Pol. J. Appl. Sci., **2018**, *4*, 51-54

THR PREDICTION OF *ESCHERICHIA COLI* O157:H7 DECLINE IN TRYPTIC SOY BROTH WITH YEAST EXTRACT TREATMENTED WITH DIFFERENT TEMPERATURES AND ACIDITY VALUES USING A PATHOGEN MODELING PROGRAM

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Abstract: The aim of this study was to assess the sensitivity of *Escherichia Coli* O157:H7 towards temperature and acidity. *E. Coli* O157:H7 is regarded to be a pathogenic microorganism producing verotoxins called Shiga toxins which provoke hemorrhagic diarrhea in people. There is a strong need to design and develop highly successful methods of enabling the elimination of *E. Coli* O157:H7 from food. The present research evaluates the tools of predictive microbiology based on the USDA Agricultural Research Service Pathogen Modeling Program which is a very useful software package available at http://pmp.errc.ars.usda.gov. Strains were inoculated to reach a number of approximately 9 $\log_{10}$ bacteria/gram, acidified with lactic acid to achieve pH 7.0, 5.5 or 4.0, and then they were exposed to a specific temperature: 55°C, 58°C or 62.5°C. The decline in the number of *E. Coli* O157:H7 cells can be achieved due to the application of different acidity values in combination with different temperatures which are inserted into the predictive microbiological software package. The decline in a number of *E. Coli* O157:H7 cells depends on the temperature and pH value. The acidity has a huge influence on the survival of *E. Coli* O157:H7 cells. At pH 7.0, the decline in a value of 1.00 $\log_{10}$ cfu/ml is achieved after 10.38 minutes of holding in 55°C, while at pH 5.5 the decline in a value of 1.00 $\log_{10}$ cfu/ml is achieved after 7.24 minutes, and at pH 4.0 the decline in a value of 1.00 $\log_{10}$ cfu/ml is achieved after 3.52 minutes. The program estimates the microbiological risk of presence of food borne pathogens in food products. It brings many benefits for the food industry and makes it possible to control each production process through the application of successful combination of temperature and acidity values to completely destroy the pathogen in food.

Key words: *Escherichia Coli* O157:H7, Pathogen Modeling Program, acidity values, temperature values

DOI: 10.34668/PJAS.2018.4.2.02

Introduction

Escherichia Coli O157:H7 is a pathogen which produces Shiga toxins (verotoxins) and poses a huge risk for people's health and lives. Its toxins are similar to the ones which are produced by *Shigella dysenteriae* type 1. This bacteria belongs to the family of *Enterobacteriaceae*, and it produces Shiga toxins. For this reason it is often called Shiga toxin-producing *E. Coli* (STEC). This bacteria is also called verotoxin or verocytotoxin-producing *E. Coli* (VTEC). It is responsible for causing anything from uncomplicated diarrhoea to haemorrhagic colitis which can develop into haemolytic uremic syndrome (HUS). There are many cases of infections caused by this pathogen in humans which have been reported by EFSA (2014). The presence of this pathogen in food and animals is detected and reported annually by EU Member States to the European Commission and EFSA which is regulated by Directive 2003/99/EC [1]. EFSA's Biological Hazard Panel announced in 2007 that monitoring the presence of *E. Coli* in animals and food should be focused on *E. Coli* O157:H7 as this serotype is the predominant cause of severe human infections in the EU. The examination should also be extended to other serotypes such as O26, O103, O91, O145 and O111. EFSA issued in 2009 a statement that *E. Coli* O157:H7 should be monitored in animals and in food. This report recommends mainly the monitoring of *E. Coli* O157:H7 in young cattle and sheep fleeces (or faeces?). The EFSA Panel on Biological Hazards provided the information that during 2007-2010, there were 13,545 confirmed human VTEC infections and 777 haemolytic uremic syndrome (HUS) cases in the EU [2]. Moreover, isolates coming from 85% of cases were not fully serotyped and, therefore, could not be classified on the base of the Karmali seropathotype concept. Seropathotype group D included 5% of isolates coming from fully serotyped cases. 14 cases (0.7%) was classified as the seropathotype group E. Isolates from 27% of cases could not be assigned. There were not any HUS cases reported for the serotypes in groups D and E, but 17 HUS cases were not assigned. The health outcome was given only for some

confirmed cases. It was reported that there were about 64% of patients presented with only diarrhoea; VTEC infection appeared in a form of HUS in 10% of cases. Only six deaths caused by STEC/VTEC were reported in the period 2006-2009. The bacteria was isolated from animals (predominantly form ruminants) as well as meat and milk thereof. The consumption of milk and meat is known to be the main sources of human infections [3].

