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Quantitative tools in microbial and chemical risk assessment

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Abstract

The popularity of biological origin food protection substances is driven by demands from consumers for natural and clean label product, increasing various food-related safety and health concerns and sustainability issues. Lactic acid bacteria (LAB) are most promising because they are a large group of beneficial microorganisms commonly used in food protection due to their ability to inhibit the growth of pathogenic bacteria and enhance food safety. Extensive scientific research has been conducted to understand the mechanisms by which LAB exert their protective effects in various food systems. Even though LAB activity against various food pathogens and spoilers is distinguished, use of cell-free supernatant (CFS) is still under investigation. This report is dedicated to present how qualitative measures can elaborate in new bacteria-origin food additive investigation. As part of the EU-FORA programme, the fellow was involved in the risk assessment tasks and projects which include gaining basic knowledge in predicative microbiology fundamentals, including different types of modelling strategies; delivering essential understanding about experimental design, knowledge in three specific software tools (MATLAB, GInaFiT and DMFit) and gained overall understanding what are the main differences while modelling growth or inactivation models. Secondary activities were included as a way to expand competences beyond qualitative measures to overall all activities done regarding risk assessment and build a strong network of food safety experts and professionals to continue engaging in risk assessment beyond fellowship programme.

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Summary

The final report of EU-FORA fellowship programme 'Quantitative tools in microbial and chemical risk assessment' hosted by National and Kapodistrian University of Athens (NKUA) supervised by Vasilis P. Valdramidis and Charalampos Proestos is dedicated to introducing fellow activities during programme implementation. Fellow is a junior scientist, first year PhD student in chemical engineering, with focus on bacteria-based antimicrobial substance investigation. Introduction part provides brief background about lactic acid bacteria (LAB) on how and why they are used in food protection with special importance to implement usage of not only whole bacteria, but also concentrated cell-free supernatants (CFS) containing metabolites and none live cells. In the work programme description aims are outlined such as the introduction to predicative microbiology fundamentals, including different types of modelling strategies, essential understanding about experimental design, training on three specific tools (MATLAB, GInaFiT and DMFit). The latter are selected according to fellow research topic and provided with overall understanding what are the main differences between modelling microbial growth, survival and inactivation. Furthermore, there descriptions on the development of different informative experimental designs were discussed. Only minor part of obtained data is presented in report, due to fact that research is ongoing even after the course and a separate scientific publication will be written with this data. Preliminary results of *L. plantarum* CFS concentrated by lyophilisation inhibitory activity were discussed. In addition, EFSA scientific opinions, concerning *Lactiplantibacillus plantarum* safety and efficacy as feed additive for various animal species and updates of the list of qualified presumption of safety recommended microbiological agents intentionally added to food or feed, released in EFSA Journal during past few years were revised, to get better understanding, what issues can occur while transiting from whole bacteria usage to use of only concentrated metabolites. Lastly, conclusions provide more insights of situation now and future prospects.

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1.   Introduction

Predictive modelling is a mathematical way to predict future outcomes by analysing patterns in input data. This type of analysis gives a good idea of what kind of events are more likely to occur and provide statistical possibilities, with a defined certainty that one or another event under given conditions may or may not appear. Predicative modelling can be used in many areas, but this programme was dedicated to its use for assessing microbial populations in various food matrices and situations, concerning food safety.

Predictive modelling is used to get knowledge for a better understanding the different processing technologies act on microorganisms and their behaviour (Stavropoulou and Bezirtzoglou, 2019). To develop an accurate predicative model input data must be of sufficient quality, which leads to the need of having adequate experimental design, providing enough data to develop and validate the model. This also requires that researchers, working in risk assessment would be well trained not only to produce laboratory work but also be capable to interpret the data correctly, select best possible models which could accurately describe particular process.

Growing consumer demand for natural and clean label products, various food-related safety and health concerns, sustainability issues, regulatory and labelling requirements are forcing science and industry to elaborate on creating and widely using biological origin food protection substances. One of the most promising options can be the lactic acid bacteria (LAB), which are a group of beneficial microorganisms commonly used in food protection due to their ability to inhibit the growth of pathogenic bacteria and enhance food safety. Extensive scientific research has been conducted to understand the mechanisms by which LAB exert their protective effects in various food systems. LAB are characterised by their ability to convert sugars into lactic acid through fermentation. The production of lactic acid creates an acidic environment in the food, lowering the pH and inhibiting the growth of pathogenic bacteria (Holzapfel et al., 1998; Zapaśnik et al., 2022). These bacteria are known to produce various antimicrobial compounds, such as bacteriocins, hydrogen peroxide and organic acids, which can inhibit the growth of pathogenic bacteria. These compounds act by disrupting the cell membranes or metabolic processes of the target bacteria (De Vuyst and Vandamme, 1994; Chen et al., 2022).

