Development of ComBase-PMP, a combined database and predictor of microbial responses to the food environment

Final Report of Food Standards Agency Project B13003

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Contents

Glossary and Abbreviations

α_0	Dimensionless quantity between 0 and 1 that describes the physiological state of spores in the Baranyi equation (see Appendix 1 for further details)
cfu	Colony forming unit
DMFit	Dynamic Modelling and Fitting (see http://www.ifr.ac.uk/safety/DMFit); a Microsoft Excel Add-In developed at IFR to fit sigmoid curves.
IFR	Institute of Food Research
NaCl	Sodium chloride (salt)
VB6	Microsoft Visual Basic 6.0 programming platform

Executive Summary

Background

In 2003, the Food Standards Agency and the Institute of Food Research from the United Kingdom and the USDA Agricultural Research Service and its Eastern Regional Research Centre from the United States signed a Concordat to collaborate in the development and maintenance of an Internet-based system called ***ComBase*** (www.combase.cc). The system allows users to browse through publicly available data on microbial responses to the food environment and provides software tools to analyse those data. The aim of the initiative is to provide food manufacturers, regulatory officers, risk assessors and food microbiology researchers around the world a common and internationally recognised pool of data and software to predict microbial responses to changes in food composition, manufacturing and storage. The Australian Food Safety Centre of Excellence joined the initiative in February 2006, in which year a new Concordat was signed.

Since then, ***ComBase*** has become the largest and most significant food microbiology database in the world, storing quantified microbial responses to the food environment. It contains data from (UK and US) government-funded research, as well as data from the literature and data-donating laboratories. It is supplied with programs that helps users to navigate and search in the database and predictive packages based on the stored raw data. The project has attracted great international acknowledgement, shown by many invitation around the world to hold workshops on its use, and data donated by many laboratories as contributions to a common goal.

The ***Combase*** web site is hosted by the Institute of Food Research (IFR), Norwich, UK. It is a hub that directs user enquiries to

(1) the actual raw data and the software navigating in the database;
(2) software tools on how to use the information in the database.

IFR has been responsible for the quality assurance of the data accumulated, and the development of the predictive software, both in terms of modelling and programming. ERRC has produced the software navigating in the database. A ComBase Executive Committee was established with yearly meetings to assist the development of the structure, function and dissemination of the initiative.

Rationale and Objectives

In this project, an internet-based platform has been developed to replace the former MAFF-funded Food MicroModel as a package integrating a much bigger database and much more sophisticated predictive modelling tools. Its easy availability and use increased the users' knowledge and confidence in predictive microbiology and contributes to improve food safety.

The objectives of this project were:

1. Enter available data in ComBase continuously.
2. Further develop and provide Technical Support to the predictive software Growth Predictor.
3. Unify four groups of publicly available datasets (FSA, ARS, EU, and Literature) to develop predictive models of growth and survival.
4. Create the ComBase web site at IFR and link it with relevant pages of FSA and other partners.

5. In collaboration with USDA-ARS ERRC, develop ComBase-PMP: a Combined Database and Predictive Modelling Program integrating browsing and predictive facilities in a single package.

Outcome/Key Results Obtained

- Growth Predictor version 2 has been launched, with improvements based on users' suggestions.
Remark: This was a stand-alone version of the predictive growth models formerly developed. However, in agreement with the FSA, the stand-alone platform was not pursued further. The technical help for installation was very time-consuming and a web-based predictor (ComBase Predictor) was developed instead, which is now available via the *ComBase Modelling Toolbox* link of the www.combase.cc web site.

- The ComBase website is linked with FSA and other partner sites, to make it easier for users to find the information most suitable to their enquiry.

- Data from European partners have been incorporated into ComBase. This was also supported by a 2-yrs project from the EU. Currently we host ca 37,000 records, and the majority of the records are full logcount curves From literature, we also put data in Combase when it represented only a growth or death rate as a response to the environment in question. Ca 2,000 records are not on the Internet yet since they were observed in dynamic environment and the ComBase Browser is not able to display dynamic environment yet.

- Using all available data (FSA, ARS, EU and literature), predictive models of growth and survival have been developed (see technical report).

- ComBase-PMP: Combined Database and Predictive Modelling Program stand-alone version was developed and made available to data donors.
Remark: By July 2006, the predictive microbiology research group at the US partner has become smaller and smaller. After departure of key expertise, its leader, Dr Mark Tamplin also left and sadly the programmer of their flagship predictive software, the Pathogen Modelling Program, died. Currently there is no prospect for PMP to be developed further and the ComBase-PMP association is not possible anymore. Therefore we omitted the reference to PMP from the integrated web-version and it has become ***ComBase Modelling Toolbox.*** This package consists of
(i) *ComBase Predictor* representing generic (i.e. based on microbial curves in broth with optimal medium) growth, non-thermal survival and thermal inactivation models, *applicable also under fluctuating temperature*;
(ii) A scenario-specific dynamic predictor, the *Perfringens Predictor* to model the growth of *Clostridium perfringens* during the cooling of beef.
(iii) The web-version of *DMFit*, which fits sigmoid curves to "log cfu vs. time" bacterial growth data.
(iv) A help system and a manual have been made freely available via the internet on the use of the above packages.
(v) ca 30 invited ComBase workshops have been held around the world.

What it means and why it is important

Similarly to genomic databases (gene banks), ComBase serves as a repository for data that can be accessed by users seeking to estimate microbial responses to various food

environments. However, even if all publicly available data are really also *accessible* (the role of ComBase Browser) it is not evident how to use the data. The Integrated ComBase environment, now also including the ComBase Modelling Toolbox, gives invaluable help, ideas, guide and examples how to process the data, which will have obvious and positive implications on the standardisation of the work of food microbiology laboratories international risk assessors, researchers and regulatory officers.

Section One: Objectives of the project

Objective 1. Launch Growth Predictor version 2.
Version 2 was launched with improvements based on users' suggestions. However, in agreement with FSA, this tool was not developed further. Growth Predictor was a stand-alone version of the predictive growth models formerly developed in the MicroBase project with FSA. This stand-alone platform required a lot of technical help when users installed it, and a web-based predictor (ComBase Predictor) was developed instead, which is now available via the ComBase Modelling Toolbox link of the www.combase.cc web site.

- Objective 2. Develop an integrated web environment with links to FSA and other partner sites.
The www.combase.cc address is today the most frequently visited web site at IFR, getting hundreds of world-wide hits every day (excluding automated search engines like Yahoo, Google, etc). It is a hub of Knowledge Transfer making it easier for users to find the information most suitable to their enquiry.

- Objective 3. Incorporate data from supporting partners into ComBase.
This was also supported by a 2-yrs project from the EU. Currently ComBase hosts ca 37,000 records, and the majority of the records are full logcount curves. From literature, we also put data in Combase when it represented only a growth or death rate as a response to the environment; these represent some 5,000 records. Ca 2,000 records are not on the Internet yet since they were observed in dynamic environment and the ComBase Browser is not able to display dynamic environment yet.

- Objective 4. Use all available data (FSA, ARS, EU, Literature), predictive models of growth and survival.
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(iii) The web-version of DMFit, which fits sigmoid curves to "log cfu vs. time" bacterial growth data.
(iv) A help system and a manual have been made freely available via the internet on the use of the above packages.

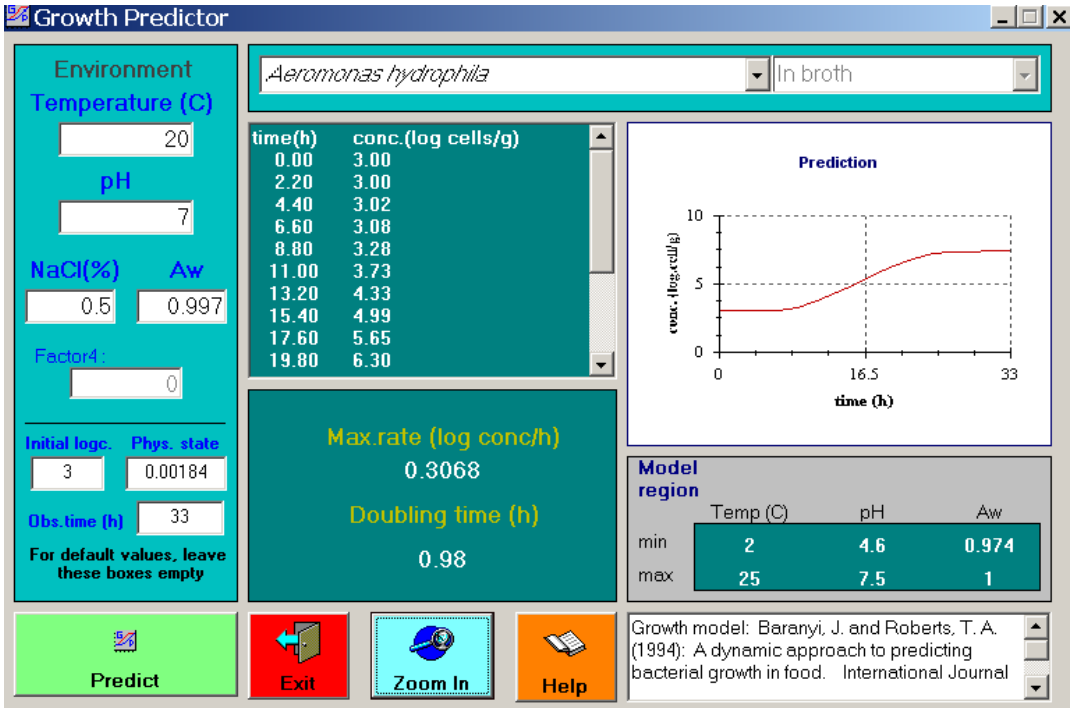
Below we give details of these objectives. Most results are summarised in the user manual on a CD enclosed to this report; we don't repeat those here.

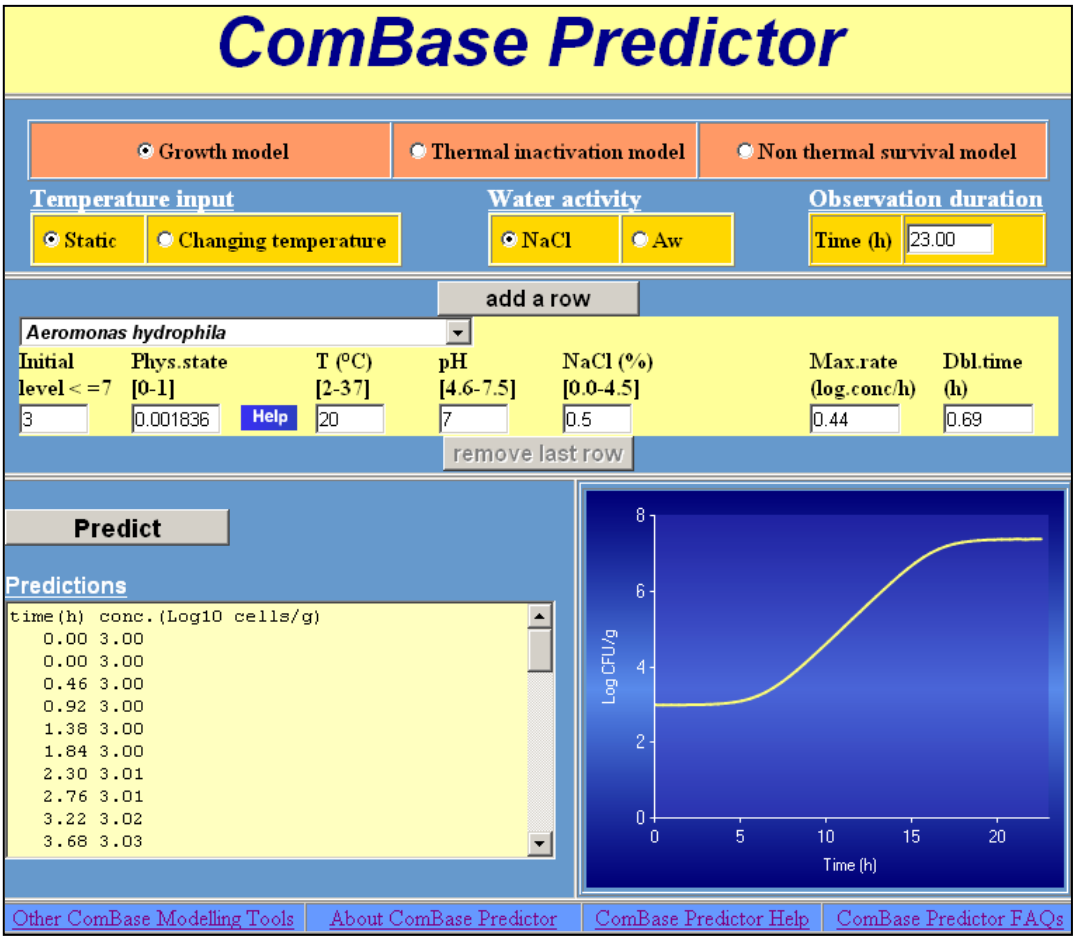
Section Two: Objective 1. Launch Growth Predictor version 2

Growth Predictor version 1 was sent to users (Unilever, US and UK food consultants) and based on their suggestions, some modifications were devised to make it more user-friendly. Its Version 2 is now available at
http://www.ifr.ac.uk/Safety/GrowthPredictor/
and also it is enclosed to this report on a CD. However, still many problems have remained with its installation. It was written in Visual Basic 6.0 and it needed the MSCHART.OCX file that is a standard Windows system file but, by default, not part of the installation. Therefore, if the user did not have administrator right, he/she could not install it, even if we enclosed it in the installation package. Another problem was that Visual Basic 6 was not supported anymore, but upgraded to Visual Studio, but the migration was far from seamless. Even today we have difficulties to modify VB6 programs in Visual Studio.
Therefore, in 2005, we agreed with the FSA project officer (Geraldine Hoad) that we don't develop the final product in VB6 but in .Net (dotNet). This means that the user can get prediction only via the internet. This may make it less attractive feature for those who has no or slow internet connection but this is only question of time when Internet will be common and fast. On the other hand, in the long run, we won't have installation problem anymore, all upgrade can be done on the server

As a result, the web-based ComBase Predictor was developed instead, which is now available via the ComBase Modelling Toolbox link of the www.combase.cc web site. The user manual of the program and the program itself is on the CD enclosed to this report.

