A KINETIC MODEL OF BRANCHING GROWTH OF PLANT HAIRY ROOT

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Introduction

To produce valuable plant-derived metabolites, hairy root (hereafter HR) has become of interest as a candidate for *in vitro* culture of plant cells or organs. HR is transformed by a soil bacterium, *Agrobacterium rhizogenes*, involving integration of the root-inducing plasmid into plant cell genome.[7] The resultant root generally exhibits active propagation in a phyto-hormone-free medium and a metabolite content comparable to the original plant root.[7,8]

In a previous paper,[9] we reported a bioreactor system suitable for HR from horseradish. To design and control the HR culture system, growth kinetics must be characterized on the basis of its characteristic nature of proliferation. The aim of this work is to propose kinetic modeling and expression of the HR growth process.

1. Experimental

The HRs used were induced from carrot (*Daucus carota*), horseradish (*Armoracia lapathifolia*), senna (*Cassia torosa*) and pak-bung (*Ipomoea aquatica*) with *A. rhizogenes*.[5,8,9]

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The HR cultures were done with an Erlenmeyer flask using a hormone-free Murashige-Skoog medium[4] as described previously.[8,9] Dry cell mass was determined by drying the HR at 353 K for 24 h in the Erlenmeyer flask cultures.

2. Modeling of HR Growth

For the modeling of HR growth, the following assumptions were made (see **Fig. 1**). A) The HR grows by one-dimensional extension at root tip meristem (growing point, denoted by GP) with length L_G. B) The binary division of GP occurs within negligible time on HR branching. Each GP grows and forms

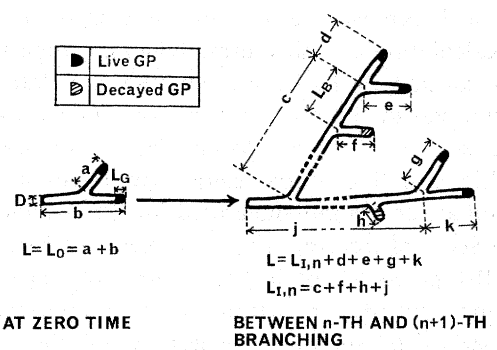

Fig. 1. Schematic drawing of HR branching growth

"highly branched HR". C) Environmental factors such as shear stress cause GP decay. D) The root is regarded as a cylinder with diameter D and length L.

On the basis of assumption A), we introduce a linear growth law for one GP:

$$dL/dt=\mu L_G \tag{1}$$

where μ is the specific elongation rate of GP. The time until next branching (division) of GP, Δt, is given by

$$\Delta t=\int_0^{L_B} dL/(\mu L_G) \tag{2}$$

Between the n-th and $(n+1)$-th branching of GP ($t_{I,n}\leqq t<t_{I,n+1}$), the initial conditions are

$$t=0 \quad : \quad L=L_0, \quad N=N_0 \quad (n=1) \tag{3}$$

$$t=t_{I,n} \quad : \quad L=L_{I,n}, \quad N=N_{I,n} \quad (n\geqq 2) \tag{4}$$

where the subscripts 0, I and n denote zero time, initial time for n-th branching and n-th branching of GP, respectively. The decay rate of GP is expressed as follows.

$$dN/dt=-k_dN \tag{5}$$

When i of GPs decay at $t=\theta_i$,

$$\theta_i=(1/k_d)\ln\{N_{I,n}/(N_{I,n}-i)\}+t_{I,t} \tag{6}$$

and hence

$$\left.\begin{array}{ll} t_{I,n}\leqq t<\theta_1 & : \ N=N_{I,n} \\ \theta_1\leqq t<\theta_2 & : \ N=N_{I,n}-1 \\ \vdots & \vdots \\ \theta_m\leqq t<t_{I,n+1} & : \ N=N_{F,n}=N_{I,n}-m \\ & \quad i=0,\ 1,\ 2,\ \cdots,\ m \end{array}\right\} \tag{7}$$

At the $(n+1)$-th branching, the initial number of GPs, $N_{I,n+1}$, is calculated from Eq. (8).

$$N_{I,n+1}=2N_{F,n} \tag{8}$$

Thus, overall root length L can be obtained by integrating Eq. (1) with respect to N of GPs.

Here, we adopt a Monod-type equation for μ.

$$\mu=\mu_{max}S/(K_S+S) \tag{9}$$

If HR cell mass yield is assumed to be constant, cell mass formed is expressed by a limiting substrate concentration decreased as follows.

$$\Delta X=-Y_X\cdot\Delta S\cdot V \tag{10}$$

Dry cell mass can be calculated from Eq. (11).

$$X=\rho\pi(1-W_c)LD^2/4 \tag{11}$$

3. Results and Discussion

So far, no kinetic expression has been reported for HR growth. As mentioned above, we presented the branching growth model, based upon the linear growth law. This law is often recognized for fungal growth pattern.[6] The filamentous growth of fungus seems essentially similar to that of the HR.

