EFFECT OF SORBED WATER ON THE DEATH RATE OF WASHED *SERRATIA MARCESCENS*

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In a previous paper (Monk *et al.*, 1956) on the effect of water on the death rate of *Serratia marcescens*, a particularly lethal effect was found at a water content of about 50 per cent in the mixture of cells and additives. Since the vapor pressure characteristics of the cells and each of the components was not known in detail it was not possible to determine how much of this water was in the cells and whether the rapid loss of viability was due to a toxic effect of some component.

This paper describes an experimental determination of the logarithmic death rates of this organism after rewetting to various water contents following lyophilization of washed cells. It was hoped that measurements of the stability in the absence of salts and other materials ordinarily present or deliberately added would indicate if different degrees of wetting would in itself be toxic to bacteria.

MATERIALS AND METHODS

Serratia marcescens strain 8UK was washed off tryptose agar with distilled water after 18–24 hr growth at 31 C, washed once and resuspended in distilled water at a concentration of about 10^{11} cells per ml. One half ml of this suspension, containing 30 mg of solids, was shell-frozen in one leg of the exposure apparatus illustrated in figure 1. The system was evaucated to about 100 μ pressure and the water sublimed into the second leg held in a dry ice and alcohol bath. The sample appeared dry and had warmed to room temperature after about 20 min, but the drying was continued for a total time of about 1 hr, after which the viable recovery was about 50 per cent. During the freeze drying the pressure indicated by a McLeod gauge did not rise over 2,000 μ. Usually eight samples were dried concurrently each day by this process, then rewetted, and a known amount and the viability were measured after various times of storage at room temperature (24–26 C). All viable counts were calculated as a percentage of the original count before drying and are the result of triplicate plating on nutrient agar following 10-fold dilutions in distilled water.

The rewetting was carried out by two processes:

(1) Since Stevens (1956, *unpublished data*) has recently measured the vapor pressure of *Serratia marcescens*, 8UK, it was possible to obtain a known water content by exposing the cells to a known water vapor pressure. Constant low vapor pressures were obtained by holding the ice trapped from the drying process at temperatures of about −80 C or −20 C. Higher vapor pressures were obtained by putting various salts into the trap before freeze-drying so that upon warming to 25 C a mixture of saturated salt solution and solid salt would result. The cells would then absorb water until their vapor pressure equaled that maintained by the mixture.

(2) At higher water contents these methods of wetting were too slow, since the vapor pressure of the cells approached that of water. To obtain rewetting above about 30 per cent moisture the water was added directly. Following freeze-drying the leg containing the ice was removed and replaced by the leg containing the dried sample. A known amount of water (10–500 λ from a microburette) was frozen in a clean leg which was then attached to the other side. Upon evacuating the system and cooling the sample to −80 C the ice quickly sublimed onto the bacterial cells. The apparatus was then warmed to 25 C so that the cells became wet. There was no evidence that cooling the dry sample lowered its viability. Of each group of eight samples, two were used to determine the water content by drying in vacuum at about 100 C.

Preliminary experiments showed that the presence of air and changes in temperature significantly altered the results at some moisture contents. In all the experiments described here the samples were stored in vacuum at room temperature during the exposure to water or water vapor.

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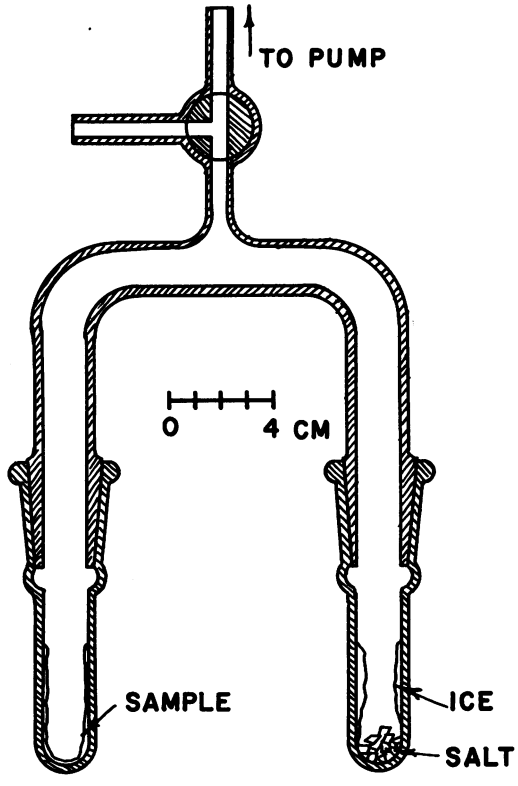

Figure 1. Diagram of the all-glass system used to freeze-dry and rewet the bacteria.