Fig. 1. Example screens from Growth Predictor and ComBase Predictor, describing the growth for *Aeromonas hydrophila* at the default conditions (temperature 20 C, pH 7, NaCl 0.5%).





Section Three: Objective 2. Develop an integrated web environment with links to FSA and other partner sites

Combase, www.combase.cc has become a predictive microbiology knowledge transfer hub with hundreds of world-wide hits every. Fig. 2, shows the available main submenu points:

Browser – Where users can access more than 35 thousand datasets describing the microbial response to a specific environment;

Submit data – Where procedures for data donations are explained (note that ca 20% of all data was received this way;

ComBase Predictor **–** This menu point will be replaced by the more general *ComBase Modelling Toolbox*, where users can access ComBase Predictor, as a generic package; Perfingens Predictor, as a (food-hazard) scenario-specific tool; DMFit as a modelling tool. The list should continue further in the future.

Support **–** Various documents to help the user's enquiry;

Training – Where users can get information from training opportunities. (ca 30 ComBase workshops were held worldwide, funded by the hosts; most of them overbooked).

FAQ – Frequently Asked Questions (and answers)

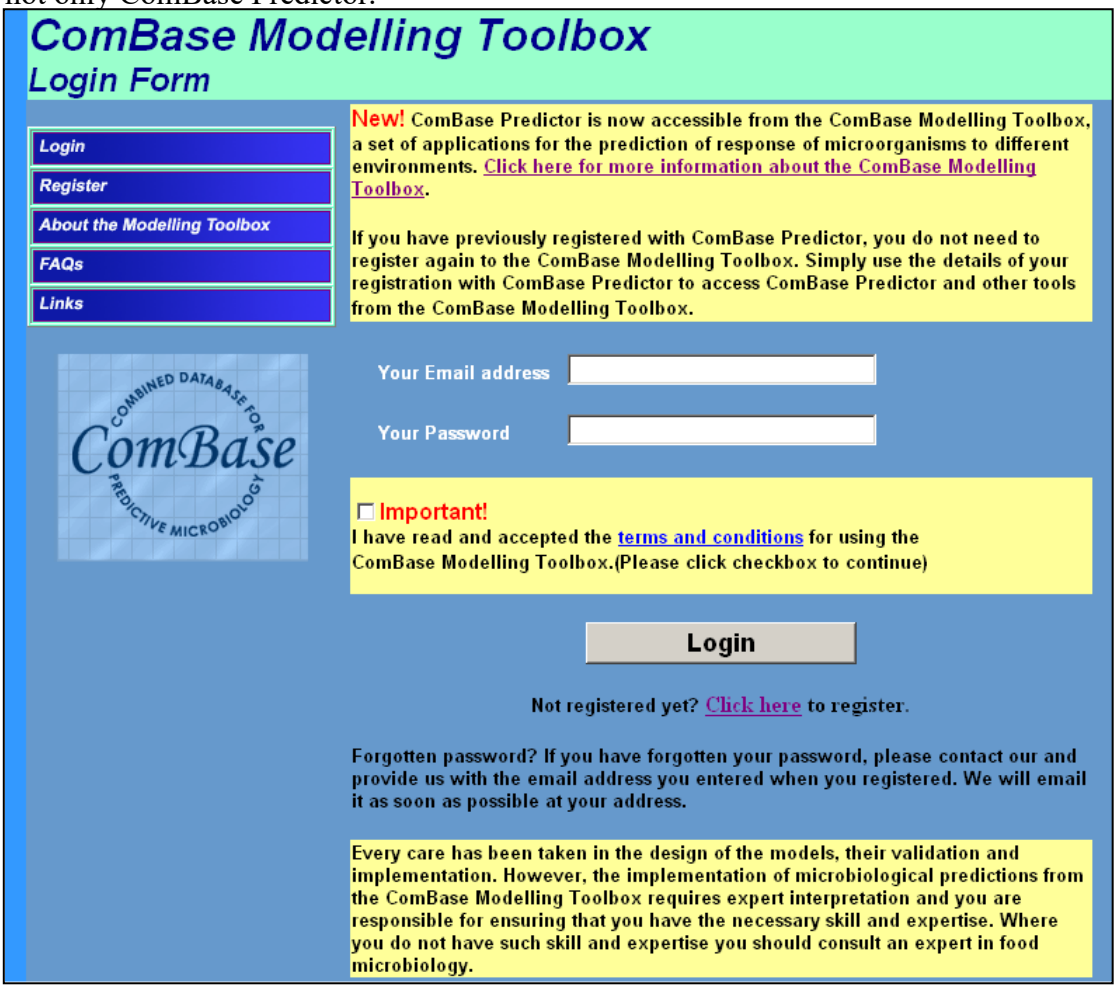
About **–** Basic information on Combase

Links – Useful links to the members of the ComBase Partnership (FSA, among them) and twin organisations.

The best demonstration of the power of the hub is trying it on the Internet.

Fig. 2. Opening screen of www.combase.cc.

Fig. 3. The new ComBase Modelling Toolbox will host many useful software tools, not only ComBase Predictor.



Section Four: Objective 3. Incorporate data from supporting partners into ComBase

During the project, the amount of data stored in ComBase increased by more than 50% and currently it has ca 37 thousand records, though from these only 35 thousand are available since the remaining were generated under dynamic conditions (mostly in fluctuating temperature) and the current browser is not able to accommodate that situation yet. The majority of the records are full logcount curves. From literature, we also put data in Combase when it represented only a growth or death rate as a response to the environment; these represent some 5,000 records.
The most significant data donors were:

Institution	**Contribution**
Dept. Food Micro.; Univ. Cordoba, Spain	Listeria in vegetables; ca. 500 rec.
Dept of Ind. Microbiol., Univ. Complutense	OD-derived rates of spoilage organisms; ca 1000 rec.
Budapest University, Hungary.	Listeria growth in presence of LAB; ca 50 rec.
Dpt Nutr. y Brom. III. Univ.Complutense, Spain	Pathogens, spoilage; mainly in MA; viable count curves and doubling times, by OD; ca 2000 rec.
Danish Institute of Fisheries Research	Spoilage organisms in broth and seafood; ca 200 rec.
INRA, Avignon. France	Growth and survival of various pathogens; ca 400 rec.
Agricultural University of Athens. Greece	Spoilage organisms, mainly in olives; ca 2000 rec.
Technical University of Bratislava, Slovakia	Pathogens and spoilage, in broth and milk ca 50 rec.
Public Health Laboratory Services - UK	Pathogens at low water activity; ca 100 rec.
Metropolitan University. London UK	Spoilage organisms in broth and food; ca 500 rec.
University of Reading. UK	Pathogens in broth, inactivation and survival; ca 100 rec.
Unilever Research Sharnbrook. UK	Pathogens in food; ca 200 rec.
Campden and Chorlywood FRA. UK	Spoilage organisms; ca 500 rec.
TNO, Holland	Lactic acid bacteria in broth and food; ca 500 rec.
Veterinary University of Viennna, Austria	Spoilage organisms in broth and food; ca 200 rec.
Instituto Zooprfilatico Sperimentale Brescia, Italy	In cheese and salami; ca 1500 rec.

These partners have received the full dataset on a CD, accompanied by validation and modelling tools developed at IFR.

Also, if workshop participants requested the full database, they got the same CD. Those who got the full version, were asked to sign a document saying that the data will not be used for commercial purposes.

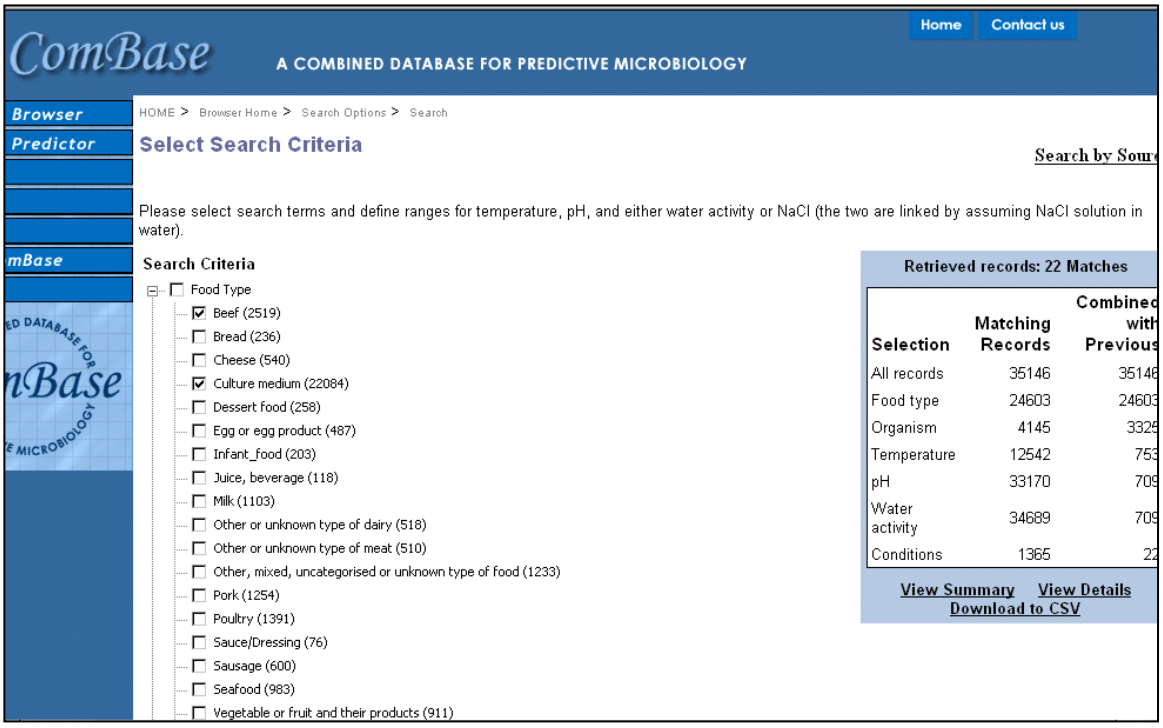
Section Five: Objective 4. Use all available data (FSA, ARS, EU, Literature), predictive models of growth and survival

This was the main objective of the project. Originally it was planned by the USDA-ARS-ERRC partner, that the product will be named as *ComBase-PMP: Combined Database and Predictive Modelling Program*. The second part of the name was used to keep the PMP abbreviation that had gained an established image in the US. However, by July 2006, the predictive microbiology research group at the US partner has lost its key members and no replacement has been made. The result is that their product the stand alone package PMP was not been developed further and the PMP has been loosing its reputation these days. This is why we agrees that we just keep the name Combase, even if it is actually not only a database. From the ComBase hub (www.combase.cc) there are two major links: the actual database, accessible by the *Combase Browser* (developed and hosted by the US partner), and the *Modelling Toolbox* (developed in this project at IFR) containing more and more (currently three) software to analyse the data. Until approval, the Modelling Toolbox can be tested at http://ifrsvwwwdev/maftest/ComBasePredictor/Login.aspx?ReturnUrl=%2fmaftest%2fComBasePredictor%2fDefault.aspx

A stand-alone integrated database + predictor was also developed and made available to data donors of the project. This can be found on the CD enclosed to this report (CBP.EXE).

The ComBase database is now accessible via a new user interface of USDA-ARS ERRC. Its main advantage is the more advanced query functionality (Fig 4.)

Fig. 4 Search in the ComBase database.



When the users select the dataset they are interested in, they can see an overview of the selection, and they can browse them one by one as shown in Fig. 5.
A major new feature here, that the measured curve can be analysed right on the spot. This was developed at IFR in this project and represents one of the most powerful and popular functionalities of ComBase.
Namely, the displayed curve can be either fitted (Fig. 6) by the model of Baranyi and Roberts (which is the most commonly used primary model, also programmed in DMFit) or can be compared with the predicted curve (Fig. 7) generated by the ComBase Predictor. The difference between the observed and predicted curve gives a visualisation of the performance of the predicted models and increases the confidence it their use. These features are increasingly used in higher education and training, mainly to demonstrate the meaning of 'safe' estimations (the predictions are based on pure culture measurements in broth with optimum media, therefore they are generally overestimate the observations in common food).

Fig. 5 Displaying a specific record from ComBase. The query of the user (see Fig 4) resulted in 366 records, from which the plot shows the details of the 20th. The curve can be fitted and compared with predictions by using the circled links (see Fig. 6).

