Quantitative Microbiology Tools and Applications

Mark Tamplin

Centre of Food Safety & Innovation
Tasmania Institute of Agriculture
University of Tasmania
Outline

• Overview of Quantitative/Predictive Microbiology
• Benefits of QM/PM
• Types of models
• Producing models
• Examples of QM/PM tools
• Model applications
• ComBase
Predictive Microbiology
Predictive models

Represent condensed knowledge, which

- describe microbial behavior in different environments
- help us better understand and manage the ecology of foodborne microorganisms

\[
\frac{dx}{dt} = \frac{q(t)}{q(t) + 1} \cdot \mu_{\text{max}} \cdot \left(1 - \left(\frac{x(t)}{x_{\text{max}}}\right)^m\right) x(t)
\]
Predictive microbiology

Assumes microbial behavior is:

• reproducible
• quantifiable by characterizing environmental factors
Drivers and Benefits of Quantitative Microbiology
Food Safety Modernization Act

Food Safety Objectives

\[ H_0 + \Sigma I + \Sigma R \leq FSO \]
A successful risk management system relies on information about how environmental conditions affect the behavior of microbial hazards.

...this information reduces uncertainty.
...and equally important

**prescriptive** → **outcome-based**

**flexibility**
Benefits

- Producing Food Safety/HACCP Plans
- Identifying Preventive Controls and Critical Limits
- Designing challenge studies
- Developing regulatory standards
- Minimizing microbiological testing
- Identifying factors that control microbial viability (e.g. temp, aw, pH, and others)
Other associated benefits

• Predictive microbiology brings together persons with diverse but complimentary skills, including microbiologists, technologists, mathematicians, engineers, statisticians and other disciplines.

• Excellent approach for capacity-building
How can we be sure that we are producing the most effective models?
Technical Aspects of Applied Research

- Research problem
- Experimental design
- Data generation
- Data analysis
- Publication
Outcomes of Applied Research

Interacting with all end-users of the model (defining the intended outcomes)

Determining the necessary resources

Research

Communicating with end-users
Types of Predictive Models
Steps in Model Production

• Primary

• Secondary

• Tertiary
Experimental design

**Extrinsic factors**
- temperature
- atmosphere (e.g. packaging gas, humidity)

**Intrinsic factors**
- food matrix
- pH
- water activity
- additives (e.g. NaCl, acidulants)
Growth
Kinetic parameters

- **Lag phase**
  - *lag phase duration*

- **Growth**
  - *growth rate*

- **Stationary phase**
  - *maximum population density*

![Graph showing growth over time](image-url)
SECONDARY MODELS
Change in parameter(s) as a function of environmental change
Measuring Model Performance
(validation)

![Graph showing the relationship between the square root of QR and temperature (°C) for pasteurized crab model compared to fresh crab.](image)
Probabilistic models

Growth/No-growth boundaries
(e.g. product development)
Growth/No-Growth

Adapted from Ross
Growth/No-Growth

Adapted from Ross
Growth/No-Growth

Adapted from Ross

More risk
TERTIARY MODELS

Growth Model

[Static | Dynamic]

Aeromonas hydrophila

Init. level 3
Phys. state 1,2n-3
Temp (°C) 20
pH 7
Aw 0.952

Max. rate (log. conc./h) 0.433
Dbl. time (hours) 0.696

Plot custom points
GR (log cfu/h) = -0.0146 + 0.0098T - 0.0206L - 0.2220D - 0.0013TL - 0.0392TD + 0.0143LD + 0.0001T^2 + 0.0053L^2 + 2.9529D^2
Examples of common model interfaces

Food Spoilage and Safety Predictor (FSSP)

ComBase
Pathogen Modeling Program
Pathogen Modeling Program

Growth of Listeria monocytogenes in Ground Ham Containing Sodium Lactate and Sodium Diacetate

**Input Conditions**

- **Sodium Lactate**: 1.0%
- **Sodium Diacetate**: 0.05%

**Modeled Parameters**

<table>
<thead>
<tr>
<th>Temp (C)</th>
<th>GR (log cfu/h)</th>
<th>LPD (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.000</td>
<td>110.5</td>
</tr>
</tbody>
</table>
Food Spoilage and Safety Predictor

Time-Temperature Integration Software

- Food Spoilage and Safety Predictor (FSSP)
  - Relative rate of spoilage (RRS) models
  - Microbial spoilage models (MSM)
  - Psychrotolerant Lactobacillus spp. (LAB)
  - Histamine formation models
- Listeria monocytogenes in chilled seafood and meat products
  - Growth of L. monocytogenes
    - Effect of temp., atmosphere, salt, smoke, pH, nitrile and organic acids (acetic/diacetate, benzoic, citric, lactic and sorbic acid)
  - Growth boundary of L. monocytogenes
- Listeria monocytogenes and lactic acid bacteria (LAB)
- Listeria monocytogenes and lactic acid bacteria (LAB) in cottage cheese
- Generic growth models
Food Spoilage and Safety Predictor
Case Studies
Examples of models to assist with food safety decisions

