Modeling the Antimicrobial Effect of Lactate on the Growth and Survival of *Listeria monocytogenes* on Ready-to-Eat Seafood

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Listeria monocytogenes

- The genus *Listeria* includes 6 different species (*L.* monocytogenes, *L. ivanovii*, *L.* innocua, *L.* welshimeri, *L.* seegligeri, and *L. grayi*).

- Both *L. ivanovii* and *L. monocytogenes* are pathogenic for mice, but only *L. monocytogenes* is consistently associated with human illness

Listeria monocytogenes

- Causes septicemia, abortion and encephalitis in humans and more than 40 animal species, but is also common in environment
- Ubiquitous in the environment, can survive for prolonged periods in the environment (apparently outside a host)
- Human listeriosis can occur as epidemic and sporadic cases
- Affects predominantly elderly and immuno-compromised people, pregnant women and newborns.

Listeria monocytogenes

- Approx. 2500 human cases/year in the U.S., resulting in about 500 deaths/year

- *L. monocytogenes* is one of the major microbial contaminants of ready to eat food, such as smoked salmon

Factors Controlling *Listeria* in smoked fish

- Smoking
- pH
- Water activity
- Competitive microorganism
- Preservatives: antimicrobial compounds

Antimicrobial compound used:

Purasal P Opti.Form 4, (PURAC America Inc.)

- Potassium lactate (PL)/ sodium diacetate (SD) ratio of 14:1 (56% PL and 4% SDA)

- Its antimicrobial effect on growth and survival of *L. monocytogenes* as a function of temperature has not been modeled in either microbiological food products or broth.

Main objectives

- To determine the antimicrobial effect of Purasal P Opti.Form 4 on *L. monocytogenes* in ready to eat smoked fish

- To develop a model for the growth and survival of *L. monocytogenes* as a function of Purasal P Opti.Form 4 concentrations

Sample preparation

5g smoked salmon slice

↓

Cut into two pieces

↓

Applied Opti.Form 4

↓

Inoculated with *Listeria* (10^2)

