ENRICHMENT OF OLIGOTROPHIC BACTERIA AT MICROSITES OF SOIL¹

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Responses of oligotrophic bacteria at microsites of soil to substrates at various concentrations were studied by means of capillary tubes. As a measure of dominance of oligotrophic bacteria, an index δ was proposed as follows;

$$\delta = (\text{DNB}_{\text{count}} - \text{NB}_{\text{count}}) / \text{DNB}_{\text{count}}$$

where NB and DNB count are plate count on the conventional nutrient broth (NB) and its 100-fold dilution (DNB) respectively. The value of the index varied widely with the site and most frequently observed between 0 and 0.1 or 0.9 and 1.0, which is interpreted by an assumption that a population developed in a capillary tube consists of offspring of one or few of the original cells. The assumption is consistent with the J-shaped cell density histogram which is approximated by the Poisson distribution. Bacterial response to substrate is expressed by the mean δ value, three types of responses being observed; glucose and succinate were effective for the enrichment of oligotrophic bacteria, glutamate for zymogenic ones, and nutrient broth affected favorably either the former or the latter depending on its concentration. Based on these results a probabilistic view is proposed on bacterial behavior at microsites of soil.

In the soil biological system, the dynamic equilibrium of zymogenic and autochthonous or oligotrophic bacteria seems to play a key role. Although the enhancement of the former by exogenous substrates is generally recognized, the response of the latter to such substrates is not clear. Studies on enrichment of oligotrophic bacteria will provide basic information on their behavior in soil. For such study, diluted nutrient broth (DNB) organisms that can grow on a 100-fold dilution of the conventional nutrient broth but not on the full strength one (1–3) are expected to be effectual, since the number of their cells can be followed by the plate method.

¹ Abbreviations used in this text are as follows: NB, the conventional nutrient broth; DNB, a 100-fold dilution of NB.

Microhabitats of soil microorganisms are discrete, and microbial cells distribute very heterogeneously (4). Consequently, their behavior should be examined at each microsite. For this purpose, capillary tubes retaining substrate solution may be useful. The present report describes responses of DNB organisms to various substrate solutions in capillary tubes stood in soil and proposes a probabilistic view of microbial behavior at microsites of soil.

MATERIALS AND METHODS

Capillary tube. Capillary tubes were prepared from glass tubing by heat fusion and cut up into pieces of about 10 cm in length. They were between 0.08 and 0.30 mm in inside diameter, most being between 0.10 and 0.20 mm.

Soil. About 100 g of paddy soil taken from Oomagari farm of Tohoku National Agricultural Experiment Station was put in a 1 l beaker after being homogeneously mixed. The soil was submerged and the top water was adjusted to 2 cm in depth. During the experiment the beaker was closed by aluminium foil to avoid water loss due to vaporization and kept at 30°.

Enrichment culture. A series of experiments generally involved the use of 15 to 30 capillary tubes. Capillaries were, after being subjected to dry heat sterilization, stood in a filter-sterilized substrate solution until the capillary tubes were filled with the solution. After the exteriors of the capillaries were washed with deionized water, they were made into a bundle and let to stand, putting one side of their tips in the surface layer of the submerged soil (about 5 mm in depth) and kept at 30°.

Determination of original population. A set of sterilized capillaries retaining sterilized water was placed with one side of their tips in the surface of the submerged soil for about 10 min and then taken out. Their exteriors were washed by water and then sterilized using cotton soaked with toluene or xylene. Soil particles retained at the tips of capillary tubes were removed by blowing from the other side into one ml of sterilized water in a test tube of 1 cm in diameter. Four test tubes containing soil particles were placed together in the cup of a sonic oscillator (Kubota Insolator Model 200 M) containing 10 ml of water and subjected to the oscillation of 40 W for 3 min. Bacterial cells thus dispersed were counted by the plate method as described later.

