INFLUENCE OF SURFACE TENSION UPON THE GERMINATION OF BACTERIAL SPORES

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In the bacteriological literature of recent years, much attention has been devoted to the study of surface tension in relation to vegetative development. Despite widespread interest in this subject the relative importance of surface forces in bacterial growth remains unsettled. Misinterpretation of results appears to have led to exaggeration of the significance of this factor in many cases. Nevertheless, it seems clearly established that the vegetative development of some types of bacteria is profoundly influenced by variations in the surface energy relationships of their environment. Evidence of this nature, together with the fact that previous researches upon surface tension were confined exclusively to vegetative cells, seemed to be sufficient justification for including this factor in a study of spore germination.

If spore germination were dependent upon diffusion of nutrient or stimulating substances through the cell membrane, we might reasonably expect to find that surface tension would exert a definite influence upon germination, for, as Frobisher (1926) has pointed out, fluids of very low surface tension may under certain conditions be in more intimate contact with objects in suspension, and might therefore facilitate contact between the essential constituents and cell contents. Solutions of low surface tension in general penetrate or seep into microscopic spaces more rapidly than do fluids of high surface tension. Moreover, according to Billard and Dieulafe (1904), osmosis and diffusion are materially accelerated under certain conditions by lowering the surface tension of the solutions involved.

METHODS

Except where otherwise indicated the methods in this study were identical with those previously described (Curran, 1931). The same strain of *B. mycoides* was used.

Culture medium. Plain infusion agar adjusted to pH 7.0 constituted the basic medium.

Germination technique. The technique employed in studying surface tension involved the use of a solid substratum. Briefly, the procedure used was as follows: melted agar was poured into a petri dish having a minimum crowning at the center. Upon solidification of the medium and drying of moisture of condensation, small blocks approximately 5 mm. square were cut in the test and control agar with a small sterile knife. A small loopful of the spore suspension was then spread evenly upon the surfaces of the two blocks. Allowing one to three minutes for the culture film to dry, the inoculated surfaces were placed face downward near the center of a clean sterile coverslip. The squares of agar were contiguous but not in direct contact. The coverslip was then suspended over a sterile straight-sided hanging drop cell, and the mount sealed by means of melted paraffin to prevent drying.

The rest of the procedure was similar to that already described.

Surface tension depressant. A 1 per cent solution of neutral sodium cleate (Merck) was used to reduce the surface tension of the culture medium. Sterilization was accomplished in the autoclave. The final reaction of the medium containing 0.1 and 0.5 cc. of this solution was approximately neutral. The higher concentrations produced a slight alkalinity.

Surface tension measurements. All the surface tension determinations were made with a du Nöuy tensiometer. Graded amounts of the sterile stock solution of oleate were added aseptically to 10 cc. quantities of the sterile agar and thoroughly mixed. The media, with and without depressant, were stored in the refrigerator until ready for use. This method of mixing depressant and media eliminated possible hydrolysis of the reagent during sterilization. By this method also, surface equilibrium was established before the measurements were made. The im-

portance of this factor in colloidal solutions has been emphasized by du Noüy (1924).

When the surface tension measurements were to be made, two tubes of agar—one oleate and the other plain agar—were dissolved in the Arnold sterilizer. The tubes containing the melted agar were then immersed in a hot water bath and the temperature reduced to 55°C. Using a sterile pipette, 1 cc. of the test solution was delivered upon the center of a flamed sterile watch glass. An interval of one-quarter to one-half of a minute was allowed to elapse, following which two or three determinations were made. Usually no difficulty was experienced in obtaining close checks with this procedure. The surface tension measurements were made between 50° and 52°C. When the measurements upon both solutions were completed, the agar was poured into separate petri plates and the procedure carried out according to the directions previously outlined.

Glassware. All glassware used in this experiment was cleaned by treatment with hot acid dichromate solution, and rinsed once with tap water and twice with distilled water,—the latter obtained by distillation in Pyrex containers in the presence of alkaline permanganate.