↓

Pieces were folded

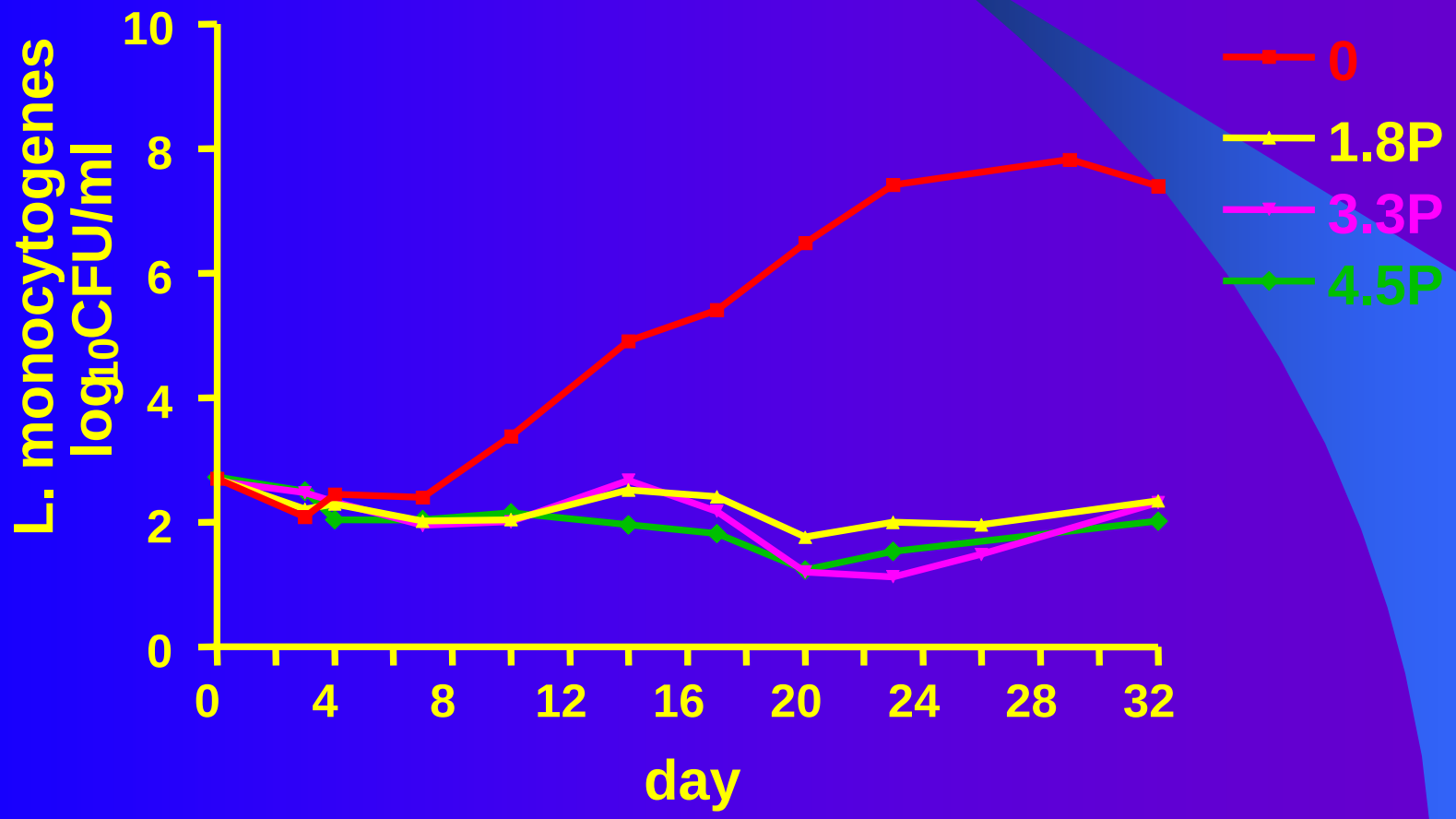
↓

Vacuum packed

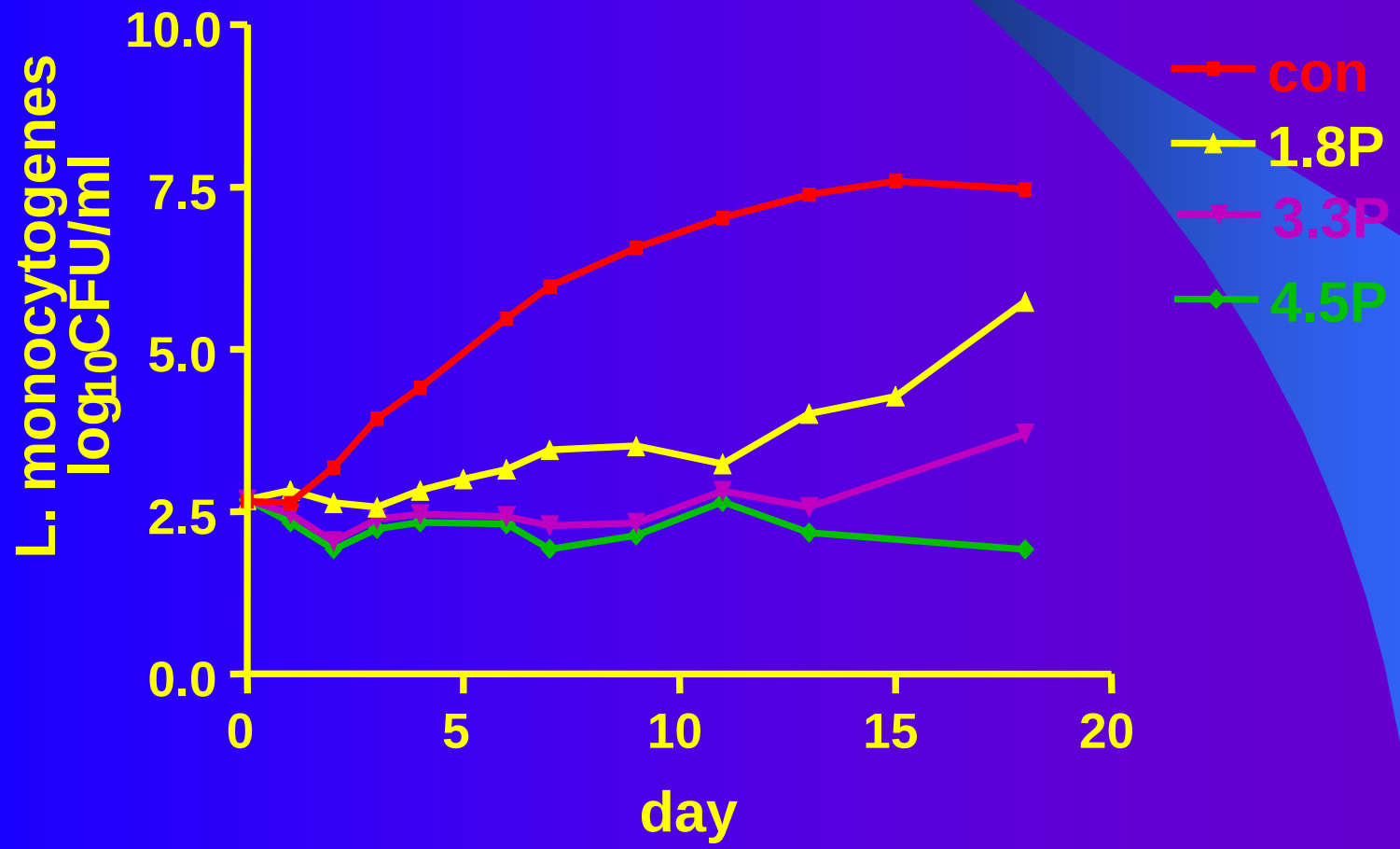
↓

Stored

Effect of Opti.Form 4 on the growth of *L. monocytogenes* at 4 ºC



Effect of Opti.Form 4 on the growth of *L. monocytogenes* at 10 ºC



Introducing the concept of predictive modeling

- In an effort to reduce the likelihood of contaminated food products, the concept of predictive modeling of bacterial growth becomes a prominent research topic among food microbiologists
- Predictive models allow estimating shelf-life, microbiological safety of foods
- Can provide an insight on how certain environmental variables affect the growth/survival profile of pathogenic or spoilage organisms

What is Predictive Modeling?

- Predictive modeling, involves mathematical equations which have been used extensively to describe microbial behavior under various environmental factors.

Levels of predictive models

1- Primary level models which describe changes of microbial numbers with time.

2- Secondary level models summarize the effect of environmental conditions on parameters in the primary growth and survival models

3- Tertiary level models that combine the two first levels

Objectives

- To study the antimicrobial effects of different concentrations of **Purasal P Opti.Form 4** on growth and survival of *L. monocytogenes* in broth as a function of pH and temperature.

- To develop primary models that describe growth and survival of *L. monocytogenes*