- USDA *Clostridium perfringens* cooling model
- Meat & Livestock Australia Refrigeration Index
- *Vibrio parahaemolyticus* in oysters

What pathogen-food combination are important in India?
How can food companies validate the effects of temperature deviation when cooling meat primals, without a lot of product testing?
Perfringens Predictor

• Previous regulation was highly prescriptive
• Sampling plans and testing were not cost-effective
• An outcome-based model was developed through a government-industry partnership
• Accepted criteria of <1 log growth of *C. perfringens* after the cooling profile
ComBase Perfringens Predictor

- 0.29 log growth
- <1 log growth

- pH [5.3-8.1]
  - 6.2

- NaCl [%] [0-4]
  - 3

- Temperature
- Logi

- Time [h]:
  - 0.00: 70.00
  - 0.50: 65.00
  - 1.00: 60.00
  - 1.50: 55.00
  - 2.00: 50.00
  - 2.50: 45.00
  - 3.00: 40.00
  - 3.50: 35.00
  - 4.00: 30.00
  - 4.50: 25.00
  - 5.00: 20.00
  - 5.50: 15.00
  - 6.00: 10.00
Boxed primals and trim destined for export

How can meat cooling profiles be accessed so that product can be more quickly exported?
The meat industry wanted to package hot-boned beef trim for export.

Australian export regulation required carcases to be cooled to 7°C in < 24 hours

A more flexible and less prescriptive approach was developed.

A predictive model was produced and validated via a government-industry-university partnership.

The Refrigeration Index predicts potential growth of *E. coli* based on a growth model.
RI now part of Australian food safety law for meat

Export Control (Meat and Meat Products) Orders 2005
Welcome to the
Refrigeration Index Calculator
Version 2.0.1836.19881

Select the product type:
- Carcase
- Boxed Trim
- Primal where the slowest cooling point is lean
- Primal where the slowest cooling point is fat OR a mixture OR you're not sure
- Offal
- Recovered meat products

The starting temperature is hot (as for initial cooling of a carcase):
- Yes
- No

Specify other parameters and information:
- Temperature measurement interval: 15 min
- Date of data collection: 15/03/2005
- Description of product, processing conditions, etc.:
The predicted E. coli growth during chilling determines whether the product is "acceptable" (< 1 log potential growth).
Benefits

- Australian Centre for International Economics showed a benefit-cost ratio of 11.5
- $161.7 million increase in Australia’s GDP over a 30-year period
- $281 million in social benefits over the 30-year period
Problem: How can companies reduce uncertainties in supply chains?
V. parahaemolyticus

2 -> 3% salt

Water temperature (°C)

V. parahaemolyticus density in oyster (Vp/g)
Model development

- *V. parahaemolyticus* growth kinetics measured from 4 - 30ºC
- Growth (>15ºC) and death rates (<15ºC) determined
- Models tested (validated) against naturally-occurring Vp
Oyster Refrigeration Index

The Australian Seafood CRC Oyster Refrigeration Index is a predictive model that estimates the growth and survival of V. parahaemolyticus and total viable count (TVC) bacteria in Pacific oysters (Crassostrea gigas).

Temperature is a key factor for controlling V. parahaemolyticus growth and this tool helps oyster companies design and monitor supply chains to maximise both oyster safety and quality. The Oyster Refrigeration Index can be especially useful for companies that have long supply chains and those exporting to countries that have maximum V. parahaemolyticus and TVC limits.

The model predictions were field-tested with Pacific oysters which contained natural populations of V. parahaemolyticus. The tools demonstrated that the model provided “tail-safe” predictions for V. parahaemolyticus growth in Pacific oysters over a temperature range of 4 to 30°C.

After registering, you can access both a web-based and Excel® downloadable version of the V. parahaemolyticus and TVC models.

We hope you find this tool useful. If you have technical questions or wish to provide us with feedback, please see the “Contact us” link below.

- Login
- New user? Register to use the predictor
- Documents and Downloads (User Guide and Excel® versions)
- Contact Us
- Acknowledgments
- Funding sponsors
- Disclaimer

Integrating Sensors and Predictive Models
Currently, predictive models are not commonly used in real-time (or even retrospectively), due to lack of data capture.

Sensors are a solution.
Integration of Time Temperature Indicator (TTI) sensors with predictive models for consumer-direct delivery of food products.
\[ v_{growth} \text{ rate} = 0.0303 \times (\text{temp} - 13.37) \]
ComBase
(www.combase.cc)
Access ComBase

ComBase is the world’s largest, freely-accessible database of quantified microbial responses in diverse food environments.

Freely accessible | 60,000 + records | 42,000 + users

A Web Resource for Quantitative and Predictive Food Microbiology

- A systematically formatted database of quantified microbial responses to the food environment with more than 50,000 records
- ComBase Predictor and Food Models – to predict the growth and inactivation of microorganisms in food

News, Events and Jobs

- 10th International Conference on Predictive Modelling in Food
Goals

- Support the development of science-based risk management systems by
  
  o Engaging with the international food microbiology community
  
  o Providing robust data that describe how food safety and spoilage organisms respond to food environments.
Applications

- Growth/thermal and non-thermal inactivation
- Shelf-life
- Hazard identification
- Product development
- Process deviations
Data Submission

ComBase needs your data
The real success of ComBase is dependent upon the goodwill of those providing data to further populate the database. ComBase is always looking to expand its database with the addition of growth and inactivation curves particularly within food matrices.

