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論 文

Calorimetric Characterization of the Inhibitory Action of Antimicrobial Drugs and a Proposal of Bacteriostatic / Bactericidal Index

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A theory to quantitatively characterize the antimicrobial action of drugs on the growth activity of microbes was developed. Growth thermograms calorimetrically observed for the microbial growth cultures in the presence of drugs were analyzed on the developed theory and it was shown that their characteristic changes in pattern can be successfully described in terms of the bacteriostatic and bactericidal effects. In order to quantify this nature in drug actions, an index term "bacteriostatic / bactericidal index, *SCI*" was proposed to define a degreee of bacteriostatic action relative to that of bactericidal action and the *SCI* values were obtained for various antimicrobial drugs. It was concluded that the method is useful for the quantitative characterization as well as for the design and the development of antimicrobial drugs.

1. Introduction

The antimicrobial actions of chemical compounds (drugs) can often be divided into bacteriostatic and bactericidal actions and understanding the details of these characteristic actions is essentially important for proper and precise characterization of the drug. However, it seems that these two actions have not been strictly distinguished and that in many cases drugs are believed to act bacteriostatically up to a certain concentration, whereas they act bactericidally above that concentration. Thus almost all the drugs are often considered to have simultaneously both bacteriostatic and bactericidal actions.

In this paper, some theoretical aspect in the action of antimicrobial drugs is discussed and a method to quantitatively characterize the drug action based on the microbial calorimetry technique is described.

2. Materials and Methods

The calorimetric data for the inhibitory action of different antimicrobial drugs employed in the present paper were taken from the works reported previously by the present authors group.[1-6] For the detection of microbial growth activity was used the multiplex batch calorimeter.[1,7] All of the measurements were performed at 30 ℃ and the inhibitory parameters of antimicrobial drugs against microbial cells were determined by the method described in the previous works.[1,8-11]

3. Results

3.1 Growth rate constant and growth retardation

Fig.1. shows the observed growth thermograms (or $g(t)$ curves in author's notation) for the growing culture of *S. cerevisiae* at 30 ℃ in GPB medium containing various

Abbreviations used: propylparaben, *p*-hydroxybenzoic acid propyl ester / Q15, cis-isomer of 1-(-3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane-chloride, N-(3-chloroallyl)-hexammonium chloride, Quarternium 15 or Dowicil 200 as a commercial name / IDU, imidazolidinyl urea, Germal 115 as a commercial name / Triclosan, 2,4,4'-Trichloro-2'-hydroxydiphenylether / DMH, 1,3-dimethylol-5,5-dimethylhydantoin, 1,3-bis-(hydroxymethyl)-5,5'-dimethyl-2,4-imidazolidinedione, Dimethyloldimethylhydantoin or DMDM hydantoin / GPB medium, glucose-peptone broth medium.

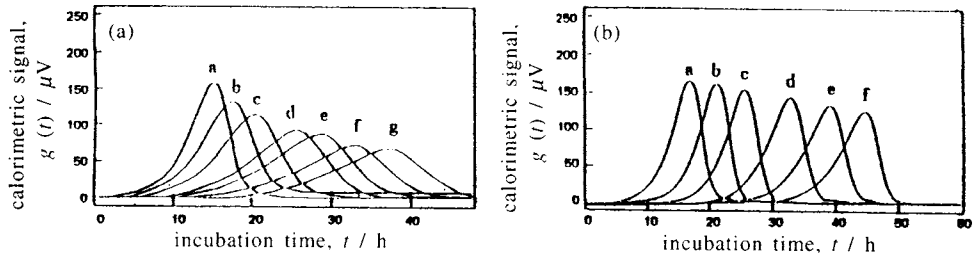

Fig.1 Growth thermograms observed for the growing culture of *Saccharomyces serevisiae* at 30 ℃ in brain-heart infusion media containing various amounts of (a) propylparaben and (b) IDU. The 7 curves shown in (a) correspond from left to right to the concentration of propylparaben: a, 0; b, 0.0072; c, 0.0102; d, 0.0132; e, 0.0144; f, 0.0156; g, 0.0168 % (w/v) and the 6 curves shown in (b) correspond from left to right to the concentration of IDU: a, 0; b,0.0040; c, 0.0056; d, 0.0088; e, 0.0128; f,0.0160 % (w/v).