RESULTS

In order that the first method of wetting be meaningful it is necessary to show that the bacteria take up water rapidly until the equilibrium value is reached. The moisture content was measured during the process of freeze-drying and rewetting by determining the dry weight concentration on different samples carried through various fractions of the process. Figure 2 shows the loss in water due to freeze drying and the subsequent gain after the trapped ice and KNO_3 was raised to room temperature in order to establish a 94 per cent relative humidity. Within the accuracy of this method the final moisture content agrees with the value of 30.5 per cent found by Stevens. It is also apparent from figure 2 that the bacteria are spread out sufficiently to allow moisture equilibrium to be attained quickly during the experiment, and similar results were obtained at other vapor pressures.

The results of all the experiments are given in table 1. The logarithmic death rate, k, is defined as in the earlier paper (Monk *et al.*, 1956):

$$k = (1/t) \log_e N_0/N$$

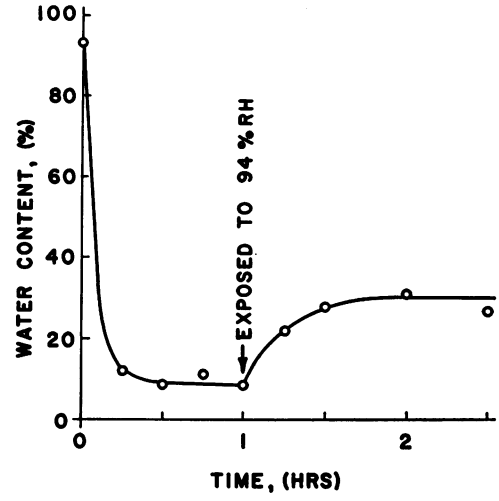

Figure 2. The change in water content of the bacterial suspension during freeze-drying and subsequent rewetting due to sorption of water at a vapor pressure corresponding to 94 per cent relative humidity.

TABLE 1

The effect of sorbed water on the death rate of washed Serratia marcescens

Method of Wetting	Number of Samples	Water Content (% of Total Weight)	Death Rate, k (Hr^{-1})	Standard Error in k (hr^{-1})
(1) −80 C	8	0.0	0.039	0.034
(1) −20 C	12	2.0	0.026	0.064
(1) LiCl, 15% RH	11	5.2	0.014	0.059
(1) $CaCl_2$, 31% RH	12	7.4	0.13	0.077
(1) $Mg(NO_3)_2$, 52% RH	8	11.0	0.13	0.058
(1) NaCl, 75% RH	21	18.0	0.81	0.23
(2) 0.015 ml H_2O	12	26.0	3.6	0.28
(1) KNO_3, 94% RH	15	31.0	3.9	0.31
(2) 0.020 ml H_2O	13	37.0	4.2	0.55
(2) 0.025 ml H_2O	10	43.0	3.1	0.45
(2) 0.038 ml H_2O	13	53.0	1.9	0.36
(2) 0.050 ml H_2O	11	60.0	0.59	0.072
(2) 0.100 ml H_2O	8	75.0	0.10	0.12
(2) 0.500 ml H_2O	6	92.0	0.10	0.03
(1) KNO_3, 94% RH (dialyzed)	14	31.0	4.3	0.89

where N was the number of viable cells in the sample t hr after N_0 were in moisture equilibrium. In all experiments the viability appeared to decrease exponentially with time.

The death rate was calculated as the negative

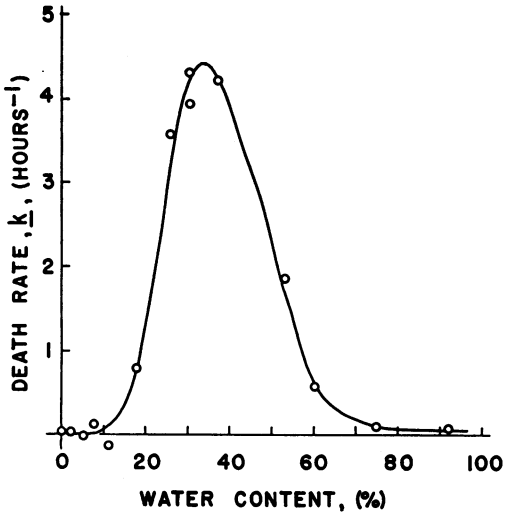

Figure 3. The logarithmic death rate of washed *Serratia marcescens* after rewetting to various water contents.