RESULTS

Owing to the negative nature of the findings, the data are presented in one condensed table (table 1). The figures represent the average of many experiments in which this type of result was consistently obtained. Reduction in the surface tension of 7 dynes from an initial measurement of 50 dynes exerted no significant effect upon the germination of the spores so cultured. The rate of germination and the total percentage of spores which germinated were essentially the same in both the test and control media. The slight discrepancy is entirely within the limits of error of the method employed. From this table it is seen that germination began after about forty-five minutes incubation under favorable conditions. Cell division, however, was not observed until the third hour. In the highest concentrations of oleate, vegetative multiplication was delayed an hour or more.

The fourth column presents the data secured when the surface

tension of the media was depressed to 35.1 dynes. In this case a very slight reduction in the rate of germination is manifest, but the total percentage of cells which germinated was practically the same in both cases.

A still further depression of the surface tension of the culture media yielded the results given in column 5. Reference to this table reveals a definite retardation in the germination rate and an appreciable reduction in the number of cells which germinated

TABLE 1
Showing the effect of surface tension upon the germination of the spores of B. mycoides

TIME FROM IN- OCULATION	GERMINATION					
	No oleate added; 50.3 dynes per cm.	0.2 cc. 1 per cent oleate to 10 cc. agar; 43.4 dynes per cm.	0.5 cc. 1 per cent oleate to 10 cc. agar; 35.1 dynes per cm.	1.0 cc. 1 per cent oleate to 10 cc. agar; 32.8 dynes per cm.	2.0 cc. 1 per cent oleate to 10 cc. agar; 31 dynes per cm.	3 cc. 1 per cent oleate to 10 cc. agar; 30.7 dynes per cm.
minutes	per cent	per cent	per cent	per cent	per cent	per cent
30	0	0	0	0	0	0
45	8	8	5	4	2	2
60	44	43	30	14	5	4
75	82	79	63	38	11	9
90	93	89	72	67	24	21
120	94	91	80	79	45	32
150	95	93	84	81	55	33
180	96	94	85	82	59	34
240	96	95	91*	85*	61	36
300			94*	88*	65*	39
330					67*	42*

* Estimated.

during the period of observation. The retarded rate of germination is particularly pronounced during the initial hour and one-half of culture. Vegetative growth proceeded more slowly in the media of lowered surface tension, with the result that a longer time elapsed before the germinated spores attained the filament stage.

The depression in surface tension was carried to 31.0 dynes and 30.7 dynes in columns 8 and 7, respectively. The results in general are similar to those in the previous column, but indicate much greater retardation in rate of germination and reduction of viable cells.

The results of these experiments are plotted in figure 1. These curves bear a rather close resemblance to the vegetative growth curve. As shown in this figure, germination begins slowly, then passes into a short period during which the percentage rate of increase is logarithmic in nature. It then falls off rather sharply, following which further increases are gradual, extending over relatively long periods of time.

FIG. 1. GERMINATION OF THE SPORES OF *B. MYCOIDES* IN MEDIA OF DIFFERENT SURFACE TENSION

In the interpretation of these data the question may arise as to our justification in ascribing to surface tension observed effects when the possible chemical action of the depressant has not been excluded.

Sodium oleate employed as the surface tension depressant was

chosen as the compound which most nearly approaches the ideal substance. Except in relatively high concentrations, it is practically inert from the standpoint of nutrition and toxicity, and can be obtained in almost pure state.

Frobisher (1926) found a very slight growth of *A. aerogenes* in aqueous solution of sodium oleate, but it would seem illogical to attribute any significant nutritional effect on this basis, particularly in view of the fact that his triply distilled water supported some slight multiplication.