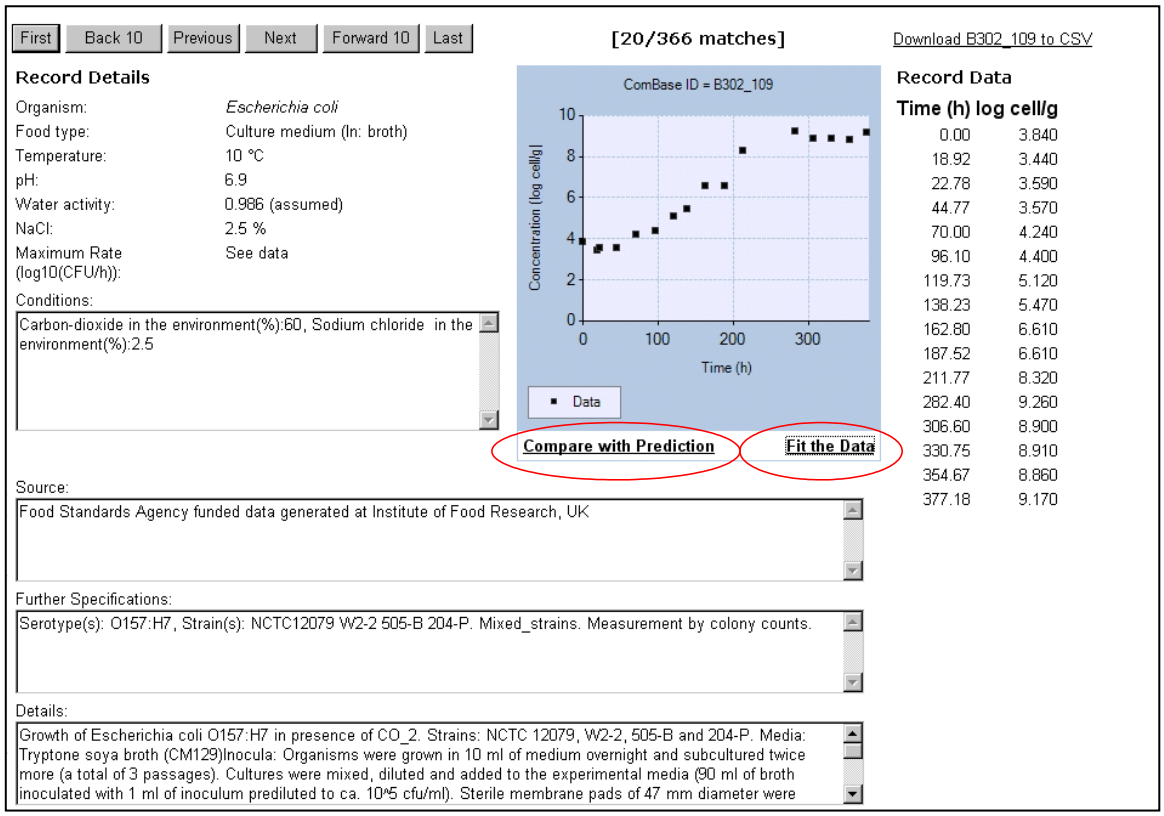


Fig. 6. The record selected from the Combase database (Fig. 5) can be fitted right on the spot, by means of our new, web-based version of the DMFit curve fitting program.

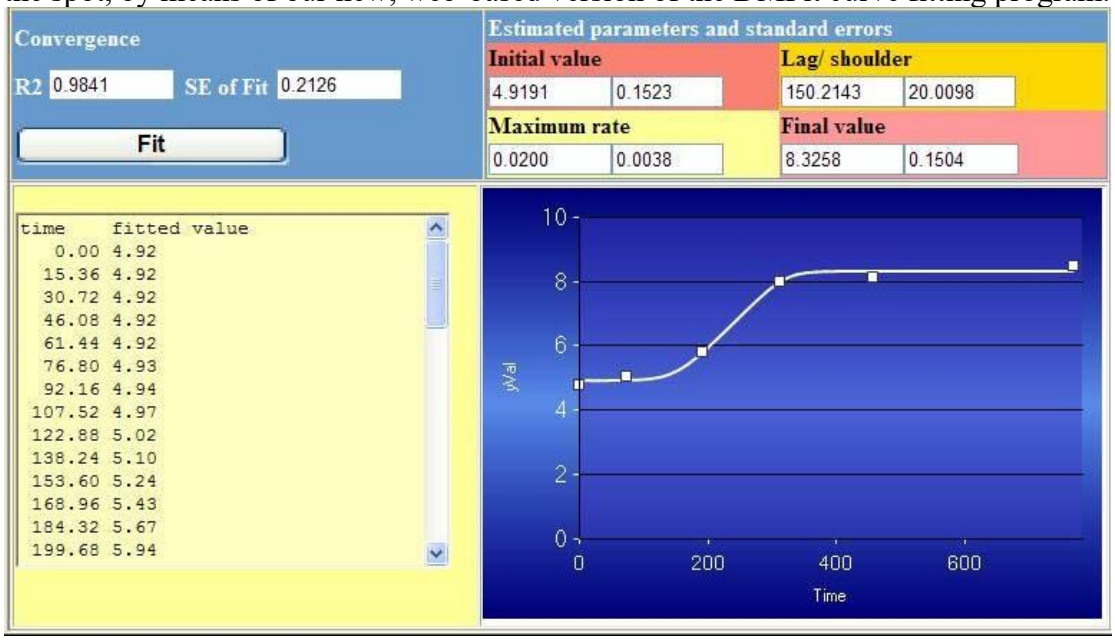
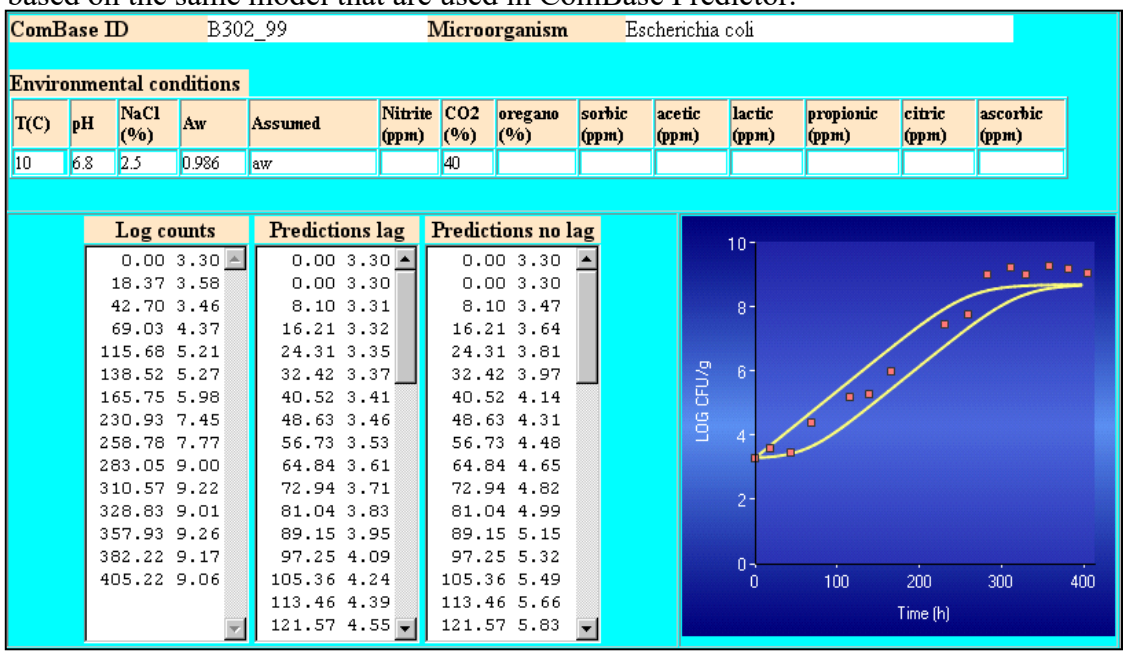


Fig. 7. The record selected from the Combase database can be compared with predictions right on the spot. The predicted curves (with and without lag time) are based on the same model that are used in ComBase Predictor.



By ComBase Predictor, users can compare the growth / survival of four organisms, and/ or under four different combinations of the environmental factors, as shown in Figs 8 and 9:

Fig. 8. Growth of various organisms under the same conditions (temp 15°C, pH 7) in three days.

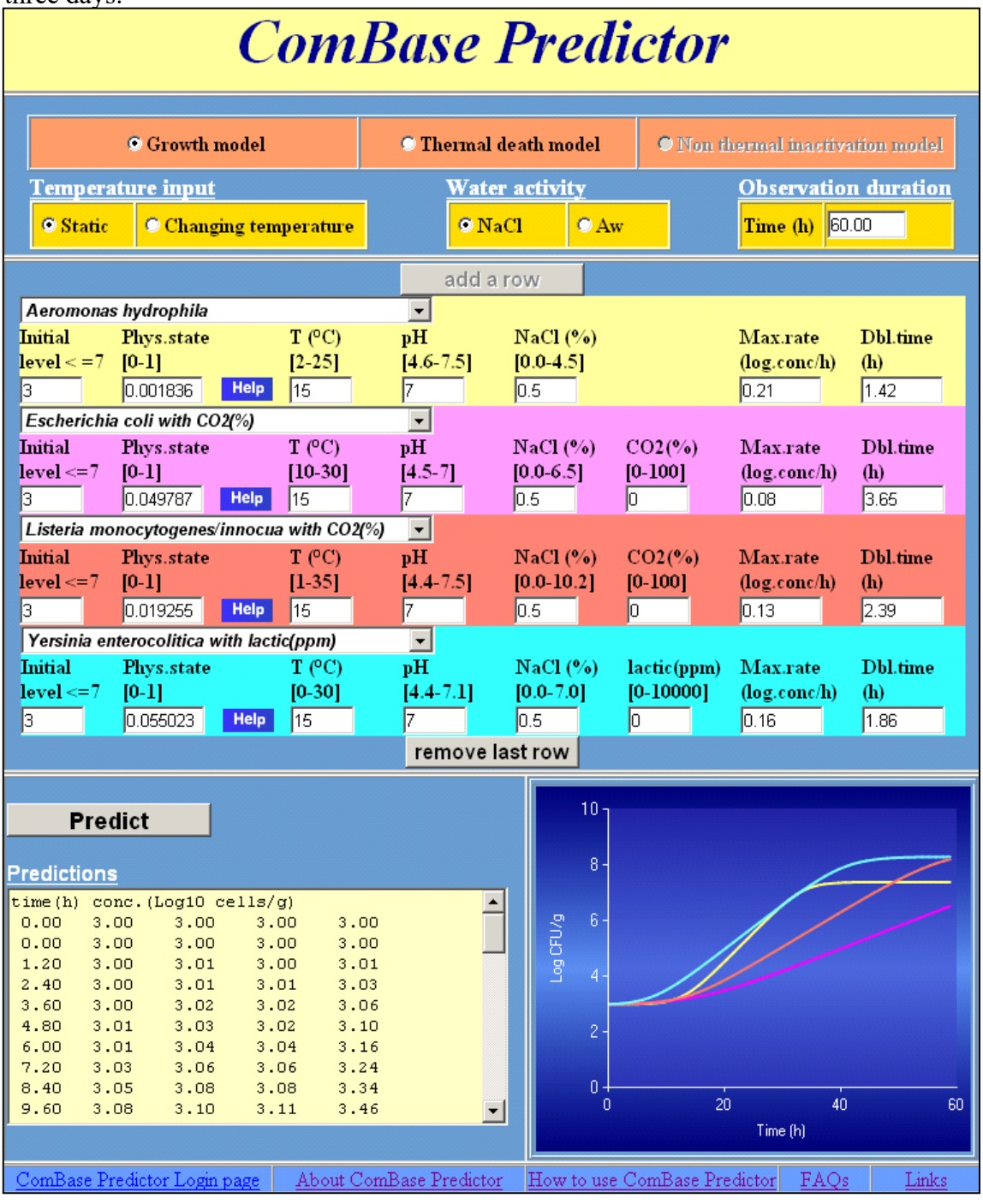
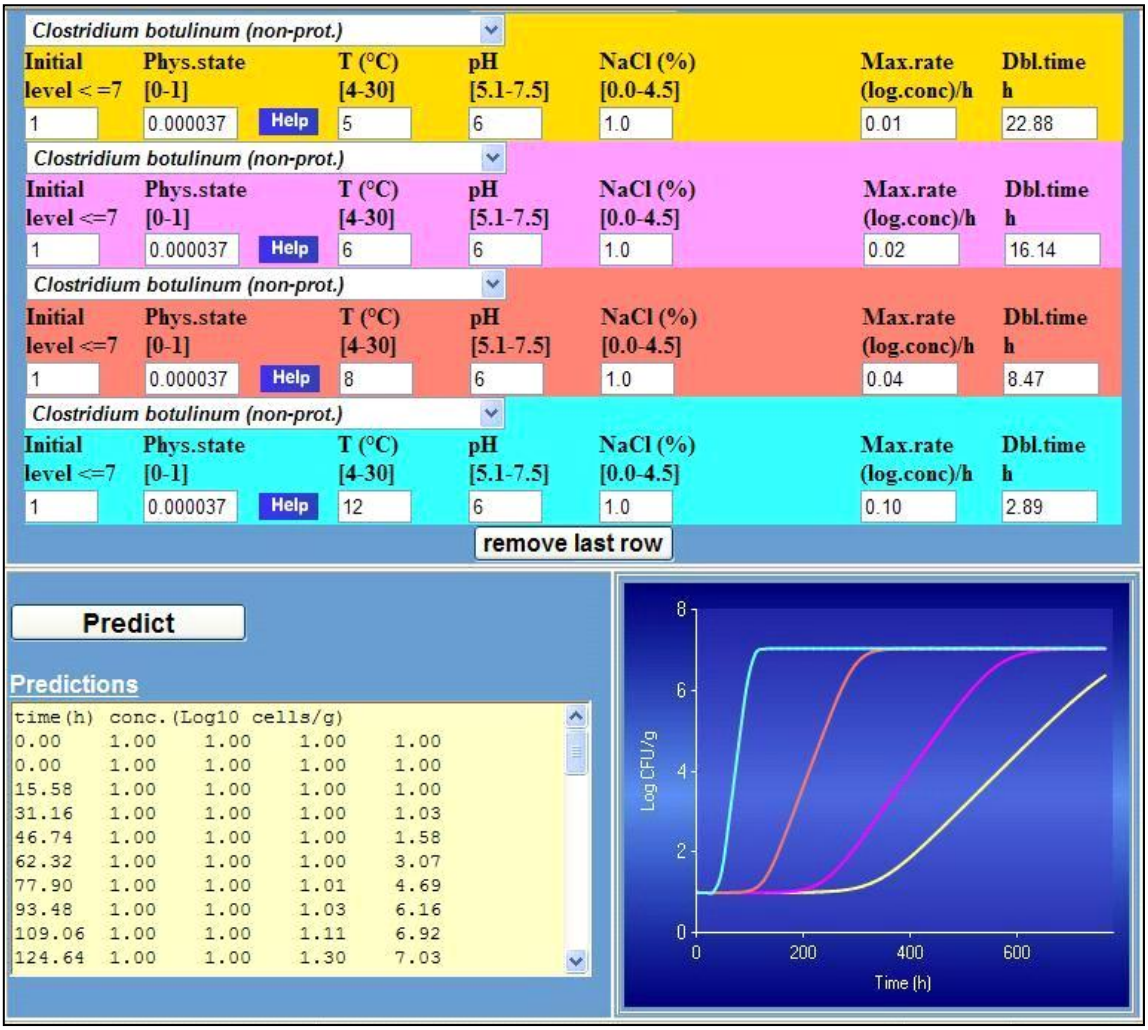


Fig. 9. Effect of temperature on the growth of non-proteolytic *C. botulinum* at pH 6, NaCl 1% .

