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Modeling Growth Kinetics of Lactic Acid Bacteria for Food Fermentation

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Abstract Modeling the growth kinetics of lactic acid bacteria (LAB), one of the most valuable microbial groups in the food industry, has been actively pursued in order to understand, control, and optimize the relevant fermentation processes. Most modeling approaches have focused on the development of single population models. Primary single population models provide fundamental kinetic information on the proliferation of a primary LAB species, the effects of biological factors on cell inhibition, and the metabolic reactions associated with cell growth. Secondary single population models can evaluate the dependence of primary model parameters, such as the maximum specific growth rate of LAB, on the initial external environmental conditions. This review elucidates some of the most important single population models that are conveniently applicable to the LAB fermentation analyses. Also, a well-defined mixed population model is presented as a valuable tool for assessing potential microbial interactions during fermentation with multiple LAB species.

Keywords: lactic acid bacteria, fermentation, modeling, growth kinetics, microbial interaction

Introduction

Lactic acid bacteria (LAB), the primary microbial group of commercial starter cultures, have long played a central role in the production of cheese, yoghurt, and wines, as well as many other traditional food products prepared through the fermentation of meats, sausage, fish, cereals, and vegetables. LAB can acidify raw materials rapidly through the transformation of fermentable sugars into organic acids, mainly lactic acid (1). LAB can biosynthesize many interesting and important functional metabolites including natural antimicrobials (1), aromatic compounds (2), exopolysaccharides (3, 4), low-calorie polyols (5), and oligosaccharides (4). In addition, LAB can reduce the toxic or antinutritive factors that naturally exist in foods, such as lactose, galactose, raffinose, stachyose, verbascose, protease inhibitors, phytic acid, tannins, cyanogenic glucosides, and biogenic amines (1). These beneficial characteristics of LAB provide fermented food products with enhanced microbial safety, longer shelf-life, improved texture, higher sensorial quality, and more health-promoting substances.

Mathematical models have been used extensively to generate better understanding and control of the complex fermentation processes associated with LAB. When developing models, most studies have focused on single population models, obtained from modeling the growth kinetics of a single LAB species. Single population models may be categorized into two groups: primary models and secondary models (6). Primary models are generally built to describe cell proliferation under given environmental conditions. Although a large variety of growth models have been proposed in predictive microbiology, only a few of them have been tested for LAB fermentations. Biokinetic models for metabolic reactions coupled with cell proliferation, which include substrate utilization and the generation of functional metabolites, are also classified as primary models. Secondary models are constructed to assess the effects of relevant environmental factors (e.g., temperature, pH, ionic strength, ethanol, organic acids) on primary model parameters, generally maximum specific growth rate (μ_{max}). The criteria that must be considered when developing models were proposed by Rosso *et al.* (7) as follows: simplicity (minimum number of parameters), convenient applicability, good quality of fit with experimental data, biological significance of parameters, and easy estimation of initial parameters.

An important factor that has not been included in single population models is the influence of background microflora and associated microbial interactions, namely interspecies interactions (8). Interspecies interactions cannot be neglected either in the case of food fermentation, in which a considerable number of mixed microbial species are often involved, or in the case of biological preservation, in which competitive microbial growth is a major concern (8). Such interactions may primarily cause two typical phenomena that are observed during LAB fermentation: the disappearance of certain strains in original starter cultures (1) and the dominance of secondary flora, conferring unique flavor characteristics to the final products, particularly during the maturation step (9). As a result, mixed population models, in which the effects of interspecies interactions on microbial growth are taken into account (10), may therefore have to be adopted for a better analysis of LAB fermentations.

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This review is organized as follows: First, single population models, including those that have already been tested for LAB fermentations, as well as those that are alleged to be useful in predictive microbiology but have not yet been tested for the fermentations, are elucidated systematically, and a generalized form of the growth kinetic model is presented. Then, a simple underlying mixed population model developed in predictive microbiology and ecology is briefly presented as a convenient tool for assessing interspecies interactions during LAB fermentations.