Determination of enriched population. The exteriors of capillaries taken out of the beaker were washed with water and then sterilized by toluene or xylene. Each capillary was numbered from smaller to larger in diameter. A part of each capillary was cut off at a point of about 1 cm from the tip kept in soil and one droplet of enriched culture solution was sampled from the cut end by blowing. With 30 capillaries, the volume of water droplets thus taken was determined by the weighing of a piece of filter paper soaked up the water droplet. The mean volume of droplets and the standard deviation was $1.33 \pm 0.43 \mu\text{l}/\text{drop}$. After an appro-

priate dilution, cell number was counted by the plate method using two kinds of media. One is the conventional nutrient broth (NB) (2) solidified by 1% of 1st grade agar (Wako Pure Chemical Industry, Ltd.). The other is a 100-fold dilution of NB medium, which was solidified by 1% of Difco Agar Noble (5). Plates were incubated at 27° for several days in the case of NB medium and 10 days or more in the case of DNB medium. The count obtained with the former will be called NB count and that with the latter DNB count. The number of DNB cells was obtained by subtracting the NB count from the DNB count and, if the NB count exceeded the DNB count, the number was put at 0.

RESULTS

Definition of DNB index, δ

The degree to which DNB organisms dominate in a bacterial population is expressed by DNB index δ , defined as follows;

$$\delta = (\text{DNB}_{\text{count}} - \text{NB}_{\text{count}}) / \text{DNB}_{\text{count}} \quad (1)$$

DNB count was usually larger than NB count excepting the case where nutritionally rich solution was used for enrichment culture. For simplification we put

$$\delta = 0 \quad (2)$$

when NB count exceeds DNB count. The value of the index will thus vary between 0 and 1.

Table 1. δ Value and ratio of DNB organisms to the isolates.

Test No.	Number of isolates (A)	Number of DNB organisms (B)	B/A	δ Value
1	25 (116)	1	0.04	0
2	29 (34)	7	0.24	0.04
3	22 (168)	12	0.55	0.46
4	22 (42)	16	0.73	0.54
5	19 (115)	17	0.89	0.95
6	10 (14)	10	1.00	0.95
7	22 (24)	22	1.00	1.00
8	30 (41)	0	0	0
9	16 (17)	1	0.06	0.11
10	27 (87)	19	0.70	0.70
11	29 (65)	28	0.97	0.97
12	29 (90)	29	1.00	1.00
13	28 (213)	0	0	0.01
14	30 (95)	3	0.10	0.05
15	25 (93)	20	0.80	0.94

Organisms in Tests No. 1 to 7 were isolated from capillaries with NB/100, those in No. 8 to 12 from capillaries with NB/1,000 and those in No. 13 to 15 from capillaries with NB/10,000. Organisms were isolated from DNB plate, the colony number of which is indicated in parentheses.

To examine whether the index really reflects the degree of dominance of DNB organisms in a bacterial population, organisms were isolated from DNB plates of various capillary samples and their growth tested on NB medium. Results of the test coincided with δ value as a whole, although some discrepancies were observed (Table 1).

Bacterial population originally given at the tip of capillary tube

In the capillary enrichment culture the inoculum consists of bacterial cells within the tip of each tube with soil particles. Bacterial population in soil particles at the tips of 24 lengths of capillary was determined. The mean cell density estimated by DNB medium was 854 cells/droplet with the standard deviation of 1,021. The mean value of DNB index of populations and the standard deviation were respectively 0.72 and 0.14.

Bacterial population developed in capillary tubes

Using single substrate solution of glutamate, succinate, glucose, ammonium sulfate and phosphate at various concentrations, enrichment culture experiments were carried out and their results are given in Table 2. Effects of mixed substrates

Table 2. The mean cell density and the mean δ value of populations in capillaries retaining single substrate solution.