LAB can also compete with pathogenic bacteria for nutrients and adhesion sites in the food matrix, thereby preventing the attachment and colonisation of harmful bacteria. This competitive exclusion mechanism helps maintain the safety and quality of the food product (Jacobsen, 1997; Siedler et al., 2020). Additionally, LAB have been shown to modulate the immune response in the human gut, enhancing the body's defence mechanisms against pathogens. This immunomodulatory effect contributes to the overall protection against foodborne infections (Vinderola and Ouwehand, 2018).

Alongside the development of methods for predictive microbiology, genome sequencing used to identify microorganism species and the analysis and identification of their produced metabolites, there is a growing interest in the activity of not only live bacteria, but also secondary metabolites isolated from them and used against food pathogens and spoilers. LAB are capable to produce various organic acids, such as lactic acid, acetic acid, and propionic acid, which contribute to the preservation of food by significantly reducing pH and creating an acidic environment unfavourable for the growth of most pathogens and spoilers (Li et al., 2017). Other peptides or proteins, that can disrupt the membrane integrity of target bacteria or interfere with their cellular processes, leading to growth inhibition are called bacteriocins. Several LAB bacteriocins, such as nisin and pediocin, have been extensively studied for their application in food preservation (Cotter et al., 2005).

This training programme was dedicated to instructing the fellow on how predictive modelling can expand knowledge about bacterial and fungal responses to substances (in this case – secondary cell-free metabolites from unique type of LAB – *L. plantarum*) used to supress their growth and even to examine the probability of microbial recovery after treatment. During this research activity, *L. plantarum* from KTU bacteria collection was selected as reference strain to investigate whether cell-free secondary metabolites can be as active as live bacteria. *L. plantarum* is well known for producing bacteriocin plantaricin – it is an antimicrobial substance that exhibit inhibitory activity against various bacteria, including pathogenic and spoilage organisms.

1.1.   Description of work programme

The EU-FORA training programme 'Quantitative tools in microbial and chemical risk assessment' was designed to provide training in the use and development of predictive models for assessing the bacterial/fungal responses for survival, growth and the probability of microbial recovery. Assessments include but not

limited to microbial responses under different food processing/preservation scenarios. Primarily, use and applications were planned to be performed by programming in MATLAB. After taking into consideration fellow's basic knowledge in statistics, mathematics, programming and other skills, as well as additional simplified tools were also introduced in the training course. This was done in order to provide useful and purposeful knowledge, that the fellow would be able to implement independently after the course. Analysis and interpretation of data could be used as inputs in microbial/chemical risk assessments studies.

1.2. Aims

The aims of EU-FORA training programme 'Quantitative tools in microbial and chemical risk assessment' was:

- to introduce fellow to predicative microbiology fundamentals, including different types of modelling strategies;
- to deliver essential understanding about experimental design;
- to provide basic knowledge on how to use some specific tools, selected according to fellow research topic;
- to give overall understanding what are the main differences while modelling growth or inactivation models.

As attending fellow was not very familiar with predicative modelling weekly meetings, organised and supervised by Vasilis P. Valdramidis, were held to ensure that all occurring questions would be addressed and clarified during periods while fellow was at sending organisation and daily meetings were held while staying at hosting site.

1.3. Additional activities for network widening

Mobility programs, such as EU-FORA is a great opportunity to introduce fellows to different work groups, participate in various events to improve multidisciplinary co-working and co-research activities. To meet these goals during fellowship programme, fellow was introduced to several secondary activities, described in Appendix A.

2. Data and methodologies

2.1. Data

Input data used to test the functionalities of specific tools was provided by supervisor Vasilis Valdramidis' research group. These data were used to understand the development of the experimental design, generation and processing results by doing practical exercises with real case data, received by methods included in work programme. Additional data for analysis were obtained from planned experiments at the fellow's sending organisation. The report presents only partial data, as all these outcomes are planned to be included in a scientific publication based on the data obtained.

