Short Report

Effects of the Agar Concentration on Colony Formation of a Soil Bacterium

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The plating technique is a useful tool in ecological studies. Recently one of the authors proposed a model simulating the appearance of colonies on a plate in relation to incubation time (Hattori, 1985; 1988). The model involves three parameters; N_{∞} which is the number of colonies expected to be eventually formed, λ which is the rate of increase in the number of colonies and tr which is the time period before the appearance of the first colony.

Colony formation may be affected by the agar concentration used for the solidification of the plating medium and also by the procedure in which cells were inoculated to the medium (pour-mixing or surface-smearing of cells). In the present report we studied the effects of these factors on the values of the three parameters of the FOR model using three kinds of agar and a bacterium isolated from soil.

The test organism was a Gram-negative rod-shaped bacterium isolated from grassland soil at Kawatabi in Miyagi Prefecture. The medium was a 100-fold dilution of the nutrient broth which contained 1% meat extract, 1% peptone and 0.5% NaCl. The medium was solidified by Wako Agar (the 1st grade, Wako Chemical Industry), Agar Noble (Difco) or Purified Agar (Oxoid). The parameter values were estimated as described previously (Ishikuri et al, 1984).

As described in Table 1 the agar concentration

affected on the parameter values. Although it was difficult to find out any defined relationship between the value of N_{∞} or λ and the agar concentration, a linear relationship was recognized between the tr value and the agar concentration (Fig. 1).

The results in Table 1 were obtained with pour-mixed cells. In following experiments we studied the effect of the agar concentration by using surface-smear cells, where we used only Agar Noble. Results were summarized in Table 2. We recognized again a linear relationship between the tr value and the agar concentration and the slope with surface-smear cells was steeper than that of the pour-mixed cells.

Fig. 1. Linear relationships between the tr values and the agar concentration. Opened and closed circles indicate, respectively, the relationship obtained with Agar Noble and Wako Agar, and closed squares that with Purified Agar. Data in Table 1 were used.

Table 1. Effects of the agar concentration on parameter values for pour-mixed cells*

Agar % (w/v)	N_{∞}	λ (hr ⁻¹)	tr (hr)
Agar Noble			
0.80	213.3±18.3	0.27±0.05	29.8±0.5
1.00	222.8±12.2	0.29±0.05	30.7±0.2
1.25	225.0±15.3	0.27±0.03	31.0±0.9
1.50	203.5± 8.9	0.32±0.03	32.8±0.3
2.00	218.4±25.8	0.31±0.07	34.6±1.1
Wako Agar			
1.00	155.6±15.9	0.35±0.06	33.5±0.2
1.50	170.0±17.1	0.38±0.04	36.5±0.2
1.75	163.5±14.8	0.37±0.04	36.8±0.2
2.00	155.8±14.4	0.36±0.01	38.7±0.3
2.25	164.4±11.8	0.21±0.04	38.9±0.5
Purified Agar			
1.00	365.7±26.4	0.29±0.03	32.2±0.4
1.50	357.4±13.3	0.38±0.06	35.5±0.2
2.00	355.3±14.1	0.26±0.04	36.5±1.1

* Each experiment was carried out with 5 replicate plates and the value of each parameter for respective plates was estimated. Figures in the table, respectively, the mean and the standard deviation. Because the cell suspension used was prepared with a different culture for different kind of agar, the values of N_{∞} for one kind of agar can not be compared with those for another kind.

Table 2. Effects of the agar concentration on parameter values for surface-smeared cells*

Agar concentration % (w/v)	N_{∞}	λ (hr ⁻¹)	tr (hr)
(Surface-smeared cells)			
1.00	100.0±13.9	0.51±0.06	29.2±0.6
1.50	102.3±18.6	0.48±0.04	32.3±0.6
2.00	102.8±12.3	0.41±0.05	34.9±1.0
(Pour-mixed cells)			
1.00	107.1±16.8	0.31±0.05	30.9±0.9

* Agar Noble was used throughout the experiment. Each experiment consisted of 20 replicate plates and each parameter value for respective plates was estimated. In the table the mean and the standard deviation for each experiment were shown. The same cell suspension was used for both surface-smeared and pour-mixed cells.

The results indicated that the agar concentration affected the tr value for both pour-mixed and surface-smeared cells. Since the parameter tr is connected to the growth rate of the organism (Ishikuri and Hattori, 1987), the effect may reflect the limitation of the growth rate by the nutrient

diffusion in the medium; an increase in the agar concentration makes the medium more viscous and the diffusion rate of nutrient becomes slower. The effect on the nutrient diffusion becomes more remarkable in the case of surface-smeared cells, which will result in a steeper relationship between

the t_r value and the agar concentration.

The parameters other than t_r may be connected to stochastic processes such as the initiation of cell cycle and the interruption of cell division of a clone (Hattori, 1985; 1988). Further repeated experiments will be required to define the effect of the agar concentration.

References

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