Substrate	Concentration	Incubation time (days)	Number of capillaries	Mean cell density ^a ($\times 10^3$ cells/droplet)	Mean δ value
Glutamate ^c	100 mM	6	20	4.42 $\pm$ 3.28 ^b	0.14 $\pm$ 0.22 ^b
	10 mM	9	18	4.28 $\pm$ 4.78	0.05 $\pm$ 0.14
	1 mM	8	17	4.86 $\pm$ 3.06	0.17 $\pm$ 0.33
	100 μ M	8	19	8.38 $\pm$ 8.04	0.18 $\pm$ 0.23
	10 μ M	12	20	2.22 $\pm$ 2.18	0.44 $\pm$ 0.38
	1 μ M	11	25	2.16 $\pm$ 2.22	0.67 $\pm$ 0.34
	100 nM	11	20	2.38 $\pm$ 2.08	0.63 $\pm$ 0.30
Succinate ^c	100 mM	14	19 (5) ^d	1.27 $\pm$ 1.35	0.35 $\pm$ 0.42
	10 mM	13	23 (11)	1.75 $\pm$ 2.18	0.31 $\pm$ 0.38
	1 mM	16	20	3.88 $\pm$ 2.90	0.40 $\pm$ 0.32
	100 μ M	12	30	3.91 $\pm$ 3.18	0.85 $\pm$ 0.25
	10 μ M	13	30	4.34 $\pm$ 3.72	0.93 $\pm$ 0.15
Glucose	10 mM	7	25	2.44 $\pm$ 3.63	0.80 $\pm$ 0.31
	10 μ M	7	15	5.18 $\pm$ 6.33	0.88 $\pm$ 0.20
	10 nM	14	23	1.15 $\pm$ 1.41	0.92 $\pm$ 0.17
(NH ₄) ₂ SO ₄	50 mM	9	20	13. 5 $\pm$ 14. 8	0.64 $\pm$ 0.36
	50 μ M	9	16	1.29 $\pm$ 1.00	0.95 $\pm$ 0.05
Phosphate ^e	100 μ M	10	10	7.52 $\pm$ 7.82	0.68 $\pm$ 0.37

^a Cell density is expressed by DNB count.

^b The standard deviation.

^c Sodium salts were used.

^d Figure in parentheses indicates the number of capillaries, δ values of which were not obtained as their DNB counts were too small.

^e KH₂PO₄-Na₂HPO₄ buffer at pH 7.0 was diluted appropriately.

Table 3. The mean cell density and the mean δ value of populations in capillaries retaining mixed substrate solution.

Substrate	Concentration or dilution	Incubation time (days)	Number of capillaries	Mean cell density ^a ($\times 10^8$ cells/droplet)	Mean δ value
Nutrient broth	1	7	27	101.9 + 144.2 ^b	0.09 + 0.20 ^b
	1/10	14	38	72.8 + 91.8	0.31 + 0.37
	1/100	8	18	10.7 + 9.8	0.51 + 0.41
	1/100	22	10	14.6 + 8.0	0.65 + 0.29
	1/1,000	8	20	11.8 + 11.7	0.55 + 0.35
	1/1,000	22	10	18.2 + 14.6	0.50 + 0.35
	1/10,000	16	30	3.76 + 4.54	0.73 + 0.28
Succinate	100 μ M	14	25	1.19 + 1.11	0.79 + 0.33
+ (NH ₄) ₂ SO ₄	5 μ M				
Succinate	1 mM	10	18	4.20 + 3.49	0.45 + 0.33
+ (NH ₄) ₂ SO ₄	50 μ M				
Glucose	1 mM	7	25	4.90 + 5.57	0.58 + 0.36
+ Phosphate	100 μ M				
Succinate	100 μ M	7	9	11.7 + 14.1	0.59 + 0.40
+ (NH ₄) ₂ SO ₄	50 μ M	13	9	13.5 + 15.1	0.51 + 0.34
+ Phosphate	10 μ M				
Glucose	100 μ M				
+ (NH ₄) ₂ SO ₄	100 μ M	6	10	5.13 + 6.19	0.76 + 0.31
+ Phosphate	10 μ M				

^a Cell density is expressed by DNB count.^b The standard deviation.

on enrichment culture were also observed using nutrient broth or a combination of an organic compound and inorganic compounds at a variety of concentrations, some of the results of which are described in Table 3. It is notable that both cell density and the value of the index varied widely with all cases.