Objectives (cont.)

- To develop secondary models for effects of temperature (4-37ºC), pH and **Purasal P Opti.Form 4 (0.0-4.5%)** on specific growth rate of *L. monocytogenes* in broth.

Enumeration

- At selected times 50 µl of cultures was spiral plated on Tryptose agar plates and incubated at 37°C for 24 hours.

- Bacterial colonies were counted with automated colony counter (Q count, Spiral Biotech Inc. Norwood, MA).

- All samples were duplicated and the means were plotted at each sampling time to generate the growth curves

Primary Model

Growth ***Curve fitting*** was generated for each experiment using two different functions as follow:

- Baranyi model
- Buchanan three-linear phase

Baranyi model

$$y(t) = y_o + \mu_{max} A(t) - \frac{1}{m} \ln\left(1 + \frac{e^{m\mu_{max}A(t)} - 1}{e^{m(y_{max} - y_0)}}\right)$$

$$A(t) = t + \frac{1}{v} \ln\left(\frac{e^{-vt} + q_o}{1 + q_o}\right)$$

where y(t) = ln x(t) with x(t) the cell concentration (CFU/ml)

y_o = ln x_o, y_{max} = ln(x_{max}), x_o being the initial and x_{max} the asymptotic cell concentration, respectively

μ_{max} is the maximum specific growth rate (1/h)

m is a curvature parameter to characterize the transition from exponential phase

and v is the rate of increase of the limiting substrate q, generally assumed to be equal to μ_{max}

Buchanan three-linear phase

- Buchanan et al. (1997) described bacterial growth in a very simple three-linear phase model. Lag phase:
- For $N_t = N_o$

