INTRODUCTION

Fungi very often contaminate foods, various human living environments, industrial materials and cultural properties and cause many problems in human life, health, industry, various environments, historical heritage and so on. To control those fungi, therefore, a variety of physical, chemical and biological techniques have been applied. For their effective application, any systematic approach may be expected, considering how fungi grow in various environments and how they survive antifungal treatments. Different methods have been used so far to evaluate quantitatively fungal growth and survival and, in fact, fungal growth on the agar plate as well as in liquid medium has been expressed in several mathematical models (e.g., Koch, 1975; Bartnicki-Garcia et al., 1989; Prosser and Trinci, 1979; Edelstein et al., 1983; Fujikawa and Itoh, 1996; Farina et al., 1997; Boswell et al., 2003; Mitchell et al., 2004; Kotov et al., 2005; Balmant et al., 2015; Abdullah et al., 2016; Dagnas et al., 2017).

However, how the growth kinetics of sublethally stressed fungi can be evaluated seems to have not been reported, although for bacteria several papers have been published (e.g., Takano and Tsuchido, 1982; Hills and Mackey, 1995; Chawla et al., 1996; Silva-Angulo et al., 2014; Sibanda and Buys, 2017; Tsuchido and Sakamoto, 2018). Although no kinetic assay was performed, using two xerophilic fungi, the influence of sugars, salts, pH, and water activity in the medium on the heat-stressed conidia of Cladosporium cladosporioides and these two mode injuries were evaluated.

Key words: Double subculture method / Injury / Stress / Fungal spore / Growth kinetics.

A Modified Double Subculture Method for the Two-Mode Injuries Evaluation in a Stressed Fungal Spore Population

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Received 25 December, 2019/Accepted 14 February, 2020

In order to evaluate injury of a stressed fungal spore population, a modification of formerly presented double subculture method, which consists of both the conventional plate count method and the growth delay analysis method, was proposed. In this method, an apparent logarithmic growth kinetics was assumed and the previous kinetic model was improved to be able to estimate injured subpopulations in two different modes containing early occurring growth-independent and late occurring growth-dependent injuries, called the $\lambda$ and $\mu$ injuries, respectively. Based on the kinetic theory developed here, this novel method was applied to heat-treated conidia of Cladosporium cladosporioides and these two mode injuries were evaluated.

Key words: Double subculture method / Injury / Stress / Fungal spore / Growth kinetics.
solid and liquid media) method, has been proposed for the comprehensive evaluation of overall injury of a bacterial stressed population. However, the effect of injury sometimes appear seems not only as a prolongation of lag period but also as a reduced growth rate of regrowth phase after a prolonged lag period resulting from exposure to a stress is decreased. In such case, this method cannot present any quantitative information on contribution of each of these injuries.

In this study, using fungal spores, we propose a modified DiVSaL method as one of the double subculture method to estimate injured subpopulation generated by exposure to a sublethal or lethal stress, considering kinetic models consisting of two distinct injury modes.

**MATERIALS AND METHODS**

**Fungal strain, cultivation and conidium preparation**

*Cladosporium cladosporioides* NBRC6358 conidia were used in this study. The fungus was cultivated on the Difco Potato Dextrose Agar (PDA, Bekton Dickinson and Co.) plates (pH5.1) at 28°C for 7 d. The resultant formed conidia were harvested with spreader, washed twice by filtration with sterilized gauze and with 50μm Nylon mesh filter (Filcon S, Becton Dickinson, USA) and then resuspended in sterile 50mM phosphate buffer saline (PBS) at pH7.4 containing 2% of glucose. The suspension containing conidia (about 2 X 10⁷ per ml) was used immediately. The percentage of germinatable conidia among the prepared suspension is about 80%, although varied with experiment.

**Heat treatment**

An aliquot (0.1 ml) of the conidium suspension was injected into a test tube containing 0.9 ml of fresh PBS buffer prewarmed to 45°C in an incubator. During heating period samples were taken out from the flask and then diluted serially with PBS (pH7.4) buffer in test tubes.