Single population models

Primary models for cell proliferation The simplest realistic approach to modeling microbial proliferation in batch monoculture with time is to use the first order growth kinetic model.

$$\frac{dN}{dt} = \mu N \tag{1}$$

where, N is the concentration of a microbial population at time t and μ is the specific growth rate of the population.

When microbial cells are introduced to a new environment, the specific growth rate should increase in monotone as the cells adjust to the new external conditions until the maximal value (μ_{max}) is reached at the end of the lag phase, and should subsequently decrease as the inhibitory factors to cell growth accumulate in the environment until the growth rate ultimately drops to zero at the end of the exponential phase. Taking both the cell adjustment and inhibition into account, Baranyi and Roberts (11) reformed Eq. 1 as follows:

$$\frac{dN}{dt} = \mu_{max} f_A f_I N \tag{2}$$

where, f_A is an adjustment (or adaptation) function and f_I is an inhibition function. The values of both functions range between 0 and 1. If neither the cell adjustment nor the cell inhibition is considered ($f_A = f_I = 1$), Eq. 2 reduces to the well-known Malthus' Law: $\frac{dN}{dt} = \mu_{max} N$. If cell inhibition is expressed as a function of cell concentration (N) only, Eq. 2 becomes one of the logistic-type growth models (Table 1).

Table 1. Logistic-type microbial growth models

	Model		Eq. no.	Reference
$f_A = 1$	Richards	$\frac{dN}{dt} = \mu_{max}\left[1 - \frac{N}{N_{max}}\right]^n N$	(3)	(12)
	Logistic (Verhulst-Pearl)	$\frac{dN}{dt} = \mu_{max}\left[1 - \frac{N}{N_{max}}\right] N$	(4)	(12)
	Hyper-Gompertz	$\frac{dN}{dt} = \mu_{max}\left[\ln\left(\frac{N_{max}}{N}\right)\right]^n N$	(5)	(12)
	Ordinary Gompertz	$\frac{dN}{dt} = \mu_{max}\left[\ln\left(\frac{N_{max}}{N}\right)\right] N$	(6)	(12)
	Modified logistic	$\log\frac{N}{N_0} = \frac{A}{\left\{1 + \exp\left[\frac{4\mu_{max}}{A}(t_{lag} - t) + 2\right]\right\}}$	(7)	(13)
	Modified Gompertz	$\log\frac{N}{N_0} = A\exp\left\{-\exp\left[\frac{\mu_{max} e}{A}(t_{lag} - t) + 1\right]\right\}$	(8)	(13)
$f_A \neq 1$	Baranyi and Roberts	$\begin{cases} \frac{dN}{dt} = \mu_{max}\left[\frac{q}{1+q}\right]\left[1 - \frac{N}{N_{max}}\right] N \\ \frac{dq}{dt} = \mu_{max} q \end{cases}$	(9)	(11)
	Fujikawa	$\frac{dN}{dt} = \mu_{max}\left[1 - \frac{N_{min}}{N}\right]^n \left[1 - \frac{N}{N_{max}}\right] N$	(10)	(14)

$A = \log(N_{max}/N_0)$;
n = an adjustment factor (≥ 0);
N_0 = initial cell concentration;
N_{max} = maximum cell concentration at stationary phase or carrying capacity of environment;
N_{min} = minimum cell concentration, which is a bit smaller than N_0;
q = a dimensionless variable characterizing the physiological state of cells;
t_{lag} = lag time;
μ_{max} = maximum specific growth rate.