How to submit your data
ComBase data must be formatted in a specific way before they can be included in ComBase.

We strongly encourage you to contact Mark Tamplin at mark@combase.cc, before formatting your data.

We provide a ComBase demo for Excel that includes an Excel demo file of data and macros that allows you to check if your data format has the proper syntax. Please unzip the file and open it in Excel 2007 or higher. The zip file includes a manual in PDF.
ComBase Browser
Tutorial - Browser
Tutorial - Browser
Record statistics

Record views and downloads
**Bacillus cereus in broth**

**Matrix**
- Culture medium
- NaCl: 0.997 (assumed)
- pH: 7

**Source**
- Choma (et al.), 2000: Effect of temperature on growth characteristics of Bacillus cereus T7415

**Conditions**

**Properties**

**Further specifications**
- Strain(s): T7415

**Details**
- No details specified

**Measurement**
- By colony counts

**Record views/downloads**
- Viewed: 6 times
- Downloaded: 2 times

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**Max. rate (log conc/h)**

![Graph showing Max. rate (log conc/h) fit data](chart)

**Prediction**

**Fit**

![Graph showing log CFU/g vs. Time (h)](data)
Bacillus cereus in broth

**Matrix**
Culture medium

**Temperature (°C)**
30

**AW NaCl (assumed)**
0.997

**pH**
7

**Source**
Chema et al., 2006: Effect of temperature on growth characteristics of Bacillus cereus 12415

**Condition**

**Properties**

**Further specifications**
Strain(s): 12415

**Details**
No details specified

**Measurement**
By colony counts.

**Record views/downloads**
Viewed: 6 times
Downloaded: 2 times

*since september, 2017

**Max rate (log conc/h)**

**Fit data**

**Baranyi and Roberts Model (no lag) [fit]**

- **R² (no lag)**: 0.971
- **SE of Fit**: 0.287
- **Initial value**: 2.194 ± 0.732
- **Max. Rate**: 0.0379 ± 0.00402
- **Final Value**: 6.7 ± 3.92
ComBase Predictor
Growth Models
Growth Models
Customized data
Customized data
Thermal inactivation
Non-thermal inactivation
Links to other model resources

Other Predictive Microbiology Tools

ComBase has no responsibility for the accuracy of these Tools. They have been developed independently of ComBase and support is provided by the tool developers.

- **Seafood Spoilage Predictor**, a software for the prediction of the shelf life and growth of bacteria in different fresh and lightly preserved seafoods. Can be used also when the effect of product temperature profiles recorded over time by data loggers.
- **Pathogen Modelling Program**, a package of models that can be used to predict the growth and inactivation of foodborne pathogens under various environmental conditions.
- **E. coli fermented meat model**, a predictive model for the inactivation of *Escherichia coli* in fermented meats.
- **E. coli SafeFerment**, predicting verotoxin producing *Escherichia coli* in fermented meats.
- **GlosFIL**, an Excel Add-in to fit various models to bacterial inactivation curves.
- **Microbial Responses Viewer (MRV)**, a database consisting of microbial growth/no growth data derived from ComBase.
- **MicroLibro**, an online tool to predict the growth of pathogens in a variety of vegetables. It also includes a risk assessment module.
- **MLA Refrigeration Index Calculator**, to predict the expected log growth of *E. coli* on meat as affected by temperature and other environmental factors.
- **Risk Ranger**, a simple food safety risk calculation tool aiding to estimate the relative risks at different product, pathogen and processing combinations.
- **Salmonella Predictions**, probabilistic and kinetics models are combined to give predictions on the concentration of *Salmonella* spp. at any stage of the pork chain under fluctuating pH, Aw and/or temperature.
Help Functions

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- Pathogen Modelling Program, a package of models that can be used to predict the growth and inactivation of foodborne pathogens under various environmental conditions.
- E. coli fermented meat model, a predictive model for the inactivation of Escherichia coli in fermented meats.
- E. coli SafeFerment, predicting Verotoxin producing Escherichia coli in fermented meats.
- GnuFit, an Excel Add-in to fit various models to bacterial inactivation curves.
- Microbial Responses Viewer (MRV), a database consisting of microbial growth/no growth data derived from ComBase.
- MicroHibro, an online tool to predict the growth of pathogens in a variety of vegetables. It also includes a risk assessment module.
- MLA Refrigeration Index Calculator, to predict the expected log growth of E. coli on meat as affected by temperature and other environmental factors.
- Risk Ranger, a simple food safety risk calculation tool aiding to estimate the relative risks at different product, pathogen and processing combinations.
- Salmonella Predictions, probabilistic and kinetics models are combined to give predictions on the concentration of Salmonella spp. at any stage of the pork chain under fluctuating pH, Aw and/or temperature.
Thank you for your attention.