2.2. Investigation of inhibitory activity

Preparation of *Lactiplantibacillus plantaru*m metabolites:

bacteria were grown at three different temperatures (+25, 37 and 45°C) for 72 h at two different initial pH (3.5 and 7.0). After incubation cell-free supernatant (CFS) was collected and lyophilised.

PDA media was enriched with different quantities (5% and 10%) of a concentrated metabolites mixture. On the hardened medium, mycelial discs (10 mm in diameter) of each isolate, taken from the edge of cultures of fungi isolates grown for 7 days, were placed mycelial downwards in the centre of each Petri dish. The plates were incubated in a thermostat at $25 \pm 2°C$ for 7 days. Every 24 h, the growth of the isolate was measured with a ruler in two perpendicular directions until the growth of the test isolate in the control plate reaches the edge of the plate. The suppression coefficient (SC) was calculated according to the formula.

$$sc = (a-b)/a \times 100\%, \tag{1}$$

a − diameter of isolate in Petri plate on the control media (mm), b − diameter of isolate in Petri plate on media with CFS (mm).

Suppression coefficient sensitivity is divided into three main levels, given in Figure 1.
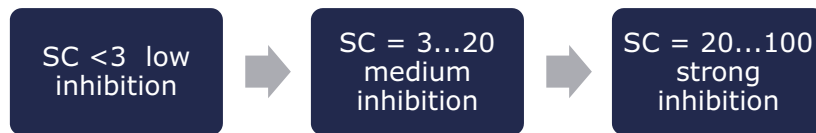
Figure 1: Suppression coefficient sensitivity levels

2.3. Methodologies on certain software usage

Two data processing strategies were used while implementing work programme. First strategy was to use MATLAB software with premade code, adjusting it to fit the needs and purposes of the research. Second strategy was to use Excel with additional add-ins like GInaFiT or DMFit, which allows to have better support for users, not yet very familiar with the predicative microbiology topic.

2.3.1. MATLAB

MATLAB is incredibly versatile tool used in many different scientific fields, such as engineering, data analysis, algorithm development and others. Specifically in microbiology this software can be used to analyse data, visualise it (as given in Figure 2) or create scripts. For this programme implementation MATLAB was used with prewritten code, allowing to simulate different scenarios in real cases. One of real-case analysis was to simulate and determine growth or no growth of certain bacteria inoculum size under certain concentration of nanoparticles.
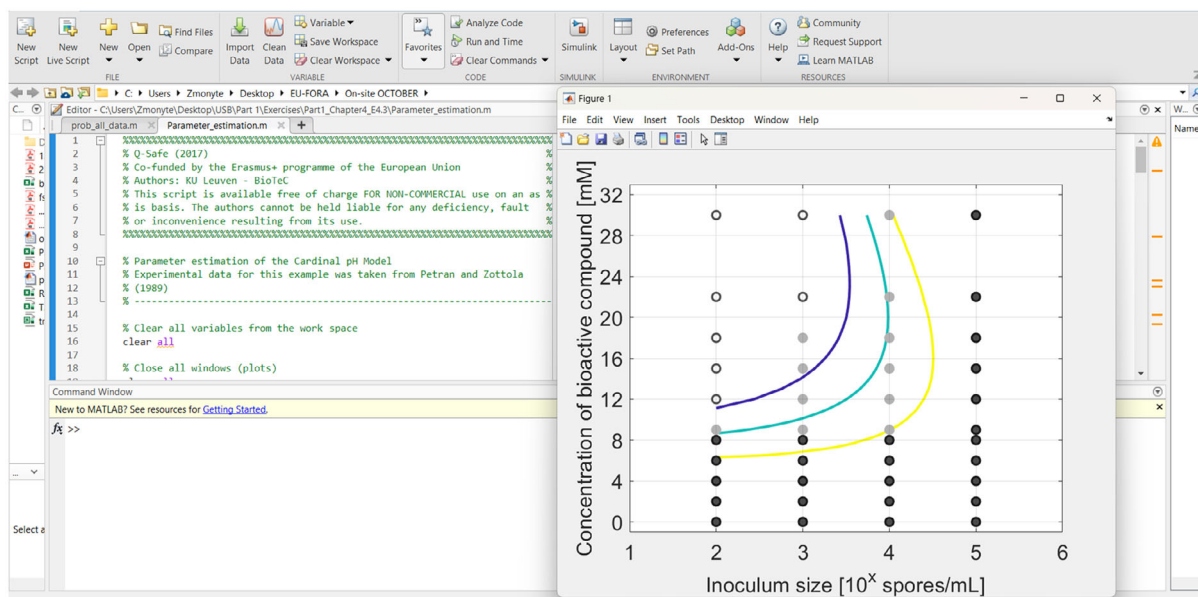
Figure 2: Hands-on MATLAB training example

As shown in Figure 2, hands-on trainings with MATLAB were carried out by fellow and supervisor. This type of research can provide valuable information about efficacy of any chemical or non-chemical treatment to survival rate of certain microorganism to certain treatment conditions.