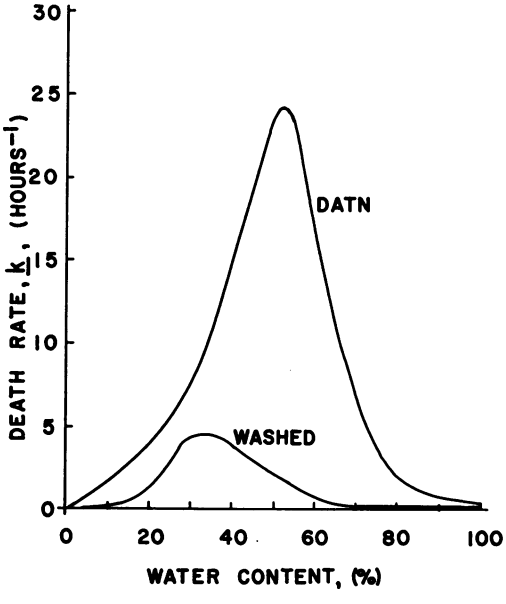

Figure 4. Comparison of the death rate of washed cells with that for cells in the presence of dextrin, thiourea, ascorbic acid, and ammonium chloride (DATN). (Monk *et al.*, 1956).

slope of a line fitted by the method of least squares to the experimental points giving the logarithm of the survival after various times. The number of samples includes all of those measured in a number of experiments carried out under identical conditions.

In order to show that residual salt was not causing the great sensitivity to water, two experiments were carried out on cells that had been dialyzed 2–4 days against distilled water at about 4 C. About 50 per cent of the cells survived the dialysis and died at the same rate as undialyzed cells. The final dialyzate had an electrical conductivity essentially equal to that of the distilled water used.

The average death rates from table 1 have been plotted as a function of water content in figure 3, and in figure 4 the present data are compared with those obtained earlier when dextrin, thiourea, ascorbic acid, and ammonium chloride were present.

DISCUSSION

The data presented in table 1 and figure 2 indicate that this species of bacteria is particularly sensitive to water concentrations of about 33 per cent and that the effect is not due to residual salt since the dialyzed cells died at the same rate as the undialyzed cells when rewet to nearly the most lethal water concentration. This most lethal amount of water is seen to be only about one eighth of the amount found in a normal cell containing about 80 per cent water. As seen in figure 4 the death rate of washed cells is lower at all water contents than the corresponding rate in the presence of a mixture of dextrin, ascorbic acid, thiourea, and ammonium chloride. Since the vapor pressure characteristics of these additives are not known it is not possible to determine the amount of water associated with the cells in the presence of these toxic additives. It remains to be demonstrated that these equilibrium death rates can be applied to cases in which the water content changes very rapidly in air as in an aerosol and that similar rates will be obtained when water is removed from a suspension.

The dependence of stability on water content can be explained qualitatively in a number of ways but a dearth of supporting evidence exists for all. For purposes of discussion most of the explanations can be grouped into three general classes.

(*1*) *Physical.* Water (33 per cent) is sufficient to bring the cell back to only about one third of its normal volume, assuming that it is collapsed and without voids. Perhaps the increased surface forces are sufficient to distort the structure of macromolecules or of a gross cellular organization. The increased surface-to-volume ratio may cause

local surface denaturation. If different parts of the cell are more hygroscopic than others, nonuniform swelling may occur with resultant distortions.

(*2*) *Denaturation.* At least one important substance in the cell may be sensitive to sorbed water. This does not explain the mechanism of denaturation but may localize the problem.

(*3*) *Metabolic.* Some steps in normal cell processes may be wet to activity preferentially while other less hygroscopic steps remain dormant when limited amounts of water are available. This could result in depletion of a necessary substance or production of an excess of a toxic material.

These suggestions can be tested in many ways and it is hoped that further experimental work can be reported later.

ACKNOWLEDGMENT

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SUMMARY

Serratia marcescens is shown to die at a maximum rate when in the presence of only a small amount of sorbed water, about one eighth of the normal intracellular water. The logarithmic death rate is about 4.5 hr^{-1} at 33 per cent water concentration, but is negligible below 15 per cent and above 70 per cent. Several possible explanations are discussed.

REFERENCES

MONK, G. W., ELBERT, M. L. STEVENS, C. L., AND McCAFFREY, P. A. 1956 The effect of water on the death rate of *Serratia marcescens.* J. Bacteriol., **72,** 368–372.