Figure 1 gives J-shaped histograms of cell density data with both DNB and NB media of experimental series whose mean cell densities were less than 10⁴ DNB counts/droplet. In this case NB counts 288 lengths (37%) of capillary were less than 100 cells/droplet. The number of DNB cells was also less than 100 cells/droplet with 152 lengths (19%) of capillary. Similar J-shaped histograms were obtained with cell density data of narrower or wider capillaries, the set of which was made by taking 5 lengths of capillary according to the capillary number of every series. It was assumed that cell density data may be approximated by the Poisson distribution. The goodness of fit of the distribution was tested by the chi-square test in Table 4. At the 5% level of significance the hypothesis was not rejected (6). Very small correlations between numbers of DNB and NB cells (Table 5) also supported the hypothesis.

Considering that the Poisson distribution is an approximation of a frequency distribution when the number of events is large and the probability of occurrence

Table 4. Fitting the Poisson distribution.^a

1. Glutamate (100, 10, 1, 0.1 mM)						3. Succinate (100, 10, 1 mM)						5. Glucose (100 mM, 100 μ M, 100 nM)						
<i>NB cells</i>		$(\times 10^3 \text{ cells/droplet})$				<i>NB cells</i>		$(\times 10^3 \text{ cells/droplet})$				<i>NB cells</i>		$(\times 10^3 \text{ cells/droplet})$				
Range		0-6	6-12	12-18	18-24	24<	Range		0-4	4-8	8-12	12<	Range		0-6	6-12	12-18	18<
Observed		45	22	3	1	3	Observed		43	10	1	2	Observed		57	3	1	2
Expected		45	22	7			Expected		43	14	3		Expected		57	6		
Total		74, $\lambda'=0.50$, $df=2$, $\chi^2=0$				Total		62, $\lambda'=0.33$, $df=2$, $\chi^2=1.98$				Total		63, $\lambda'=0.10$, $df=1$, $\chi^2=0$				
<i>DNB cells</i>		$(\times 10^3 \text{ cells/droplet})$				<i>DNB cells</i>		$(\times 10^3 \text{ cells/droplet})$				<i>DNB cells</i>		$(\times 10^3 \text{ cells/droplet})$				
Range		0-1.2	1.2-2.4	2.4-3.6	3.6<		Range		0-4	4-8	8-12	12<	Range		0-2.8	2.8-5.6	5.6-8.4	8.4<
Observed		63	8	1	2		Observed		40	13	8	1	Observed		46	10	2	5
Expected		59	14	3			Expected		40	17	4	1	Expected		43	16	7	
Total		74, $\lambda=0.23$, $df=2$, $\chi^2=3.34$				Total		62, $\lambda=0.43$, $df=3$, $\chi^2=4.94$				Total		63, $\lambda=0.38$, $df=2$, $\chi^2=4.70$				
2. Glutamate (1, 0.1 μ M)						4. Succinate (100, 10 μ M)						6. Nutrient broth (NB)						
<i>NB cells</i>		$(\times 1.2 \cdot 10^3 \text{ cells/droplet})$				<i>NB cells</i>		$(\times 10^3 \text{ cells/droplet})$				<i>NB cells</i>		$(\times 10^5 \text{ cells/droplet})$				
Range		0-1.5	1.5-3.0	3.0-4.5	4.5<		Range		0-0.8	0.8-1.6	1.6-2.4	2.4<	Range		0-1	1-2	2-3	3<
Observed		35	6	3	1		Observed		54	1	1	4	Observed		16	7	4	3
Expected		33	10	4			Expected		55	6			Expected		16	10	4	1
Total		45, $\lambda'=0.30$, $df=2$, $\chi^2=3.63$				Total		60, $\lambda'=0.09$, $df=1$, $\chi^2=0.22$				Total		30, $\lambda'=0.65$, $df=3$, $\chi^2=4.90$				
<i>DNB cells</i>		$(\times 1.2 \cdot 10^3 \text{ cells/droplet})$				<i>DNB cells</i>		$(\times 10^3 \text{ cells/droplet})$				<i>DNB cells</i>		$(\times 10^4 \text{ cells/droplet})$				
Range		0-0.8	0.8-1.6	1.6-2.4	2.4<		Range		0.4	4-8	8-12	12<	Range		0-0.5	0.5-1.0	1.0<	
Observed		29	9	1	6		Observed		38	14	7	1	Observed		23	2	2	
Expected		25	14	7			Expected		38	18	8		Expected		23	4		
Total		45, $\lambda=0.55$, $df=2$, $\chi^2=3.23$				Total		60, $\lambda=0.47$, $df=2$, $\chi^2=2.69$				Total		27 ^b , $\lambda=0.16$, $df=1$, $\chi^2=0.20$				