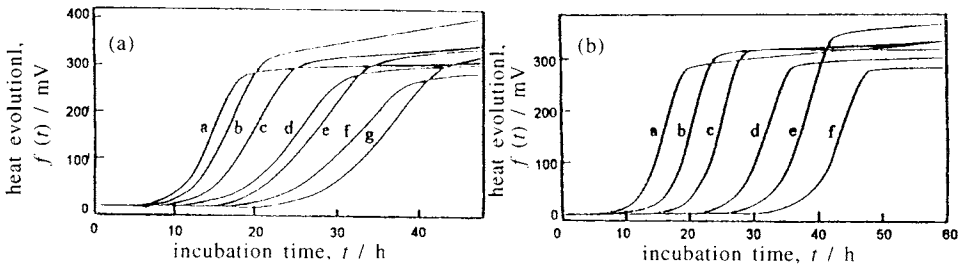

Fig.2 Growth curves of *Saccharomyces cerevisiae* obtained for the culture with antimicrobial drugs, (a) propylparaben and (b) IDU. The each curves were obtained from the growth thermograms given in Figs.1(a) and (b) by calculation using eq. (1) and therefore the drug concentrations are the same for those given in Fig.1.

amounts of propylparaben **(a)** and IDU **(b)**. It can be seen that changes in thermogram patterns are quite different for the effect of two drugs, *i.e.*, while in **(a)** the growth thermogram broadens with increasing the drug concentration, in **(b)** the basic shape of the thermograms remains unchanged, except for the parallel shift toward longer incubation times. These changes in patterns are also clear when the g (t) curves are converted to the actual heat evolution curves as shown in **Fig.2(a)** and **2(b)**.

The actual heat evolution curves (or f (t) curves in author's notation) were obtained by the following equation[1,8-12]

$$f(t) = g(t) + K \int g(t)\, dt \qquad (1)$$

where K is the heat conduction constant of the calorimetric unit.

It should be important that the f (t) values calculated by eq. (1) are well correlated with the number of microbial cells and that the f (t) curves thus obtained correspond to the growth curves calorimetrically observed.[1,10,11]

The results shown in **Fig.2** indicate that although the inhibition of growth activity is seen in both (a) and (b), the changes in pattern are remarkably different. Since the slope of initial portions in both g (t) and f (t) curves corresponds to the growth rate of microbial cells, the results given in **Figs.1(a)** and **2(a)** clearly show that propylparaben affects the growth rate constant of the yeast, whereas this is unaffected by IDU as understood from the changes in pattern shown in **Figs.1(b)** and **2(b)**.

In principle, the *bacteriostatic action* can be defined as the one that inhibits growth activity, but does not kill microbial cells. Accordingly, it may be assumed from **Figs.1(a)** and **2(a)** that the action of propylparaben on the yeast cell is characterised by a bacteriostatic nature. On the other hand, *bactercidal action* can be defined as the one that kills microbial cells, while the rest of the cells that survived the drug action continue to have a normal growth activity. Based on this fact, as shown in **Figs.1(b)** and **2(b)**, IDU is considered to have bactericidal rather than bacteriostatic effect.

If the initial cell population (or the inoculum size) is expressed as N_0 and the growth rate constant as μ, the