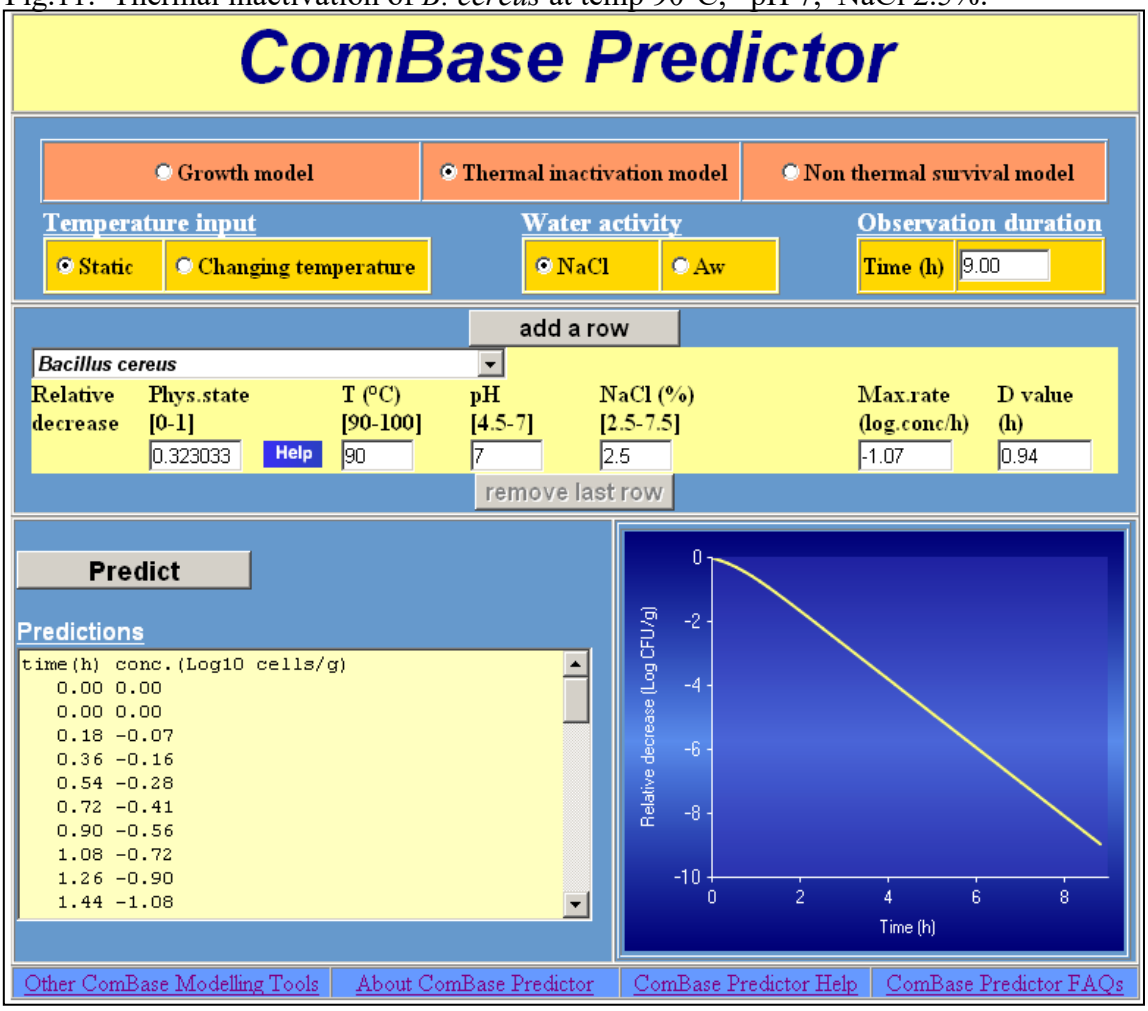


Major progress have been made in the extension of the models. Now, ComBase Predictor can be used to estimate growth / inactivation curves under dynamically changing temperature, too, as shown in Fig 10.

Fig. 10. *S. aureus* and *E. coli* under fluctuating temperatures at pH 5.9, NaCl 1.5%.

Also, thermal inactivation and non-thermal survival curves can be generated according to new models created in the last year of the project (Fig. 11)

Fig.11. Thermal inactivation of *B. cereus* at temp 90°C, pH 7, NaCl 2.5%.



The models in ComBase Predictor are based on the data that are summarised in the tables 1-3. Their mathematical background has been published and summarised in the project report of a previous FSA-funded project.

Table 1. Summary of raw datasets (observed "logcount v. time" curves) selected to develop growth models. Initial datasets: observed growth curves used to generate the growth models in the previous version of ComBase Predictor.

		(3 factors*)		(4th factor**)	
Growth	**Extended**	**No. of curves in the initial set**	**No. of newly included curves**	**No. of curves in the initial set**	**No. of newly included curves**
Aeromonas hydrophila	**Yes**	125	**33**	-	**-**
Bacillus cereus with CO2(%)	**Yes**	86	**109**	58	**-**
Bacillus licheniformis	No	53	**-**	-	**-**
Bacillus subtilis	No	68	**-**	-	**-**
Clostridium botulinum (non-prot.)	No	52	**-**	-	**-**
Clostridium botulinum (prot.)	No	77	**-**	-	**-**
Clostridium perfringens	No	51	**-**	-	**-**
Escherichia coli with CO2(%)	**Yes**	80	**89**	78	**-**
Listeria monocytogenes / innocua with CO2(%)	**Yes**	251	**279**	75	**9**
Listeria monocytogenes / innocua with nitrite(ppm)	**Yes**	251	**279**	63	**118**
Listeria monocytogenes / innocua with lactic(ppm)	**Yes**	251	**279**	129	**-**
Listeria monocytogenes / innocua with acetic(ppm)	**Yes**	251	**279**	52	**-**
Staphylococcus aureus	No	93	**-**	-	**-**
salmonellae with CO2(%)	**Yes**	146	**113**	23	**-**
salmonellae with nitrite(ppm)	**Yes**	146	**113**	28	**-**
Shigella flexneri with nitrite (ppm)	**Yes**	-	**193**	-	**125**
Yersinia enterocolitica with CO2(%)	**Yes**	282	**35**	31	**-**
Yersinia enterocolitica with lactic(ppm)	**Yes**	282	**31**	77	**-**
Brochothrix thermosphacta	No	44	**-**	-	**-**
Pseudomonas spp	**Yes**	-	**144**	-	**-**

* 3 factors: temperature, pH and Aw

** 4th factor: additional facor (either CO_2 or lactic acid or acetic acid or nitrite)

Table 2. Summary of raw datasets (observed “logcount v. time” curves) selected to develop thermal inactivation models. Initial datasets: observed growth curves used to generate the growth models in the previous version of ComBase Predictor.

		(3 factors*)		(4th factor**)	
Thermal inactivation	**Extended**	**No. of curves in the initial set**	**No. of newly included curves**	**No. of curves in the initial set**	**No. of newly included curves**
Bacillus cereus	No	41	-	-	-
Clostridium botulinum (non-prot.)	No	35	-	-	-
Escherichia coli	**Yes**	51	**21**	-	-
Listeria monocytogenes/innocua	**Yes**	67	**149**	-	-
Salmonellae	**Yes**	61	**8**	-	-
Yersinia enterocolitica	**Yes**	82	**4**	-	-
Brochothrix thermosphacta	**Yes**	-	**53**	-	-

* 3 factors: temperature, pH and Aw
** 4th factor: additional factor (either CO_2 or lactic acid or acetic acid or nitrite)

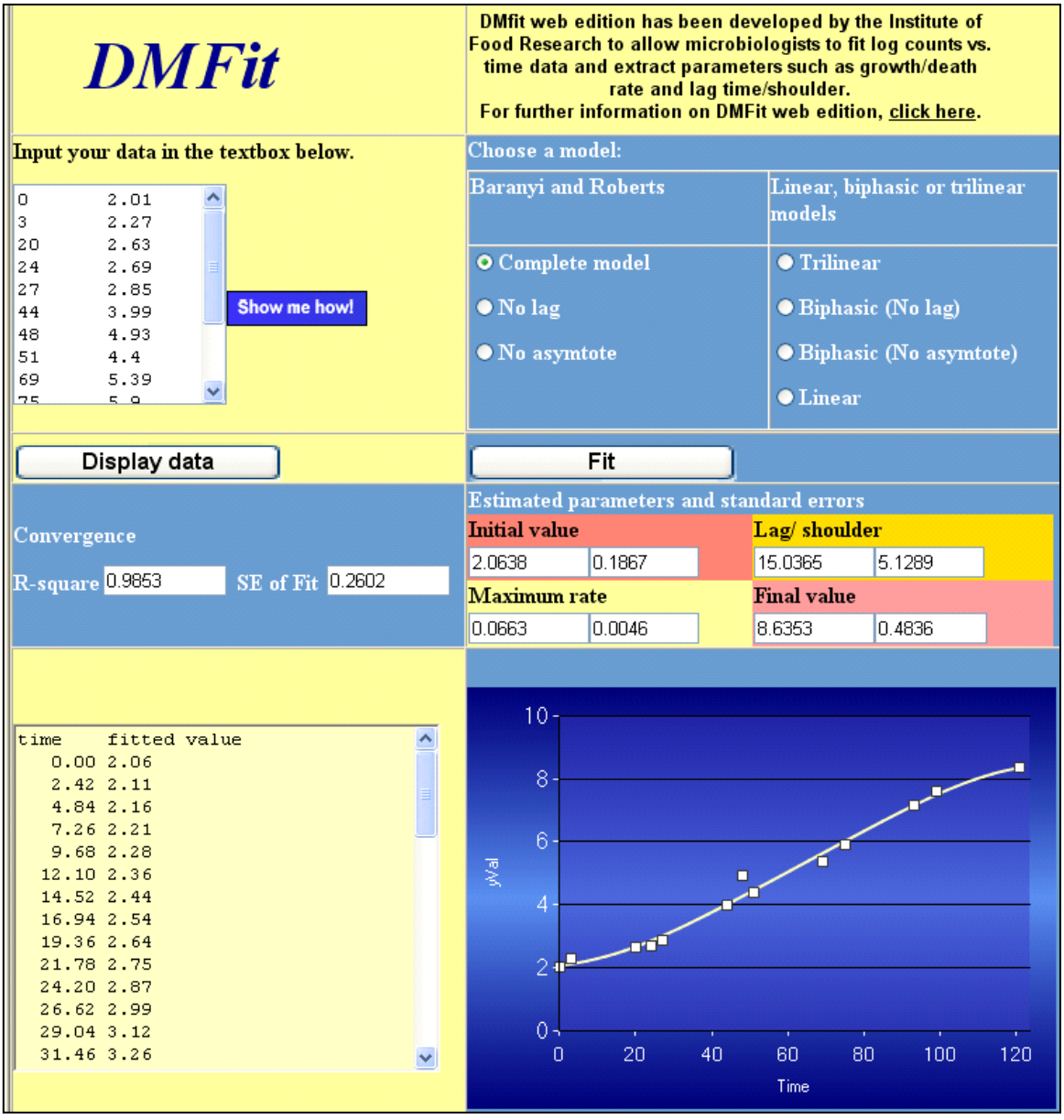
Table 3. Summary of raw datasets (observed “logcount v. time” curves) used to develop non-thermal survival models.

NEW non-thermal survival models	**No. of observed survival curves selected**
Listeria monocytogenes/innocua	**194**
salmonellae	**102**

* 3 factors: temperature, pH and Aw
** 4th factor: additional factor (either CO_2, lactic acid, acetic acid or nitrite)

As mentioned, other features of the ComBase Modelling Toolbox is the Perfringens Predictor, a result of another FSA-funded project, and the DMFit web edition (Fig. 12), which is an extra plus, originally not in the objectives, but completed on users' demand. Users can do predictive modelling tasks completely on the internet now, using freely available software packages.