7. Nutrient broth (NB/10)				8. Nutrient broth (NB/100, NB/1,000)				9. Nutrient broth (NB/10,000)				
NB cells ($\times 10^4$ cells/droplet)				NB cells ($\times 10^3$ cells/droplet)				NB cells ($\times 10^3$ cells/droplet)				
Range 0-0.5 0.5-1.0 1.0-<				Range 0-0.8 0.8-1.6 1.6-2.4				Range 0-3 3-6 6-9 9-<				
Observed	26	8	5	Observed	49	5	4	Observed	25	3	1	1
Expected	25	11	3	Expected	45	11	2	Expected	25	5	2	1
Total	39, $\lambda' = 0.43$, $df = 2$, $\chi^2 = 2.19$			Total	58, $\lambda' = 0.25$, $df = 2$, $\chi^2 = 5.63$			Total	30, $\lambda' = 0.20$, $df = 2$, $\chi^2 = 1.8$			
D/NB cells ($\times 10^4$ cells/droplet)				D/NB cells ($\times 10^3$ cells/droplet)				D/NB cells ($\times 10^3$ cells/droplet)				
Range 0-0.3 0.3-0.6 0.6-<				Range 0-0.6 0.6-1.2 1.2-1.8				Range 0-2 2-4 4-<				
Observed	29	8	2	Observed	44	13	1	Observed	22	5	3	
Expected	30	8	1	Expected	44	12	2	Expected	21	8	2	
Total	39, $\lambda = 0.25$, $df = 2$, $\chi^2 = 1.03$			Total	58, $\lambda = 0.28$, $df = 2$, $\chi^2 = 1.08$			Total	30, $\lambda = 0.37$, $df = 2$, $\chi^2 = 1.67$			

^a Data of experiments described in Tables 2 and 3 were used. Data of DNB cells were obtained by subtraction of NB count from DNB count. The frequencies in the tails of the distribution were pooled. Expected value was calculated using the equation, $P(x) = e^{-\lambda} \lambda^x / x!$, where x is the category (cell density range) number and λ is a parameter, the value of which is assumed in each case. In the case of NB cells, λ' is used instead of $\lambda(6)$.

When the degree of freedom (df) is 1, $P[\chi^2 \geq 3.84] = 0.05$. When $df = 2$, $P[\chi^2 \geq 5.99] = 0.05$ and, when $df = 3$, $P[\chi^2 \geq 7.82] = 0.05$.

^b DNB counts of three capillaries were not obtained.

Fig. 1. A histogram of cell density data.

Data were taken from various experimental series with the mean cell density less than 10^4 cells/droplet. Cell density was indicated by its number/droplet. A shows DNB count and B NB count.

is small, one may assume that a bacterial population in a capillary mainly consists of offspring of one or few original cells, even though about 800 cells on an average were initially found at the tip of each capillary. The assumption is consistent with the observation that only one or a few types of colony appeared on the same plate.

The variance of cell density (σ^2) was larger than the mean value with all series (Tables 2 and 3). It is recognized that, if the population is contagious, the variance is larger than the mean (6). Analogously, one may suppose that the probability of finding the offspring of each original cell in an enriched population, although small, does not remain the same but varies with the physiological group to which the cell belongs.

The mean cell density itself varied with the degree of dilution of nutrient broth, although no appreciable difference was observed between values with NB/100 and NB/1,000 (Table 3). A value of 72×10^3 cells/droplet with NB/10 will be 55×10^6 cells/droplet, for the mean volume of a droplet is $1.3 \mu\text{l}$. When soil bacteria were grown on NB/10 in test tubes with an inoculum of one drop of diluted soil suspension, the mean maximal growth was 106×10^6 cells/ml, the value of which is not far from that obtained with capillary tubes.

