CASE 8

A Detection Method for Thermoresistant Bacteria

Abstract: In cultivating bacteria, time is regarded as a signal factor. Since a certain number of bacteria exist before starting, we used a reference-point proportional equation with the ninth hour from the start as a reference time. On the other hand, because the real number of bacteria was unknown, the numbers diluted to 1/5 and 1/25 were chosen as signal factors to be used for measurement. In other words, using dilution ratio as the signal, we calculated an SN ratio whose true values were unknown.

1. Introduction

Thermoresistant bacteria form heat-resisting spores in the body that survive in the food after cooking and rot heat-treated food. In order to detect a specific bacterium causing food poisoning, we developed a method of detecting poison produced by a bacterium or technique using an antigen–antibody reaction. However, since a heat-resistant bacterium is a common decomposing bacterium, a method of detecting cultivated bacteria is widely used. In this case, a key issue was to accelerate bacteria as quickly as possible and to detect them accurately. If the speed of occurrence of bacteria is slow, accuracy in detection is reduced. In contrast, poor accuracy of detection leads to a larger error in confirmation of occurrence. This relationship between a measuring method and a measured result cannot be separated in technological development, and each should be analyzed individually. Whereas cultivation of bacteria is considered a growth-related phenomenon, detection is a measurement- or SN ratio–related issue. In our study we dealt with both in a single experiment.

In cultivating bacteria, time is regarded as a signal factor (Figure 1). Since a certain number of bacteria exist before starting, we used a reference-point proportional equation with the ninth hour from the start as a reference time. On the other hand, because the real number of bacteria was unknown, the numbers diluted to 1/5 and 1/25 were chosen as signal factors to be used for measurement. In other words, using dilution ratio as the signal, we calculate an SN ratio whose true values were unknown (Figure 2). In addition, on the assumption that the pH of the food had already been adjusted to control microbes, it was considered as the noise factor:

N_1: diluted solution, pH 7

N_2: diluted solution, pH 3

Following our objective of study, we proceeded with the following data analysis:

1. To perform parameter design for bacteria cultivation, we calculated the SN ratio of the reference-point proportional equation using time as a signal factor (analysis 1).
2. By computing the SN ratio for bacteria detection at each point of time, we used bacteria dilution ratio as a signal factor (analysis 2).

Using the results obtained from both, we chose the optimal configuration for cultivation and detection methods for bacteria.

2. SN Ratio for Cultivation Conditions for Bacteria (Analysis 1)

As a data example, the data in the first row of an L_{18} orthogonal array are shown in Table 1. To

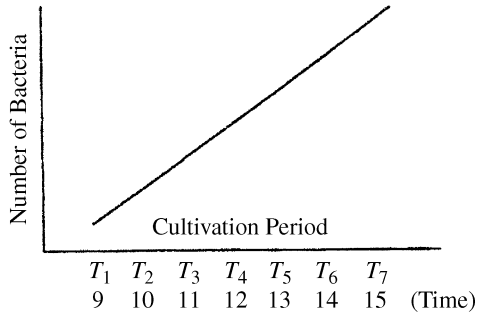

Figure 1
Cultivation of bacteria using time as the signal

compute the SN ratio using a reference-point proportional equation whose reference point is set to T_1, we created an auxiliary table (Table 2).

Total variation:

$$S_T = 79.5^2 + 147.5^2 + \cdots + 7.5^2 + 8.5^2 = 603{,}265 \quad (1)$$

Effective divider:

$$r = 0^2 + 1^2 + \cdots + 6^2 = 91 \quad (2)$$

Linear equation:

