.0 (Wykres 4 a, b – 5 a, b). Stwierdzono, że modele uzyskane z badań na konkretnym produkcie różnią się od tych uzyskanych na pożywkach mikrobiologicznych.(Wykresy 1a, b – 5a, b) Modele prognostyczne, które mogą być przydatne w kreowaniu mikrobiologicznej jakości powinny być opracowywane dla każdego rodzaju żywności.