THE WATER RELATIONS OF *VIBRIO METCHNIKOVI* AT 30°C

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Summary

For a strain of *Vibrio metchnikovi* the rate of growth in liquid media and the number of colonies forming on solid media were both greatly increased by reducing the water activity (a_w) from *c.* 0·999 to 0·995.

Below *c.* 0·995 a_w both the rates of growth and colony numbers were greater in the presence of salts than in the presence of sucrose or glucose.

I. Introduction

Vibrio metchnikovi is a pathogen for birds and some laboratory animals. In several respects it is similar to the cholera vibrio and, like this organism, it is relatively susceptible to drying (Annear 1956). The organism was found to grow luxuriantly in brain heart infusion, but in nutrient broth containing 5 g peptone and 3 g of meat extract per litre it grew very slowly and at times only after a prolonged lag phase. As the former medium, containing 37 g dry matter per litre, had a water activity (a_w) of 0·993, and the latter an a_w of *c.* 0·999 (Scott 1953), it was decided to examine the effect of adding various solutes to nutrient broth. The addition of small quantities of salts to this medium permitted greatly increased rates of growth and therefore the effects of various levels of a_w were studied in more detail. This paper reports the effects of a_w on the rate of growth and on the plate count of this organism. A wider discussion of the effect of a_w on microbial growth has been given elsewhere (Scott 1957).

II. Methods

The methods for controlling a_w by the addition of solutes, and for determining rates of growth in rocking T-tubes, were as described previously (Scott 1953). Two basal media each of *c.* 0·999 a_w were used. These were nutrient broth and brain heart infusion containing respectively 8 and 5 g of dry matter per kilogram of water. The solids for the brain heart infusion were obtained by freeze-drying a large batch prepared according to the formula in the Difco manual (9th Ed.). Solid media of virtually the same a_w were made by dissolving 8 g of agar per kilogram of water in the corresponding liquid media.

For the plate counts, dilutions were made in sodium chloride solution (0·995 a_w) and, for some experiments, in solutions of potassium chloride (0·995 a_w) or in mixed electrolytes ($NaCl:KCl:Na_2SO_4$: : 5: 3: 2 moles, 0·995 a_w). Plates were inoculated with 1 ml of the diluted suspension and, for each medium, triplicate plates were poured with *c.* 15 ml of the melted agar cooled to 45°C. The water in the inoculum was sufficient to increase the a_w of the agar at 0·970 a_w by almost 0·002 and at 0·980 a_w by *c.* 0·001. For media of higher a_w the change due to the water in the inoculum was very much less. In experiments in which different diluents were compared (Table 1) plates

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were inoculated with only 0·1 ml of the diluted suspension for each 15 ml of agar. This ensured that the diluent with the greatest solute concentration did not depress the a_w of the agar medium by more than 0·0002.

Plates were counted after incubation for 2 and 5 days at 30°C. To reduce evaporation from the medium, and, at the same time, to provide oxygen, the plates were incubated in metal containers with loosely fitting lids.

The culture studied was forwarded by Dr. D. I. Annear.

III. Results

(a) *Growth in Liquid Media*

The rates of growth in nutrient broth and brain heart infusion each adjusted to several levels of a_w with a number of solutes are shown in Figures 1 and 2 respectively. The values plotted are the means of two to four determinations. Figure 1 shows that