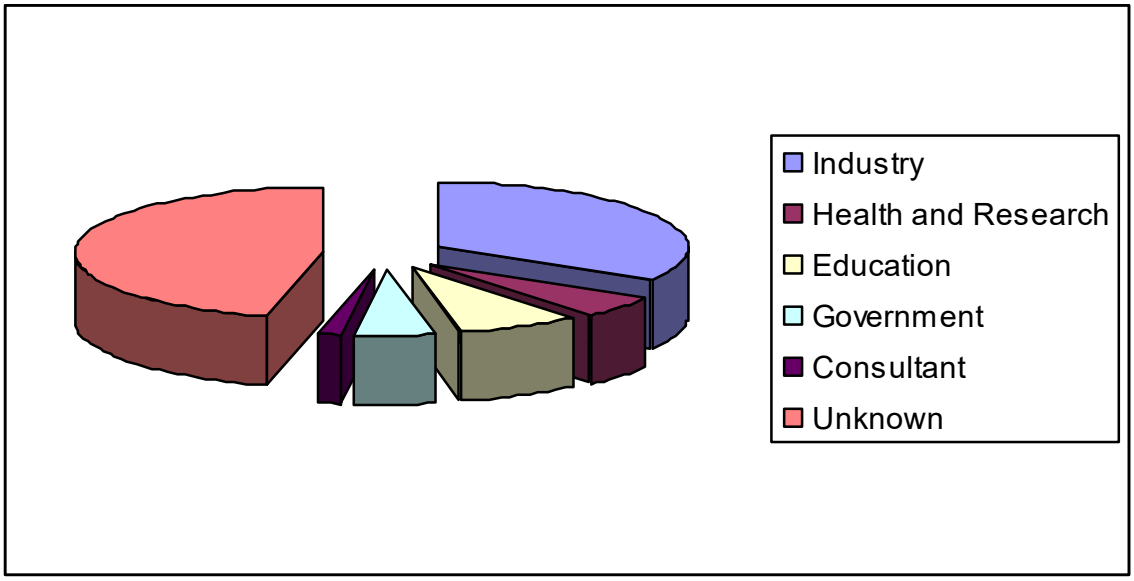
Fig. 12. DMFit web-edition. It fits complete sigmoid curves or their partial versions to data.



Section Six: Dissemination

All development are available on the Internet. Acknowledgement is shown by several publications referring to it (see the references), invited workshops (see below), many invited conference talks (among them keynote lectures) and the fact that editors of the Journal of Food Protection, a leading, refereed food science publication, call authors of accepted publications to submit their data to Combase.

Telling from the email addresses of those logging on ComBase, the distribution of users can be represented as below:



This means that the majority of users come from industry.

Section Seven: Publications and presentations

In addition to written and oral reports given to the Food Standards Agency, other publication and presentation activities are listed below

Refereed papers, proceedings and book chapters

Baranyi J. and Tamplin M. (2004). ComBase: A Common Database on Microbial Responses to Food Environments. J. Food Prot. 67, 1967–1971.

Tamplin M., Baranyi J. and Paoli G. (2003). Software programs to increase the utility of predictive microbiology information. In: Modelling Microbial responses in Foods. (Eds: R.C McKellar, X. Lu.) CRC, Boca Raton, Fla.

Baranyi, J. (2004). The ComBase Initiatve. In: Proceedings of the EU COST 920 Risk Assessment Symposium, Pamplona, SPAIN, 28-29 June, 2004. (Ed: Arie Havalar)

Le Marc Y, Baranyi J, and Ross T (2005): Mechanistic modelling of pathogen stress response. In: Understanding pathogen behaviour. (Ed: Mansell Griffiths). Woodhead Publishing, London.

Baranyi J. (2005). Quantitative microbial ecology of food. Evolution of mathematical modelling in food microbiology. Invited Editorial. Acta Alimentaria, 34, 335–337.

Le Marc Y., Pin C. and Baranyi J. (2005): Methods to determine the growth domain in a multidimensional environmental space. Int.J. Food Microbiol. 100., 3-12

Le Marc Y, Huchet V, Beczner J, Thuault D, Mészáros L, Wilson D, Farkas J, Brocklehurst T and Baranyi J. (2005). Modelling the kinetics of *Listeria innocua* and *Listeria monocytogenes* in coculture with *Lactococcus lactis*. J.Appl. Microbiol. (submitted)

Baranyi J. (2006): Using the ComBase database and associated software tools to predict microbial responses to food environments. Food Manufacturing Efficiency, 1, 9-13.

McMeekin J., Baranyi J., Zwietering M., Ross T., Dalgard P., Bowman J. and Kirk M. (2006). Information systems in food safety management. Int. J. Food Microbiol. 112, 181–194

Popular articles:

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Baranyi J. and Pin C. (2003). ComBase – a combined database on bacterial responses to food environments. Invited workshop associated with SAFEPORK: the 5th Conference on the safety of pork; Crete, Greece, 1 October, 2003. (Lead by J. Baranyi)

Baranyi J. (2003). Computational tools for food microbiology. International course of the Master Programme “Food Safety of Animal Products" organised by University of

Bologna. (Invited lecture series).

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Baranyi, J. (2004). Predictive Microbiology Tools and ComBase. Workshop on the Annual Meeting of the American Society for Microbiology, New Orleans, 23-26 May.

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Baranyi J. (2004). ComBase – a combined database on bacterial responses to food environments. Invited workshop associated with FoodMicro 2004, Portoroz, Slovenia, 15 September, 2004.

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Baranyi J. (2006). ComBase – a combined database on bacterial responses to food environments. Workshop and seminars on the invitation of the University of La Sabana, Bogotá, Colombia. October 2006. The same time: Invited talk to undergraduates.

Baranyi J. (2006). Modelling Microbial Interactions. Seminar at University of Colorado State. November 2006.

Baranyi J. (2006). ComBase – a combined database on bacterial responses to food environments. Workshop and seminars on the invitation of the University of North London. May 2007.

Section Eight: Deliverables achieved

All the project deliverables have been achieved as shown below.

D1	***Growth Predictor* version 2** will have been improved and supplied with user-friendly help file and manual; also accompanied by continuous technical support.	31.12.2003	31.12.2003	Yes
D2	Create the ***ComBase* web-site** at IFR and link it with relevant pages of FSA and other partners.	30.09.2004	30.09.2004	Yes
D3	Include all available data from European partners in *ComBase*.	31.03.2005	31.03.2005	Yes
D4	Using all available data (FSA, ARS, EU, literature), **develop basic predictive models** of growth and survival.	31.12.2005	31.12.2005	Yes
D5	Using all the available data, **develop advanced predictive models**, with uncertainty measures and options of dynamic environment, of growth and survival; thereby upgrading all the predictive models in *ComBase*.	31.03.2006	31.03.2006	Yes
D6	Supply both the desktop and the internet version of the database with the new models, creating ***ComBase-PMP***: Combined Database and Predictive Modelling Program.	31.03.2006	31.03.2006	Yes; the name is different
D7	Dissemination activities, papers, leaflets, workshops.	30.06.2006	30.06.2006	Yes

Conclusions

According to Prof Tom McMeekin, Director of Australian Food Safety Centre of Excellence, "*...properly supported… ComBase can be a watershed in the development of Predictive Microbiology and its applications.*" (McMeekin, T.A.(2003). An Essay on the Unrealized Potential of Predictive Microbiology. In: McKellar, R.C. and Lu, X (eds): Modelling Microbial responses in Foods. CRC, Boca Raton, Fla., USA, pp 231-235). This project was a demonstration what direction it needs to go to successfully reach its potentials.

Acknowledgements

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Appendix A. Modelling background

A1. Introduction

Since the early 1980s, with the advent of powerful PCs, food safety research has increasingly turned to quantitative, computational tools. The development of databases, hand in hand with mathematical and statistical processing, has become a key element in food safety. This was recognised by the UK Ministry of Agriculture Fisheries and Food, who launched their Predictive Microbiology Programme at the end of the 1980s. Work funded under this Programme produced a large amount of growth and survival data for various food-borne organisms and formed the underlying database for the *Food MicroModel* software, which predicted the growth and survival of various (mainly pathogenic) micro-organisms in food. These data now constitute the core of the database behind *ComBase Predictor*.

A2. Data

Predictive modelling, for microbiological purposes, and therefore *ComBase Predictor,* is based on the recognition that, although food microbiology experiments may have been carried out for different purposes, such as challenge tests for new product development, or establishing the properties of an emerging pathogen, the results can always be put into the broad framework of "*Microbial response* to a specific *environment* around the cells". Mathematically, this is a mapping between two spaces. Both can be characterised by one or more (possibly dynamic, i.e. time-dependent) factors depending on how detailed the description is intended to be.

Predictive models for *ComBase Predictor* are based only on output from laboratory experiments observed in culture media under well-controlled laboratory conditions. Data were not all considered with equal weight, since the aim was not to model the average outcome of those measurements, but to produce safe (conservative) predictions. Additionally, many data were obvious outliers, identified by mathematical / statistical means. However, these data were retained in the database (though not used in the creation of the models), since they can be useful to estimate the variability of microbial responses when experiments are repeated. These are the main reasons for observed growth curves being generally below predicted curves.

The raw data are stored in a Microsoft Access database, in a structure that was developed at IFR.

A3. Mathematical modelling

The need to predict bacterial kinetics in food generated a discipline that is called Predictive Microbiology in the scientific literature. It focuses on the mathematical description of the microbial responses to the environment in food. A comprehensive review of the subject can be found in Ross *et al*. (1999), which also serves as a basis for this summary.

Predictive microbiology is based on the hypothesis that growth is an intrinsic characteristic of the organism and will occur reproducibly in the same environment. Variation of cell concentration is described by a mathematical (growth or survival) curve and this is called a ***primary model***. ***Secondary models*** describe how the parameters of primary models depend on environmental factors such as temperature, pH and water activity. These are described by mathematical functions and, by interpolation, the cell concentration against time can be predicted for any combination of conditions.

A3.1 Primary models

A3.1.1 A primary model for growth and thermal inactivation

Unlike the superseded *Food MicroModel*, which predominantly used the Gompertz sigmoid function for its primary growth model, *ComBase Predictor* uses the model of Baranyi and Roberts (1994). The main differences between the two approaches are explained below.

A sigmoid primary model is commonly described by four parameters:

(1) y_0 the natural logarithm of the initial cell concentration.
This is an initial value that is specific to the history of the organism and not related to the current growth environment.

(2) λ the time in lag phase (or shoulder).

The length of the lag phase will depend on the intrinsic characteristics of the bacteria, the current environmental conditions and the history (initial physiological state) of the cells. Cells that have come from a different environment or are damaged (for example after heat treatment or freezing) may require more time to synthesise macromolecules and repair damage before they can divide than undamaged cells from a similar environment.

(3) μ_{max} the maximum specific growth rate (or death rate).

This represents the steepest slope of the curve *cell concentration (natural logarithm) v. time*. It is considered to be an intrinsic characteristic of the organism dependant on the current environment but is not affected by the cell history. Note that the specific growth rate means the increase in natural logarithm per unit time, whilst the curves are shown with a logarithm to the base 10 of the cell concentration. Thus the real specific growth rate is, in fact, about 2.3-times higher than the growth rate seen on the plot.
The doubling time (required for the population to double in the exponential phase) is calculated as:

doubling time= ln(2)/specific growth rate.

The D-value (the time required for a 1-log reduction of the bacterial population in the death phase) is obtained as:

D-value= -ln(10)/specific death rate.

Note that all time values are in hours in the software.

(4) y_{max} the natural logarithm of the maximum cell concentration (or *ymin*, the logarithm of the concentration of the resistant population in the case of a death curve)

The maximum concentration is less extensively studied in predictive microbiology than the other growth parameters because food safety risk and spoilage generally appears at much lower concentrations; therefore, it is modelled only by an average value. In the case of thermal death models, y_{min} is not modelled but considered to be $y_{min}= -\infty$.

Using the above terminology:

1. The Gompertz function overestimates the maximum specific growth rate by 10-15%.

2. The maximum specific growth rate was the main model parameter for *Food MicroModel* and is the main model parameter for *ComBase Predictor*. However, in *ComBase Predictor*, the other one is not the lag but rather the “initial physiological state”, $\alpha 0$ (see Baranyi and Roberts, 1994). It is a dimensionless number between 0 and 1;if α_0 =0, then there is no growth, and the lag time is infinite; if α_0=1, there is no lag, and growth will commence immediately. It has the same role as the inoculum size: an initial parameter, quantifying the history of the cells (this parameter can be set up by the user).
From that, the lag is a consequence, as the following formula shows:

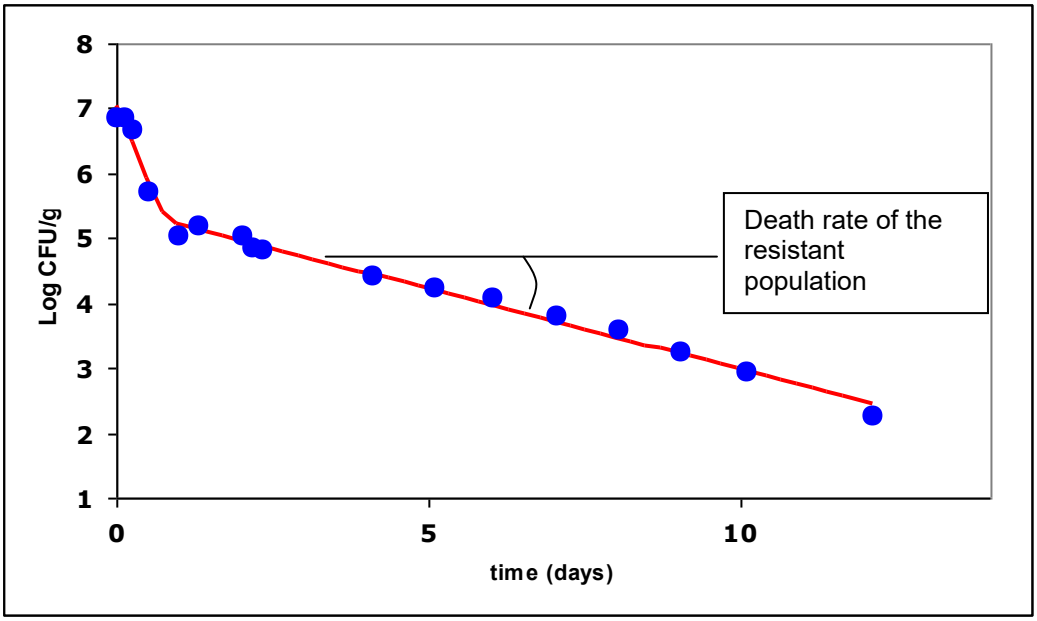
$$\lambda = -\ln(\alpha_0)/\propto_{max}$$

Because the user is rarely able to provide its value, a typical value is used as the default. Namely, if the chosen growth curves are fitted with the primary model, then the $\alpha 0$ values

obtained are modelled as function of the environmental factors (temperature, pH, water activity, possibly a fourth factor). This “secondary” model is simply a constant, taking the geometrical average of the $h_0 = -\ln(\alpha_0)$ values.

