Predictive Microbiology (theory)

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Summer School
“In Silico Methods for Food Safety”

European Food Safety Authority
THE CONCEPT

A detailed knowledge of microbial responses to environmental conditions, synthesized in a mathematical model, enables objective evaluation of processing, distribution and storage operations on the microbiological safety and quality of foods, by monitoring the environment without recourse to further microbiological analysis.
THE PRINCIPLES

- Growth, survival and inactivation of microorganisms in foods are reproducible responses.
- A limited number of environmental parameters in foods determine the kinetic responses of microorganisms (Temperature, Water activity/water phase salt, pH, Food preservatives).
- A mathematical model that quantitatively describes the combined effect of the environmental parameters can be used to predict growth, survival or inactivation of a microorganism and thereby contribute important information about product safety and shelf-life.

Roberts and Jarvis (1983)
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APPLICATIONS

- Predict the effect of product characteristics and storage conditions on microbial responses (safety and shelf-life)
- Predict effect of changes in parameters (product development)
- HACCP plans – establish limits for CCP
- Food safety objectives – equivalence of processes
- Education – easy access to information
- Quantitative microbiological risk assessment (QMRA)
- (The concentration of microbial hazards in foods may increase or decrease substantially during processing and distribution)
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TYPES OF MODELS

➢ Primary models: describing the microbial evolution (growth, inactivation, survival) as a function of time. Estimate kinetic parameters

➢ Secondary models: describing kinetic parameters as a function of influencing factors like pH, temperature, water activity, concentration of preservatives, ...

➢ Tertiary models: integrate primary and secondary models in a software tool
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STEPS IN MODEL DEVELOPMENT

- Fitting data to primary model

Translate growth curves to numbers

![Graph showing growth curves over time in log cfu/g vs. TIME (h).]
STEPS IN MODEL DEVELOPMENT

- Estimation of kinetic parameters

```
<table>
<thead>
<tr>
<th>TIME (h)</th>
<th>Log cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
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<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>
```

- Log $N_{\text{max}}$
- Log $N_0$
- Lag
- Maximum rate
### STEPS IN MODEL DEVELOPMENT

- **Primary models**

**Exponential model**

\[ \log(N_i) = \log(N_0 \times \exp(\mu_{\text{max}} \times \text{time})) \]

**Logistic model without lag**

\[ \log(N_i) = \log\left(\frac{N_{\text{max}}}{1 + \left(\frac{N_{\text{max}}}{N_0} - 1\right) \times \exp(-\mu_{\text{max}} \times \text{time})}\right) \]

**Logistic model with lag**

\[ \log(N_i) = \log(N_{\text{min}} + \frac{N_{\text{max}} - N_{\text{min}}}{1 + \exp(-\mu_{\text{max}}(\text{time} - t_i))}) \]

**Baranyi & Roberts (1994)**

\[ \log(N_i) = \log(N_0) + \frac{1}{\mu_{\text{max}}} \times \left[\text{time} + \frac{1}{\mu_{\text{max}}} \times \ln\left(\frac{\exp(-\mu_{\text{max}} \times \text{time}) + q_0}{1 + q_0}\right)\right] - \frac{1}{\ln(10)} \times \ln\left(1 + \frac{\exp\left(\frac{\mu_{\text{max}} \times \ln(1)}{\mu_{\text{max}}} \times \ln\left(\frac{\exp(-\mu_{\text{max}} \times \text{time}) + q_0}{1 + q_0}\right)\right)}{\exp(\log(N_{\text{max}}) - \log(N_0))}\right) - 1 \]

**Modified Gompertz model**

\[ \log(N_i) = \log(N_0) + (A \times \exp\left(-\exp\left[\frac{\mu_{\text{max}} \times \exp(1)}{A} \times (\text{lag} - \text{time}) + 1\right]\right) / \ln(10) \]
STEPS IN MODEL DEVELOPMENT

- Primary models

![Graph showing growth models with different equations and models over storage period (hours) with log (cfu/g) on the y-axis and storage period (hours) on the x-axis.](image)
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STEPS IN MODEL DEVELOPMENT

- Mathematical description of the effect of the environmental factors to the kinetic parameters (secondary models)
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STEPS IN MODEL DEVELOPMENT

- Secondary models

- Kinetic models
  - Polynomial and constrained linear polynomial models
  - Square-root-type models
  - Arrhenius type models
  - Cardinal parameter models
  - Gamma concept models
  - Artificial neural networks

- Growth/no growth interface models (probabilistic models)
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STEPS IN MODEL DEVELOPMENT