Table 2 shows that the mean cell density with a single organic substrate solution did not respond significantly to its concentration. Because cell proliferation may be slow in such a single substrate solution, loss by outward diffusion of the

Table 5. Correlations between numbers of NB (X) and DNB (Y) cells.^a
 $Y = a + bX$, correlation coefficient = r .

Substrate	Concentration	a	b	r
Glutamate	100, 10, 1, 0.1 mM	1,144	-0.008	-0.051
	1, 0.1 μ M	1,373	-0.042	-0.036
Succinate	100, 10, 1 mM	863	-0.038	-0.165
	100, 10 μ M	3,878	-0.443	-0.164
Glucose	10 mM, 10 μ M, 10 nM	3,962	-0.923	-0.047
Nutrient Broth	NB	2,236	0.008	0.126
	NB/10	3,242	-0.009	-0.017
	NB/100, NB/1,000	7,446	-0.100	-0.148
	NB/10,000	2,108	-0.096	-0.161

^a X , Y and a were expressed in cell number/droplet.

Data were those used for calculation in Table 4.

substrate may be among factors limiting the mean cell density in these cases. Want of some other elements may also be another factor.

Variation of the value of DNB index, δ

Large variations were also observed with the value of DNB index as seen in Tables 2 and 3. Using δ value data of experimental series, the mean δ value of which was between 0.30 and 0.70, a U-shaped δ value-frequency histogram was obtained (Fig. 2). Similar histograms were also obtained with the narrower or wider capillary set. It is notable that in every histogram, high frequency was observed at δ value ranges of 0 to 0.10 and 0.90 to 1.00.

Fig. 2. A histogram of values of DNB index (δ).

Data were taken from experimental series, the mean value of which was between 0.30 and 0.70.

Fig. 3. Histograms of values of DNB index in each class.

All sets of capillary experiments were divided into classes by their mean value. The range of the value of each class is as follows; A is from 0 to 0.10, B from 0.10 to 0.20, C from 0.30 to 0.40, D from 0.40 to 0.50, E from 0.50 to 0.60, F from 0.60 to 0.70, G from 0.70 to 0.80, H from 0.80 to 0.90 and I from 0.90 to 1.00.

To analyze the variation of δ values, all sets of capillary experiments were divided into ten classes by their mean δ values, and a δ value-frequency histogram of each class is depicted in Fig. 3, excepting the class with the mean δ value from 0.20 to 0.30 which was not observed with any experimental series. With every class, high frequency appeared exclusively within a range of 0 to 0.10 or/and 0.90 to 1.00. Further analysis showed that over 70% of frequency in each range was concentrated in the narrower range of 0 to 0.05 or 0.95 to 1.00, excepting the class with the mean value of 0.70 to 0.80 in which 67% of frequency in the range of 0.90 to 1.00 belonged to the narrower one.

These histograms may be interpreted again by the assumption that each of the

Fig. 4. Relation between the mean value of DNB index and frequencies of both end ranges in each class shown in Fig. 3.

The mean value of the index was estimated from values of all capillaries belonging to experimental series of each class. Frequency in the range from 0.90 to 1.00 is expressed by a and that from 0 to 0.10 by b .

original cells will have a small probability of succeeding in producing an appreciable amount of its offspring and, consequently, the population in a capillary tube will consist generally of only DNB or NB cells and infrequently of both DNB and NB cells at an appreciable ratio.

Figure 3 alludes to the idea that a δ value-frequency histogram may be split up into two J-shaped histograms oppositely directed. The mean δ value of each class is closely related to frequencies in both end ranges (Fig. 4), which supports the idea and suggests further that relative weight between two component histograms may determine the mean δ value.

Formation of δ value-frequency relation

Based on the above assumption, the relation between δ value and frequency will be expressed as superposition of two probability functions; $\phi(\delta)$ and $\phi'(1-\delta)$. The first refers to the probability of occurrence of a population with dominance of DNB at a degree of δ . The second is a similar one concerning dominance of NB cells. As the first approximation, values of both functions decrease uniformly with increase of δ .