When cell adjustment is not considered (f_A = 1), the familiar Richards, logistic, and Gompertz-type models (Eq. 3 to 6) are obtained. The Richards (Eq. 3) and logistic (Eq. 4) models have been used most frequently for the analysis of LAB fermentation (6, 15-22). One of the drawbacks of these models is that they cannot describe the lag phase on a semi-logarithmic plot, in which the transition between lag and exponential phases is generally observed for microbial proliferation. To overcome this shortcoming, the analytical solutions of the logistic (Eq. 4) and ordinary Gompertz (Eq. 6) models were modified by introducing a lag factor (13). The modified Gompertz function (Eq. 8) is one of the most popular growth kinetic models in predictive microbiology. However, the model has rarely been used for LAB fermentation, probably because previous studies on LAB fermentation have been carried out under conditions designed to minimize the lag phase. The lag-related problem was also solved in a more sophisticated way by taking into account the adaptation of cells during the lag phase ($f_A \neq 1$). Baranyi and Roberts (11) proposed an adjustment function based on Michaelis-Menten kinetics [$f_A = q/(1+q)$ in Eq. 9], where q is a dimensionless variable characterizing the physiological state of cells during the lag phase. The value of q is supposed to be proportional to the per cell concentration of a critical substance that is the bottle-neck in the process of cell growth. For this biological explanation of lag phase, the f_A of Baranyi and Roberts may be regarded as semi-mechanistic (23). Fujikawa *et al.* (14) developed a purely empirical adjustment function depending on cell concentration [$f_A = (1-N_{min}/N)^n$ in Eq. 10]. The authors showed that the model of Baranyi and Roberts (Eq. 9) and their new model (Eq. 10) yielded more accurate estimations of μ_{max}, t_{lag}, and N_{max} than the modified Gompertz model (Eq. 8), which overestimated values for the growth of *Escherichia coli* at various levels of N_0 (10^2 - 10^5 CFU/mL) and temperature (27.6 - 36.0°C). However, these two advanced models (Eq. 9 and 10) have not yet been tested for LAB fermentation.

Although the N-dependent inhibition functions, which force the specific growth rate in Eq. 1 to decrease monotonically during exponential growth, enable the logistic-type growth models to describe the transition from exponential to stationary phases quantitatively, the biological factors involved in cell inhibition are not explicitly represented in the functions. In the case of LAB, such inhibitory factors include depletion of essential nutrients (e.g., fermentable sugars, amino acids), production of inhibitors (e.g., organic acids, bacteriocins), and acidification of the environment (pH decrease) (6). Leroy and De Vuyst (24) proposed an inhibition function, in which the cell inhibition caused by the depletion of glucose (S), the production of undissociated lactic acid (HL), and the exhaustion of some essential nutrients in the complex nutrient source (CNS) (i.e., bacteriological peptone, Lab-Lemco powder, and yeast extract) of MRS (de Man-Rogosa-Sharpe) broth is reflected, for modeling the growth of *Lactobacillus sakei* CTC 494, a bacteriocin-producing starter for sausage fermentation. Assuming that no interaction effects occur between the three individual inhibitory actions, the inhibition function (f_I) was expressed as follows:

$$f_I = f_S f_{HL} f_{CNS} \quad (11)$$

where, f_S, f_{HL}, and f_{CNS} are the individual inhibition functions for glucose depletion, undissociated lactic acid production, and complex nutrient exhaustion, respectively, and range between 0 and 1. The structures of the individual functions are shown in Table 2. For the function

Table 2. Individual inhibition functions used as components of inhibition function (f_I) for the analysis of lactic acid bacteria fermentation

Inhibition factor	Associated chemical	Individual inhibition function	Eq. no.	Reference
Nutrient depletion	Sugar	$f_S = \frac{S}{K_S + S}$	(12)	(24)
	Complex nutrient source	$f_{CNS} = \begin{cases} 1 & if\ N < N_1 \\ 1 - a_1(N - N_1) & if\ N_1 < N < N_2 \\ 1 - a_1(N_2 - N_1) - a_2(N - N_2) & if\ N > N_2 \end{cases}$	(13)	(24)
Product inhibition	Organic acids	$f_{HA} = \left[1 - \frac{HA}{HA_{max}}\right]^n$	(14)	(24)
Acidification	Proton	$f_{pH} = \frac{(pH - pH_{min})(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_\mu)^2}$	(15)	(24)