**Viability assay**

Diluted heated conidium samples were poured onto PDA plates and the plates were incubated at 28°C for 7 d. The resulted formed colonies were counted to estimate viability (CFU per ml). In this study the viable counts obtained both before and after heat treatment were regarded as the numbers of viable germinatable conidia.

**GDA method**

Using the automated quantitative assay for microbial growth (Takano and Tsuchido, 1982; Broekaert et al., 1990; Stephens et al., 1997), conidia samples were assayed for their growth. Heated conidium samples (0.02 ml aliquots) were also inoculated into 0.18 ml of Potato Dextrose Broth (PDB) in each well of a 96-well microplate and the microplate was incubated in a microplate reader (Thermo Fisher Scientific K. K.) to monitor OD at 600 nm (OD600) to measure the growth from conidium. The resultant OD data were processed with a macro automation software (Excel 2016. Microsoft, Corp., USA) to obtain the growth delay time as well as G10 values as described previously (Takano and Tsuchido, 1982; Tsuchido et al., 1989).

**Evaluation of injured population**

Following the method reported previously (Tsuchido, 2017), the overall injured population size for the above heat-stressed conidia of *C. cladosporioides* was evaluated comprehensively by the differential viabilities obtained between plate count method and GDA method. The kinetic model for this method is described as the reduced μ model with the following Eq. (1) (Tsuchido, 2017).

\[
-\log \nu' = t' / G\nu' - t / G_{10}
\]

where all symbols are explained as below (see Theory), except for \(\nu'\) and \(G_{10}'\), which are \(\nu\) value for the estimation of the overall injury and \(G_{10}\) value for the stressed population, respectively.

Apart from this method, using its modified method developed in this study two mode injuries were evaluated individually based on the same data (see Theory in detail).

**Statistical analysis**

The experiments were performed independently at least three times and the average value with S.D. were obtained. The statistical significance was tested with Student’s \(t\) test \((p<0.05)\), using spreadsheet program in Excel 2016 Software (Microsoft Corp., USA).

**THEORY**

**Concept of two mode injuries**

In this study we assume two basal principles for fungal spore growth. One is that the spore growth at least during its early phase follows an apparent first order kinetics after a lag period in a liquid medium. The other is that in stressed spores two modes of injury are induced as described below, being growth independent and dependent injuries, which appear early during a prolonged lag period and late during the subsequent growth stage, respectively (Fig. 1).

As for the former principle, in fact, several investigators have already confirmed this growth kinetics for fungal growth (Pirt, 1967; Trinci, 1969). In the latter two-mode injury hypothesis, on the other hand, the early appearing...
injury is considered to be repaired, but whether the recovery from the late appearing injury occurs is unclear and is needed to be evidenced. Although the evaluation method for the former injury is substantially based on the model presented previously (Takano and Tsuchido, 1982) and that for the latter is newly introduced in this study.

Providing that the above two assumptions can be applied, the apparently overall growth kinetics of unstressed and stressed fungal spore populations can be expressed as Eqs. (2) and (3), and depicted schematically in Fig. 2, with curves a and b, respectively.

\[ N = N_0 \exp \left[ \mu_c (t - l) \right] \]  
\[ N = n_i N_0 \exp \left[ \mu' (t - l - \lambda) \right] \]

where \( N_0 \) and \( N \) are the numbers of germinatable spore per ml in the inoculum and after an incubation time, \( t \) (h), respectively. The parameters of \( \mu_c \) and \( \mu' \) (h\(^{-1}\)) are the specific growth rates for unstressed and stressed populations (\( \mu_c \geq \mu' \)) and \( l \) and \( \lambda \) are the assumptive lag time and its apparent increment (h), respectively. \( n_i \) is a total survived fraction among the initial viable population, \( N_0 \) (0 < \( n_i \) < 1).

