Low Temp. Sci. Ser. B 36: 57-59, 1978

Short Note

An Explanation for the Cell Concentration Dependent Survival of *E. coli* after Freeze-Thawing[1]

Kouzou TANNO
丹 野 皓 三

Abstract Higher concentration of *E. coli* cells before freezing gives them higher survival after frozen at −30°C with rate of cooling 70°C/min. It was found out that the result of the experiment fitted the following equation: $R=78.97-12.4\times10^{10}/x$, R: Percentage of recovery x: Number of *E. coli* cells in 1 ml distilled water before freezing. The second term of the right side of the equation means that the constant number (1.247×10^{9}) of cells is always killed by freezing in spite of the initial cell concentration. The explanation of this phenomenon was attempted.

It was reported that higher concentration of *E. coli* cells suspended in distilled water before freezing gave them higher survival after frozen at −30°C at the rate of cooling 70°C/min (1). I found out that the experimental data of the reported fitted following, simple and very interesting equation (Fig. 1).

$$R=78.97-12.47\times10^{10}/x$$

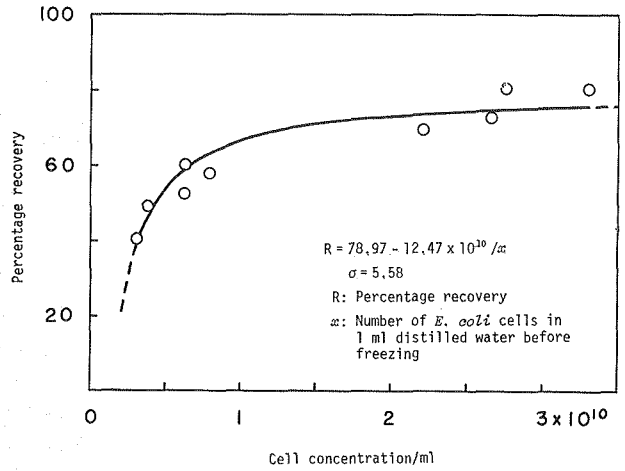

Fig. 1. Effect of cell concentration on survival after freezing at −30°C with rate of cooling 70°C/min.

[1] Received for publication October 20, 1978. Contribution No. 1990 from the Inst. Low Temp. Sci.

[2] The Institute of Low Temperature Science, Hokkaido University, Sapporo, 060

R : Percentage of recovery
x : Number of *E. coli* cells in 1 ml distilled water before freezing

These constant of the equation was obtained by the methods of least squares. The value of standard deviation is very small ($\sigma=5.58$). The second term of the right side of the equation means that the constant number (1.247×10^9) of cells is always killed by freezing in spite of the initial cell concentration.

On the other hand, when cells suspended in distilled water were frozen at $-30°C$ at the rate of cooling 4°C/minutes, the survival after thawing was constant (87.22%) independently of the suspended cell concentration (Fig. 2). The correlation coefficient was almost zero ($\gamma=0.0182$).

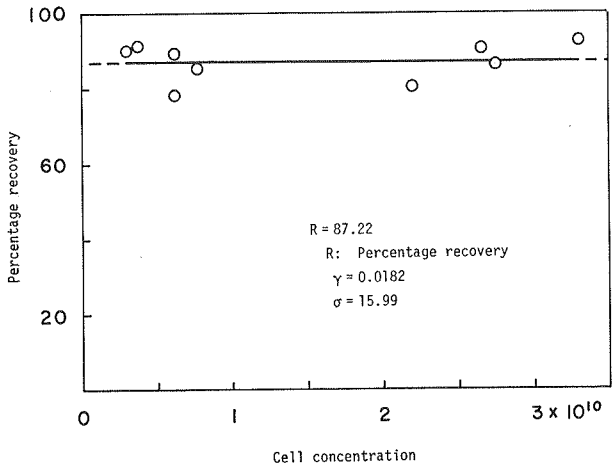

Fig. 2. Effect of cell concentration on survival after freezing at $-30°C$ with rate of cooling 4°C/min.

Why does the constant number of cells be always killed by rapid freezing? This may be explained as following. Almost all the cells in suspension may be concentrated among ice crystals in the process of freezing, but a small amount of the cells may be scattered in ice crystals. The more rapid cooling rate may give the larger probability of cells being scattered in ice crystals. The probability may be zero at very slow freezing condition. The velocity of cells being scattered in ice crystals may be in proportion to the cell concentration on the surface of growing ice crystals. As the cell concentration on the surface of growing ice crystals may be saturated soon after the initiation of freezing, the probability of cells being scattered finally in ice crystals may be constant in spite of the initial cell concentration within the limits of the reasonable cell concentration. Thus, the number of the scattered cells (L) in ice crystals may be almost constant if the other freezing conditions are the same. As the velocity of the dehydration from the scattered cells may be larger than that from the cells concentrated among ice

crystals in the process of freezing, freezing injury coused by rapid dehydration may be larger in the latter. Thus the percentage of recovery (R) is expressed by the following equation.

$$R = K_1(x-L)+K_2L/x = K_1-L(K_1-K_2)/x$$

R : Percentage of recovery
x : Number of cells suspended in 1 ml distilled water
L : The number of the scattered cells in ice crystals
K_1 : Percentage of survival in the cells concentrated among ice crystals
K_2 : Percentage of survival in the cells scattered in ice crystals

K_1 and $L(K_1-K_2)$ are constant. Therefore, the meaning of this equation is the same as the first equation. The value of L may be almost zero at slow freezing condition shown in figure 2.

The cell concentration is not main couse of the freezing injury in cells but may be one of important factors.

Literature Cited

1) Sato, T. 1954 On the mechanism of the death of bacteria by low temperature treatment. *Low Temp. Sci.*, B, **12**: 39-61.