Predictive Accelerated Stability Modeling of Probiotics using ASAP*prime*®

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Study Goals

This study tests whether probiotic stability can be predictively modeled using the Accelerated Stability Assessment Program (ASAP*prime*®), and if formulated probiotic stability in packaging is predictable from pure probiotic powder stability models.

Principles of ASAP

ASAP enables rapid shelf life assessment from short (~3 weeks) stressing studies based on the following concepts:

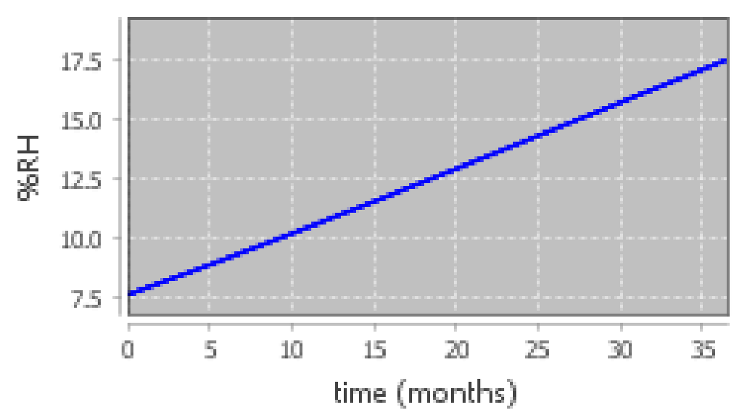
1. Time-to-fail specification (isoconversion times) rather than true rate constant
2. Moisture-modified Arrhenius equation:

$$\ln(k) = \ln(A) - E_a/(RT) + B(RH)$$

k = (initial value – specification limit)/(isoconversion time),
ln(A) = preexponential factor, E_a = activation energy, R = gas constant,
T = temperature in Kelvin, B = humidity sensitivity factor, and
RH = equilibrium relative humidity

3. Calculation of RH inside packaging as a function of time using:
   - Moisture sorption isotherms of product and desiccants
   - Moisture permeability of package (MVTR)
   - Initial water activity of product
   - External storage conditions

Example: Internal RH for 60 capsules and 1 g of silica gel desiccant in a 100-cc HDPE bottle stored at 25°C/60% RH



4. Determination of probability of achieving desired shelf life under long-term storage condition(s) in targeted packaging configuration(s)

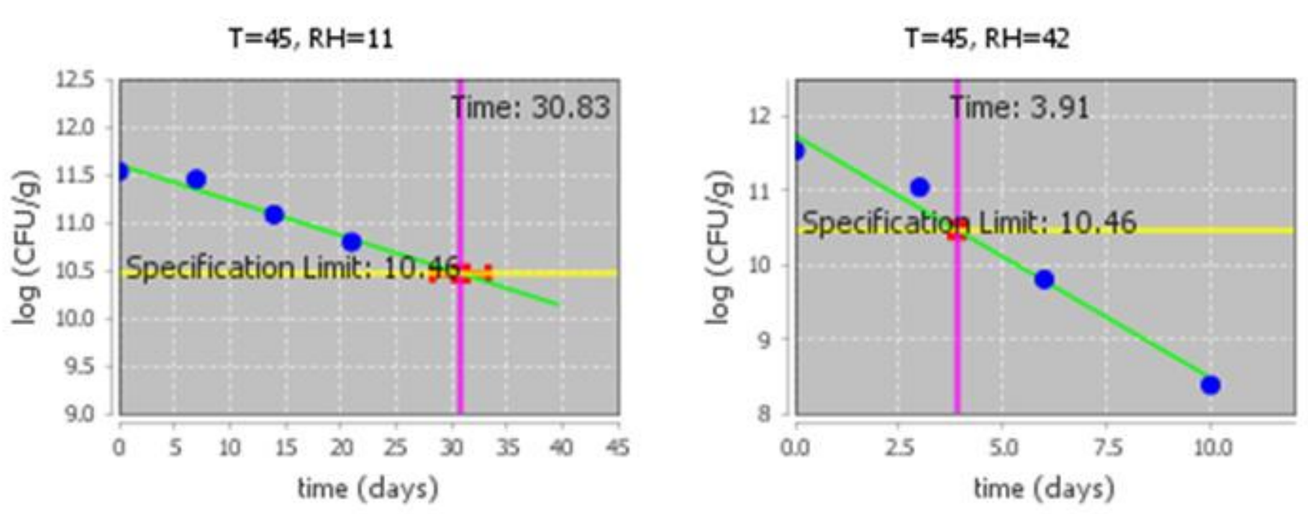
Methods

- Powders of *Lactobacillus acidophilus* and *Bifidobacterium lactis* were stressed (outside of packaging) at a range of temperature and RH conditions for up to three weeks.