Generally, the growth delay time, \( \tau \) (h), in a stressed microbial population is generated by either or both of death (\( \tau_d \)) and injury (\( \tau_i \) and \( \tau_j \)). Although injury has often been considered overall, it is assumed to include substantially two distinct modes. One is early appearing growth-independent injury, called here "the \( \mu \) injury", and the other late appearing growth-dependent injury, called "the \( \rho \) injury". To evaluate each injury mode we assumed here two hypothetical reference growth curves for the stress survived populations, one without both injury modes (\( \lambda = 0, \mu' = \mu_c \)) and the other without only \( \mu \) injury mode (\( \lambda > 0, \mu' = \mu_c \)), as depicted with curves c and d, respectively, in Fig. 2. The growth rate and the delay time referred to unstressed for the former population are set to \( \mu_0 \) and \( \tau_a \), and for the latter are \( \mu_d \) and \( \tau_d + \tau_i (= \lambda) \), respectively, when evaluated at a fixed population size, \( N_0 \), in the exponential phase of the subsequent growth. These growth curves are expressed in Eqs. (4) and (5), respectively.

\[ N = n_i N_0 \exp \left[ \mu_c (t - l) \right] \]  
\[ N = n_i N_0 \exp \left[ \mu_d (t - l - \lambda) \right] \]

The Eq. (5) introduced is based on the theory of the GDA method considering an induced injury distribution among the population, by using \( \nu \) value which is

**FIG. 1.** A schematic presentation of the two-mode injury model for a stressed fungal spore population. Black and white arrows indicate the processes occurring during and after a stress treatment, respectively.

**FIG. 2.** Schematic growth curves of stressed and unstressed conidial populations. A, OD change; B, change of assumptive viable number of conidial population to corresponding to OD change. Broken lines indicate the phantom growth curves for stressed population with the same \( \mu \) value as that of unstressed. See the text for other symbols.
functions of fraction of each differently injured subpopulation and $\lambda$, as reported previously (Takano and Tsuchido, 1982; Tsuchido, 2017).

Concerning $\mu_b$ and $\mu_a$, by definition,

$$\mu_b = \mu_a = \mu$$  \hspace{1cm} (6)

Then, when the hyphal growth from spore is evaluated at the population level of $N_a$, Eqs. (2) to (5) for curves a to d can be changed to the following Eqs. (7) to (10), respectively.

$$N_s = N_o \exp \left[ \mu_o (t_a - l) \right]$$ \hspace{1cm} (7)

$$N_s = n_o N_0 \exp \left[ \mu' (t_i' - l - \lambda) \right]$$ \hspace{1cm} (8)

$$N_s = n N_0 \exp \left[ \mu_b (t_a - l) \right]$$ \hspace{1cm} (9)

$$N_s = \nu N_0 \exp \left[ \mu_a (t_a - l) \right]$$ \hspace{1cm} (10)

From Eqs. (7) and (9),

$$N_o \exp \left[ \mu_o (t_a - l) \right] = n_o N_0 \exp \left[ \mu_b (t_a - l) \right]$$

using Eq. (6) and the relationship of $t_b - t_c = T_{s_a}$

$$\log N_o - \log n_o N_0 = - \log n_b = \mu_c T_{s_a} / 2.303$$  \hspace{1cm} (11)

From Eqs. (7) and (10),

$$N_o \exp \left[ \mu_o (t_a - l) \right] = \nu N_0 \exp \left[ \mu_a (t_a - l) \right]$$

using Eq. (6) and the relationship of $t_a - t_q = T_{s_a} + T_{t_q}$

$$\log N_o - \log \nu N_0 = - \log \nu$$

$$= \mu_c (T_{s_a} + T_{t_q}) / 2.303$$  \hspace{1cm} (12)

As in the previous paper (Takano and Tsuchido, 1982), when $G_{10} (= 2.303/\mu)$ value is introduced as the net increment of time delay for cell population to grow up to an OD of $OD_s$ corresponding to $N_s$ when the inoculum size for cultivation is reduced by one tenth, Eqs. (11) and (12) are converted to the following Eqs. (13) and (14). The left side of Eq. (14) is "the integrated viability" (Takano and Tsuchido, 1982).

$$- \log n_o = \frac{T_{s_a}}{G_{10}}$$  \hspace{1cm} (13)

$$- \log \nu = \frac{(T_{s_a} + T_{t_q})}{G_{10}}$$  \hspace{1cm} (14)