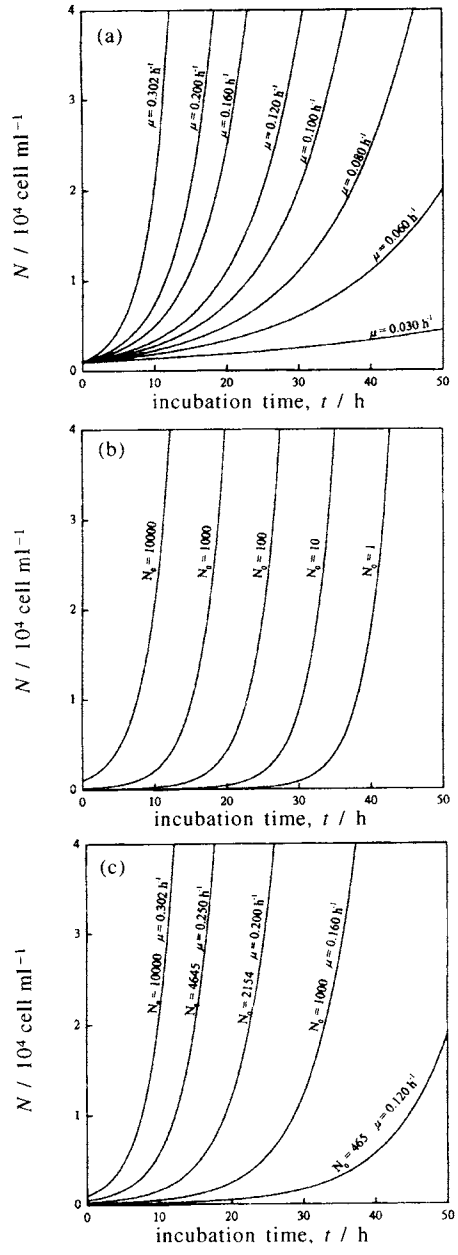

Fig.3 Microbial growth curves drawn on the basis of eq. (2). In (a) the growth rate constant μ was varied over the range of 0.030 ~ 0.302 h $^{-1}$ at the fixed initial cell population N_0 = 10,000 cell ml $^{-1}$, while in (b) the initial cell population N_0 was varied over the range 1 to 10,000 cell ml $^{-1}$ with the fixed growth rate constant μ = 0.302 h $^{-1}$. In (c) both the growth rate constant and the initial cell population were varied as given in the figure.

cell population at the incubation time t during an exponentially growing phase is given by the following equation:

$$N = N_0 \exp\{\mu(t-\tau)\} \quad (2)$$

where τ is the so-called lag time (or the induction period for growth). Using eq. (2), the theoretical growth curves were obtained by fixing the initial cell population N_0 = 10,000 cell ml $^{-1}$ and changing the growth rate constant μ in the range of 0.030 ~ 0.302 h $^{-1}$ and are shown in **Fig.3(a)**. In this case, τ was assumed to be zero just for convenience.

On the other hand, **Fig.3(b)** shows the growth curves obtained from eq. (2) by fixing μ to be 0.302 h $^{-1}$ and changing N_0 from 1 to 10,000 cell ml $^{-1}$. It will be clear that these results correspond to the initial exponential growth phase in **Figs.2(a)** and **2(b)**, respectively. Therefore, whether the action is bacteriostatic or bactericidal, it is clearly expressed by the change in the growth curve pattern and the changes presented in the growth curve patterns shown in **Fig.3** are the cases when a drug acts ideally either bacteriostatically **(a)** or bactericidally **(b)**.

However, it should be noted that many of the drugs may actually affect both the initial cell population and the growth rate constant. In this case, expected changes in the growth curve pattern are mathematically expressed as those shown in **Fig.3(c)** where the growth curves are drawn using eq. (2) by changing both the growth rate constant (μ = 0.030 ~ 0.302 h $^{-1}$) and the initial cell population (N_0 = 1 ~ 10,000 cell ml $^{-1}$). Such a situation, where the antimicrobial action of a chemical compound is expected to consist of bacteriostatic and bactericidal actions, can be more quantitatively characterized by the specific activity as measured from both the growth rate constant, μ_i/μ_m and the apparent growth retardation, $t_\alpha(0)/t_\alpha(i)$, which have been defined in the preceding works.[1,8-11] In the latter, we define the incubation time required for the microbial activity to reach a level α in the absence of the drug to be $t_\alpha(0)$ and that in the presence of the drug to be $t_\alpha(i)$ and the specific growth retardation is expressed by the ratio of these two.[1,8,9]

For a drug characterized by an ideally bacteriostatic action a drug potency curve derived from the growth rate constant μ_i/μ_m would be obtained as the one shown in **Fig.4(a; solid line)**. In this case, the use of a logarithmic scale for drug concentrations is convenient

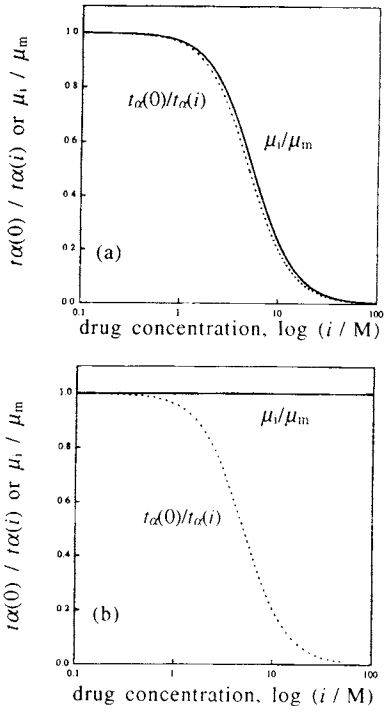

Fig.4 Drug potency curves derived from the specific growth rate constant μ_i/μ_m and the specific growth retardation $t_\alpha(0)/t_\alpha(i)$. In (a) the specific growth rate constant μ_i/μ_m changes with drug concentration i in parallel with the change of $t_\alpha(0)/t_\alpha(i)$ for an ideally bacteriostatic action, while in (b) μ_i/μ_m is constant ($=1$) for an ideally bactericidal action.

for expressing over a wide range of drug concentrations.