A3.1.2 Primary models for non-thermal inactivation

The model of Baranyi and Roberts (1994) has also been used to describe inactivation curves with sigmoid shapes (or without shoulder or tail). The model of Baranyi and Roberts is inappropriate to describe some inactivation curves which do not exhibit this sigmoid shape. For example, for some curves, the death phase has a concave shape, due to the presence of a resistant population. Concave curves or concave curves followed by tailing were modelled with respectively biphasic (see figure below) or tri-phasic models. In order not to underestimate microbiological risks, only the death rate of the resistant population was considered in the secondary modelling.



A3.2 Secondary models

When creating the secondary models, the logarithms of the specific growth rates (or death rates) were described as a function of the (possibly rescaled) environmental factors by a standard quadratic multivariate polynomial. The %NaCl values were first transformed into water activity values by the formula:

Aw=1-NaCl*(5.2471+0.12206*NaCl)/1000

(Resnik and Chirife, 1988). For the growth and the non-thermal death models, the water activity values were rescaled as in Gibson *et al*. (1994) to obtain:
b_w =sqrt(1-Aw).

For the models of *ComBase Predictor*, a standard second order polynomial modelled the effect of temperature, pH, and Aw (or b_w) values on the logarithm of the growth or death rate. This model was fitted for the rates produced by the primary models for each organism. They represent the **basic models**, which produce the “worst case” predictions, since further “fourth factors”, such as CO_2 or nitrite content, only decrease these growth rates. (Note that other subsidiary conditions are also recorded in the database, but not taken into account for the predictions).
Currently only one “fourth factor” can be taken into account in any particular model. If the value of the fourth factor is 0, then the prediction produced by the basic three-factor model is obtained. This feature is currently lacking in any other predictive microbiology programme.

A4. Model performance

Although modelling lag time would be preferable, to date, validated predictive models have been developed on growth rates. This is because the lag phase is technically difficult to study, so quantitative data is lacking, and lag is more difficult to model as it depends not only on the current conditions but also on the history (or initial physiological state) of the cells.

To demonstrate the goodness of fit of the models, observed rates were plotted against predicted, for each model, in log-scale (see Appendix C). The deviation of the points from the 45° equality line demonstrates the accuracy of the models. As a rule of thumb, the current performance of three-factor (core) predictive models is about 30-35 %, which means that the average error of prediction for the exponential growth / death rates is about this fraction of the respective predicted value.
One basic principle of empirical modelling is that one should not extrapolate, i.e. predictions should not be made outside the region of observations.

The limits of the growth and thermal death models for each environmental factor for each organism are given in the Table A1-A3 below:

Table A1. The limits of each environmental factor in each growth model. The primary variables are temperature, pH and water activity (Aw); the fourth factor (ef4) can be either CO2 , nitrite, or organic acids, but not a combination of more of them.

Model	**$Temp_{min}$**	**$Temp_{max}$**	**pH_{min}**	**pH_{max}**	**Aw_{min}**	**$ef4_{max}$**
Aeromonas hydrophila	2	37	4.6	7.5	0.974	0
Bacillus cereus with CO2(%)	5	34	4.9	7.4	0.94	60
Bacillus licheniformis	13	34	4	7.6	0.907	0
Bacillus subtilis	10	34	4.3	7.8	0.933	0
Clostridium botulinum (non-prot.)	4	30	5.1	7.5	0.974	0
Clostridium botulinum (prot.)	14	40	4.7	7.2	0.954	0
Clostridium perfringens	15	52	5	8	0.971	0
Escherichia coli with CO2(%)	10	42	4.5	7.5	0.961	100
Listeria monocytogenes/innocua with CO2(%)	1	40	4.3	7.5	0.924	100
Listeria monocytogenes/innocua with nitrite(ppm)	1	40	4.4	7.5	0.924	200
Listeria monocytogenes/innocua with lactic(ppm)	1	40	4.4	7.5	0.924	20000
Listeria monocytogenes/innocua with acetic(ppm)	1	40	4.4	7.5	0.924	10000
Staphylococcus aureus	7.5	30	4.3	7.1	0.907	0
salmonellae with CO2(%)	7	40	3.9	7.4	0.973	100
salmonellae with nitrite(ppm)	7	40	3.9	7.4	0.973	200

Shigella flexneri with nitrite (ppm)	15	37	5.5	7.5	0.971	1000
Yersinia enterocolitica with CO2(%)	-1	37	4.4	7.2	0.957	80
Yersinia enterocolitica with lactic(ppm)	-1	37	4.4	7.2	0.957	10000
Brochothrix thermosphacta	0	30	5.5	7	0.95	0
Pseudomonas spp	0	20	5	7.4	0.961	0

Notes: For each organism and model the upper limit of Aw = 1.
The fourth environmental factor is denoted by ef4, for which the minimum is always 0.

Table A2. The limits of each environmental factor in each thermal inactivation model. The primary variables are temperature, pH and water activity (Aw).

Model	**$Temp_{min}$**	**$Temp_{max}$**	**pH_{min}**	**pH_{max}**	**Aw_{min}**	**Aw_{max}**
Bacillus cereus	90	100	4.5	7	0.954	0.986
Clostridium botulinum (non-prot.)	80	95	4.1	7.3	0.971	1
Escherichia coli	54.5	64.5	4.2	8	0.947	1
Listeria monocytogenes/innocua	60	68	4.2	7	0.943	1
Salmonellae	54.5	65	4	7.1	0.997	1
Yersinia enterocolitica	52	60	4.2	7	0.961	1
Brochothrix thermosphacta	40	55	5	7	0.989	1

Table A3. The limits of each environmental factor in each non-thermal survival model. The primary variables are temperature, pH and water activity (Aw); the fourth factor (ef4) can be either CO2, nitrite, or organic acids, but not a combination of them.

Model	**$Temp_{min}$**	**$Temp_{max}$**	**pH_{min}**	**pH_{max}**	**Aw_{min}**
Listeria monocytogenes/innocua	0	20	3.5	7	0.793
Salmonellae	0	40	4.3	7.5	0.781

Note: For each organism and model the upper limit of Aw = 1

Note that it is possible that at certain conditions (for example high salt), both growth and survival predictions can be generated. The reason for this is that the interpolation region is not well definable in multi-dimension. The most advanced methods to overcome this difficulty is that of Le Marc et al (2005) but the implementation of that method in ComBase Predictor would require much more effort than what was the scope of this project. In a possible continuation of the project, the solution of this problem should be one of the main priorities. As a temporary measure, when the conditions of temperature, pH and water activity are also included in a growth model, a error message appears and the user is invited to continue with “Growth model”.

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Appendix B. Source of data to generate models

B1 Growth models

***Aeromonas hydrophila* (3 factors)**

Core model (temperature, pH, Aw): 125 rates from the Food Micro Model data set, 31 rates from Palumbo et al. (1991), 2 rates from Golden et al. (1989)

References

Palumbo S. A., Willimas A.C., Buchanan R.L. and Phillips J.G. 1991: Model for the aerobic growth of *Aeromonas hydrophila* K144. J. Food Prot. 54, 429-435.

Golden D.A., Eyles M. J., and Beuchat L. R. 1989: Influence of modified atmosphere storage on the growth of uninjured and heat injured *Aeromonas hydrophila*. Appl. Environ. Microbiol. 55(11), 3012-3015.

***Bacillus cereus* with CO2(%)**

Core model (temperature, pH, Aw): 86 rates from the Food Micro Model data set, 76 rates from the Institute of Food Research (Norwich, UK), 33 rates from London Metropolitain University.

CO_2 data: 58 rates from the Food Micro Model data set.

***Bacillus licheniformis* (3 factors)**

Core model (temperature, pH, Aw): 58 rates from the Food Micro Model data set.

***Bacillus subtilis* (3 factors)**

Core model (temperature, pH, Aw): 68 rates from the Food Micro Model data set.

***Clostridium botulinum* (non-prot.) (3 factors)**

Core model (temperature, pH, Aw):52 rates from the Food Micro Model data set.

***Clostridium botulinum* (prot.) (3 factors)**

Core model (temperature, pH, Aw): 77 rates from the Food Micro Model data set.

***Clostridium perfringens* (3 factors)**

Core model (temperature, pH, Aw): 51 rates from the Food Micro Model data set.

***Escherichia coli* with CO2(%)**

Core model (temperature, pH, Aw): 80 rates from the Food Micro Model data set, 72 data from Buchanan et al. (1992) and 17 data from Glass et al. (1992).

CO_2 data: 78 rates from the Food Micro Model data set.

References

Buchanan R.L., Klawitter, L. A. 1992. The effect of incubation temperature, initial pH and

sodium chloride on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiol. 9: 185 - 196

Glass K. A., Loeffelholz J. M., Ford J. P., and. Doyle M. P. 1992. Fate of Escherichia coli O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58(8), 2513 - 2516

***Listeria monocytogenes/innocua* with CO2(%)**

Core model (temperature, pH, Aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

CO_2 data: 75 rates from the Food Micro Model data set, 9 data from the Institute of Food Research, Norwich.

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y. H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

Le Marc Y. Développement d'un modèle modulaire décrivant l'effet des interactions entre les facteurs environnementaux sur les aptitudes de croissance de *Listeria*. Thèse de doctorat. Université de Bretagne Occidentale, France.

***Listeria monocytogenes/innocua* with nitrite(ppm)**

Data (temperature, pH, Aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

Nitrite data: 75 rates from the Food Micro Model data set, 118 data from Buchanan and Phillips (1990).

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y. H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

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***Listeria monocytogenes/innocua* with lactic(ppm)**

Data (temperature, pH, Aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

Lactic acid data: 129 rates from the Food Micro Model data set.

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y.H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

Le Marc Y. Développement d'un modèle modulaire décrivant l'effet des interactions entre les facteurs environnementaux sur les aptitudes de croissance de *Listeria*. Thèse de doctorat. Université de Bretagne Occidentale, France.

***Listeria monocytogenes/innocua* with acetic(ppm)**

Data (temperature, pH, Aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

Acetic acid data: 52 rates from the Food Micro Model data set.

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y. H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

Le Marc Y. Développement d'un modèle modulaire décrivant l'effet des interactions entre les facteurs environnementaux sur les aptitudes de croissance de *Listeria*. Thèse de doctorat. Université de Bretagne Occidentale, France.

***Staphylococcus aureus* (3 factors)**

Core model (temperature, pH, Aw): 93 rates from the Food Micro Model data set.

salmonellae with CO2(%)

Core model (temperature, pH, Aw): 146 rates from the Food Micro Model data set, 9 data from Bovill et al. (2000) and 104 data from Oscar (1999).

CO_2 data: 75 rates from the Food Micro Model data set.

References

Bovill R., Bew J., Cook N., D'Agostino M., Wilkinson N. and Baranyi J. 2000. Predictions of growth for *Listeria monocytogenes* and Salmonella during fluctuating temperature. Int. J. Food Microbiol. 59, 157-165.

Oscar T.P.1999. Response surface models for effects of temperature, pH, and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth. J. Food Protect. 62,106-111.

salmonellae with nitrite(ppm)

Core model (temperature, pH, Aw): 146 rates from the Food Micro Model data set, 9 data from Bovill et al. (2000) and 104 data from Oscar (1999).

Nitrite data: 28 rates from the Food Micro Model data set.

References

Bovill R., Bew J., Cook N., D'Agostino M., Wilkinson N. and Baranyi J. 2000: Predictions of growth for *Listeria monocytogenes* and Salmonella during fluctuating temperature. Int. J. Food Microbiol. 59, 157-165.

Oscar T.P.1999: Response surface models for effects of temperature, pH, and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth. J. Food Protect. 62, 106-111.

***Shigella flexneri* with nitrite(ppm)**

Core model (temperature, pH, Aw): 193 data from Zaika et al. (1992).

Nitrite data: 125 rates from Zaika et al. (1992).

Reference

Zaika L. L., Phillips J. G. and Buchanan R.L. 1992. Model for aerobic growth of *Shigella flexneri* under various conditions of temperature, pH, sodium chloride and sodium nitrite concentrations. J. Food Protect. 55, 509-513.

***Yersinia enterocolitica* with CO2(%)**

Core model (temperature, pH, Aw): 282 rates from the Food Micro Model data set, 12 rates from Alber et al. (1992) and 23 data from Badhuri et al. (1995).

CO_2 data: 31 rates from the Food Micro Model data set.

References

Alber S. A. and Schaffner D. W. 1992. Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. Appl. Environ. Microbiol. 58, 3337-3342.

Bhaduri S., Buchanan R. L. and Phillips J. G.1995. Expanded response surface model for predicting the effects of temperatures, pH, sodium chloride contents and sodium nitrite concentrations on the growth rate of *Yersinia enterocolitica*. J. Appl. Bacteriol. 79,163-170.

***Yersinia enterocolitica* with lactic(ppm)**

Core model (temperature, pH, Aw): 282 rates from the Food Micro Model data set, 12 rates from Alber and Schaffner (1992) and 23 data from Badhuri et al. (1995).

Lactic acid data: 77 rates from the Food Micro Model data set.