a_1, a_2 = slopes on the plot of f_{CNS} vs. N when $N_1 < N < N_2$ and $N > N_2$, respectively;
HA = concentration of an undissociated organic acid;
HA_{max} = concentration of an undissociated organic acid, above which growth ceases;
K_S = limiting substrate concentration at which the specific growth rate is half its maximum value;
N_1, N_2 = critical cell concentrations at which the nutrient inhibition pattern changes from non-inhibition to the first inhibition phases, and from the first inhibition to the second inhibition phases, respectively;
pH_{max} = the highest pH value that allows cell growth;
pH_{min} = the lowest pH value that allows cell growth;
pH_μ = pH value at which μ is maximum;
S = concentration of a fermentable sugar.

of f_S, Monod's equation (Eq. 12) was used. For the function of f_{HL}, Eq. 14, which was proposed as a secondary model by Passos *et al.* (25), was adopted. This function is able to relate the concentration of HL to the cell inhibition caused by the presence of HL molecules as they cross the cell membrane and cause a drop in the intracellular pH (23). The inhibitory action of HL is also associated with the decrease in the pH of the medium due to the accumulation of HL and the consequent protonation of lactate (L^-) and other dissociated organic acids (A^-) present in the medium (23, 24). These additional inhibitory effects of HL were minimized by maintaining a constant pH in the medium during the experiments, but were further considered in the authors' subsequent work (24), in which the individual inhibition functions for acetic and citric acids based on Eq. 14 and the inhibition function for decreases in the medium pH (Eq. 15) were incorporated into Eq. 11 as well. The concentration of undissociated organic acids (HA) was estimated using the following Henderson-Hasselbalch equation.

$$HA = \frac{H^+ L}{H^+ + K_A} \tag{16}$$

where, H^+ is the concentration of protons, L is the total concentration of lactic acid (= HA + A^-), and K_A is the dissociation constant of organic acids ($10^{-3.86}$ M for lactic acid). For the function of f_{CNS}, a three-step equation (Eq. 13) was proposed. This N-dependent function divides growth inhibition into three phases, a non-inhibition phase (f_{CNS} = 1) and two subsequent inhibition phases. The function was able to describe a strong reduction in the growth rate of *L. sakei* CTC 494 during fermentation, which could not be sufficiently explained by inhibition due to glucose depletion and HL production. However, the assumption of a discontinuous inhibition pattern on the basis of a sudden shortage of essential nutrients seems to be awkward, because the behavior of the microbial population as a whole is expected to change smoothly (23). Leroy and De Vuyst (24, 26) successfully employed Eq. 11 as an inhibition function for modeling the growth of *L. sakei*, although they did not consider the cell adaptation (f_A = 1). Care should be taken when applying Eq. 11 to other microbes and fermentation processes, because possible correlation between the individual inhibitory actions may seriously interfere with the modeling.

Primary models for metabolic reactions Modeling the metabolic reactions occurring during cell proliferation, including sugar utilization, lactic acid generation, and bacteriocin production, is also crucial to understanding and controlling LAB fermentation processes (Table 3). The consumption of fermentable sugars by LAB has been successfully described by the well-known Pirt's model (Eq. 17), in which the cell yield factor ($Y_{N/S}$), an overall stoichiometric coefficient equal to the total cell mass (or cells) produced divided by the total mass of sugar consumed, and the specific maintenance rate for the sugar (m_S) are included (6, 15-19, 21, 24, 26, 27). The generation of lactic acid by LAB can be estimated from the sugar consumption with the yield factor for the conversion of sugar into lactic acid ($Y_{L/S}$) using Eq. 18 (6, 15-19, 21, 22, 24, 26, 27). The value of $Y_{L/S}$ is often not significantly influenced by environmental conditions such as temperature, pH, and salt, and is close to 1.0 g-lactic acid/g-glucose for homofermentative LAB such as *L. sakei* and *L. curvatus*, which convert all glucose into lactic acid (16, 17, 22, 24, 26). Equation 19, obtained by combining Eq. 17 and 18, can be also used to predict the rate of lactic acid generation (27). The production of bacteriocin by LAB can be predicted by using Eq. 20, which describes the rate of change in bacteriocin activity in the medium (6, 18, 22, 24, 26). Equation 20 denotes that the production of bacteriocin increases with cell yield, ceases when the stationary growth phase is reached, and then decreases mainly due to the adsorption of bacteriocin molecules to the cells (22).