E. Coli O157:H7 was recognized to be a food-borne pathogen in 1982 after the appearance of approximately 16 documented outbreaks connected with food consumption such as ground beef and raw milk [4]. It was also determined in 1991 that *E. Coli* O157:H7 contained in apple cider was responsible for haemorrhagic colitis in Massachusetts [5]. Another outbreak connected with apple cider consumption contaminated with *E. Coli* O157:H7 was recorded in 1980 in Canada [6]. Such results prove that *E. Coli* O157:H7 may show tolerance to acidic conditions. The experiment of Zhao et al. [5] revealed that the pathogen was able to grow and persist in apple cider (pH 3.6 to 4.0). Another study carried out by Brackett et al. [7] showed that the treatment of raw beef with hot sprays of acetic, citric, and lactic acids at 55°C did not influence the survival of *E. Coli* O157:H7. The research carried out by Cutter and Siragusa [8] also showed that organic acids used to wash carcass did not cause the complete inactivation of *E. Coli* O157:H7 from beef tissues. Moreover, the survival of *E. Coli* O157:H7 was also observed in fermented dairy products [9] which meant that the pathogen showed tolerance to organic acids.

It is widely known that the parameters examined in this paper such as temperature and pH have an influence on the behaviour of this pathogen in the environment. The types of food which could be potentially infected with *E. Coli* O157:H7 include raw milk and dairy products. This bacteria can spread from a cow's udders to its milk. It is very important for milk to be pasteurized. The pasteurisation process destroys live bacteria cells. Other products which can be infected include raw fruit and vegetables such as alfalfa sprouts, unpasteurized apple cider and other unpasteurized juices that may come in contact with infected animal faeces [10].

Due to the fact that *E. Coli* O157:H7 is considered to be a food-borne pathogen which is able to survive in acidic conditions, there is a need to assess the antibacterial activity of food-associated organic acids against this pathogen [11]. The aim of this study was to assess the growth, survival and death characteristics of the pathogen which were affected by temperature based on the USDA Agricultural Research Service Pathogen Modeling Program (PMP70). The special tasks were focused on the assessment of the pH needed to achieve in order to have the complete inhibition of *E. Coli* O157:H7 at different temperatures (55°C, 58°C and 62,5°C) in a liquid medium acidified with lactic acid and to analyse the decline in population in acidified media [12,13].

This study concentrates on the assessment of different temperatures in combination with pH values on the survival of *E. Coli* O157:H7 in tryptic soy broth with 0.6% yeast extract (TSBYE). The prediction of a decline in the number of cells is determined based on Pathogen Modeling Program (PMP70). Strains were inoculated to reach a number of approximately 9 $\log_{10}$ bacteria/gram, acidified with lactic acid to achieve pH 7.0, 5.5 and 4.0; next they were exposed to a specific temperature values: 55°C, 58°C and 62.5°C. The decline in the number of cells was measured in minutes. The aim of this study was to assess the sensitivity of *E. Coli* O157:H7 towards temperature and acidity.

Material and methods

The influence of different temperatures and acidity values on *Escherichia Coli* O157:H7 survival was examined using parameters from Pathogen Modeling Program (PMP70). The present research evaluates the tools of predictive microbiology based on the USDA Agricultural Research Service Pathogen Modeling Program (PMP70) which contains a very useful software package available at http://pmp.errc.ars.usda.gov.