In contrast, the situations with bactericidal drugs are quite different. If we assume that the growth rate constant of microorganisms is not affected by the bactericidal action, the above defined specific growth activity equals 1, irrespective of the drug concentration i ($\mu_i = \mu_m$ and therefore $\mu_i/\mu_m = 1$). This feature is graphically represented in **Fig.4(b; solid line)** and this relation holds for a wide range of drug concentration as long as at least a single cell survived. Thus, the difference in drug actions, whether they are bacteriostatic or bactericidal, can be easily distinguished, if the specific growth activity μ_i/μ_m is plotted against drug concentration i.

As mentioned above, in the case of a drug that exhibits ideally bactericidal action the surviving bacterial cells that escaped the drug action should be perfectly unaffected (healthy) and should have the same growth

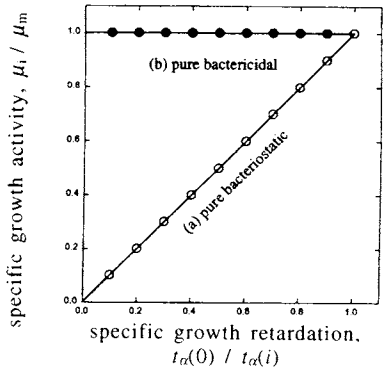

Fig.5 Graphic representation of the relation between μ_i/μ_m and $t_\alpha(0)/t_\alpha(i)$. (a) is for pure bacteriostatic action and (b) is for pure bactericidal action.

rate constant as that of the microbial cells in the absence of the drug. In such a condition the observed growth curves would be the one shown in **Fig.3(b)** characterised by a change in pattern that shows a parallel shift toward longer incubation time with increasing the drug concentration, while their shape remains unaffected.[5] This means that the effect of a drug with purely bactericidal action is reflected in the growth curve by a retardation in growth, while the growth rate remains constant.

It should be noted, however, that an apparent retardation in growth is also observable with antimicrobial drugs exhibiting bacteriostatic action as easily understood from **Fig.3(a)**. In other words, the apparent retardation in growth is a property that is common for cultures affected by both the bacteriostatic and bactericidal actions. Based on this, the apparent growth retardation can be used to draw a drug potency curve.[1,8,10,11]

As will be discussed later (section 3.3), the specific activity based on the apparent growth retardation can be determined from incubation times required for the growth activity attaining a definite level $f'(t) = \alpha$ according to the procedure reported earlier.[1,8-11] The specific growth retardation $t_\alpha(0)/t_\alpha(i)$ is related to the specific growth activity μ_i/μ_m by the following equation:[1,8]

$$t_\alpha(0)/t_\alpha(i) = (\mu_i/\mu_m) \cdot [(\ln \alpha - \ln A'_0 N_0)/(\ln \alpha - \ln A'_i N_i)] \quad (3)$$

where A'_0 and A'_i are the constants and N_i is the initial cell population (the inoculum size) for the growth culture in the presence of an antimicrobial drug.

Since no cell is killed in the case of drugs

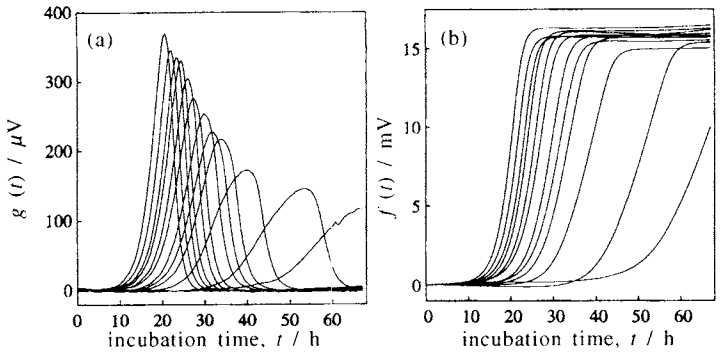

Fig.6 An example of model experiments for bacteriostatic action. Calorimetric mesurement was conducted on the growing culture of Saccharomyces cerevisiae at 30 ℃ grown in media containing various amounts of ethanol at concentration 0 ~ 5.92 %. The growth thermograms observed are shown in (a) and the corresponding growth curves obtained by calculation using equation (1) are shown in (b). The ethanol concentrations for the each curves from left to right: 0.00; 0.45; 0.89; 1.33; 1.77; 2.20; 2.63; 3.05; 3.47; 4.30; 5.12 and 5.92 %. Being in strong contrast to the results shown in Fig.8, the inhibition is apparently characterised by the bacteriostatic nature.