$$L_1 = (0)(79.5) + \cdots + (6)(346.5) = 6178.5$$

$$L_2 = 2965.5 \qquad L_3 = 2018.0$$

$$L_4 = 167.0 \qquad L_5 = 590.5 \qquad L_6 = 133.5 \quad (3)$$

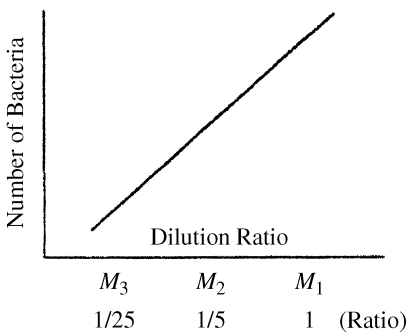

Figure 2
Measurement of bacteria using dilution of bacteria as the signal

Table 1
Measured data of number of bacteria in experiment 1

		M_1	M_2	M_3
T_1	N_1	496	119	11
	N_2	337	35	8
T_2	N_1	564	137	23
	N_2	425	50	12
T_3	N_1	612	153	27
	N_2	480	63	13
T_4	N_1	663	164	34
	N_2	525	76	14
T_5	N_1	710	171	40
	N_2	558	86	15
T_6	N_1	746	180	41
	N_2	578	92	17
T_7	N_1	763	186	41
	N_2	605	96	18

Table 2
Calibrated data for reference-point proportional equation

		M_1	M_2	M_3
0	N_1	79.5	42.0	1.5
	N_2	−79.5	−42.0	−1.5
1	N_1	147.5	60.0	13.5
	N_2	8.5	−27.0	2.5
2	N_1	195.5	76.0	17.5
	N_2	63.5	−14.0	3.5
3	N_1	246.5	87.0	24.5
	N_2	108.5	−1.0	4.5
4	N_1	293.5	94.0	30.5
	N_2	141.5	9.0	5.5
5	N_1	329.5	103.0	31.5
	N_2	161.5	15.0	7.5
6	N_1	346.5	109.0	31.5
	N_2	188.5	19.0	8.5
Linear equation	N_1	L_1	L_3	L_5
	N_2	L_2	L_4	L_6

Variation of proportional term:

$$S_\beta = \frac{(L_1 + L_2 + L_3 + L_4 + L_5 + L_6)^2}{6r} = 266{,}071.08 \quad (4)$$

Variation of difference between proportional terms by dilution:

$$S_{M\beta} = \frac{(L_1 + L_2)^2 + (L_3 + L_4)^2 + (L_5 + L_6)^2}{2r} - S_\beta = 222{,}451.65 \quad (5)$$

Variation of differences between proportional terms due to noise:

$$S_{N\beta} = \frac{(L_1 + L_3 + L_5)^2 + (L_2 + L_4 + L_6)^2}{3r} - S_\beta = 55{,}826.82 \quad (6)$$

Error variation:

$$S_e = S_T - S_\beta - S_{M\beta} - S_{N\beta} = 58{,}915.45 \quad (7)$$

Error variance:

$$V_e = \frac{S_e}{38} = 1550.41 \quad (8)$$

Total variance:

$$V_N = \frac{S_{N\beta} + S_e}{39} \times 2942.11 \quad (9)$$

SN ratio:

$$\eta = 10 \log \frac{(S_\beta - V_e)/2r}{V_N} = -3.06 \quad (10)$$

Sensitivity:

$$S = 10 \log \frac{S_\beta - V_e}{2r} = 31.62 \quad (11)$$

3. SN Ratio for Measurement of Number of Bacteria (Analysis 2)

Using the data of time T_1 in Table 1, we calculated the SN ratio for using the bacteria dilution ratio. Table 3 summarizes each datum divided by 416.5, the average number of bacteria at M_1.

Table 3
Calibrated data

		1	0.2	0.04
T_1	N_1	1.191	0.286	0.026
	N_2	0.809	0.084	0.019

Total variation:

$$S_T = 1.191^2 + 0.286^2 + \cdots + 0.019^2 = 2.163 \quad (12)$$

Effective divider:

$$r = 1^2 + 0.2^2 + 0.04^2 = 1.042 \quad (13)$$

Linear equations:

$$L_1 = (1)(1.191) + \cdots + (0.04)(0.026) = 1.249$$

$$L_2 = 0.827 \quad (14)$$

Variation of proportional term:

$$S_\beta = \frac{(L_1 + L_2)^2}{2r} = 2.068 \quad (15)$$

Variation of difference between proportional terms:

$$S_{N\beta} = \frac{L_1^2 + L_2^2}{r} - S_\beta = 0.086 \quad (16)$$

Error variation:

$$S_e = S_T - S_\beta - S_{N\beta} = 0.009 \quad (17)$$

Error variance:

$$V_e = \frac{S_e}{4} = 0.002 \quad (18)$$

Total variance:

$$V_N = \frac{S_{N\beta} + S_e}{5} = 0.019 \quad (19)$$

SN ratio:

$$\eta = 10 \log \frac{(S_\beta - V_e)/2r}{V_N} = 17.216 \quad (20)$$

Sensitivity:

$$S = 10 \log \frac{S_\beta - V_e}{2r} = -0.04 \quad (21)$$

We also computed each value for T_2 to T_7 and then proceeded with the same calculation for experiments 2 to 18.

4. Optimization of Cultivation and Detection Methods and Confirmatory Experiment

Table 4 shows the control factors for the design of experiments on cultivation and detection of bacteria. Type of bacterium, *A*, was allocated as an indicative factor. For control factors, we chose type of dilution solution, *B*; type of medium, *D*; and amounts of elements *E* to *H*, which are assumed to facilitate growth of bacteria (added to medium). On the other hand, bench time *C*, measured in terms of the number of days, with bacteria maintained at 10°C after being heated up.

As below, we showed the response graphs for measurement of the number of bacteria (analysis 2). Figure 3 shows the response graphs for factor *A* only. Since analysis is conducted for each time level, there will be response graphs of T_1 to T_7 for other factors.

We obtained the following identical optimal configurations for analyses 1 and 2:

$$\textit{Analysis 1:}\quad A_1B_3C_1D_3E_2F_1H_2$$

$$\textit{Analysis 2:}\quad A_1B_3C_1D_3E_2F_1H_2$$

Under both optimal and current configurations, we performed a confirmatory experiment. The results are shown in Table 5.

5. Results of Experiment

According to the results of the confirmatory experiment shown in Table 5, we discovered that satisfactory reproducibility in the gain of the SN ratio cannot be obtained. However, since the trend toward increasing SN ratio with respect to time in the results of analysis 2 was similar to that of the estimation, we concluded that our experimental results were fairly reliable. On the other hand, a number of peaks and V-shapes in the response graphs for the control factors were regarded as problems to be solved in a future study.

Despite several remaining problems, the following improvements were achieved:

1. We reduced the experimentation time by two days because the bench time allocated as one of the control factors was not necessary. More-

Table 4
Control and indicative factors and levels

Factor	Level 1	Level 2	Level 3
A: type of bacterium (*B. subtilis*)	*A*	*B*	—
B: dilution solution	Sterile water	Phosphate buffer solution	Peptone–phosphate buffer solution
C: bench time (days)	0	1	2
D: type of medium	Standard	Glucose tryptone	Trypticase soy
E: amount of catalase (μg/mL)	0	50	100
F: amount of lysozyme (μg/mL)	0	0.01	0.1
G: amount of sodium pyruvate (%)	0.00	0.10	0.20
H: amount of alanine (μg/mL)	0	20	40