- Secondary models

Square root type model

\[
\sqrt{\mu_{\text{max}}} = b \times (T - T_{\text{min}})
\]

\[
\sqrt{\mu_{\text{max}}} = b \times (T - T_{\text{min}}) \times (1 - \exp(c(T - T_{\text{opt}})))
\]
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STEPS IN MODEL DEVELOPMENT

- **Secondary models**

**Square root type model**

\[
\sqrt{\mu_{\text{max}}} = b \cdot (T - T_{\text{min}}) \cdot \sqrt{a_w - a_{w,\text{min}}}
\]

\[
\sqrt{\mu_{\text{max}}} = b(T - T_{\text{min}}) \times \frac{\%CO_2_{\text{max}} - \%CO_2}{\%CO_2_{\text{max}}}
\]

\[
\mu = b \cdot (a_w - a_{w,\text{min}}) \cdot (pH - pH_{\text{min}}) \cdot (pH - pH_{\text{max}}) \cdot (T - T_{\text{min}})^2
\]

\[
\sqrt{\mu_{\text{max}}} = b \cdot (T - T_{\text{min}}) \cdot (1 - \exp(c(T - T_{\text{max}}))) \cdot \sqrt{(a_w - a_{w,\text{min}})(1 - \exp(d(a_w - a_{w,\text{max}})))}
\]
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STEPS IN MODEL DEVELOPMENT

- Secondary models

Gamma concept models

Many factors that affect microbial growth rate act independently, effect of each can be represented by a discrete term that is multiplied by terms for the effect of all other growth rate affecting factors.

\[ \mu = f(temperature) \times f(a_o) \times f(pH) \times f(organic \ acid) \times f(\text{other}_1) \times f(\text{other}_2) \times \ldots \times f(\text{other}_n) \]

The effect on growth rate of any factor can be expressed as a fraction of the maximum growth rate (i.e., the rate when that environmental factor is at the optimum level).

\[ \gamma = \frac{\text{Growth rate at actual environmental conditions}}{\text{Growth rate at optimal environmental conditions}} \]
STEPS IN MODEL DEVELOPMENT

- Secondary models

Gamma concept models

\[ \mu_{\text{max}} = \mu_{\text{max opt}} \cdot \gamma(T) \cdot \gamma(a_w) \cdot \gamma(pH) \]

\[ \gamma(T) = \left( \frac{T - T_{\text{min}}}{T_{\text{opt}} - T_{\text{min}}} \right)^2 \]

\[ \gamma(pH) = \frac{(pH - pH_{\text{min}}) \cdot ((pH_{\text{max}} - pH_{\text{opt}}))}{(pH_{\text{opt}} - pH_{\text{min}}) \cdot (pH_{\text{max}} - pH_{\text{opt}})} \]

\[ \gamma(a_w) = \frac{a_w - a_{w \text{ min}}}{1 - a_{w \text{ min}}} \]

\[ \gamma(CO_2) = \left( \frac{\%CO_2_{\text{max}} - \%CO_2}{\%CO_2_{\text{max}} - \%CO_2_{\text{opt}}} \right)^2 = \left( \frac{\%CO_2_{\text{max}} - \%CO_2}{\%CO_2_{\text{max}}} \right)^2 \]
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STEPS IN MODEL DEVELOPMENT

- Secondary models

Cardinal parameter model
Probability models for the ability of growth

The dependent variable is discrete (1 for growth and 0 for no growth)

Use of logistic regression with logitP transformation of the response variable

\[
\logit P = \log(P/(1 - P))
\]

where \( P \) is the probability of the outcome of interest.
logit \( P \) is described as some function \( Y \) of the explanatory variables, i.e.: 

\[
e^{Y}/(1 + e^{Y}) = P
\]
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STEPS IN MODEL DEVELOPMENT

- Validation of models

Most models are developed in laboratory media. There can be no guarantee that predicted values will match those that would occur in any specific food system. Before the models could be used in such a manner, the user would have to validate the models for each specific food of interest.