Secondary models A number of secondary models, most of which are purely empirical, have been developed to assess the dependency of μ_{max} of LAB on environmental factors, such as temperature, pH, organic acids, ethanol, and ionic strength. A simple but effective way of secondary modeling is to use the following two assumptions: (1) μ_{max} is influenced by the initial conditions of inhibitory environmental factors, and (2) the inhibitory actions of individual inhibitory factors are independent (25, 26). The first assumption can be represented by:

$$\mu_{max} = (\mu_{max})_{opt} \gamma_0 \tag{21}$$

where, $(\mu_{max})_{opt}$ is the optimal maximum specific growth

Table 3. Models for metabolic reactions coupled with cell proliferation

Metabolic reaction	Model	Eq. no.	Reference
Sugar utilization	$\frac{dS}{dt} = -\frac{1}{Y_{N/S}}\frac{dN}{dt} - m_S N$	(17)	(15)
Lactic acid generation	$\frac{dL}{dt} = -Y_{L/S}\frac{dS}{dt}$	(18)	(15)
	$\frac{dL}{dt} = \frac{1}{Y_{N/L}}\frac{dN}{dt} + m_L L$	(19)	(27)
Bacteriocin production	$\frac{dB}{dt} = k_B\frac{dN}{dt} - k_i BN \quad if\ N \geq N_B$	(20)	(24)

B = bacteriocin activity in cell medium;
k_B = specific bacteriocin production (or yield coefficient for bacteriocin on the cells produced);
k_i = apparent rate of bacteriocin inactivation;
m_L = specific maintenance rate (or maintenance coefficient) for lactic acid (= $m_S Y_{L/S}$);
m_S = specific maintenance rate (or maintenance coefficient) for sugar;
N_B = minimum cell concentration for bacteriocin production, below which the value of k_B is equal to zero;
$Y_{L/S}$ = yield coefficient for the conversion of sugar into lactic acid (= $\Delta L/\Delta S$);
$Y_{N/L}$ = yield coefficient for cells on the lactic acid produced (= $\Delta N/\Delta L$);
$Y_{N/S}$ = yield coefficient for cells on the sugar consumed (= $\Delta N/\Delta S$).

rate obtained under optimal environmental conditions in the absence of inhibitory factors, and γ_0 is the function denoting the initial inhibition due to suboptimal initial environmental conditions ($0 < \gamma_0 < 1$). According to the second assumption, we may write:

$$\gamma_0 = \gamma_T \gamma_{pH} \gamma_{HA} \gamma_{Et} \gamma_{IS} \quad (22)$$

where, γ_T, γ_{pH}, γ_{HA}, γ_{Et}, and γ_{IS} refer to the individual initial inhibition functions for temperature, pH, organic acids, ethanol, and ionic strength, respectively. If there are other initial inhibitory factors, the functions for those factors can be also added into Eq. 22 to the extent that their independencies are ensured. Some individual initial inhibition functions successfully used in the analysis of LAB are presented in Table 4. The functions of Eq. 23, 24, 25, and 32 generate parabolic curves with a maximum value equal to unity at optimal initial levels of inhibitory factors ($0 < \gamma_i < 1$). The functions of Eq. 26, 27, 29, and 30 decrease from one towards zero ($0 < \gamma_i < 1$) as the levels of initial inhibitory factors increase from zero to their maximum values for cell growth. The functions of Eq. 28 and 31 describe the growth-stimulating effects of some inhibitory factors such as acetic acid and ionic strength (25, 29, 30). The functions are unity in the absence of inhibitors (optimum conditions) and increase positively with the levels of initial inhibitory factors. When the levels exceed certain values, the functions begin to decrease and

