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Retention of Bacteria in Liquid Films at Agar Surfaces

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The number of bacteria retained by agar dipslides immersed in bacterial suspensions was dependent solely on suspension population density and was unaffected by the nutrient status of the agar surface or liquid, disturbance of the liquid, or bacterial motility and chemotaxis.

Agar immersion (dipslide) techniques are used frequently to assess the bacteriological quality of liquids (4, 5, 7, 9, 11, 12). However, most evaluation data are of a semiquantitative nature, and factors that may affect the number of bacteria enumerated by the technique have not been adequately defined. Consequently, we have examined the following factors: time of immersion, the liquid bacterial population density, nutrient status of both the suspension media and the dipslide agar, agitation of the liquid sampled, bacterial motility, and chemotaxis.

Dipslides of varying nutrient status (nutrient agar to Noble agar [Difco]) were immersed for varying times (up to 25 min) in washed cell suspensions of either a motile bacterium, *Pseudomonas* A80 (8), or a nonmotile bacterium, *Flavobacterium* 25 (10). The motile bacterium had previously been shown to display a chemotactic response to nutrient broth (Difco) by the method of Adler (1, 2). Suspension population densities varied from 10^4 to 10^9 cells/ml, and agitation was provided by a magnetic stirrer. Suspension population densities were determined on duplicate surface-spread plates of nutrient agar. Numbers of bacteria retained by dipslides were determined similarly, after maceration of the agar in saline diluent.

Figure 1 demonstrates the effect of varying times of immersion of nutrient agar dipslides in suspensions of bacteria. No increase in numbers retained by dipslides was observed for either *Pseudomonas* A80 or *Flavobacterium* 25. The effect of changes in suspension population density upon numbers of bacteria retained by dipslides was shown to be a linear relationship, which may be expressed in a form of the Freundlich empirical equation for adsorption (14):

$$\log R = \log A + n \log C \quad (1)$$

where A is a constant, n is slope of the linear relationship between $\log R$ and $\log C$, R is the number of bacteria retained by a surface, and C is the bacterial suspension population density. Values of n and $\log A$ for each experimental data set were computed by the least-squares method. Table 1 summarize these regression data. In all cases, the slope (n) of the relationship between suspension concentration ($\log C$) and the numbers of bacteria retained by dipslides ($\log R$) was found to be close to unity ($r^2 > 0.95$). This shows the relationship to be unaffected by time of immersion of the dipslide, disturbance, bacterial motility, and the nutrient status of either the suspension liquid or the dipslide agar. This evidence suggests that the retention of bacteria in liquid films on agar surfaces after immersion in bacterial suspensions is a simple dilution effect: the number retained is dependent only upon suspension population density. With undisturbed systems, which enable detection of chemotactic responses by motile bacteria toward nutrient sources (capillary tube method [3]), slopes of 2 have been obtained for equation 1 when using suspensions of *Pseudomonas* A80. Slopes of unity were obtained when suspensions of nonmotile *Flavobacterium* 25 were used or when the capillary tubes were filled with fluid containing no attractant. Such data demonstrate the effect of chemotaxis upon parameters of equation 1.

Further confirmation of the simple dilution effect for dipslides was made by comparing experimental weights of liquid retained by dipslides, with theoretical values obtained from regression data as follows: with slopes of unity, equation 1 becomes

$$\log R = \log A + \log C \quad (2)$$

Taking antilogarithms and differentiating, equation 2 becomes $dR/dC = A$. Since C expresses population density as numbers per unit volume of suspension and R is the number of bacteria retained by the dipslide, then A expresses the volume or weight of liquid retained on the agar surface after removal from

the suspension. Calculated values of A (0.006 to 0.062 g; mean, 0.028 g) obtained from values of log A in Table 1 agreed well with values obtained by weighing (0.020 to 0.060 g; mean, 0.031 g).

The persistence of the simple dilution effect does not exclude the presence of chemotaxis as a mechanism by which motile bacteria may aggregate at an immersed agar dipslide surface. However, the effect of such a mechanism is overridden after removal of that surface, since liquid adjacent to the immersed surface is replaced by liquid from the bulk suspension.

Therefore, agar immersion techniques are soundly based since agar surfaces transiently immersed in bacterial suspensions will retain a population dependent upon the bacterial population density alone. Extrapolation of these results to surfaces other than agar is not valid. However, it is of interest that similar relationships exist between bacterial population densities and retention of bacteria by tooth enamel, buccal epithelial cells (6), and chicken skin (13).

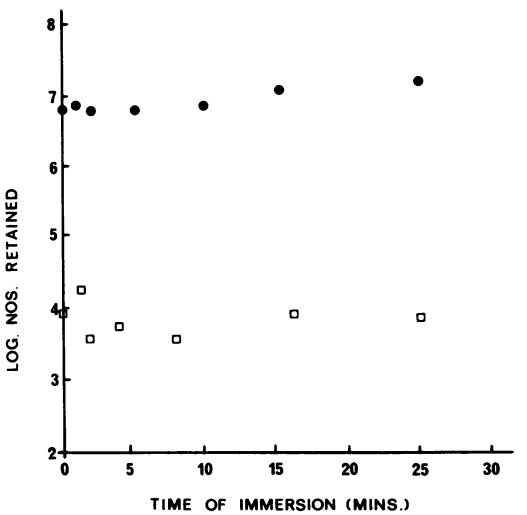

FIG. 1. *Effect of time of immersion in suspensions of bacteria upon numbers retained by nutrient agar dipslides. Symbols:* (●) *Pseudomonas A80 (suspension population density, 1.88 × 10⁸/ml).* (□) *Flavobacterium 25 (suspension population density, 8.43 × 10⁵/ml).*

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TABLE 1. *Regression data for log-log relationships of numbers of Pseudomonas A80 and Flavobacterium 25 retained by dipslides as a function of suspension population density*

Dipslide agar nutrient status	Suspension fluid nutrient status	Stirred system						Unstirred system					
		Slope (n)		y-intercept (log A)		Correlation coefficient		Slope (n)		y-intercept (log B)		Correlation coefficient	
		Pseudomonas A80	*Flavobacterium* 25	*Pseudomonas* A80	*Flavobacterium* 25	*Pseudomonas* A80	*Flavobacterium* 25	*Pseudomonas* A80	*Flavobacterium* 25	*Pseudomonas* A80	*Flavobacterium* 25	*Pseudomonas* A80	*Flavobacterium* 25
Nutrient agar	Nil	1.05	1.04	−1.42	−1.54	0.99	0.99	1.17	1.08	−2.42	−2.21	0.95	0.98
Nutrient agar	Nutrient broth	1.09	0.98	−1.97	−1.39	0.99	0.95	1.06	0.95	−1.40	−1.21	1.00	0.99
Noble agar	Nil	1.13	1.04	−2.22	−1.83	0.98	0.99	1.06	1.02	−1.53	−1.57	0.97	0.99