References

Alber S. A and Schaffner D. W. 1992: Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. Appl. Environ. Microbiol. 58, 3337-3342.

Bhaduri, S., Buchanan, R. L. and Philips J. G.1995. Expanded response surface model for predicting the effects of temperatures, pH, sodium chloride contents and sodium nitrite concentrations on the growth rate of *Yersinia enterocolitica*. J. Appl. Bacteriol. 79,163-170.

***Pseudomonas spp.* (3 factors)**

Data (temperature, pH, Aw): 12 rates from Pin et al. (1998), 70 rates from Campden and Chorlwywood Research Association, Chipping Campden and 62 rates from London Metropolitan University.

Reference

Pin C. and Baranyi J. 1998. Predictive models as means to quantify the interactions of spoilage organisms. Int.J. Food Microbiol. 41. 59-72.

***Brochothrix thermosphacta* (3 factors)**

Core model (temperature, pH, Aw): 44 rates from the Food Micro Model data set.

B2. Thermal inactivation models

***Bacillus cereus* (3 factors)**

Core model (temperature, pH, Aw): 41 death rates from the Food Micro Model data set.

***Clostridium botulinum* (non-prot.) (3 factors)**

Core model (temperature, pH, Aw): 35death rates from the Food Micro Model data set.

***Escherichia coli* (3 factors)**

Core model (temperature, pH, Aw): 51 rates from the Food Micro Model data set, 21 data from Unilever Research.

***Listeria monocytogenes* (3 factors)**

Core model (temperature, pH, Aw): 67 rates from the Food Micro Model data set, 13 data from Unilever Research; 136 data from Linton et al. (1995).

Reference

Linton, R.H., Carter W.H., Pierson M.D, Hackney C.R. and Eifert J.D. 1995. Use of a modified gompertz equation to predict the effects of temperature, pH, and NaCl on the inactivation of *Listeria monocytogenes* Scott A heated in infant formula. J. Food Protect. 59, 16-23

***Salmonellae* (3 factors)**

Core model (temperature, pH, Aw): 61 rates from the Food Micro Model data set, 8 data from ARS-Eastern Regional Research Center, Philadelphia, USA.

***Yersinia enterocolitica* (3 factors)**

Core model (temperature, pH, Aw): 86 death rates from the Food Micro Model data set.

***Brochothrix thermosphacta* (3 factors)**

Core model (temperature, pH, Aw): 53 death rates from Baranyi et al. (1996).

References

Baranyi J., Jones A., Walker C., Kaloti A. and Mackey B.M. 1996. A combined model for growth and thermal inactivation of *Brochothrix thermosphacta*. J. Appl. Env. Microbiol. 62. 1029-1035.

B3. Non thermal survival models

***Listeria monocytogenes* (3 factors)**

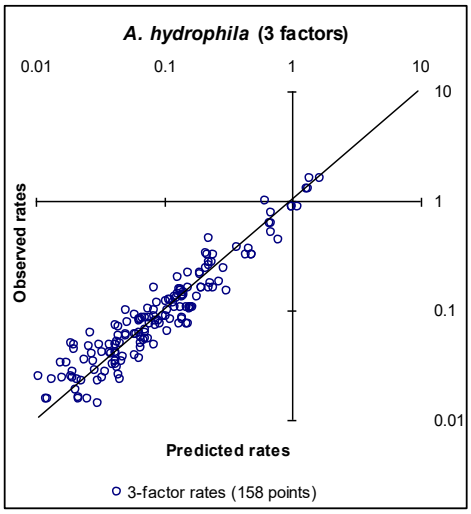
Data (temperature, pH, Aw): 194 inactivation rates from the Food Micro Model data set.

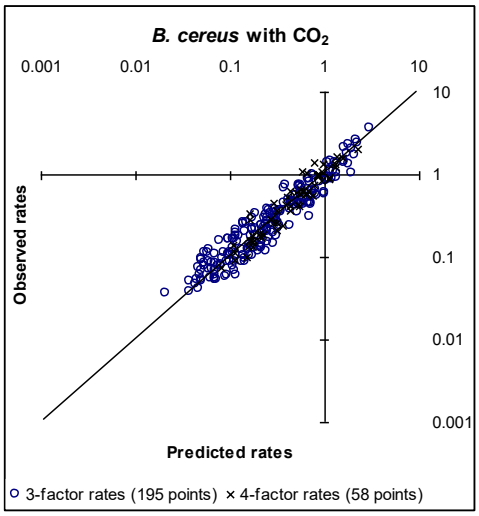
***Salmonellae* (3 factors)**

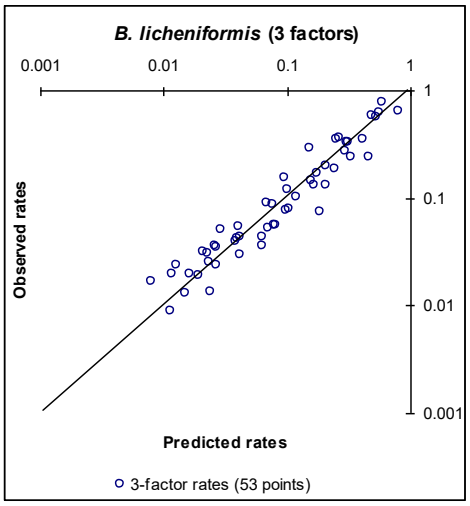
Data (temperature, pH, Aw): 102 inactivation rates from the Food Micro Model data set.

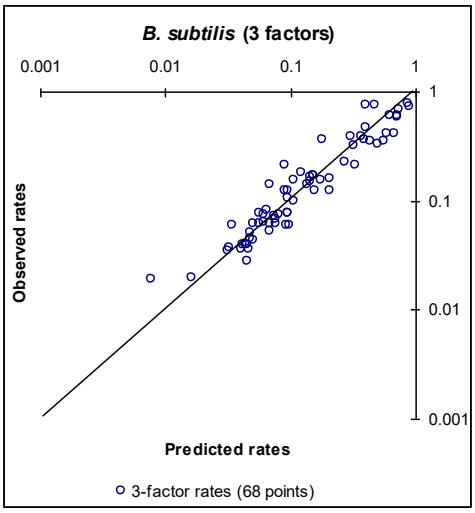
Appendix C. Goodness of fit of generated models