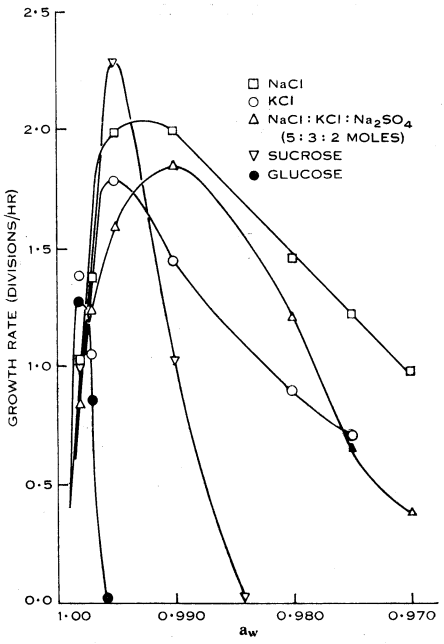

Fig. 1

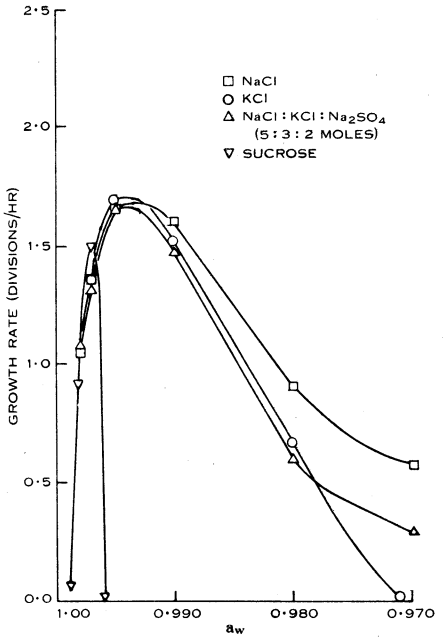

Fig. 2

Figs. 1 and 2.—Relation between rate of growth at 30°C and a_w for *Vibrio metchnikovi* in nutrient broth (Fig. 1) and in brain heart broth (Fig. 2) with added solutes.

all solutes tested caused a sharp increase in the rate of growth as the a_w was reduced below 0·999. For sodium and potassium chlorides and sucrose the maximum rates of growth occurred at 0·995 a_w. For the salts mixture the greatest rate of growth occurred at 0·990 a_w, whereas with glucose it was at 0·998 a_w. The inhibition by glucose at less than 0·997 a_w has not been explained. With the brain heart infusion (Fig. 2) similar trends with the various solutes were revealed, although stimulation in sucrose media was now greatest at 0·997 a_w, and in media containing added glucose growth was not

observed at all. Although the maximum rates of growth shown in Figure 2 are somewhat less than those in Figure 1, a greater rate of 2·4 divisions/hr was observed in brain heart infusion of 0·993 a_w and containing 37 g of dry matter per kilogram of water. This is the concentration of nutrients usually recommended for this medium, and is over 7 times the concentration used in the experiments summarized in Figure 2. For both the basal media the greatest rates of growth between 0·990 and 0·970 a_w were obtained when sodium chloride was the added solute.

(*b*) *The Plate Count*

The numbers of colonies which developed on nutrient agar adjusted to various levels of a_w with different solutes are shown in Figures 3 and 4. The inocula consisted