exhibiting ideally bacteriostatic action, and therefore the initial cell population does not change, $N_0 = N_i$, and because α is exponentially larger than A_0/N_0 or A_i/N_i, the following relation holds to a good approximation;

$$t_\alpha(0)/t_\alpha(i) = \mu_i/\mu_m \qquad (4)$$

Thus, the specific activities measured from the growth retardation and that measured from the growth rate constant should coincide and therefore the drug potency curve drawn on the basis of $t_\alpha(0)/t_\alpha(i)$ should agree with the one based on μ_i/μ_m. This situation is graphically shown in **Fig.4(A; solid and dotted lines)**.

In contrast, in the case of drugs exhibiting ideally bactericidal action, the drug potency curve drawn on the basis of $t_\alpha(0)/t_\alpha(i)$ no longer coincides with that drawn from the observed dependence of μ_i/μ_m, since the growth rate constant should not be affected by the drug and is constant irrespective of the drug concentration. This feature is illustrated in **Fig.4(B; solid and dotted lines)**.

3.2 Comparison of μ_i/μ_m and $t_\alpha(0)/t_\alpha(i)$

As shown in **Fig.4**, there is a distinct difference in the relation between the specific activity evaluated from the growth rate constant and that evaluated from the growth retardation, depending on whether a drug acts bacteriostatically or bactericidally. This characteristics can be more clearly represented by plotting μ_i/μ_m versus $t_\alpha(0)/t_\alpha(i)$.

As shown in **Fig.5(a)**, in the case of drugs exhibiting ideally bacteriostatic action, a plot of μ_i/μ_m versus $t_\alpha(0)/t_\alpha(i)$ should give a straight line with a slope of 1, since the two parameters coincide irrespective of the drug concentration as described in the preceding section. On the other hand, in the case of drugs exhibiting ideally bactericidal action, the plot should give a straight line with a slope of 0 as shown in **Fig.5(b)**, since μ_i/μ_m is constant, no matter how $t_\alpha(0)/t_\alpha(i)$ changes.

3.3 Experimental demonstration based on model systems

The features described in the above section can be demonstrated using experimental model systems. As an example, an experiment conducted with ethanol as a model antimicrobial compound will be shown.

Although it is generally understood that ethanol acts bactericidally at concentrations as high as 70 %, it is also expected to act bacteriostatically at low concentrations. This is because ethanol is known to induce structural changes in the cell membrane at a low concentration[1,13,14], without killing the cells.

The g (t) curves observed for *S. cerevisiae* cultured at 30 ℃ in media containing various amounts of ethanol at concentrations from 0 to 5.9 % are shown in **Fig.6(a)**. The corresponding f (t) curves obtained by using equation (1) are shown in **Fig.6(b)**. The growth rate constants at each ethanol concentration were determined from the f (t) curves by regression analysis according to the method described before[1,8,12] and are summarised in the second

Table 1 The effect of ethanol concentration on the growth activity of *Saccharomyces cerevisiae*.[15] The growth parameters μ_i and $t_\alpha(i)$ at each ethanol concentration were determined for the growth thermograms shown in Fig.6.

i / %(v/v)	μ_i / min^{-1}	μ_i / μ_m	$t_\alpha(i)$ / h	$t_\alpha(0)/t_\alpha(i)$
0.00	0.00644	1.000	15.67	1.000
0.45	0.00618	0.960	17.07	0.918
0.89	0.00580	0.901	18.32	0.855
1.33	0.00562	0.873	19.23	0.815
1.77	0.00541	0.841	20.61	0.760
2.20	0.00501	0.778	22.01	0.712
2.63	0.00505	0.784	24.45	0.641
3.05	0.00463	0.720	26.31	0.596
3.47	0.00442	0.687	28.17	0.556
4.30	0.00421	0.653	32.93	0.476
5.12	0.00320	0.497	46.79	0.335
5.92	0.00259	0.402	65.43	0.239

μ_m was taken to be 0.00644 min^{-1} and $t_\alpha(0)$ was taken to be 15.67 h.

column of **Table 1**.

The specific growth activity μ_i/μ_m was also calculated by taking $\mu_m = 0.00644$ min^{-1} which was obtained for the growth in the absence of ethanol ($i = 0$) and is given in the third column. From **Table 1**, It will be clear that the specific growth activity μ_i/μ_m decreases when ethanol concentration increases.

