

QUANTITATIVE STUDY OF DAMAGE AND SURVIVAL OF
BACTERIA DURING FREEZE-DRYING AND DURING
STORAGE OVER A TEN YEAR PERIOD

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A broth culture was centrifuged, the pellet taken up in double-strength skim milk, and this suspension dispensed in 0.2 ml portions into ampoules (10 mm x 35 mm). The ampoules were frozen in an ethanol-dry ice bath, were placed in a jar at room temperature connected to a vacuum pump assembly, and the frozen suspensions were dried at 70-100 micra Hg overnight. The ampoules were removed from the vacuum jar, were placed in phials (14 mm x 60 mm), and the latter sealed at 70-100 micra. Assays were carried out prior to the freezing, immediately after freezing (thawed at room temp.), immediately after the drying period, and after various intervals of storage at 8°C. The phials were opened, the ampoule was wiped with ethanol, was air dried, and then was pulverized by shaking within a bottle containing saline and several glass marbles. From this initial dilution further serial dilutions were prepared and aliquots were plated on suitable agar. Table I summarizes viability data with 12 species.

TABLE I
Viability after freezing, drying, and freeze-dried storage
(All assays represent the number of cells in the ampoule)

Species	Initial count	After freez.	After dry.	After storage for	1 wk.	8 wks.	12 wks.	10 yrs.
<i>E. coli</i>	9x10 ⁹	9x10 ⁹	8x10 ⁹	3x10 ⁸	2x10 ⁸	2x10 ⁸	2x10 ⁸	2x10 ⁸
<i>V. costi.</i>	3x10 ⁸	4x10 ⁸	----	5x10 ⁴	5x10 ⁴	2x10 ⁴	2x10 ⁴	2x10 ¹
<i>L. bifidus</i>	4x10 ⁸	3x10 ⁸	3x10 ⁸	1x10 ⁹	4x10 ⁸	3x10 ⁸	2x10 ⁷	2x10 ⁷
<i>L. fermenti</i>	7x10 ⁸	6x10 ⁸	3x10 ⁸	2x10 ⁸	1x10 ⁸	1x10 ⁸	1x10 ⁸	1x10 ⁸
<i>Ac. melano.</i>	7x10 ⁸	7x10 ⁸	5x10 ⁷	3x10 ⁷	8x10 ⁷	----	2x10 ¹	2x10 ¹
<i>Aer. aerog.</i>	5x10 ⁹	5x10 ⁹	2x10 ⁹	9x10 ⁹	1x10 ⁹	9x10 ⁸	7x10 ⁸	7x10 ⁸
<i>Ps. species</i>	7x10 ⁹	9x10 ⁹	1x10 ⁹	7x10 ⁸	4x10 ⁸	9x10 ⁸	4x10 ⁸	4x10 ⁸
<i>Ps. aerugin.</i>	4x10 ⁹	3x10 ⁹	1x10 ⁸	5x10 ⁷	6x10 ⁷	----	7x10 ⁵	7x10 ⁵
<i>Ps. chloro.</i>	1x10 ¹⁰	9x10 ⁹	8x10 ⁶	----	1x10 ⁵	6x10 ⁵	3x10 ⁵	3x10 ⁵
<i>Prot. vul.</i>	3x10 ⁹	3x10 ⁹	6x10 ⁷	5x10 ⁷	1x10 ⁷	----	5x10 ⁶	5x10 ⁶
<i>Ser. marces.</i>	1x10 ⁹	9x10 ⁸	2x10 ⁸	2x10 ⁸	1x10 ⁸	1x10 ⁷	5x10 ⁷	5x10 ⁷
<i>M. albus</i>	5x10 ⁹	5x10 ⁹	3x10 ⁹	2x10 ⁹	7x10 ⁸	7x10 ⁸	9x10 ⁸	9x10 ⁸

Freezing (with thawing) causes little, if any, loss of titer. But the subsequent manipulations cause a measurable loss of titer which varies among species. The loss of viability is either due to