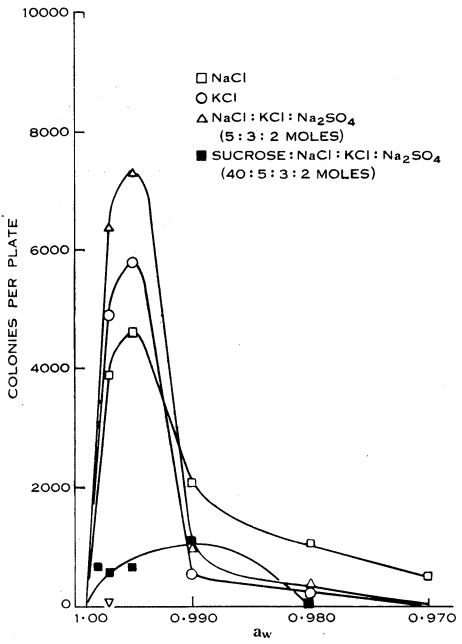

Fig. 3

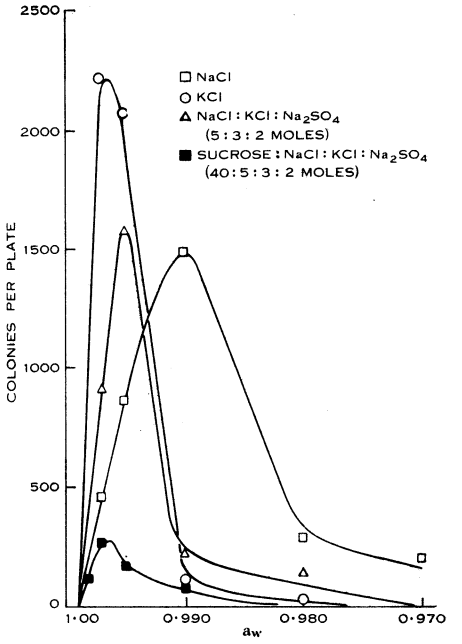

Fig. 4

Fig. 3.—Relation between plate count and a_w of nutrient agar for *Vibrio metchnikovi* grown at 0·995 a_w. Counts are means for triplicate plates inoculated with 10^{-6} ml of a culture grown for 6 hr at 30°C in nutrient broth adjusted to 0·995 a_w with KCl (0·121m).

Fig. 4.—Relation between plate count and a_w of nutrient agar for *Vibrio metchnikovi* grown at 0·975 a_w. Counts are means for triplicate plates inoculated with 10^{-6} ml of a culture grown for 18 hr at 30°C in nutrient broth adjusted to 0·975 a_w with NaCl (0·727m).

of replicate 1-ml aliquots of the same diluted suspension. It is clear that the addition of some solutes has had a large influence on the number of colonies which appeared. For instance, for the experiment summarized in Figure 3 only 24 colonies developed on the control basal medium of 0·999 a_w whereas over 7000 colonies appeared when the a_w was reduced to 0·995 with the salts mixture. In contrast to its effects on the rate of growth, sucrose has had little effect on the number of colonies. No colonies appeared

when a_w was adjusted with glucose, but over 1000 colonies were formed when a_w was controlled by a sucrose–salts mixture. For this latter mixture the maximum count occurred at 0·990 a_w whereas with the salts mixture the colony count was much greater at 0·995 a_w. For an inoculum grown at 0·975 a_w results in Figure 4 show similar large effects of electrolytes. No colonies occurred on the basal medium but over 2000 developed when the agar was adjusted to 0·995 or 0·997 a_w with potassium chloride. Addition of a salts mixture or sodium chloride produced media giving counts of *c.* 1600 and 1500 at 0·995 and 0·990 a_w respectively. When the a_w was adjusted with sucrose or glucose no colonies were found, but 267 developed on agar adjusted with the sucrose–salts mixture to 0·997 a_w. It may be seen that although grown at 0·975 a_w the

TABLE 1

EFFECT ON THE PLATE COUNT OF VIBRIO METCHNIKOVI OF VARIATIONS IN THE a_w OF THE PLATING MEDIUM AND OF THE DILUENT

The values represent the mean number of colonies on triplicate plates each inoculated with 10^{-6} ml of a saline broth culture. The culture consisted of nutrient broth adjusted to 0·975 a_w with NaCl, and incubated for 18 hr at 30°C

a_w* of Nutrient Agar Used for Plating Medium	a_w* of Diluent						
	0·999	0·997	0·995	0·990	0·980	0·975	0·970
0·999	1	1	3	5	1	2	3
0·997	45	1150	340	260	530	680	700
0·995	140	1920	1570	1860	1690	1610	2020
0·990	29	580	1630	990	650	1420	1530
0·980	0	990	810	1040	450	540	1430
0·970	0	39	220	79	300	330	400

*Controlling solute NaCl.

maximum count occurred on media adjusted to a_w between 0·990 and 0·997. Sodium chloride was the only solute permitting colony development at 0·970 a_w, but in neither experiment did this solute lead to the greatest number of colonies at 0·995 a_w. Results similar to those shown in Figure 4 have been obtained also when dilutions were made in solutions of potassium chloride or of a mixture of sodium and potassium chlorides and sodium sulphate at 0·995 a_w.