Internal validation: Comparison between predicted and observed values for data used for model development

External validation: Comparison between predicted and observed values for independent data
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STEPS IN MODEL DEVELOPMENT

- Prediction of microbial growth

Primary model

Calculation of kinetic parameters for the environment of interest

Secondary model

\[
\sqrt{\mu_{\text{max}}} = b \times (T - T_{\text{min}}) \\
\sqrt{\mu_{\text{max}}} = b \times (T - T_{\text{min}}) \times (1 - \exp(c(T - T_{\text{opt}})))
\]
Example 1
Development of a model for *Listeria monocytogenes* growth in pasteurized milk
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Example 1
Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 1. Data collection
Example 1
Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 2. Fitting growth data to a primary model
Example 1
Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 2. Estimation of kinetic parameters

<table>
<thead>
<tr>
<th>curve</th>
<th>rate</th>
<th>lag</th>
<th>y0</th>
<th>yEnd</th>
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<td>1A</td>
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<td>502.45</td>
<td>3.70</td>
<td>7.74</td>
</tr>
<tr>
<td>1B</td>
<td>0.0035</td>
<td>460.90</td>
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<tr>
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<td>0.0111</td>
<td>181.19</td>
<td>4.03</td>
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<tr>
<td>4D</td>
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<td>4.11</td>
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<td>8A</td>
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</tr>
<tr>
<td>8B</td>
<td>0.0252</td>
<td>25.24</td>
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<td>0.0973</td>
<td>7.83</td>
<td>3.63</td>
<td>8.62</td>
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</tbody>
</table>
Example 1
Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 3. Fitting kinetic parameters data to a secondary model

\[ \sqrt{\mu_{\text{max}}} = b(T - T_{\text{min}}) \]

[Graph showing the relationship between temperature (°C) and \((\mu_{\text{max}})^{0.5}\).]
Example 1
Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 3. Fitting kinetic parameters data to a secondary model

\[ \sqrt{\mu_{\text{max}}} = b(T - T_{\text{min}}) \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{\text{max}} ) (h(^{-1}))</td>
<td>0.024</td>
<td>0.023</td>
<td>0.025</td>
<td>0.988</td>
</tr>
<tr>
<td>( b )</td>
<td>-2.32</td>
<td>-3.02</td>
<td>-1.61</td>
<td></td>
</tr>
</tbody>
</table>
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Example 1
Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 4. Validation under dynamic conditions

prediction based on the square root model for the estimation of the “momentary” rate and the differential equations of Baranyi and Roberts model which were numerically integrated with respect to time:

\[
\frac{d}{dt} x = [b(T(t) - T_{\text{min}})]^2 \left( \frac{q}{q + 1} \right) \left( 1 - \frac{x}{x_{\text{max}}} \right)^m x
\]

\[
\frac{d}{dt} q = [b(T(t) - T_{\text{min}})]^2 q
\]
Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 4. Validation under dynamic conditions
Example 2

Modeling the Boundaries of Growth of Salmonella Typhimurium in Broth as a Function of Temperature, Water Activity, and pH

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Modeling the Boundaries of Growth of Salmonella Typhimurium in Broth as a Function of Temperature, Water Activity, and pH

KONSTANTINOS P. KOUTSOUMANIS,1 PATRICIA A. KENDALL,2 AND JOHN N. SOFOS1*

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MS 03-166: Received 22 April 2003/Accepted 17 August 2003
Example 2
Strains: *S. Typhimurium*  *(ATCC 70408, ATCC 14028, R-4, R-5, SF-530)*

Growth medium: TSB

Method: Optical density in microtitre plates

Storage time: 60 ημέρες

Conditions:
- pH (HCl 1N): 3.76, 3.94, 4.24, 4.45, 4.76, 4.96, 5.19, 5.47, 5.96, 6.44
- $a_w$ (NaCl): 0.997, 0.983, 0.971, 0.960, 0.948, 0.939, 0.928, 0.913, 0.900
- T: 10, 15, 25, 30, 35 °C
Example 2

Combination of conditions tested

In each conditions we record growth (1) or no growth (0)
Example 2

Combination of conditions tested

Open symbols: No Growth
Closed symbols: Growth
Example 2

Model development

Method: Logistic Regression

Model: Polynomial

\[
\text{Logit (P)} = a_0 + a_1 T + a_2 \text{pH} + a_3 b_w + a_4 T \text{pH} + a_5 T b_w + a_6 \text{pH} b_w + a_7 T^2 + a_8 \text{pH}^2 + a_9 b_w^2
\]

\[
\text{Logit (P)}: \ln[P/(1-P)]
\]

\[P: \text{probability of growth (range 0-1)}\]

\[a_i: \text{parameters to be estimated}\]