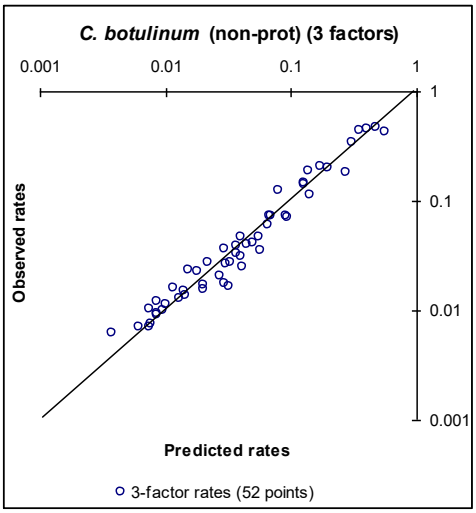
C1. Growth models

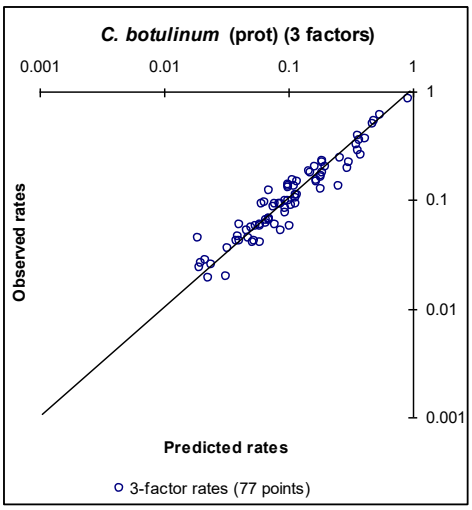


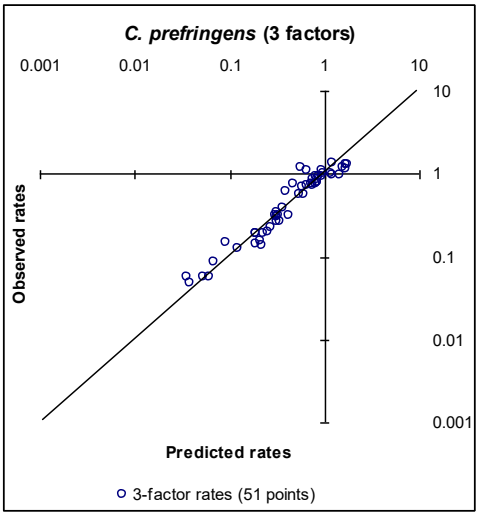


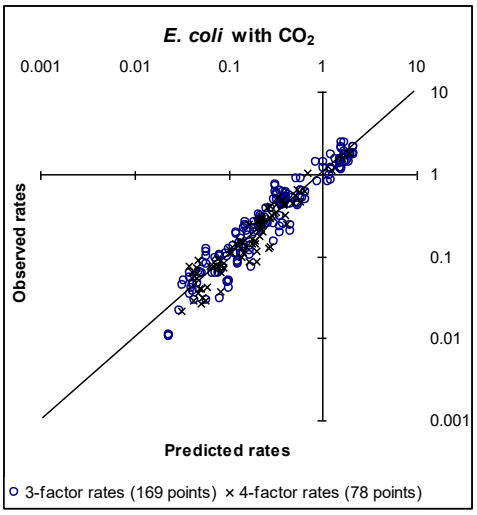


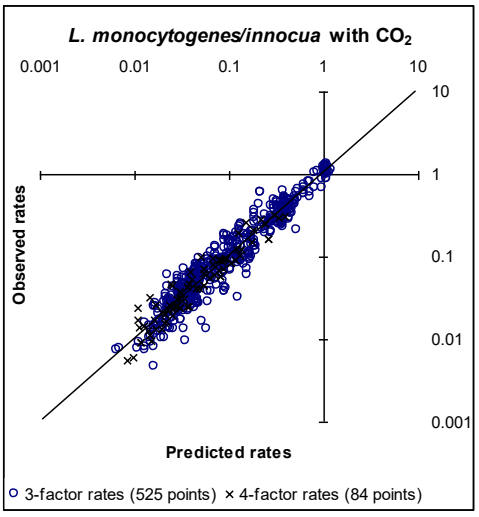


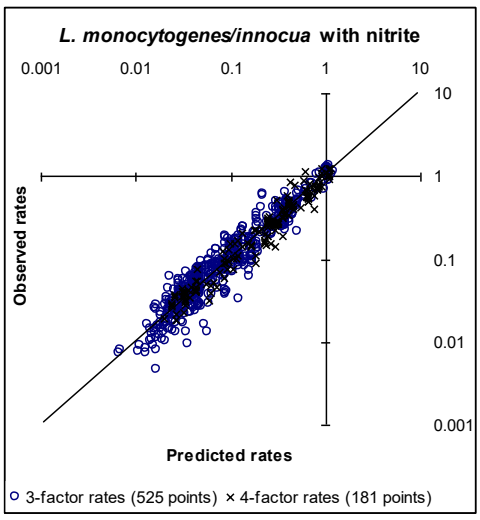


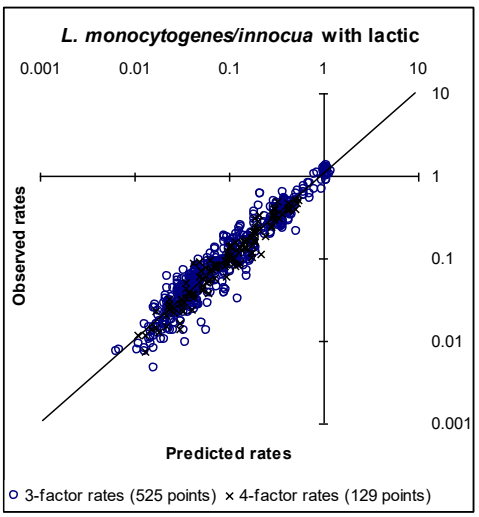


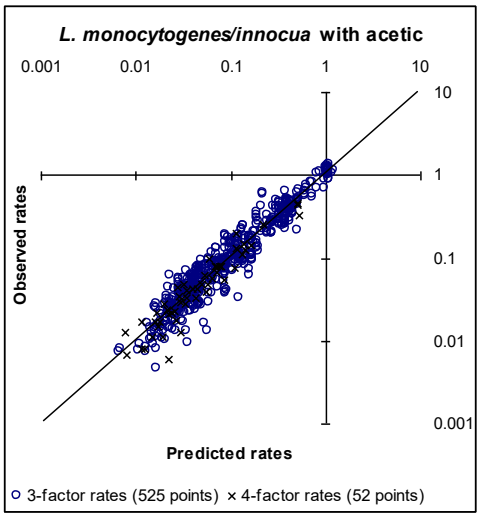

C. prefringens (3 factors)
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (51 points)

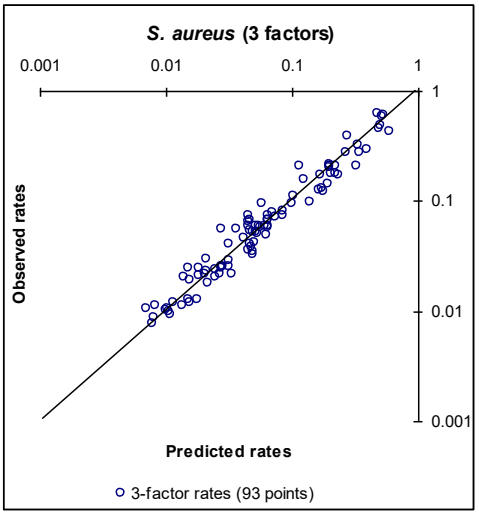

E. coli with CO2
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (169 points) × 4-factor rates (78 points)

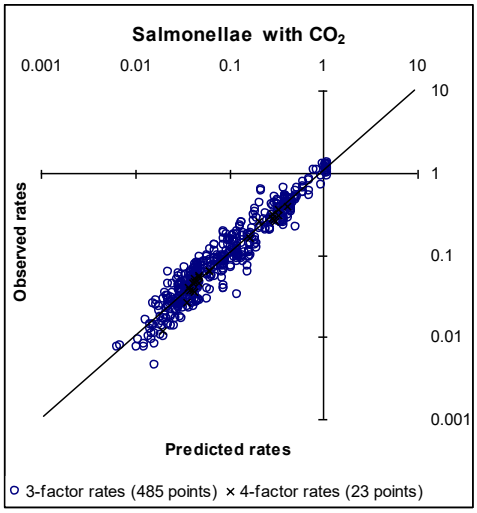

L. monocytogenes/innocua with CO2
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (84 points)

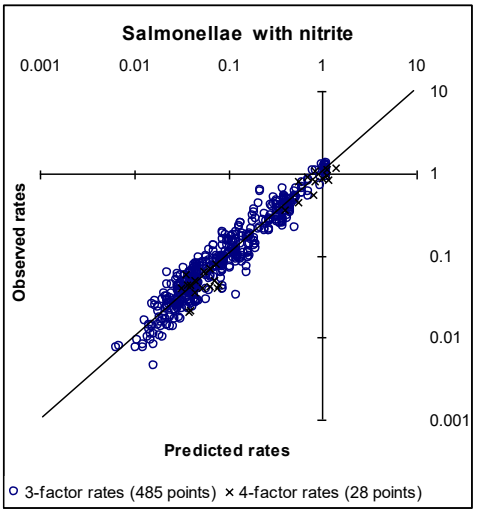

L. monocytogenes/innocua with nitrite
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (181 points)

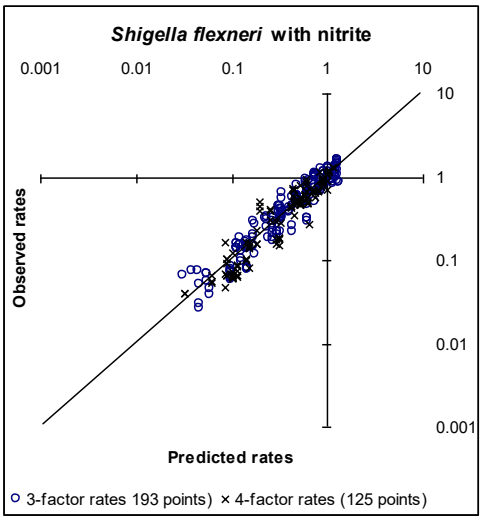

L. monocytogenes/innocua with lactic
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (129 points)

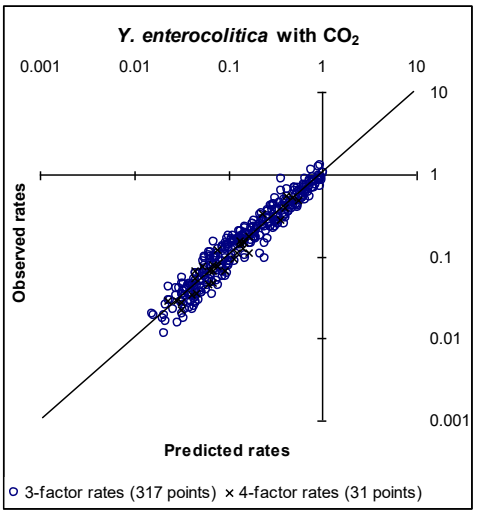

L. monocytogenes/innocua with acetic
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (52 points)

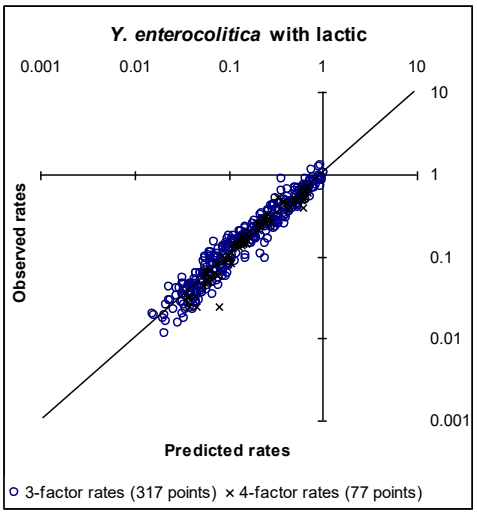

S. aureus (3 factors)
0.001
0.01
0.1
1
Observed rates
Predicted rates
3-factor rates (93 points)

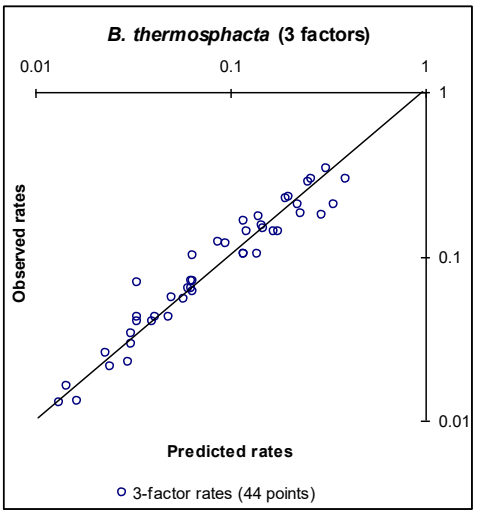

Salmonellae with CO2
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (485 points) × 4-factor rates (23 points)

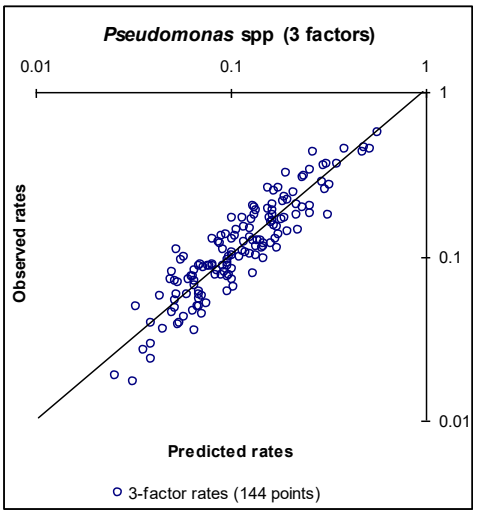

Salmonellae with nitrite
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (485 points) × 4-factor rates (28 points)


Shigella flexneri with nitrite
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates 193 points) × 4-factor rates (125 points)

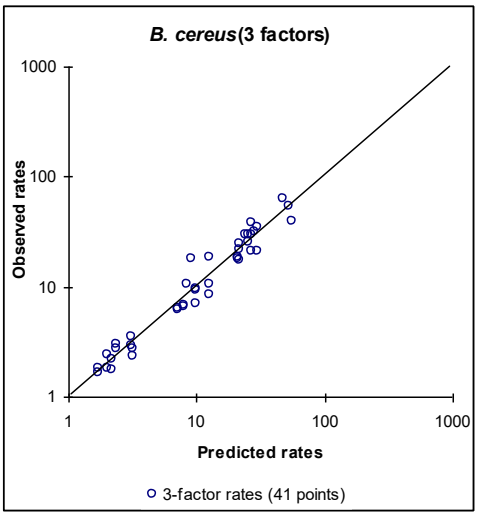

Y. enterocolitica with CO2
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (317 points) × 4-factor rates (31 points)

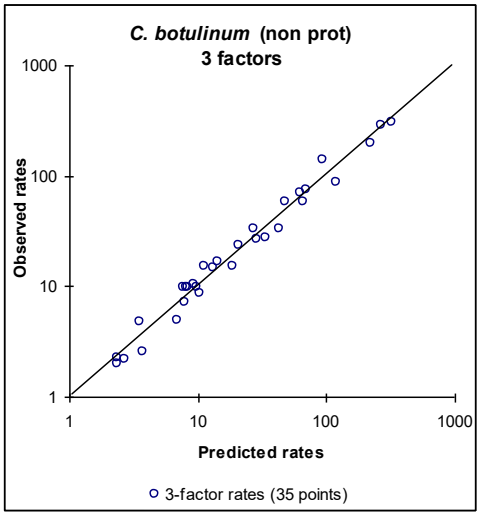

Y. enterocolitica with lactic
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (317 points) × 4-factor rates (77 points)

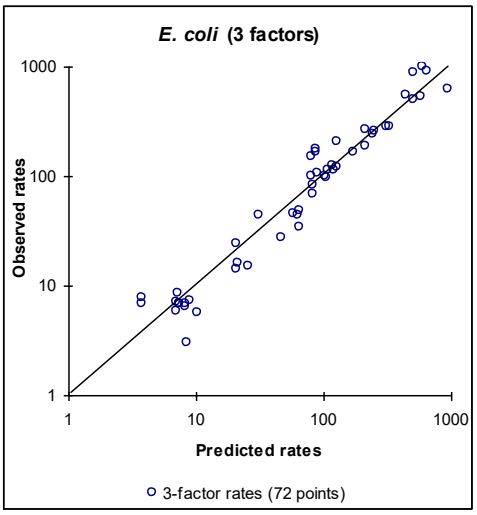


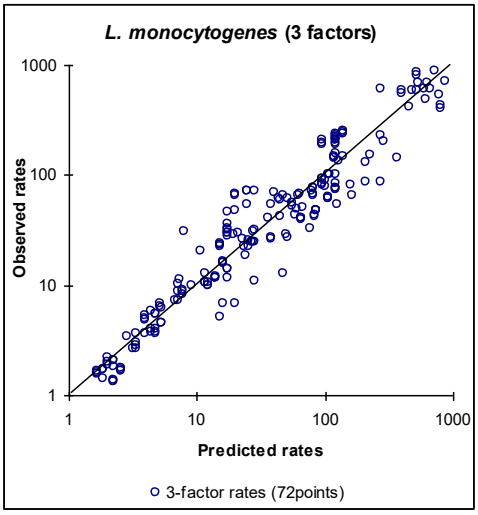


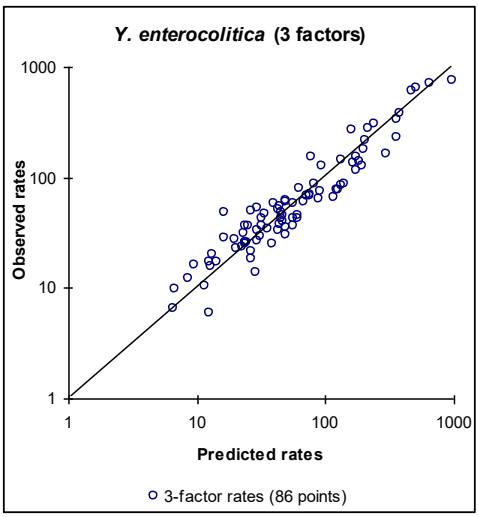
C2. Thermal inactivation models

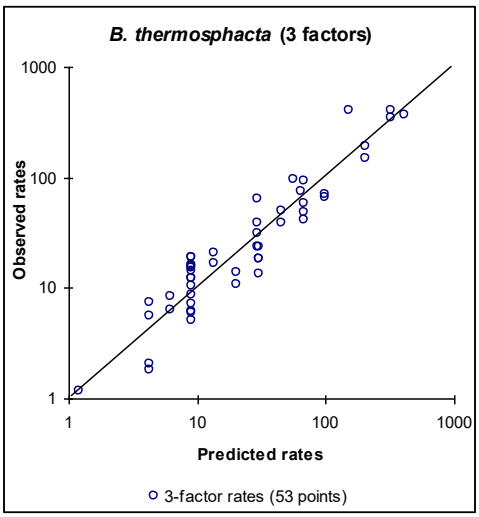




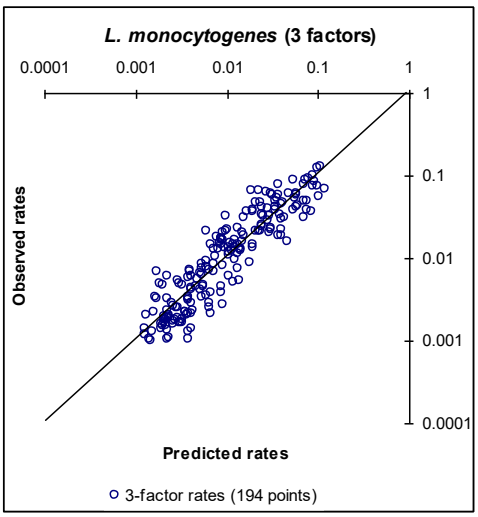


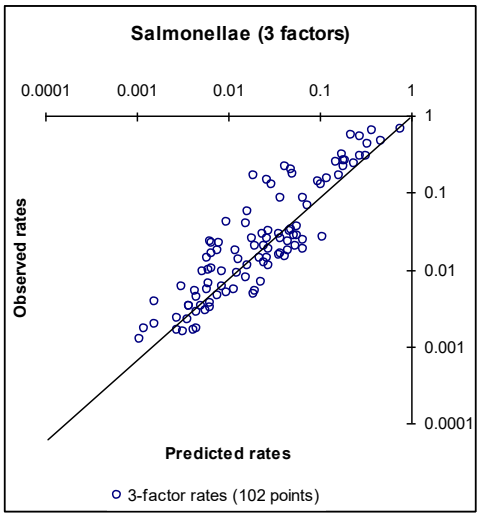






C3. Non-thermal survival models





Appendix D. List of files on the enclosed CD

1. This report — CBPtechRep.pdf
2. User manual for ComBase Predictor web version — CBPuserMan.pdf
3. ComBase Predictor stand-alone version — folder CBP
   (This needs to be copied in a working directory on the hard disk)