2.3.2. Microsoft® Excel add-ins – GInaFiT

Excel itself can help with many calculations needed to create predicative models and freeware as GInaFiT can help to fasten and simplify some of the calculations and even prevent typical mistakes in data processing. GInaFiT aims to bridge the gap between people developing predictive modelling approaches and end-users in the food industry or research groups not disposing of advanced non-linear regression analysis tools.

The tool is useful for testing 10 different types of microbial survival models on user-specific experimental data relating the evolution of the microbial population with time. The 10 model types are:

1) classical log-linear curves,
2) curves displaying a so-called shoulder before a log-linear decrease is apparent,
3) curves displaying a so-called tail after a log-linear decrease,
4) survival curves displaying both shoulder and tailing behaviour,
5) concave curves,
6) convex curves,
7) convex/concave curves followed by tailing,
8) biphasic inactivation kinetics,
9) biphasic inactivation kinetics preceded by a shoulder,
10) curves with a double concave/convex shape.

The models were originally published as Bigelow and Esty (1920), Cerf (1977), Geeraerd et al. (2000), Mafart et al. (2002), Albert and Mafart (2005), Geeraerd et al. (2005) and Coroller et al. (2006). Next to the obtained parameter values, the following statistical measures are automatically reported: standard errors of the parameter values, the sum of squared errors, the (root) mean sum of squared errors, the R2 and the adjusted R2. In addition, t4D, the time needed for a 4 log reduction of the initial microbial population, as originally proposed by Buchanan et al. (1993), is also automatically reported (for data sets covering at least four decimal reductions).

The tool can be used in two ways. On one hand, for end-users having already a qualitative idea of the general shape of their survival curves, the choice for one of the model types is obvious. On the other hand, if the end-user does not have a clear idea yet, two or more of the different model types available can be tested and compared. The time for a four decimal reduction can be useful to summarise the information present in a data set, for example, if a common survivor curve shape cannot be selected for a range of different conditions tested (Geeraerd et al., 2000).

Additionally, the tool has some built-in features testing for misuse, for example, when trying to identify a model with tailing on data not having a tail or when using a too limited number of data points (observations) in comparison with the number of parameters in the model type chosen (the number of parameters ranges from 2 to 5 for the ten model types available).

2.3.3. Microsoft® Excel add-ins – DMFit

DMFit is another free tool, that can be accessed either online (https://browser.combase.cc/DMFit.aspx) or added as Microsoft® Excel add-in. Web edition is a web-based application to fit bacterial curves where a linear phase is preceded and followed by a stationary phase. The desktop version is part of the system used in-house at the Institute of Food Research to model the time-variation of the logarithm of cell concentrations of bacterial batch cultures. DMFit can be used to visualise growth or survival data, obtain parameters estimates from data fitted to growth or survival models, calculate maximum growth or death rate, lag phase time, initial or final cell count and estimate standard errors on these parameters.

Data input is user-friendly because it is compatible with Excel spreadsheets or textfiles (both online and desktop versions). Data can be fitted to two different types of models:

a) Model of Baranyi and Roberts (1994), which describes a sigmoid bacterial curve. This model, unlike other sigmoid curves, has very close to linear mid-phase. This model has four main parameters (Initial Value, lag, maximum rate, Final Value) and two curvature parameters: mCurv and nCurv which describe the curvature of the sigmoid curve, respectively, at the beginning and at the end of the growth phase. This tool allow to describe only the growth or death at the stationary phase, if mCurv parameter is set to zero and 'no lag' model option is selected. Model can also describe only the lag phase and growth or death phase if nCurv is set to zero and 'no asymtot' model option is selected.

b) Trilinear, biphasic or linear models. Trilinear model describes a bacterial growth curve with three straight lines: the lag phase and the stationary phase are described by two horizontal straight lines. The slope of the third straight line describing the growth/death phase is called the 'maximum rate'. Biphasic model must be used when data-based curve has no lag phase or no stationary phase. Linear model can be used in case bacterial counts describe only growth or death phase.