ASAP stress conditions for *L. acidophilus* powder

Temperature (°C)	% RH (Saturated Salt Solution)	Time in Days (Repeats)
Control (2–8)	N/A	0 (5)
30	56 (Sodium Bromide)	3 (1); 10 (2); 17 (1)
35	32 (Magnesium Chloride)	14 (1); 21 (1)
40	66 (Potassium Iodide)	1 (1); 2 (1)
45	11 (Lithium Chloride)	7 (1); 14 (1); 21 (1)
45	42 (Potassium Carbonate)	3 (2); 6 (1); 10 (1)
50	2 (Cesium Fluoride)	11 (1); 15 (1); 21 (1)
55	30 (Magnesium Chloride)	1 (1); 3 (1)
60	11 (Lithium Chloride)	3 (1); 14 (1)
65	21 (Potassium Fluoride)	1 (1); 2 (1)
70	2 (Cesium Fluoride)	1 (1); 3 (1)
Total		**31 samples**

- Viability was quantified by cultural plating enumeration methods (CFU/g) and evaluated using ASAP*prime*® software.



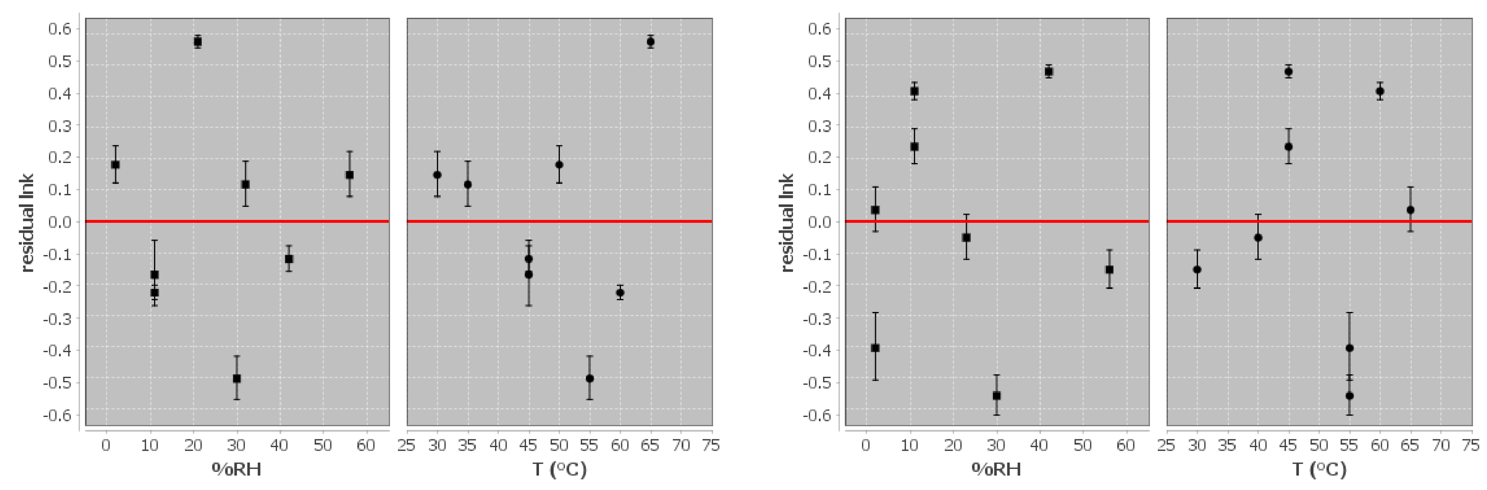
Example isoconversion plots for cell viability in *L. acidophilus* powder

- Behavior of the formulated capsule product was calculated based on pure probiotic powder results by explicitly taking into account the moisture sorption behavior of the capsule shell and excipients.

Results

ASAP*prime*®-modeled Arrhenius parameters for loss of cell viability (loss of 2.5 log (CFU/g) from initial)