It is to be noted that each of these functions itself does not refer to the overall feature of occurrence of a population with a value of δ but only to the probability of dominance of DNB or NB organisms. Then we can define the probability function Ψ concerning occurrence of a population with a value of δ ,

$$\Psi = f \cdot \phi(\delta) + (1-f) \phi'(1-\delta) \quad (3)$$

where f is a partition function which will vary with environmental factors for bacterial enrichment as well as an initial bacterial population itself. At a given condition or with an experimental series the value of f can be approximated by $a/(a+b)$ as shown in Fig. 4.

Fig. 5. A schematic expression of effects of substrate concentration on the mean value of DNB index.

Based on data in Tables 2 and 3. A, glutamate; B, glucose; C, succinate; D, nutrient broth.

Table 6. Comparison between $\lambda/(\lambda+\lambda')$ and the mean value of DNB index ($\bar{\delta}$).^a

Substrate	λ	λ'	$\lambda/(\lambda+\lambda')$	$\bar{\delta}$
Glutamate (100, 10, 1, 0.1 mM)	0.23	0.50	0.32	0.14
Glutamate (1, 0.1 μ M)	0.55	0.30	0.65	0.65
Succinate (100, 10, 1 mM)	0.43	0.33	0.57	0.36
Succinate (100, 10 μ M)	0.47	0.09	0.84	0.89
Glucose (10 mM, 10 μ M, 10 nM)	0.38	0.10	0.79	0.86
NB	0.16	0.65	0.20	0.09
NB/10	0.25	0.43	0.37	0.31
NB/100, NB/1,000	0.28	0.25	0.53	0.55
NB/10,000	0.37	0.20	0.65	0.73

^a Values of λ and λ' are taken from those described in Table 4. Mean value of DNB index is calculated from data of all capillaries retaining substrates indicated.

The linear regression between two variables is

$$\bar{\delta} = -0.22 + 1.34\lambda/(\lambda+\lambda')$$

$$r=0.963.$$

Effect of substrate concentration on the mean $\bar{\delta}$ value

Figure 5 depicts schematically effects of substrate concentration on the mean $\bar{\delta}$ value. Three types of substrate were observed; the first was more effective for the enrichment of DNB organisms (glucose and succinate), the second for NB organisms (glutamate) and the third favorably affected either DNB or NB organisms depending on its concentration (nutrient broth). Supplementation of ammonium sulfate or/and phosphate at various concentrations to succinate or glucose solution, more or less resulted in a decrease of the mean $\bar{\delta}$ value (Table 3).

Table 6 indicates that values of the parameters, λ and λ' given in Table 4 varied with substrate or its concentration, which suggests that the response of the mean δ value to various substrates may be a reflection of change in probabilities of various organisms to be enriched. A high correlation between $\lambda/(\lambda + \lambda')$ and the mean δ value support this. Then we can postulate

$$f = \lambda/(\lambda + \lambda') \quad (4)$$

as an approximation.

DISCUSSION

Soil microorganisms live in discontinuous microhabitats, and a capillary tube, the tip of which is placed into soil, enlarges the microhabitat so that we can observe separately microbial response to substrate at each microsite. The present study strongly suggests that such microbial response at a different microhabitat may not be uniform but varying, and may be regulated by the laws of probability. Approximating cell density data to the Poisson distribution, it is assumed that only one or few of the original cells succeeded in producing an appreciable amount of their offspring in a newly established population. The probability at which each of the original cells appreciably proliferates may not be uniform but varying with bacterial group or their physiological state. Such a probabilistic feature of enrichment culture at microsites of soil will furnish the key for understanding the dynamic equilibrium of the soil microbial population consisting of a variety of organisms.

DNB and NB organisms responded differently to various substrate solutions, the mechanisms of which will be studied from the standpoint of physiology. As a whole, nutritionally rich solution was favorable for enrichment of NB organisms, and poor solution resulted in dominance of DNB organisms, although large variation was observed at different microsites of soil. Dominance of DNB organisms in a population was expressed by DNB index, δ and its value-frequency relation was expressed by the sum of two probability functions of δ . Relative weight between the two functions will be determined by a function of λ and λ' which are the parameters of the Poisson distribution. The parameters will be split up into two terms; one the number of cells and the other the probability of each cell of being enriched. The latter may be related to two probabilistic processes; the initiation of cell division and its continuation.

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