**Evaluation models for the $\lambda$ and $\mu$ injuries**

For the growth-independent, early appearing injury process, therefore, as depicted in Fig. 2, when the injured and dead subpopulation sizes are expressed as $N_{(\lambda)}$ and $N_{(\mu)}$, respectively, as expressed by differences in log value in a double subculture method,

$$N_{(\lambda)} = N_0 - n N_0 = N_0 (1 - n_\lambda)$$ \hspace{1cm} (15)

$$N_{(\mu)} = n N_0 - \nu N_0 = N_0 (n_\nu - \nu)$$ \hspace{1cm} (16)

When expressed as differential log values,

$$\Delta \log N_{(\lambda)} = \Delta (\log N_0 - \log n N_0)$$ \hspace{1cm} (17)

$$\Delta \log N_{(\mu)} = \Delta (\log n N_0 - \log \nu N_0)$$ \hspace{1cm} (18)

For practical evaluation of the $\lambda$ injury, the delay time can be estimated with OD in a range of early log phase instead of $N$, and also $N_o$ and $n N_0$ are assumed to be done as CFU with the plate count method.

On the other hand, in the analysis of the growth-dependent, late appearing injury of the stressed population, we assume that the degree of the reduction from $\mu$ to $\mu'$ reflects the degree of the $\mu$ injury. For its evaluation, we define the time point estimated with the OD monitoring from the intersection of the above two growth curves by interpolation (Fig. 2B). If the OD at this point is referred to as $OD_m$, the population size corresponding to this OD is $N_m$, which is equal to $n N_0$. After this point, the $\mu$ injury can be evaluated with the $\mu$ ratio between curves b and d for the stressed population, $\mu'$ and $\mu_b (= \mu_c)$, respectively. Here, when a period of the growth delay due to the reduced $\mu$ is $T_{s_q}$. $T_{s_q} = t' - t_a$

Therefore, by converting the time on the abscissa to the log level of population on the ordinate, using a proportional relationship,

$$\frac{(t_a - t_{s_q})}{(t' - t_a)} = \frac{\log N_q - \log N_{s_q}}{\log N_q - \log N_{s_q}}$$ \hspace{1cm} (19)

where $N_q (\text{ml}^{-1})$ is the population size for the stressed population at time, $t_{s_q} (h)$.

This time ratio in the left side in Eq. (19) corresponds to the ratio of specific growth rates of injured to uninjured populations, $\mu'/\mu_c$. When the ratio of the right side is replaced with $n_\lambda$ and also the size of the stressed population having the $\mu$ injury is taken as $N_{(\mu)} (\text{ml}^{-1})$, the size of uninjured population among total survivors can be expressed as follows.

$$N_{(\mu)} = n N_0 - n N_0 = N_0 (1 - n_\lambda)$$ \hspace{1cm} (20)

$$\Delta \log N_{(\mu)} = \Delta (\log n N_0 - \log n_\lambda N_0)$$ \hspace{1cm} (21)

**Assumptions for application of the model**

It should be noted that at least a part of the population having the $\lambda$ injury may be overlapped with that having the $\mu$ injury or vice versa. It also indicated that the theory proposed here is based on the following additional assumptions. 1) Although the $\mu$ value is supposed to distribute differently with the degree of injury in the stressed subpopulation, it is regarded here as the overall growth rate of the whole population. 2) The viable
Population size is reflected by OD at least at and near Na, which corresponds to Od. 3)
Once a conidium starts to germinate, even though sublethally injured before cultivation, it continues to grow up to a population level at stationary phase in liquid medium or to form a colony on an agar medium, without death whichever late recovery occurs or not (see Fig. 1).
The λ and μ injuries are mutually independent. It is possible therefore that one spore has both injuries. 5)
In the model presented here, based on the above third assumption, the number of spores having the μ injury as well as the λ injury is included in the viable counts enumerated with the plate count method, as demonstrated in Eqs. (20) and (21).