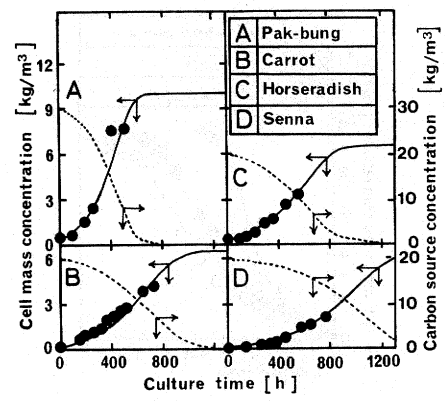

Fig. 2. Simulation of HR cultures with Erlenmeyer flasks
The lines show the calculated results using the following values of X_0 [kg], N_0 [—] and S_0 [kg/m³], respectively: 0.40, 224 and 30 (pak-bung); 0.20, 112 and 20 (carrot); 0.25, 140 and 20 (horseradish); and 0.10, 56 and 20 (senna). The values of L_0 and N_0 were calculated from a given X_0 value, based upon Eq. (11) and $N_0=L_0/L_B$.

Table 1. Parameter and constant values used for calculation

		Pak-bung	Carrot	Horse-radish	Senna
μ_{max} [h^{-1}]		0.47	0.43	0.43	0.40
K_S [kg/m³]		4.1	4.5	4.4	4.5
k_d [10^{-3} h^{-1}]	Erlenmeyer flask	4.6	4.6	4.6	4.6
	Turbine pump	—	2.4	—	—
	Air lift	—	5.3	—	—
	Rotating drum	—	6.0	—	—

The other values: $D=1.0\times10^{-3}$ m, $L_B=1.5\times10^{-2}$ m, $L_G=5.0\times10^{-4}$ m, $V=1.0$ m³, $W_c=0.85$, $Y_X=0.32$, $\rho=1.01$ kg/m³.

The model parameters μ_{max} and K_S in Eq. (9) were estimated by simulation for the Erlenmeyer flask cultures of pak-bung, carrot, horseradish and senna HRs. From the results shown in **Fig. 2**, the following μ_{max} [h^{-1}] and K_S [kg/m³] values were obtained, respectively: 0.47 and 4.1 (pak-bung); 0.43 and 4.5 (carrot); 0.43 and 4.4 (horseradish); and 0.40 and 4.5 (senna). The parameters and constants used for the calculation are summarized in **Table 1**. From the μ_{max} values, it was calculated that a GP elongates by 5–6 mm per day maximum. On the other hand, the K_S values were almost the same for the HRs tested. A wide range of K_S (0.25–7 kg/m³) was reported for callus suspension cultures.[1,2] Although it is difficult to compare the HR and callus directly, the K_S values obtained in our work are thought to be reasonable

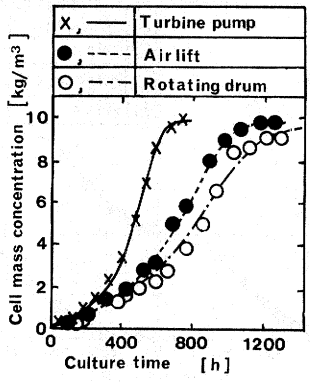

Fig. 3. Simulation of carrot HR cultures with various fermentors

The lines show the calculated results using $X_0=0.20$ kg, $N_0=112$ and $S_0=30$ kg/m^3. The other captions are the same as in Fig. 2.

for highly organized plant cells.

We have reported carrot HR cultures with various fermentors.[3] The results were compared with the growth model shown in **Fig. 3**, and the root growth processes could also be expressed in fair agreement with the experimental data. The difference in root growth rates seemed to be due to the effect of physical damage to root cells such as shear stress and collision. The k_d values for each reactor (Table 1) were considered to reflect the extent of this effect. In a turbine pump bioreactor,[3] the physical damage can be minimized because the HR is anchored and grown on a cylindrical stainless grid apart from a mechanical agitation unit. The HR growth and reactor characteristics will be discussed elsewhere.

In conclusion, we propose a kinetic model for HR proliferation, based upon the linear extension and lateral branching of the GP at root tip. The model made it possible to simulate and characterize the HR growth successfully.

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Nomenclature

D	=	diameter of HR	[m]
K_S	=	saturation constant	[kg/m^3]
k_d	=	decay rate constant of GP	[h^{-1}]
L	=	overall length of HR	[m]
L_B	=	average length between branches	[m]
L_G	=	length of GP	[m]
N	=	number of GPs	[—]
S	=	concentration of carbon source	[kg/m^3]
t	=	culture time	[h]
V	=	culture volume	[m^3]
W_c	=	water content of HR	[—]
X	=	weight of HR cell mass	[kg]
Y_X	=	HR cell mass yield for carbon source	[—]
μ	=	specific rate of elongation	[h^{-1}]
μ_{max}	=	maximum specific rate of elongation	[h^{-1}]
ρ	=	density of HR	[kg/m^3]
θ	=	time at which m PGs decay	[h]

〈Subscripts〉

F	=	value at final time for n-th branching
I	=	value at initial time for n-th branching
m	=	number of GPs decayed
n	=	n-th branching of GP
0	=	value at zero time

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