Even though tool use most common units, users must pay attention that by default time is given in hours, bacteria counts in log10 cfu/g and that leads to maximum growth or death rate given as log10cfu/g per h. User after loading data must ensure, that units are aligned accordingly.

3. Inhibitory activity and main points in risk assessment

3.1. CFS inhibitory activity results

Lactiplantibacillus plantarum MI-LPI recently has shown promising activity by inhibiting growth of various food pathogens, such as bacteria or yeast with specific inhibition effectiveness on Candida albicans (Riešuté et al., 2022). But mentioned activity was determined by using live lactic acid bacteria in model systems. *L. plantaru*m from unique Kaunas University of Technology collection is known for producing plantaricin, which has potential antifungal properties. The aim of this investigation was to use CFS to investigate excreted metabolites activity. In order to have higher concentration of excreted metabolites CFS was lyophilised and dry powders were used for experiments. The yeast species used in the study were selected based on the highest potential results in the live bacteria study and the moulds were isolated from organic wheat grown in Lithuania.

CFS inhibitory activity was tested on three different yeast (*Candida albicans, Saccharomyces cerevisiae, Rhodotorula rubra*) and six fungi (identified from organically grown wheat) (*Penicillium commune, Penicillium roqueforti, Penicillium griseofulvum, Fusarium oxysporum, Fusarium solani, Aspergillus cristatus*) and nine bacteria (*Bacillus cereus, Bacillus subtilis subsp. Spizizenii, Enterococcus faecalis, Escherichia coli, Klebsiella aerogenes, Listeria monocytogenes (serotype 4B), Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella enterica subsp. Enterica serovar* Typhimirium).

Metabolites from cultivation on neutral pH media showed no measurable inhibition, and samples cultivated in acidic pH at 45°C also showed no measurable inhibition. Inhibitory activity on different fungi was detected, but SC value was lower than 3. Medium and strong inhibition was identified on yeast samples. The highest inhibition was identified in *Candida albicans* when treated with a 10% metabolite additive in media. This result supports the idea, that CFS with *L. plantarum* metabolites can be considered as yeast growth inhibitor. As neutral pH samples did not show measurable inhibition, further investigation and determination of active metabolites is needed to eliminate hypothesis, that activity is based only on organic acids influencing the acidic pH. Research on CFS inhibitory activity on nine selected bacteria are being continued and newly gained knowledge on tools are being used to analyse data obtained. After completion of additional experiments scientific publication will be released.

3.2. Safety concerns and risk assessment done by EFSA

Just as in other industries, food sector experiences influence of different trends. For instance, usage of various bacteria for food safety or other technological purposes is one of them. Safety questions and concerns about *Lactiplantibacillus plantarum* use in food and feed is already circulating in EFSA opinions.

EFSA periodically releases updated list on generic pre-evaluation of the safety of microorganisms, intended to use in food and feed chains (EFSA BIOHAZ Panel, 2023). These statements are based on an assessment of published data for each agent, with respect to its taxonomic identity, the body of relevant knowledge and safety concerns. Safety concerns identified for a taxonomic unit (TU) are, where possible, confirmed at the species/strain or product level and reflected by 'qualifications'. These continuing publications provide updated and current knowledge not only to The Panels in EFSA, but also researchers and industry.

During past few years several opinions were dedicated to investigating safety of *Lactiplantibacillus plantarum* in various forms and terms of use. Also, EFSA opinions support one health approach and consider safety in the aspect of animal, human and environment health. Summarised opinions are given below.

In September of 2022 scientific opinion provided conclusions on the efficacy of two technological additives to improve ensiling of forages consisting of *Lactiplantibacillus plantarum* strains ATCC 55058 and ATCC 55942, respectively, for all animal species (EFSA FEEDAP Panel, 2022a). Request confirmed, that both additives were intended to be used for all forages and for all animal species and minimum bacteria concentration was given at 5×10^6 CFU/kg forage. In previous scientific opinions The Panel could not conclude decision on safety and efficacy due to insufficient data. But in this case, applicant provided supplementary information, which included enough evidence, that the addition of *L. plantarum* strains ATCC 55058 or ATCC 55942, have the potential to improve the ensiling process by reducing protein degradation in all type of forages as indicated by the reduction of ammonia production.