Table 4. Individual initial inhibition functions for secondary modeling

Inhibitory factor	Individual initial inhibition function	Eq. no.	Reference
Temperature	$\gamma_T = \frac{(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]}$	(23)	(7)
	$\gamma_T = \left[\frac{ce}{p}\right]^p (T_{max}-T)^p e^{-c(T_{max}-T)}$	(24)	(28)
pH (or H^+)	$\gamma_T = \frac{(pH-pH_{min})(pH-pH_{max})}{(pH-pH_{min})(pH-pH_{max})-(pH-pH_{opt})^2}$	(25)	(7)
	$\gamma_{H^+} = \left[1-\frac{H^+}{H^+_{max}}\right]^n$	(26)	(25, 29)
Organic acids	$\gamma_{HA} = \left[1-\frac{HA}{HA_{max}}\right]^n$	(27)	(25, 29)
	$\gamma_{HA} = \left[1+\frac{b_{HA}HA}{K_{HA}+HA}\right]\left[1-\frac{HA}{HA_{max}}\right]^n$	(28)	(25, 29)
Ethanol	$\gamma_{Et} = \left[1-\frac{Et}{Et_{max}}\right]^n$	(29)	(28)
Ionic strength	$\gamma_{IS} = \left[1-\frac{IS}{IS_{max}}\right]^n$	(30)	(28)
	$\gamma_{IS} = \left[1+\frac{b_{IS}IS}{K_{IS}+IS}\right]\left[1-\frac{IS}{IS_{max}}\right]^n$	(31)	(29)
	$\gamma_{IS} = \frac{IS(IS-IS_{max})}{IS(IS-IS_{max})-(IS-IS_{opt})^2}$	(32)	(28)

b_{HA}, b_{IS} = stimulation coefficients for organic acids and ionic strength, respectively;
c, p = adjustment factors;
Et = initial percent of ethanol at which the fermentation begins;
Et_{max} = initial percent of ethanol above which growth ceases;
H^+_{max} = initial concentration of protons above which growth ceases;
IS = initial ionic strength at which the fermentation begins;
IS_{max} = initial ionic strength above which growth ceases;
IS_{opt} = initial ionic strength at which M_{max} is optimal;
K_{HA}, K_{IS} = inhibition coefficients for organic acids and ionic strength, respectively;
pH_{opt} = initial pH at which M_{max} is optimal;
T = initial temperature at which the fermentation begins;
T_{max} = initial temperature above which growth ceases;
T_{min} = initial temperature below which growth ceases;
T_{opt} = initial temperature at which M_{max} is optimal.

reach zero at the maximum levels of inhibitory factors for cell growth. By substituting Eq. 21 into Eq. 2, a general form of the single population microbial growth kinetic model can be expressed as follows:

$$\frac{dN}{dt}=(\mu_{max})_{opt}\gamma_0 f_A f_I N \tag{33}$$

Mixed population models

Several mathematical approaches have been proposed in predictive microbiology to analyze the microbial interactions among different species (10, 31-39). In this paper, the modified Lotka-Volterra model developed by Dens *et al.* (31) for two-species competition for a limited amount of resources in homogeneous food products is presented as a useful example of a mixed population model, because it has been mathematically well studied so that it could be applied to the analysis of LAB communities in a convenient and sophisticated manner. The basic Lotka-Volterra model for two interacting species, which is an extension of the logistic model (Eq. 4), can be written as follows:

$$\begin{aligned}\frac{dN_1}{dt}&=\mu_{max1}\left[1-\frac{N_1+\alpha_{12}N_2}{N_{max1}}\right]N_1\\ \frac{dN_2}{dt}&=\mu_{max2}\left[1-\frac{N_2+\alpha_{21}N_1}{N_{max2}}\right]N_2\end{aligned} \tag{34}$$