\[b_w = (1-a_w)^{0.5}\]
## Example 2

### Model parameter estimation

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>DF</th>
<th>Estimate</th>
<th>St. error</th>
<th>Chi-square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>-438.1</td>
<td>65.7</td>
<td>44.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>5.465</td>
<td>0.89</td>
<td>37.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$b_w$</td>
<td>1</td>
<td>233.5</td>
<td>84.6</td>
<td>7.62</td>
<td>0.0058</td>
</tr>
<tr>
<td>pH</td>
<td>1</td>
<td>128.0</td>
<td>19.9</td>
<td>41.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$b_w \times pH$</td>
<td>1</td>
<td>-235.6</td>
<td>45.0</td>
<td>27.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temperature $\times$ pH</td>
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<td>-0.236</td>
<td>0.06</td>
<td>14.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>Temperature$^2$</td>
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<td>-0.074</td>
<td>0.01</td>
<td>40.9</td>
<td>&lt;0.0001</td>
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<td>$b_w^2$</td>
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<td>23.8</td>
<td>&lt;0.0001</td>
</tr>
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<td>pH$^2$</td>
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<td>-5.186</td>
<td>1.25</td>
<td>17.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Example 2

Growth boundaries prediction

\(T=25 \, ^\circ C\)

\[P=0.1\]

\[P=0.5\]

\[P=0.9\]
Predictive Microbiology

Example 2

Growth boundaries prediction

$aw = 0.967$
Example 2

Growth boundaries prediction
Risk Assessment

Risk Assessment Stages

Hazard Identification: what biological, chemical and physical agents are we dealing with and with which foods is it associated?

Hazard Characterization: what illness can be caused, associated in relation to dose and population?

Exposure Assessment: how likely it is that an individual or a population will be exposed to a microbial hazard and what numbers of organisms are likely to be ingested?

Risk Characterization: the integration of the above resulting in the probabilities of illness
Exposure Assessment

Environmental data for the food chain
(time, temperature, pH, aw ...)

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Starting Point (Available Data)

Concentration at consumption time
Important Aspects of Risk Assessment

Variability represents a true heterogeneity of the population that is a consequence of the physical system and irreducible (but better characterized) by further measurements.

Uncertainty represents the lack of perfect knowledge of a parameter value, which may be reduced by further measurements.
Variability (Example)

We all want to move to the 5th floor using the elevator in groups of 5 (randomly selected) people.
The weight limit of the elevator is 480 kg.

Estimate the chance of exceeding the weight limit.

Deterministic method (variability is not taken into account)

Average individual weight = 70 kg

5 persons x 70 = 350 kg < 480 kg

The weight limit is not exceeded.
Exposure Assessment

Variability (Example)

Stochastic method (variability is taken into account)

![Histogram showing normal distribution of weight in kg]
Exposure Assessment

Variability (Example)

Stochastic method *(variability is taken into account)*

Random selection of 5 values

Repeat 100000 (iterations)

Sum the 5 values

Monte Carlo Simulation

Limit

\[ P(>480) = 3 \times 10^{-4} \]
Exposure Assessment

Uncertainty (Example)

Stochastic method

Uncertainty: We don’t know the weight limit of the elevator

Expert Opinion: Min:450, Max:550 Most likely:500
Predictive Microbiology in a Risk-based approach

- The use of predictive microbiology in a Risk-based approach has different demands than “traditional” predictive microbiology.

- “Traditional” predictive models are developed and validated to produce point estimates of microbial population level.
Traditional Predictive Microbiology

Point estimates

Log (Nt) vs Time
In a Risk-based approach however, microbial populations should be expressed in terms of probability (for example to predict the probability distribution of the microbial concentration at the time of consumption).
Predictive Microbiology in a Risk-based approach

Probability Distributions
Applications of predictive models used in a Risk-based approach should take into account both uncertainty and variability. This can be achieved by the use of stochastic modeling where the parameters affecting microbial growth can be introduced as distributions.
Sources of variability in microbial behaviour

- Storage temperature
- Product characteristics (pH, $a_w$ etc)
- Storage time
- Physiological state
- Contamination level
- Individual cell heterogeneity
- Strain-to-Strain differences

LogCFU/g vs Time vs Concentration at consumption
Individual cell heterogeneity (Noise) in microbial growth

Modeling Colonial Growth of Single Bacterial Cells:
The output of the method is a video for each individual cell

Colonial growth of Salmonella single cell at 25 °C

Stochasticity in Colonial Growth Dynamics of Individual Bacterial Cells

Konstantinos P. Koutsourakis, Alexandra Lianou
Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece
The reason for Individual cell heterogeneity (Noise) in microbial growth is the stochastic gene expression.
Individual cell heterogeneity (Noise) in microbial growth

Colonial growth of Salmonella single cell at 25 °C
Sources of variability in microbial behaviour

variability is extremely important in risk assessment
Predictive Microbiology (theory)

Questions?

For future questions you can contact me kkoutsou@agro.auth.gr

Summer School “In Silico Methods for Food Safety”