- Exponential growth phase:
- For $N_t = N_o + \mu(t-t_{LAG})$

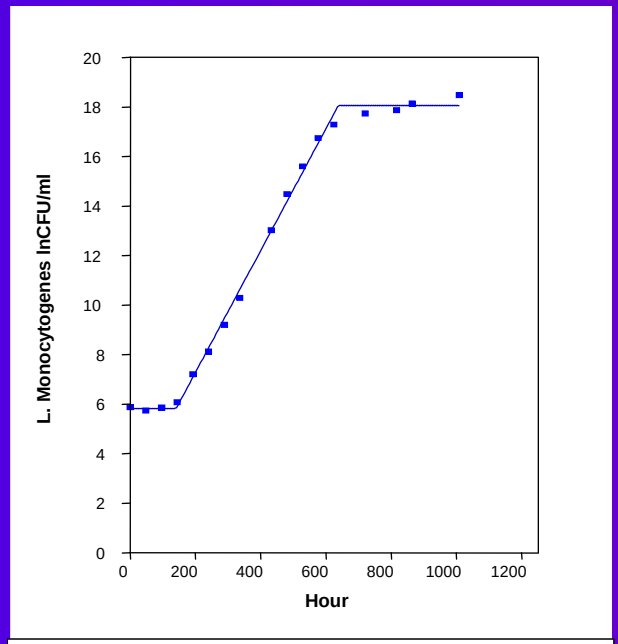
- Stationary phase:
- For $N_t = N_{MAX}$

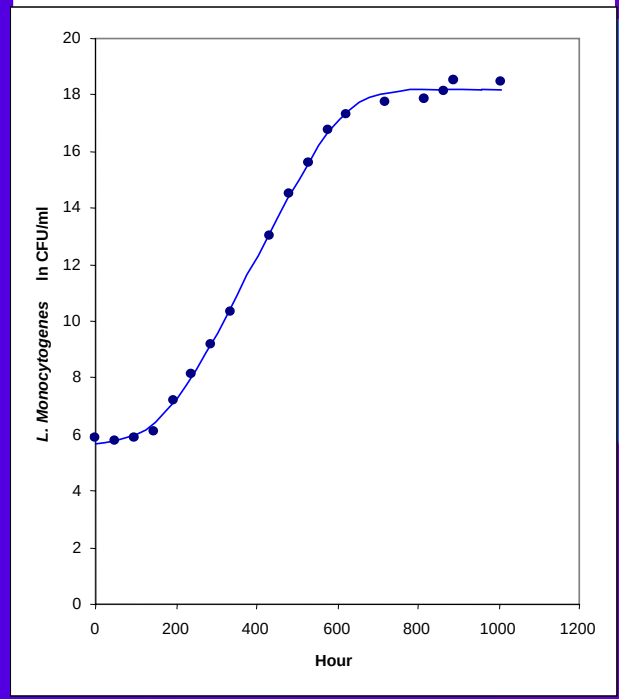
- Where N_t is the log of the population density at time t ; N_o the log of the initial population density; N_{MAX} the log of the maximum population density; t the elapsed time; t_{LAG} the time when the lag phase ends; t_{MAX} the time when the maximum population density is reached; and μ is the specific growth rate (log cfu ml^{-1} h^{-1}).

Curve fitting

- The growth curves were iteratively fitted to the corresponding function using a GraphPad PRISM® for Buchanan model

- DMFit version 2.0, an Excel add-in for fitting sigmoid curves, for Baranyi model





Conclusion

- At pH 5.5 and all temperatures a listeriostatic effect of Purasal P Opti.Form 4 has been observed at all tested levels
- At pH 6.0, addition of 1.8 % reduced the growth rate of *L. monocytogenes*, while 3% and 4.5% completely inhibited the growth over the six temperatures studied
- At pH 6.5 and 7.0 the efficacy Purasal P Opti.Form 4 has been reduced

Future Research

- To develop secondary response surface models for effects of temperature, pH and different concentrations of Purasal P Opti.Form 4 on lag time and specific growth rate of *L. monocytogenes* in broth

- To develop and validate tertiary model for potential growth or survival of *L. monocytogenes* as a function of temperature, pH, and different concentrations of Purasal P Opti.Form 4

- These models can be integrated to the pathogen model program (PMP)

Thank You