Avery (1918), Ayers, Rupp and Johnson (1923), Frobisher (1926), and Day and Gibbs (1928) utilized sodium oleate to study surface tension and their results indicate that with the exception of the particularly fastidious pneumococcus, and a very few strains of *Streptococcus pyogenes*, there was no appreciable restraining action in concentrations of 0.1 per cent and less. The work of Day and Gibbs (1928) reveals even a slight stimulative action in 0.1 per cent concentration upon the growth of *L. acidophilus* and *L. bulgaricus*. The observation of Ayers, Rupp and Johnson (1923) that sodium oleate is converted to an insoluble, inert form in the presence of acid would not invalidate the results herein reported because of the absence of any significant quantity of fermentable sugar in the germination medium.

Our own observations indicated that the vegetative cells of *B. mycoides* grew abundantly in nutrient solutions containing oleate in the highest concentrations used in the germination studies. Aside from these facts, however, the negative nature of the results obtained in the lowest concentrations of oleate, should constitute ample proof of the absence of significant chemical activity in these media.

DISCUSSION

The results described in the present paper indicate rather clearly that surface tension is not a significant factor in the germination of the spores of *B. mycoides* under normal circumstances. Organic acids, esters and other substances produced in the growth of many bacteria may, under certain conditions, reduce the surface tension of their solutions. Nevertheless the

depression in surface tension induced by purely metabolic activities would be considerably less than that produced by the lowest concentrations of oleate used in these studies. Within the conditions of the experiments the rate of germination was practically unaffected when the surface tension was reduced to 43.4 dynes, while a measurement of 35.1 dynes served only slightly to retard the rate of germination.

The total number of spores which germinated during the period of observation was not appreciably reduced until the surface tension was depressed to 32.8 dynes and, then, only slightly. With a longer period of observation it is not improbable that this slight difference would have entirely disappeared.

While germination proceeded quite as well with the surface tension reduced 7 dynes, as in the control, there is no evidence of stimulation. If the permeability of the spore envelope and diffusion of the nutrient fluid into the cell were increased, they exerted no appreciable stimulus to the germination process. It is conceivable, of course, that other interacting factors may have masked possible effects upon permeability and diffusion.

The rather marked decrease in the germination rate and reduction in viable cells evident in columns 4 and 5 was possibly due, in part at least, to the chemical activity of the depressant, for in these cases the oleate was present in concentrations of 0.2 and 0.3 per cent, respectively. However, if the lowered germination rate and total percentage germination were due primarily to the chemical factor, one should expect a far greater retardation in column 5, in which the concentration of oleate was more than 33 times greater than in column 4. While the difference is definite it appears to follow more closely the smaller change in surface tension than the concentration of the depressant. However this may be, the principal point which we believe this work demonstrates is that the spores of this organism are comparatively insensitive to relatively wide variations in surface tension. Reduction in the surface tension representing 15 dynes produced but slight influence upon the germination process, while, with the maximum possible depression, 50 per cent of the spores germinated within six hours.

In the interpretation of these data it must be recognized that the results reported, in common with those of other investigators, are based upon measurements of the surface tension at the air-medium interface which may or may not represent the surface energy relationship at the organism-medium interface.

The facts brought out in this study may be briefly summarized as follows:

1. A reduction in surface tension to 43.4 dynes from an initial of 50 dynes has no appreciable effect upon either the rate of germination or the total percentage of viable spores.
2. The development of spores in media the surface tension of which is depressed to 35.1 dynes is only slightly affected. The rate of germination is slightly retarded and the total percentage of germinating cells is somewhat less over a five-hour period.
3. At 32.8 dynes the rate of germination is markedly retarded but the total percentage of viable spores is only slightly reduced.
4. Below 32.8 dynes, germination is attended by a material decrease in both rate and total germination.
5. With the surface tension reduced to 30.7 dynes about 50 per cent of the spores are able to germinate within six hours.
6. Depression of the surface tension can not be relied upon to prevent or accelerate spore germination under ordinary conditions.
7. Surface tension is not a significant factor in the germination of the spores of *B. mycoides*.

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