Similar scientific opinion was released in September 2021. This was also requested from European Commission, but in this case concentration and strain was different. In scientific opinion safety and efficacy of *Lactiplantibacillus plantarum* when used as a technological additive intended to improve ensiling of forage intended to use in all types of forage and for all animal species with minimum concentration of 1×10^8 CFU/kg (EFSA FEEDAP Panel, 2021a). In this case, strain identity was also clearly established with no signs of antimicrobial resistance. The Panel concluded that use of the strain as additive can be considered to be safe for animals, humans and environment. Furthermore, any signs of skin or eye sensitisation, but mentioned as possible respiratory sensitiser. Similar conclusions were given in two scientific opinions released in June of 2021 (EFSA FEEDAP Panel, 2021b,c).

Upon request from European Commission scientific opinion was released by EFSA in March 2023 (EFSA FEEDAP Panel, 2023a). In this case, *L. plantarum* was used as acidity regulator incorporated in oat-derived products, carrot root-derived products and coconut flesh-derived products at minimum inclusion level of 8.0×10^{10} CFU/kg with intention to use mentioned products in feed for horses, dogs, cats and pet rabbits. The reference strain has no signs of resistance to certain antibiotics used in humans or in veterinary. The Panel concluded that in this particular use this particular strain is considered as safe for target species, consumers of horse meat and environment. Regarding user safety, no evidence of skin or eye irritation was found, but taking into consideration the proteinaceous nature of additive, special precautions for respiratory sensibilisation were given.

This year the scientific opinion was published in EFSA Journal. This was done by following request from European Commission to deliver scientific opinion on the feed additive consisting of *L. plantarum* and *L. reuteri* (AQ02) as a zootechnical feed additive for suckling piglets (EFSA FEEDAP Panel, 2023b). Even though in previous opinion FEEDAP concluded that mentioned additive was considered safe for target species, consumers and the environment, new conclusion was not so straightforward. The Panel drawn attention to possible respiratory irritations and insufficient data to conclude on skin or eye irritation potential. Based on provided data The Panel could not conclude on the efficacy of the additive.

Another opinion, released in May 2023 considering the assessment of the application for renewal of *Lactiplantibacillus plantarum* DSM 23375, a technological additive to improve ensiling of fresh material for all animal species was requested (EFSA FEEDAP Panel, 2023c). In this case, the applicant provided evidence, that additive is already on the market and complies existing conditions of authorisation. The Panel concluded, that *L. plantarum* DSM 23375 remains safe for all animal species, consumers and environment under authorised conditions of use. It is important to notice, that This Panel also could not provide conclusions on user safety and the skin sensitisation potential of the additive. Similar conclusions in similar situation when renewal of authorisation was needed was also released earlier in EFSA Journal on January of 2022 (EFSA FEEDAP Panel, 2022b).

4.   Conclusion

Exploration and implementation of novel microorganism-based food protection substances require to focus effort in well planned and funded research. Predictive microbiology can be an incredibly useful tool used to optimise laboratory research and providing more knowledge about growth or death rates which leads to process optimisation towards best possible results. Even though *L. plantarum* demonstrated tremendous potential in food and feed applications, further investigations are still needed. In past few years EFSA concluded several opinions on *L. plantarum* usage in feed, but usage possibilities in food are still under investigation. Furthermore, the transition from live bacteria to concentrate of metabolites cannot be done without adequate safety measures.

Fellow will continue research on *L. plantarum* metabolites inhibitory activity at the sending organisation. Next steps will aim at better understanding the mechanism and efficacy of metabolites was *in silico* investigation of possible compounds and will be followed by identification of these compounds in CFS. Furthermore, regarding the shown inhibitory activity against spoilers, investigation of metabolites efficacy in model food matrix will be done.

Qualitative models cannot always provide sufficient data for proper risk assessment so quantitative tools are essential part of adequate risk assessment of any kind. In this work programme microbial and chemical risk assessment became very close one to another. The main target was CFS which already was without any living bacteria cells, but this cannot be taken into consideration as only chemical risk source as the substances were obtained directly from certain bacteria species (*L. plantarum*). During fellowship implementation all aims were addressed. Fellow was introduced to predicative microbiology fundamentals by participating in lectures, workshops with supervisor and self-

learning activities. Delivered understanding about experimental design was used for laboratory research planning and implementation. Furthermore, three specific tools were combined to better understand differences between different models, data fitness for purpose and differences between growth and inactivation models in general.