The specific activity based on the apparent growth retardation can also be determined from incubation times required for the growth activity attaining a definite level $f'(t) = \alpha$ according to the procedure described earlier[1,8,9] (see section **3.1**). The values of $t_\alpha(i)$ and the specific growth retardation $t_\alpha(0)/t_\alpha(i)$ determined are presented, respectively, in the fourth and fifth columns of **Table 1**. In this case, the value of α was chosen to be 100 μV so that $t_\alpha(0)$ is sufficiently larger than the lag time τ which is negligibly small under the present experimental condition. In another word, the value of α can be any figure, as long as it is in the range of the logarithmic growth phase. It will be clear that with increasing the ethanol concentration, the specific growth retardation $t_\alpha(0)/t_\alpha(i)$ decreases.

In **Fig.7(a)** the plot of μ_i/μ_m versus $t_\alpha(0)/t_\alpha(i)$, both being determined for the inhibitory action of ethanol, is shown in open circles. Obviously the plot gave a straight line with a steep slope and that the two indices, μ_i/μ_m and $t_\alpha(0)/t_\alpha(i)$, are simultaneously changed,

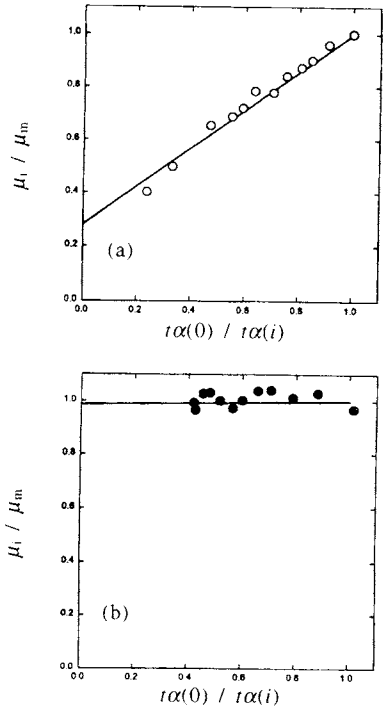

Fig.7 Plot of μ_i/μ_m versus $t_\alpha(0)/t_\alpha(i)$. The values of μ_i/μ_m and $t_\alpha(0)/t_\alpha(i)$ were taken from Table 1 in (a) and from Table 2 in (b).

indicating that ethanol is of bacteriostatic nature under the studied conditions. The slope of the plot obtained by regression analysis was $a = 0.721$. In this case the regression was made on the equation;

$$\mu_i/\mu_m = a\{t_\alpha(0)/t_\alpha(i) - 1\} + 1 \qquad (5)$$

where a is the slope.

As for the demonstration experiment of the bactericidal action, a hypothetical culture system was employed. Instead of performing an experiment with drugs exhibiting ideally bactericidal action, the growth curves of the yeast were observed by changing the initial cell population (inoculum size) in the culture system. The growth thermograms (g (t) curves) and the corresponding f (t) curves (calorimetrically obtained growth curves) are shown in **Figs.8(a)** and **8(b)**, respectively.[15]

The changes in the growth thermograms and the growth curves are in strong contrast to those given in **Figs.6(a)** and **6(b)** which were obtained with ethanol as an antimicrobial drug. The result given in **Fig.8** clearly indicates that the changes in patterns of the f (t) and

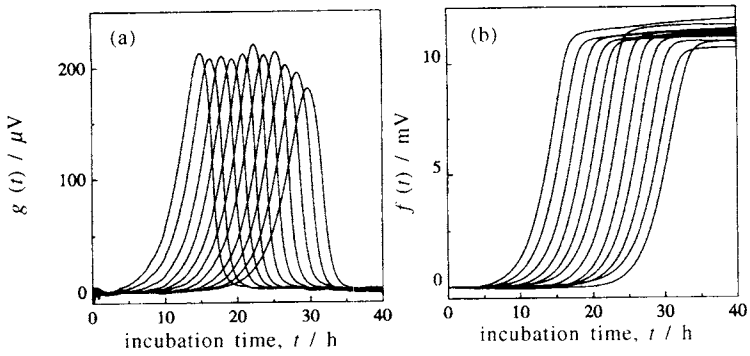

Fig.8 An example of model experiments for bactericidal action. A calorimetric measurement was conducted on the growing culture of *Saccharomyces cerevisiae* at 30 ℃ with the various initial cell population from 1.1×10^3 to 2153×10^3 cell ml $^{-1}$. Instead of changing the concentration of a drug having purely bactericidal action, the initial cell population (the inoculum size) was varied by quantitatively diluting the cell suspension.