RESULTS AND DISCUSSION

The growth delay of a microbial population exposed to a sublethal or lethal stress seemed to appear chronologically in two different patterns. One is due to a growth arrest necessary for recovery from the growth-independent λ injury before regrowth and the other is due to a reduced growth rate in the regrowth as the growth-dependent μ injury (Fig. 1). In case of bacteria, stressed cells or spores start vegetative growth (after germination and the subsequent outgrowth for spores) at almost the same rate as the unstressed ones after a prolonged lag period. In fungi, however, such a stressed cell or spore population may often grow at a reduced rate, compared to unstressed population, according to our preliminary observations.

We applied the kinetic analysis method proposed with the theory described above to C. cladosporioides conidia heat-treated at 45°C for different periods to evaluate the two mode injuries presented. To get total survivors before and after heat treatment, N0 and niN0, in the whole population, the conventional plate count method with PDA was used in this study. And the populations of the λ and μ injuries, Nλ(ni)I and Nμ(ni)I, respectively, were estimated with the differences in viabilities obtained from between the growth kinetic analysis and the plate count method.

The growth patterns of unheated and heated conidial populations were demonstrated in Fig. 3A. The conidia seemed to germinate and then subsequently appearing hyphae grew logarithmically in early to middle stage of cultivation, although decelerated growth can be seen in middle to late stage. Therefore, during the early growth phase objective of the kinetic analysis in this study, the conidial growth was confirmed to follow apparently first order kinetics assumed above. This principle in fungi has already been reported by other researchers (Pirt, 1967; Trinci, 1969) and thus the growth kinetics for elongation and branching of fungal hyphae developed from conidia at least during its early growth phase is apparently similar to that for bacteria, as expressed with Eq. (2).

Based on this confirmation, we applied the GDA method to evaluate the injured population of heat-stressed conidia in the following study. Before the evaluation of the λ injury using these curves, the growth curves of serially 10-fold diluted samples of unheated and heated populations were obtained to confirm a linear relationship between the inoculum size and the
growth delay time, as previously reported (Takano and Tsuchido, 1982) (Fig. 3B). From the slopes of the lines obtained, $G_{10}$ values were calculated to be 12.1, 15.6, and 16.7 h for conidia heated for 2, 5, and 8 min, respectively, whereas 12.6 h for unheated. After heat treatment at 45°C for 5 and 8 min, significant differences in $G_{10}$ values were detected with those treated conidia referred to untreated. Different factors relating to the nature of injury and repair may be possibly involved in such an increase in $G_{10}$ value (namely a decrease in $\mu$ value) in stressed population. We discuss this below in close relation to the $\mu$ injury. From this result, in order to estimate the overall injury of the stressed population, we determined to adopt the DiVSaL method with the variable $\mu$ model rather than the constant $\mu$ model (Tsuchido, 2017).

By kinetic analyses of the CFU data as well as the above growth curves, we estimated the total survivors ($n_0N_0$) and evaluated both $\lambda$ ($N_{(r)}$) and $\mu$ injuries ($N_{(g)}$) (Figs. 4A and 4B). These results indicate that heat treatment of C. cladosporioides conidia causes much $\lambda$ injury but less $\mu$ injury, which was found significantly only at 8 min of heating at 45°C (Fig. 4B). On the other hand, the overall injury evaluated with Eq. (1)
demonstrated a pattern more close to that of the $\lambda$ injury but with relatively large variations in the estimated value as indicated with the S.D. bars in the figure (Figs. 4A and 4B).

These two mode injuries are presumed to be generated by stress action on different intracellular sites. Early occurring growth-independent $\lambda$ injury, is probably due to damages to basal functions essential for cell or spore repair and survival, such as energy production, cell membrane biogenesis, and transcription and translation systems. On the other hand, late occurring growth-dependent $\mu$ injury, may result from dysfunction of the basal growth system critical for the germ tube formation and the subsequent hyphal growth, such as cell wall synthesis and DNA replication. Furthermore, the carryover of metabolic disturbance or imbalance still remaining even after recovery from the $\lambda$ injury, such as the accumulation of denatured or aggregated proteins and their relating stress proteins, shortage of essential nutrients and undesirable byproduct generation, might also be additional factors. These injuries may also be responsible for a reduction in $\mu$ value (an increase in $G_{10}$ value) in growing hyphae described above.