where, N_i is the concentration of species *i*, μ_{maxi} is the maximum specific growth rate of species *i*, N_{maxi} is the maximum concentration of species *i* (or carrying capacity of the environment for species *i*) in the absence of other species, and α_{ij} is a coefficient of interaction representing the effects of species *j* on the growth of species *i*. The interaction can be categorized into four classes depending on the value of α_{ij} (40): (1) mutualistic interaction when $\alpha_{ij} < 0$, (2) commensal interaction when $\alpha_{12} < 0$ and $\alpha_{21} \cong 0$ or when $\alpha_{12} \cong 0$ and $\alpha_{21} < 0$, (3) parasitic interaction when $\alpha_{12} < 0$ and $\alpha_{21} > 0$ or when $\alpha_{12} > 0$ and $\alpha_{21} < 0$, and (4) competitive interaction when $\alpha_{ij} > 0$. In the case of no interaction ($\alpha_{ij} = 0$), Eq. 34 reduces to the logistic growth model (Eq. 4) for each single species. The basic Lotka-Volterra model was expanded by Dens *et al.* (31) into the following form using the model of Baranyi and Roberts (Eq. 9) to account for the influence of lag phase on the interaction.

$$\begin{aligned}\frac{dN_1}{dt}&=\mu_{max1}\left[\frac{q_1}{1+q_1}\right]\left[1-\frac{N_1+\alpha_{12}N_2}{N_{max1}}\right]N_1\\ \frac{dN_2}{dt}&=\mu_{max2}\left[\frac{q_2}{1+q_2}\right]\left[1-\frac{N_2+\alpha_{21}N_1}{N_{max2}}\right]N_2\end{aligned} \tag{35}$$

where, q_i is a dimensionless variable characterizing the physiological state of species *i* ($dq_i/dt = \mu_{maxi}q_i$). If no interaction occurs ($\alpha_{ij} = 0$), Eq. 35 reduces to the model of Baranyi and Roberts (Eq. 9) for each single species. For a very large value of q_i, the equation reduces to Eq. 34.

Liu *et al.* (10) investigated the interactions of six naturally occurring spoilage microorganisms during the storage of sliced pork shoulder at 5°C by comparing the values of α_{ij} estimated for each pair of the microorganisms; the microorganisms included LAB, coliforms, Pseudomonads, *Brochothrix thermosphacta*, *Salmonella*, and yeasts. The comparison showed that LAB and yeasts had high antagonistic activity against the other four microorganisms and that yeasts were highly antagonistic against LAB. This approach clearly depicted a simple and explicit way of assessing microbial interactions. However, the following two important issues were not sufficiently discussed by the authors: First, the values of μ_{maxi} and N_{maxi} reported in Liu *et al.* (10) were obtained not under monospecific growth conditions, but under natural mixed growth conditions using the modified Gompertz function (Eq. 8). Second, the estimated α_{ij} values may not be the intrinsic values of the corresponding microbial pairs in the given conditions, because of the presence of other microorganisms.

One major advantage of using the modified Lotka-Volterra model (Eq. 35) is that this model system can be easily characterized by the well-known phase plane method without actually solving the system numerically. The phase plane method enables one to investigate how the populations of two interacting species in a given environment become stabilized and change around the steady-state points. For Eq. 35, four steady-state points can be obtained by setting dN_i/dt equal to zero (31);

(i) trivial steady-state point, $(N_{1e}, N_{2e}) = (0, 0)$,
(ii) N_2 extinction point, $(N_{1e}, N_{2e}) = (N_{max1}, 0)$,
(iii) N_1 extinction point, $(N_{1e}, N_{2e}) = (0, N_{max2})$,
(iv) coexistence point, (N_{1e}, N_{2e})

$$=\left(\frac{N_{max1}-\alpha_{12}N_{max2}}{1-\alpha_{12}\alpha_{21}},\frac{N_{max2}-\alpha_{21}N_{max1}}{1-\alpha_{12}\alpha_{21}}\right)$$