Table 2 The effect of the hypothetically bactericidal action on the growth of Saccharomyces cerevisiae.[15] The growth parameters μ_i and $t_\alpha(i)$, were determined for the growth thermograms with different initial cell populations shown in Fig.8.

initial cell population 10^3 cell ml^{-1}	μ_i / min^{-1}	μ_i / μ_m	$t_\alpha(i)$ / h	$t_\alpha(0) / t_\alpha(i)$
2153	0.00621	0.968	11.08	1.015
1077	0.00660	1.029	12.75	0.882
538	0.00648	1.012	14.25	0.789
269	0.00667	1.040	15.92	0.707
135	0.00665	1.038	17.08	0.658
67.3	0.00642	1.002	18.75	0.600
33.6	0.00623	0.972	19.92	0.565
16.8	0.00642	1.001	21.75	0.517
8.4	0.00661	1.031	23.42	0.480
4.2	0.00658	1.027	24.75	0.454
2.1	0.00619	0.966	26.42	0.426
1.1	0.00636	0.993	26.75	0.421

μ_m was taken to be 0.00621 min $^{-1}$ and $t_\alpha(0)$ was taken to be 11.08 h.

g (t) curves are characterized by a parallel shift with respect to the incubation time and that the decrease in the initial cell population due to the hypothetically bactericidal action simply results in a retardation in growth, with the growth rate constant being constant.

The growth rate constant μ_i, the apparent growth retardation $t_\alpha(i)$ and the specific growth activity, μ_i/μ_m and the specific growth retardation, $t_\alpha(0)/t_\alpha(i)$, were also determined from each of the f (t) curves shown in **Fig.8(b)** by applying the same procedure as described above and are summarised in **Table 2**. Since this is a demonstration experiment with a hypothetically bactericidal action, the inoculum size N_i are prepared by quantitatively diluting the cell suspension as given in the first column of **Table 2**, instead of changing the drug concentration.

In this case the values of $t_\alpha(i)$ were obtained by taking the value of α to be 85 μV. For the calculation of specific growth activities, the values of $\mu_m = 0.00621$ min $^{-1}$ and $t_\alpha(0) = 11.08$ h which were obtained with the maximum inoculum size $N_0 = 2.153 \times 10^3$ cell ml $^{-1}$ were employed.

The relationship between the two specific activities obtained are shown in closed circles in the form of a plot of μ_i/μ_m versus $t_\alpha(0)/t_\alpha(i)$ in **Fig.7(b)**. It can be seen that the plot gives a straight line with the slope of almost zero, being in agreement with the theoretical consideration described in the previous sections.

3.4 A proposal of bacteriostatic/bactericidal index: *SCI*

The results given in the preceding sections obviously support that the theoretical consideration stated above is reasonable and indicate that this type of procedure is applicable to other drugs exhibiting antimicrobial actions. Hence, the slope of the plot in **Fig.7** is proposed as an index to show the degree of bacteriostatic action relative to that of bactericidal action, and is named the bacteriostatic/bactericidal index or simply *SCI*. According

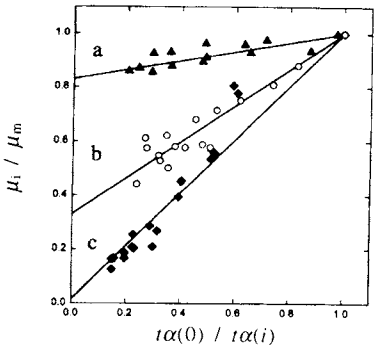

Fig.9 Some examples of the *SCI* plot observed for three different drugs. The plots are for the inhibitory action of (a) Quaterniumu 15 on *Kleb. pneumoniae*, (b) sodium benzoate on *Asp. oryzae* and (c) *p*-hydroxybenzoic aicd propyl ester on *Kleb. pneumoniae* at 30 ℃.

to the definition given here, the value for the inhibitory action of ethanol on *S. cerevisiae* shown above as the model experiment is *SCI* = 0.721. Some other plots of μ_i/μ_m versus $t_\alpha(0)/t_\alpha(i)$ obtained for different drugs, named *SCI* plots hereafter, are given in **Fig.9**. The *SCI* plots, clearly give different slopes for the different drugs. The values of *SCI* evaluated as the slope of each plot are listed in **Table 3**, together with the results obtained for some other antimicrobial drugs so far investigated in the author's research group.