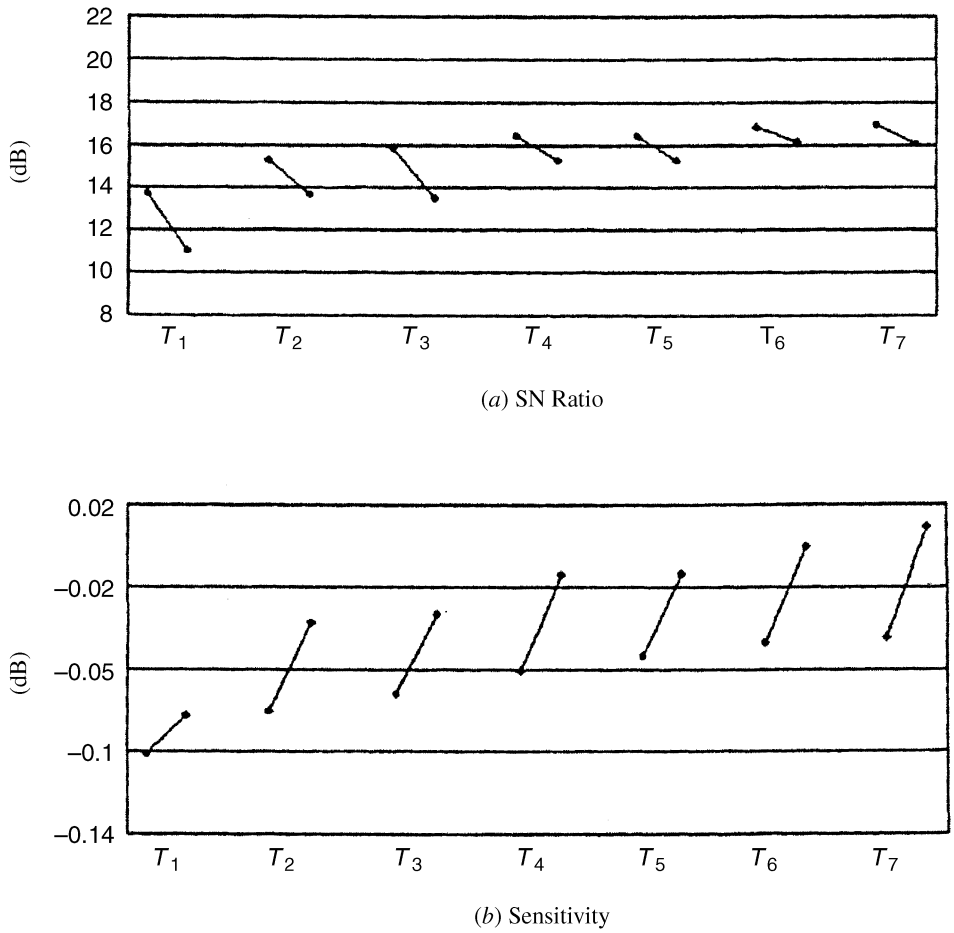

Figure 3
Analysis 2: Response graphs for factor *A*

over, whereas five days are used as a cultivation period in our current process, we found such a long-time cultivation not meaningful for experimentation. Therefore, we finished an inspection that normally takes one week in only two days.

2. The improvement above contributed not only to reducing the cost but also to taking countermeasures more swiftly. If we can detect the occurrence of bacteria sooner, we can more retrieve defective products immediately, and thereby alleviate the risk of inflicting damage on customers.
3. For detectability of bacteria, we obtained improvement of 15 dB in the SN ratio (1/32 of the current variability) regarding a cultivation condition of bacteria when time was selected as a signal factor, whereas the bacteria dilution ratio was approximately 21 dB (1/122 of the current variability) after 15 hours.

The resulting economic benefits were computed as follows:

Table 5
Confirmatory experiment (dB)

Analysis 1			Configuration		
			Optimal	**Current**	**Gain**
SN ratio		Estimation	12.85	−14.01	26.87
		Confirmation	−2.53	−17.58	15.05
Sensitivity		Estimation	31.02	28.62	2.40
		Confirmation	30.84	23.30	7.54
Analysis 2			Configuration		
			Optimal	**Current**	**Gain**
Sn Ratio	T_1	Estimation	29.67	5.33	24.34
		Confirmation	20.01	7.61	12.40
	T_2	Estimation	37.77	5.08	32.69
		Confirmation	22.20	7.88	14.32
	T_3	Estimation	35.47	6.54	29.93
		Confirmation	23.84	7.87	16.44
	T_5	Estimation	40.66	6.33	34.33
		Confirmation	25.65	7.68	17.97
	T_6	Estimation	43.20	6.32	36.88
		Confirmation	26.75	7.69	19.05
	T_7	Estimation	42.77	6.40	36.37
		Confirmation	28.55	7.70	20.85
Sensitivity	T_1	Estimation	−0.19	−0.14	−0.05
		Confirmation	−0.07	−0.10	0.02
	T_2	Estimation	−0.13	−0.10	−0.03
		Confirmation	−0.04	−0.08	0.04
	T_3	Estimation	−0.12	−0.08	−0.04
		Confirmation	−0.03	−0.06	0.03
	T_4	Estimation	−0.10	−0.04	−0.05
		Confirmation	−0.03	−0.05	0.02
	T_5	Estimation	−0.08	−0.02	−0.06
		Confirmation	−0.02	−0.04	0.02
	T_6	Estimation	−0.07	−0.0	−0.07
		Confirmation	−0.02	−0.02	0.01
	T_7	Estimation	−0.06	−0.01	−0.05
		Confirmation	−0.01	−0.02	0.01