Although the results in Figure 4 indicate that cells grown at 0·975 a_w gave the greatest plate count at *c.* 0·995 a_w, the result may have been a consequence of diluting the cells at 0·995 a_w prior to plating. Accordingly, cells were diluted in sodium chloride solutions at seven concentrations between 0·999 and 0·970 a_w and subsequently plated on six agar media covering the same a_w range. Results of a typical experiment are given in Table 1, from which it may be seen that for all seven diluents the greatest numbers of colonies developed when the a_w of the agar was close to 0·995. In other words, the probability that a cell would form a colony was greatest at *c.* 0·995 a_w even for cells grown and diluted in more concentrated solutions of sodium chloride. It is

shown also in Table 1 that dilution in 0·999 a_w sodium chloride results in over 90 per cent. mortality. Similar trends have been shown in other experiments, using cells harvested during the logarithmic growth phase as well as during the stationary phase. There is, however, reason to believe that different cultures grown under apparently similar conditions may nevertheless vary in their susceptibility to lysis on dilution. In one exceptional experiment with stationary phase cells grown at 0·975 a_w it was found that diluents of 0·997 and 0·995 a_w were, like the 0·999 a_w diluent, markedly destructive, whereas dilution in more concentrated solutions caused no destruction. In further tests with the same series of diluents and agar media, destruction was confined to the 0·999 a_w diluent.

IV. Discussion

Comparison with results obtained for some other bacteria shows that *Vibrio metchnikovi* is relatively exacting in its osmotic and ionic requirements. For instance, in nutrient broth reduction of the a_w from 0·999 to 0·995 caused a five-fold increase in the rate of growth (Fig. 1), whereas with salmonellae (Christian and Scott 1953) and *Staphylococcus aureus* (Scott 1953) the same change in a_w increased the growth rate by only 10 and 20 per cent. respectively. The contrast between these bacteria and *V. metchnikovi* is even more striking when the relation between a_w and the plate count is considered. For salmonellae and staphylococci the plate count showed a considerable independence of a_w whereas for *V. metchnikovi* the probability that a cell would form a colony was extremely sensitive to relatively minor variations in the osmotic and ionic conditions of the plating medium.

Although the present experiments do not explain the osmotic sensitivity of *V. metchnikovi*, it is of interest to point out that some other organisms appear to have comparable properties. For instance, Koser, Breslove, and Dorfman (1941) reported that the growth of several species of *Brucella* was greatly stimulated by the addition of several electrolytes including sodium chloride. The calculated a_w of their basal medium No. 4 was close to 0·999, and the optimum concentration of sodium chloride reduced this to between 0·995 and 0·993.

The rate of growth of all the *Brucella* strains was increased by the addition of salts to the basal medium, and two strains of *Brucella suis* did not grow unless the amount of added sodium chloride was sufficient to reduce the a_w to about 0·997. Mager (1955) has reported that the growth of *Neisseria perflava* and *Pasteurella tularensis* was stimulated by various salts and sugars, the optimum concentration in each case corresponding to 0·997–0·995 a_w. Rodwell (1956) has shown that the growth of *Asterococcus mycoides* was sensitive to the tonicity of the medium when serum was replaced by other factors. These few organisms which are osmotically sensitive are also pathogenic to animals, and it may be that the parasitic existence in the closely controlled environment of the host has led to a loss of properties conferring resistance to osmotic change. With *A. mycoides* the lack of a rigid cell wall is an obvious factor which may lead to osmotic fragility. Further work would be needed to show whether the properties of the cell wall are a cause of the requirements manifested by *V. metchnikovi*, and the abovementioned *Brucella*, *Neisseria*, and *Pasteurella* strains.

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