The modified DiVSaL method proposed here may have an advantage in more elaborate kinetic analysis of cellular injury in fungi, because the introduction of the two-mode injury concept, the $\lambda$ and $\mu$ injuries, improved the fidelity of measured data, as demonstrated with smaller S.D. values in comparison with the overall injury analysis evaluated by the method proposed previously (Tsuchido, 2017).

However, this method has substantial or possible disadvantages as well. In the construction of the improved kinetic model, being different from the previous works (Takano and Tsuchido, 1982; Tsuchido, 2017), we simplified the kinetic growth model without taking care of distributions of $\lambda$ and $\mu$ values among the conidial population and regarded as the total injury for the whole conidial population. Therefore, the introduction of statistical analysis of such an injury distribution may be necessary for further improvement. In the enumeration, in addition, only germinatable conidia may be counted for the injury evaluation. Although, as one of the DS method, the DiVSaL method consisting of the plate count method and the GDA method is applicable to fungal spores, in case of fungal hypha, the former method cannot be applied. In such case, however, the plate count method can be replaced with the most probable number (MPN) method using a liquid medium instead, that is also available for bacteria, as the DiVLaL (differentiation viabilities between liquid and liquid media) method, using liquid system in both. Further, if the samples are turbid because of the presence of insoluble materials, the growth of fungal population should be measured with an alternative technique other than the OD monitoring. Even though technically available, it should be noted that such an alternative method cannot be applied if the prerequisite assumptions presented above are not satisfied.

In conclusion, even though the proposed method has some difficulties and restrictions in the application at present, it is a kind of macroscopic method for injury evaluation with growth kinetic analysis, which has a merit of application concept close to shelf life evaluation of foods. With such an advantage, it is expected to be applied to design fungal control strategy on the basis of cell injury mode and mechanism analyses.

REFERENCES

Abdulh, N. A. H., Nayan, N. A., Kamaludin, N. H. I., Idris, Z. M. and Tompang, M.F. (2016) Cell growth kinetics of Aspergillus oryzae in industrial natural rubber effluent serum. ARPN J. Eng. Appl. Sci., 11, 2687-2692.

Balmant, W., Sugai-Guerios, M. H., Coradin, J. H., Krieger, N., Furigo Jr., A. and Mitchell, D. A. (2015) A model for growth of a single fungal hypha based on well-mixed tanks in series: Simulation of nutrient and vesicle transport in aerial reproductive hyphae. PLOS One, 10 (3), e0120307. Doi:10.1371/journal.pone.0120307, pp. 22.

Bartnicki-Garcia, S., Hergert, F. and Gierz, G. (1989) Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. Protoplasma, 153, 46-57.

Beuchat, L. R. (1984) Injury and repair of yeasts and moulds. In The Revival of Injured Microbes (Andrew, M. H. E. and Russell, A. D., ed.), pp.293-308. Academic Press, London (1984).

Beuchat L. R. and Pitt J. I. (1990) Influence of solute, pH, and incubation temperature on recovery of heat-stressed Wallemia sebi conidia. Appl. Environ. Microbiol. 56, 2545-2550.

Beuchat L. R. and Pitt J. I. (1990) Influence of water activity and temperature on survival of and colony formation by heat-stressed Chrysosporium fanincola aleuriospores. Appl. Environ. Microbiol. 56, 2951-2956.

Boswell, G. P., Jacobs, H., Davidson, F. A., Gadd, G. M. and Ritz, K. (2003) A positive numerical scheme for a mixed-type partial differential equation model for fungal growth. Appl. Mathemat. Comput., 138, 321-340.

Broekaert, W. F., Terras, F., R., G., Cammue, B. P. A. and Vanderleyden, J. (1990) An automated quantitative assay for fungal growth inhibition. FEBS Microbiol. Lett., 69, 55-80.

Chawla, C. S., Chen, H. and Donnelly, C.W. (1996) Mathematically modeling the repair of heat-injured Listeria monocytogenes as affected by temperature, pH, and salt concentration. Intl. J. Food Microbiol., 30, 231-242.