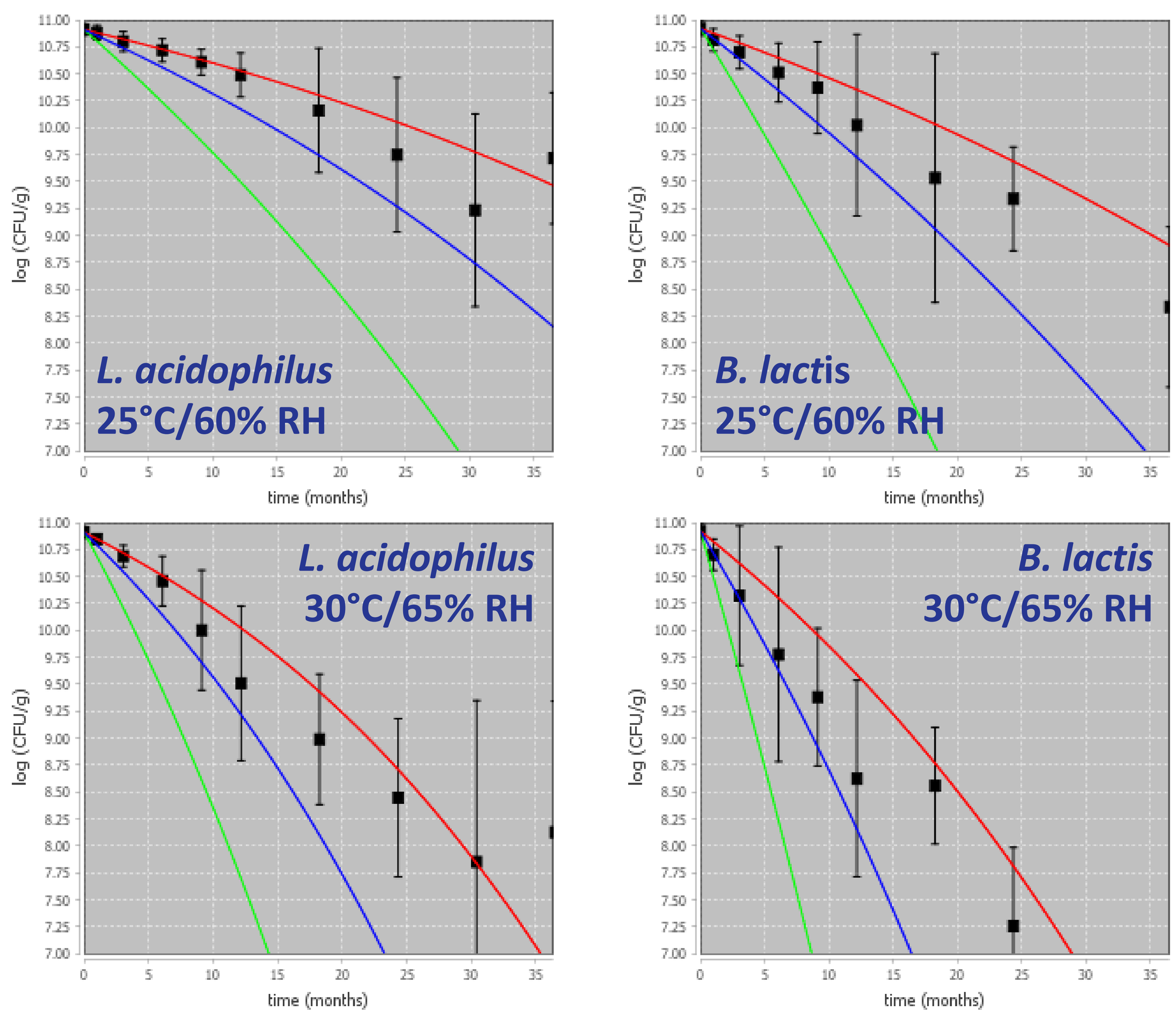
Strain	Specification limit log (CFU/g)	ln A	E_a (kcal/mol)	B	R^2	Q^2
L. acidophilus	9.0	38.5 ± 4.9	26.8 ± 3.2	0.066 ± 0.010	0.94	0.82
B. lactis	9.5	41.2 ± 7.1	28.1 ± 4.7	0.052 ± 0.014	0.88	0.72



Residuals plots of fit to the Arrhenius model for loss of cell viability in *L. acidophilus* (left) and *B. lactis* (right) powder

Component
Probiotic powder
Microcrystalline cellulose
Magnesium stearate
Silicon dioxide
Vcaps® size 0 HPMC

Composition of probiotic capsule products



Long-term stability data (error bars ± 1σ) for cell viability (log (CFU/g)) of capsule products packaged in 100-cc HDPE (HIS) bottles containing 60 capsules and 1 g of silica gel desiccant overlaid on loss predicted by ASAP*prime*®. Blue line: mean predicted behavior. Green and red lines: 90% confidence interval.

Conclusions

- ASAP*prime*® predictive stability models based on three weeks of aging at a designed range of temperature and RH conditions (open) accurately predict the observed long-term stability of two bacterial strains.
- Models generated for the pure probiotic powder are applicable to formulated and packaged capsule products by explicitly calculating the in-package RH as a function of time: excipients and capsule shells predominately affect moisture balance and do not directly impact the probiotic stability.
- Accelerated stability modeling of probiotic shelf life using ASAP*prime*® enables rapid determination of shelf life under different storage conditions, packaging requirements, overage needs, and impact of shipping excursions.

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2023