4. Discussion

Regarding the molecular mechanism of drug action, when a drug specifically acts on or binds to a specific site of a microbial cell or subcellular components, an exhibition of either the bacteriostatic or bactericidal effect can be expected. For example, when an antibiotic such as penicillin irreversibly binds to a specific binding site on a cell surface and, as a consequence, inhibits the cellular growth, it is understood that the drug acts bactericidally. On the other hand, when a drug binds reversibly to one of the enzymes involved in a metabolic pathway, resulting in inhibition of the cellular growth by suppressing its catalytic function, it is assumed that the drug act bacteriostatically, provided all the cells stopped growing. However, in many cases, chemical compounds that actually exhibit antimicrobial action are considered to have two or more types of activities. For example, ethanol inhibits the catalytic activity of some enzymes and simultaneously induces structural changes in the cell membranes, influencing various cellular processes.[11,13,14] The quantitative index *SCI* proposed here that indicates the degree of bacteriostatic and bactericidal actions of a certain drug will certainly be useful for understanding the characteristics of drug potency.

Table 3 *SCI* values of some antimicrobial drugs.

drug	microbe	*SCI*
ethanol	*S. cerevisiae*	0.72
p-hydroxybenzoic acid methyl ester	*K. pneumoniae*	0.86
p-hydroxybenzoic acid ethyl ester	*K. pneumoniae*	0.99
p-hydroxybenzoic acid propyl ester	*K. pneumoniae*	0.99
p-hydroxybenzoic acid butyl ester	*K. pneumoniae*	1.02
sodium benzoate	*Asp. oryzae*	0.67
isopropyl methylphenol	*K. pneumoniae*	0.69
Quaternium-15	*K. pneumoniae*	0.17
imidazolidinyl urea	*K. pneumoniae*	0.06
Triclosan	*K. pneumoniae*	0.34
DM hydantoin	*K. pneumoniae*	0.17
boric acid	*S. serevisiae*	0.76
benzeneboronic acid	*S. cerevisiae*	0.94
4-methyl benzeneboronic acid	*S. cerevisiae*	0.94

As shown in **Table 3**, different *SCI* values were obtained for different compounds. For example, the *SCI*s for *p*-hydroxybenzoic acid alkyl esters are all very close to 1.0. This result indicates that these 4 drugs are definitely of the bacteriostatic nature. In contrast, *SCI*s of Q15, IDU and DMH were found to be 0.17, 0.06 and 0.17, respectively, being close to 0. Therefore, it is possible to note that these compounds are antimicrobial drugs having a highly bactericidal action, although not ideal. In contrast to the above drugs, ethanol was found to have a *SCI* value of 0.72. This result indicates that ethanol, which is often used for disinfection, has both the bacteriostatic and bactericidal properties, the former being slightly more than the latter. The similar situation was also seen with boric acid, which has often been used as a weakly acidic disinfectant (*SCI* = 0.76). Furthermore, it is interesting to note that *SCI*s of both benzene boronic acid and 4-methyl benzeneboronic acid were 0.94, indicating that the bacteriostatic action is enhanced by the introduction of benzene ring in boronic acid. It should be noted that in this case the mechanism of drug action appears to be changed by the modification with benzene ring.

Thus, it will be clear that the value of *SCI* given as the slope of the *SCI* plot as shown in **Figs.7** and **9** differ from drug to drug, depending on their nature of molecular action and the index term *SCI* defined here will be effectively used to characterize the antimicrobial action of various drugs. It can also be concluded that the method presented in this study will be useful for the design and the development of antimicrobial drugs.

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要　旨

微生物の増殖活性におよぼす抗微生物薬剤の作用について，それを定量的に把握するための理論的な考察を行った。熱測定法で観測される微生物の増殖サーモグラムのパターンが薬剤の存在下で変化する様子を薬剤の静菌作用，殺菌作用という2つの観点で特徴づけることができることを示した。すなわち，理想的な静菌効果を示す薬剤の作用においては増殖速度定数の低下で眺めた場合の増殖活性の変化が，増殖の時間遅れで見た場合の変化と良く一致するのに対し，理想的な殺菌効果と示す薬剤の作用においては，増殖活性の変化が増殖の時間遅れのみによって観測されることをモデル実験系で示した。さらに，静菌効果と殺菌効果を合わせ持つ薬剤に対して，その作用を定量化するため，静・殺菌指数という新しい指標（*SCI*）を定義し，それをいくつかの抗微生物薬剤について実際に求めて示すとともに，ここに提案した方法が薬剤作用の定量化ならびに新しい抗微生物薬の設計に有効であると結論した。

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