Streamlined Inspection due to Time Reduction

If we assume that

$$\begin{aligned}\text{inspection cost} = {} & (\text{sample cost per inspection}) \\ & + (\text{labor cost}) \\ & + (\text{machine running cost})\end{aligned}$$

where the daily labor cost is 10,000 yen and the machine running cost is 155 yen, we obtain the following costs:

Current configuration:

$$235 + (10{,}000)(5) + (155)(5) = 51{,}010$$

Optimal configuration:

$$221 + (10{,}000)(2) + (155)(2) = 20{,}531$$

The cost benefit per inspection is 30,479 yen.

Risk Aversion due to Time Reduction

Now suppose that it takes two days from the point when the production of a product is completed to the point when it starts to be sold at a shop. If the optimally produced product contains a harmful bacterium, despite being in process of shipment, we can withdraw it shortly after checking the result of inspection. Then we assume that the loss in withdrawal costs 1 million yen. On the other hand, under the current configuration, the product reaches a consumer. In this case, we suppose that the loss to a consumer amounts to 150 million yen per person for the death, or 100,000 yen for hospitalization (as the number of victims is derived from a certain past record).

$$\textit{Current:}\quad \frac{(180 \text{ persons} \times 100{,}000 \text{ yen}) + (3 \text{ persons} \times 150 \text{ million yen})}{183}$$

$$= 2{,}557{,}377 \text{ yen/product}$$

$$\textit{Optimal:}\quad \frac{1 \text{ million yen}}{183}$$

$$= 5{,}264 \text{ yen/product}$$

Thanks to time reduction, we can slash the social loss by $2{,}557{,}377 - 5264 = 2{,}552{,}113$ yen/product.

Loss Function of Detectability of Bacteria

Now we assume that if 100,000 harmful bacteria exist in a certain product, a person who eats it will die, and the resulting loss totals 150 million yen (for loss of life). On the other hand, the loss due to discard of a product is 300 yen.

$$A_0 = 150 \text{ million yen}$$

$$A = 300 \text{ yen}$$

$$\Delta_0 = 100{,}000 \text{ products}$$

The tolerance is

$$\Delta = \sqrt{\frac{A}{A_0}}\,\Delta_0 = \sqrt{\frac{300}{150{,}000{,}000}}\,(100{,}000) = 141.42$$

Supposing that the current average number of bacteria is 100, we have the following loss function:

$$L = \frac{300}{(141.42)^2}\,(100^2) = 150 \text{ yen}$$

Converting the gain of 20.85 dB into an antilog number, we obtain 121.62. Then, the improvement achieved is $(150)(1/121.62) = 1.23$. Therefore, the current loss is 150 yen, whereas the optimal is 1 yen. As a result, we can reduce the loss by $150 - 1 = 149$ yen.

This study took only six days to complete. In our conventional process, we have had an enormous amount of time loss through reiterated trials and errors, and moreover, always doubted whether we could arrive at a conclusion. If research work can be completed within a short period by taking advantage of quality engineering, we believe that many achievements can be obtained for other inspection methods.

Reference

Eiko Mikami and Hiroshi Yano, 2001. Studies on the method for detection of thermoresistant bacteria. *Proceedings of the 9th Quality Engineering Symposium*, pp. 126–129.

This case study is contributed by Eiko Mikami and Hiroshi Yano.