Dagnas, S., Gougouli, M., Ohno, B., Koutsoomanis, K. P. and Membre, J. -M. (2017) Quantifying the effect of water activity and storage temperature on single spore lag times of three moulds isolated from spoiled bakery products. Intl. J. Food Microbiol., 240, 75-84.

Edelstein, L., Hadar, Y., Chet, I., Henis, Y. and Segel, L. A. (1983) A model for fungal colony growth applied to Sclerotium rolfsii. J. Gen. Microbiol., 129, 1873-1881.
Farina, J. I., Tonetti, G. R., Perotti, N. I. (1997) A mathematical
model applied to the fungal colony growth of Sclerotium
rolfsii. Biotechnol. Techniques, 11, 217-219.
Fujikawa, H. and Itoh, T. (1996) Thermal inactivation curve
of Aspergillus niger spores. Appl. Environ. Microbiol., 62,
3745-3749.
Hills, B. P. and Mackey, B. M. (1995) Multi-compartment
kinetic models for injury, resuscitation, induced lag and
growth in bacterial cell populations. Food Microbiol., 12,
333-346.
Koch, A. L. (1975) The kinetics of mycelial growth. J. Gen.
Microbiol., 89, 209-216.
Kotov, V., Anishchenko, I., Sirenko, I. and Reshetnikov, S.
(2005) Statistical analysis of structural and kinetic char-
acteristics of fungal colony growth with Trichoderma viride
Pers.: S. F. Gray. Microbiol. Res., 160, 273-278.
Mitchell, D. A., von Meien, O. F., Krieger, N. and Dalanter, R.
D. H. (2004) A review of recent developments in modeling
of microbial growth kinetics and intraparticle phenomena
in solid-state fermentation. Biochem. Eng. J., 17, 15-26.
Pirt, S. J. (1967) A kinetic study of the mode of growth of
surface colonies of bacteria and fungi. J. Gen. Microbiol.,
47, 181-197.
Prosser, J. I. and Trinci, A. P. J. (1979) A model for hyphal
growth and branching. J. Gen. Microbiol., 111, 153-164.
Sibanda, T. and Buys, E. M. (2017) Resuscitation and growth
kinetics of sub-lethally injured Listeria monocytogenes
strains following fluorescence activated cell sorting (FACS).
Food Res. Int., 100, 150-158.
Silva-Angulo, A. B., Zanini, S. F., Rodrigo, D., Rosenthal, A.
and Martinez, A. (2014) Growth kinetics of Listeria innocua
and Listeria monocytogenes under exposure to carvacrol
and the occurrence of sublethal damage. Food Control.,
37, 336-342.
Stephens, P. J., Joynton, J. A., Davies, K. W., Holbrook, R.,
Lappin-Scott, H. M., and Humphrey, T. J. (1997) The use
of an automated growth analyser to measure recovery times
of single heat-injured Salmonella cells. J. Appl. Microbiol.,
83, 445-455.
Stevenson K. E. and Graumlich T. R. (1978) Injury and recov-
er of yeast and molds. Adv. Appl. Microbiol., 23, 203-217.
Takano, M. and Tsuchido, T. (1989) Availability of growth
delay analysis for the evaluation of total injury in stressed
bacterial population. J. Ferment. Technol., 60, 189-198.
Trinci, A. P. J. (1969) A kinetic study of the growth of
Aspergillus nidulans and other fungi. J. Gen. Microbiol.,
57, 11-24.
Tsuchido, T., Koike, T. and Takano, M. (1989) A modified
assessment of growth inhibition from growth-delay time in
a cell population exposed to an environmental stress. J.
Ferment. Bioeng., 67, 132-134.
Tsuchido, T. (2017) A novel double subculture method and its
theory for the enumeration of injured cells in stressed micro-
bial population. Biocontrol Sci., 22, 131-135.
Tsuchido, T. and Sakamoto, J. J. (2018) Characteristics,
occurrence, detection and enumeration of injured microor-
ganisms. Nippon Shokuhin Kagaku Kogaku Kaishi (Jpn.
J. Food Sci. Technol.), 65, 73-79 (in Japanese).