Recoveries of Salmonella ndolo from desiccates exposed to 100° C under various conditions with respect to desiccant, desiccant temperature and atmosphere

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SUMMARY

Desiccates of Salmonella ndolo dried in sodium glutamate have been exposed to 100° C. in air or *in vacuo*; in the presence of wet or dry P_2O_5 ; and with desiccant maintained at either 25° or 100° C. Of the eight treatments thus compared, rapid killing occurred where the desiccate was heated *in vacuo* and in the presence of dry P_2O_5 maintained at 100° C. There was little difference among the death-rates of the other seven treatments. Sublimation of the P_2O_5 with resultant attack on the desiccate would appear to be responsible for the rapid killing in the treatment where it was observed.

INTRODUCTION

In a study on the survival of dried bacteria at 100° C. (Annear & Bottomley, 1965) it was shown that recoveries were much modified by the condition of the phosphorus pentoxide which was used as a desiccant in the ampoules to produce dry atmospheres. In vacuo and where the P_2O_5 was maintained in the dry state, a relatively rapid killing occurred, associated with browning and bubbling of the desiccate. In vacuo and with the surface of the P_2O_5 wetted to generate phosphoric acid, these events did not occur, nor did they occur in air with either wet or dry P_2O_5 .

It seemed highly probable that the effects obtained with dry P_2O_5 in vacuo were due to sublimation of that compound and that they might be reduced if the P_2O_5 was kept cool during the heating of the desiccates. A comparison of survival was therefore made between two sets of desiccates; in one set the P_2O_5 was exposed to the same temperature as the desiccates (100° C.), while in the other the P_2O_5 was maintained near ambient temperature (ca. 25° C.). In each set the effects of wet and dry P_2O_5 were tested and also the effects of storage in air and storage in vacuo. In all, therefore, eight treatments were compared.

METHODS

A suspension of the test organism Salmonella ndolo (NCTC 8700) was made in 20% sodium glutamate and single-drop volumes of it were dried *in vacuo*. Small tubes of wet or dry P_2O_5 were included in the ampoules which were sealed either

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in vacuo or in air. Details of these procedures have been described previously (Annear & Bottomley, 1965). The desiccates were heated in an atmosphere of free steam and Fig. 1 shows the arrangement by which the two temperatures for the P_2O_5 were obtained and that by which the desiccates were held at the one temperature (100° C.). The constrictions in the ampoules were flattened so that in the upper set the P_2O_5 tubes were prevented from occluding the orifice between the body and neck of the ampoule.

Viable counts were made on blood-agar plates by methods also previously described (Annear, 1965).

RESULTS AND DISCUSSION

The results of treatments 5–8 (Table 1) confirmed those previously obtained, namely that with dry P_2O_5 in vacuo (treatment 8) a relatively rapid killing associated with browning of the desiccates was obtained, and that between the

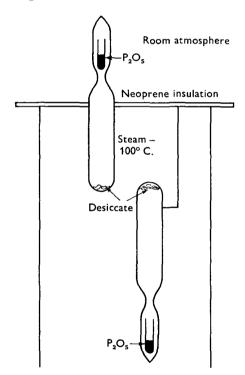


Fig. 1. Arrangement for maintaining desiccates at 100° C. and desiccant at either 100° or 25° C.

recoveries in the other three treatments there was little difference. Where the P_2O_5 was maintained at 25° C. (treatments 1–4) there were no large differences, although the recoveries from desiccates held *in vacuo* were somewhat higher than from those held in air.

The comparison of most interest in this investigation is that between treatments 4 and 8, and the results strongly indicate, as suggested, that the more rapid

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killing of the organism in treatment 8 is due to P_2O_5 , which sublimes more freely *in vacuo* than it does in air and more freely at the higher temperature than the lower.

In blank experiments it was shown by colorimetric methods that in conditions obtaining in treatments 1–7 only traces of phosphate were detected on the walls of the ampoules, whereas for conditions obtaining in treatment 8 tests for phosphate were strongly positive.

Table 1. Recoveries of Salmonella ndolo from desiccates exposed to 100° C. under eight sets of conditions with respect to desiccant condition, desiccant temperature and atmosphere

| - | P_2O_5 at 25° C. | | | | $P_2O_5 at 100^\circ C$ | | | |
|--------------------|-----------------------------------|-------------|-----------------------------------|-----------|-----------------------------------|-------------|-----------------------------------|-------------|
| Treatment | Wet P ₂ O ₅ | | Dry P ₂ O ₅ | | Wet P ₂ O ₅ | | Dry P ₂ O ₅ | |
| | Air 1 | Vac. 2 | Air 3 | Vac. 4 | Air 5 | Vac. 6 | Air 7 | Vac. 8 |
| Days at 100° C. | | | | | | | | |
| 3 | 6.1* | 6.5 | $6 \cdot 2$ | 6.6 | 6 ·0 | $5 \cdot 9$ | $6 \cdot 1$ | $2 \cdot 2$ |
| 5 | 4 ⋅8 | 5.1 | 4 ·8 | 5.5 | 4 ·8 | 4.4 | 4 ·8 | < 1 |
| 7 | 4 ·0 | 4.8 | $4 \cdot 0$ | 4.7 | $3 \cdot 8$ | 4 ·0 | 3.7 | < 1 |
| 9 | $2 \cdot 9$ | $4 \cdot 0$ | $2 \cdot 9$ | 4.1 | $2 \cdot 3$ | $2 \cdot 6$ | $2 \cdot 6$ | < 1 |

Initial number of organisms per tube = 6.0×10^9 . Recovery immediately after drying = 5.9×10^9 .

* Recovery expressed as log₁₀.

It seems most unlikely from a consideration of the results as a whole that P_2O_5 plays any role other than that of a desiccant at ambient temperature. However, it would be of interest to make some critical comparisons between this compound and other systems suitable for controlling low humidity levels in bacterial desiccates.

The highest recoveries obtained in these experiments reveal the remarkable heat-tolerance that can be conferred on some vegetative bacteria when dried and held under conditions of extreme desiccation.

REFERENCES

ANNEAR, D. I. (1965). Effect of heat during drying on survival of bacteria in desiccates. Australian Journal of Experimental Biology and Medical Science 43, 665.

ANNEAR, D. I. & BOTTOMLEY, G. A. (1965). Survival of bacteria in desiccates at 100° C. in dry atmospheres. Nature, London 206, 1373.