According to the phase plane method, the local stability properties of each steady-state point in the N_1N_2-plane can be determined by linearizing Eq. 35, which is a set of two first-order nonlinear non-autonomous (time-dependent) differential equations, followed by computing the eigenvalues of the Jacobian matrix at each steady-state point. The mathematics associated with the method is presented in detail elsewhere (31). The results of this stability analysis are summarized in Table 5. The trivial steady-state point behaves as an unstable node in the N_1N_2-plane, at which all the solution curves of Eq. 35 are in outward direction. The N_1 and N_2 extinction points can be either stable nodes, to which the solution paths converge, or unstable saddle points, which the solution curves bypass, depending on the sign of $(N_{max1} - \alpha_{12}N_{max2})$ and $(N_{max2} - \alpha_{21}N_{max1})$, respectively. The coexistence point can also be either a stable node or an unstable saddle point according to the conditions specified in Table 5. This coexistent point does not exist if the values of $(N_{max1} - \alpha_{12}N_{max2})$ and $(N_{max2} - \alpha_{21}N_{max1})$ have opposite signs.

Conclusions

Mathematical models applicable to the analysis of LAB

Table 5. Stability properties of four steady-state points in N_1N_2-plane for the modified Lotka-Volterra model

Steady-state point (N_{1e}, N_{2e})	Conditions	Stability property
(1) Trivial steady-state point (0, 0)	Always	Unstable node
(2) N_2 extinction point (N_{max1}, 0)	$N_{max2} - \alpha_{21}N_{max1} > 0$	Unstable saddle point
	$N_{max2} - \alpha_{21}N_{max1} < 0$	Stable and attractive node
(3) N_1 extinction point (0, N_{max2})	$N_{max1} - \alpha_{12}N_{max2} > 0$	Unstable saddle point
	$N_{max1} - \alpha_{12}N_{max2} < 0$	Stable and attractive node
(4) Coexistence point $\left(\frac{N_{max1} - \alpha_{12}N_{max2}}{1 - \alpha_{12}\alpha_{21}}, \frac{N_{max2} - \alpha_{21}N_{max1}}{1 - \alpha_{12}\alpha_{21}}\right)$	$\begin{cases} 1 - \alpha_{12}\alpha_{21} > 0 \\ N_{max1} - \alpha_{12}N_{max2} > 0 \\ N_{max2} - \alpha_{21}N_{max1} > 0 \end{cases}$	Stable and attractive node
	$1 - \alpha_{12}\alpha_{21} < 0$ $N_{max1} - \alpha_{12}N_{max2} < 0$ $N_{max2} - \alpha_{21}N_{max1} < 0$	Unstable saddle point

fermentations have been developed mostly on the basis of single population growth. A generalized form of the single population growth kinetic model can be expressed as Eq. 33. Logistic-type deterministic models have been used extensively to describe the proliferation of a single LAB species during fermentation. Among those models, the model of Baranyi and Roberts (Eq. 9) or Fujikawa's model (Eq. 10), in which an adaptation function is incorporated, has been known to be very accurate; however, they have not been applied to the analysis of LAB fermentations yet. To understand the influence of nutrient depletion, inhibitor production, and medium acidification on the cell inhibition during the course of fermentation, a combined-type inhibition function (Eq. 11) can be used instead of the N-dependent inhibition functions of the logistic-type growth models. The metabolic reactions that occur during the fermentation, such as sugar utilization, lactic acid generation, and bacteriocin production, can also be simply described. The influence of the initial levels of environmental factors, such as temperature, pH, organic acids, ethanol, and ionic strength, on the maximum specific growth rate of LAB has been successfully assessed with a combined-type initial inhibition function (Eq. 22). Potential microbial interactions must be carefully considered when multiple LAB species are involved during the fermentation. The modified Lotka-Volterra model (Eq. 35), a mathematically well developed mixed population growth kinetic model, could achieve such a goal in a simple and refined manner.

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