In the Pathogen Modeling Program (PMP70) three isolates of *E. Coli* O157:H7 coming from retail chicken (301C), retail pork (240P) and retail beef (505B) were used. The cultures were incubated in brain heart infusion and stored in brain heart infusion-glycerol (50:50, vol/vol) at -80°C. In order to prepare inoculum for the further experiment in the test media, these cultures were activated through two successive transfers in tryptic soy broth with 0.6% yeast extract (TSBYE; Difco) at 37°C for 24 h. The suitable solutions of lactic acid concentrations (Sigma Chemical Co., St. Louis, Mo.) were prepared, filter sterilized, and then used to acidify batches of TSBYE to achieve pH 7.0, 5.5 or 4.0. The temperatures used to analyse the decline of *E. Coli* O157:H7 at each pH value were the following: 55°C, 58°C and 62,5°C. The amount of lactic acid which was added to reach the proper pH was measured and used to calculate the concentration of acid in TSBYE for each test pH [14].

Results and discussion

The results achieved from Pathogen Modeling Program are presented in Table 1.

Statistical analysis

Quantitative and qualitative testing results were recorded in an Excel spreadsheet and the descriptive data analysis was performed using the software Stata 8.0 (Stata

Corporation, College Station, Texas, USA). A comparison of the enumeration results was performed using Poisson regression. The significant differences between the results obtained for different combination of pH and temperature were not observed.

Table 1: Log decline of *Escherichia Coli* O157:H7 after its treatment with the different combination of temperature and acidity values with different holding times to achieve the decline at the level of 8.00 $\log_{10}$ cfu/ml.

pH = 7.0			pH = 5.5			pH = 4.0		
55^{0}C	58^{0}C	62.5^{0}C	55^{0}C	58^{0}C	62.5^{0}C	55^{0}C	58^{0}C	62.5^{0}C
8.00 $\log_{10}$ cfu/ml after 83.07 minutes	8.00 $\log_{10}$ cfu/ml after 29.41 minutes	8.00 $\log_{10}$ cfu/ml after 4.36 minutes	8.00 $\log_{10}$ cfu/ml after 57.89 minutes	8.00 $\log_{10}$ cfu/ml after 24.75 minutes	8.00 $\log_{10}$ cfu/ml after 3.91 minutes	8.00 $\log_{10}$ cfu/ml after 28.15 minutes	8.00 $\log_{10}$ cfu/ml after 14.54 minutes	8.00 $\log_{10}$ cfu/ml after 3.40 minutes

All the above data were achieved on the basis of different parameters of temperatures and acidity values introduced into the modeling program, which enabled the decline of *E. Coli* O157:H7 to be predicted when treated with different combination of the above-mentioned parameters.

It can be summed up that the decline in a number of *E. Coli* O157:H7 cells remains in a close correlation with the temperature and pH value [15]. The acidity has a huge influence on the survival of *E. Coli* O157:H7 cells. The sweeter the environment is, the lower the is the observed decline. At pH 7.0, the decline in a value of 1.00 $\log_{10}$ cfu/ml is achieved after 10.38 minutes of holding in 55°C, while at pH 5.5 the decline in a value of 1.00 $\log_{10}$ cfu/ml is achieved after 7.24 minutes, and at pH 4.0 the decline in a value of 1.00 $\log_{10}$ cfu/ml is achieved after 3.52 minutes. The properly designed treatment conditions appeared to have a huge antimicrobial activity towards *E. Coli* O157:H7 [16–18]. The purpose of transferring data from the forecasting program was to check the possibilities of the deactivation of *E. Coli* O157:H7 cells under different combinations of pH and temperature. The achieved results might be useful in controlling the behaviour of this bacteria in different food products conditions. However, there is a potential risk that this bacteria might produce toxins in those incubation conditions, so further examination needs to be carried out in order to verify such a possibility.

Conclusion

Predictive microbiology constitutes a significant role in the microbiological assessment of risk associated with the potential presence of spoilage and pathogenic microorganisms in food. It is gaining more and more popularity nowadays. It is regarded to be a useful and promising area of food microbiology [19–22]. Predictive microbiology makes it possible to assess the growth of different pathogens after treating them with different temperatures and acidity values [23–26]. The modeling programs should be used as a standard research tool enabling the control of spoilage and pathogen microorganisms in the food industry [27–30].

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Received: 2018
Accepted: 2018