5. Recommendations

As fellowship programme is not only about gaining specific knowledge on various steps in risk assessment but also expanding fellows circle of contacts, follow up meetings, organised in any convenient way for both – fellow and supervisor, might be a good practice to maintain created working groups in different organisations.

6. Documentation provided to EFSA

1) Quantitative tools in microbial and chemical risk assessment. July 2023. KTU & NKUA

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Abbreviations

CFS	cell-free supernatant
DM	dynamic modelling
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
KTU	Kaunas University of technology
LAB	lactic acid bacteria
NKUA	National and Kapodistrian University of Athens
QPS	qualified presumption of safety
SC	suppression coefficient

Appendix A – Secondary activities

Additional relevant activities and learning opportunities completed by fellow:

1) 'Induction training of the European Food Risk Assessment Fellowship Programme', EFSA premises, 5–23 of September 2022
2) Lecture sessions provided by hosting site, NKUA premises, October – November, 2022
   Lecture - 'A History of Predictive Microbiology'
   Lecture - Predictive Microbiology Fundamentals'
   Lecture - 'Predictive Microbiology Fundamentals'
   Lecture - 'Experimental Design 1'
   Lecture - 'Kinetic Models'
   Lecture - 'Experimental Design 2'
   Lecture - 'QMRA Model - Scenario Analysis'
   Lecture - 'Parameter Estimation'
   Lecture - 'Applied Modelling'
   Lecture - 'Non-Linear Regression in Excel'
   Lecture - 'Predicting Microbial Behaviour during Food Storage in Risk Assessment'
   Lecture - 'Binomial Distribution'
   Lecture - 'Risk Assessment'
   Lecture - 'MATLAB - Getting Started'
   Lecture - 'MATLAB - Parameter Estimation'
   Lecture - 'MATLAB - Simulations'
   Lecture - 'Life Cycle Assessment'
   Lecture - 'Integrating Process Modelling approaches in Microbial Modelling'
   Lecture - 'Modelling Effect of Process and Formulation on Microbial Level in Food'
   Lecture - 'Thermal Inactivation: Application of Models'
   Lecture - 'Partitioning'
   Lecture - 'Implementation of Microbial Risk Assessment Model'
   Lecture - 'Building a Microbial Risk Assessment Model'
   Lecture - 'Basics of Multiscale Modelling in Predictive Microbiology'
3) 'Module 1 training of the European Food Risk Assessment Fellowship Programme', held online, 28th of November – 2nd of December 2022
4) 'Risk Assessment Research Assembly', organised by EFSA in Berlin, 7th of December 2022
5) Participating in organisational committee of 5th congress of Baltic microbiologists, September 2022 – October 2023 (conference will be held in 11–13th of October in Vilnius)
6) Course in English 'Science communication to society' 1 ECTS, Kaunas, October-December 2022
7) Participation in course in national language (Lithuanian) 'Introduction to variance analysis with SPSS', Kaunas, 18th and 31st of January 2023
8) Course in English 'Principles of academic and scientific writing in English' 1 ECTS, Kaunas, March-April 2022
9) Course in English 'Digital tools for scientific writing and publishing' 1 ECTS, Kaunas, March-April 2022
10) Participation in course in national language (Lithuanian) 'Validation and verification of research methods', Kaunas, 2nd of March 2023
11) 'Module 2 training of the European Food Risk Assessment Fellowship Programme', held online, 20–27th March 2023
12) Participation in IAFP's European Symposium on Food Safety with poster 'Investigation antifungal properties of L. plantarum metabolites concentrated by lyophilisation', Aberdeen, 3–5th of May, 2023
13) 'FunShielf4Med' seminar 'Identification of Mycotoxins', Institute of Technology of Agricultural Products, Athens, 16th of May 2023
14) Visit at laboratory of Food Quality Control and Hygiene of Agricultural University of Athens, 18th of May 2023
15) 'Module 3 training of the European Food Risk Assessment Fellowship Programme', EFSA premises, 12-16th of June 2023

16) Training School 'Modelling the effects of low pH and other stresses on foodborne micro-organisms to improve food and drink quality' of the COST Action CA18113 EuroMicropH, 'Understanding and exploiting the impacts of low pH on micro-organisms' NKUA, Athens, 28-30th of June 2023