Protection Branch Report of Test No. 16-67

Recovery of Vegetative Bacteria from Eccofoam FP and Diatomaceous Earth

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At the request of Lawrence B. Hall, Planetary Quarantine Officer, NASA, a brief investigation was undertaken to determine whether vegetative bacteria can (1) survive the polymerization process of a plastic and (2) survive for short periods thereafter in plastic or other materials. This Laboratory showed early in its spacecraft sterilization studies that the bacterial contamination recovered from the inside of electronic components was frequently a vegetative cocci [1]. The work was qualitative since it has not been possible to determine the total number of viable microorganisms present inside a component. It is not technically feasible to completely pulverize or dissolve a component and liberate all entrapped microorganisms without killing an unknown number. The period that vegetative bacteria will survive in the components has not been specifically tested. Previous tests in this Laboratory did show that B. subtilis spores can survive for more than two years in Eccofoam FP plastic. Since diatomaceous earth was found to offer protection for spores against dry heat [2], it also was included in these tests.

Thus, this investigation was designed to include the following criteria: (1) a high concentration of vegetative cells incorporated in a solid to insure recovery if a reasonable percentage survive a specific time: (2) the use of a test organism that has relatively low survival in a dry state at room temperature; and (3) a test span of one to three weeks.

The results obtained for the recovery of vegetative cells from Eccofoam FP and diatomaceous earth are given herein.

MATERIALS AND METHODS

Eccofoam FP

About a billion lyopholized powdered Serratia marcescens cells were mixed into ten grams of Eccofoam FP* (polyurethane) then one gram of Catalyst 12-6 was added to the mixture. After curing one day at room temperature, one-fourth of the sample was cut into small pieces and divided

* Manufactured by Emerson & Cumings, Inc., 869 Washington St., Canton, Mass.

among three 0.05% Tween 20 blanks containing glass beads. The samples were shaken ten minutes then assayed for viable count by the pour plate method with trypticase soy agar as culture medium. Plates were incubated at 32 C for 24 hours before colony counts were made. This procedure was repeated 7, 14, and 21 days later.

Diatomaceous Earth

The lyopholized powdered S. marcescens cells were suspended in water previously boiled to dissipate the chlorine. Ten milliliters of this suspension (9×10^6/ml) were mixed with $1\frac{1}{2}$ grams of diatomaceous earth. The thick-moist mixture was packed into a 2-ml syringe previously modified by cutting the tip flush to the bottom of the barrel [27]. Immediately, the mixture was extruded in 1/2 cc portions (pellets) on to a glass plate, severing the portions with a scapel. The pellets were allowed to air dry one day. Then each of four pellets were placed in a 0.05% Tween 20 blank containing glass beads and shaken 10 minutes. The samples were assayed for viable count in the manner described above.

RESULTS AND DISCUSSION

The results summarized in Table I indicate that viable vegetative cells of S. marcescens entrapped in Eccofoam FP or diatomaceous earth can be recovered after various periods of time. Since small, but not micron sized pieces of Eccofoam FP were assayed, it is probable that most of the viable bacteria entrapped in the plastic were not liberated or recovered. However, a significant number of organisms was recovered to indicate that vegetative bacteria entrapped in a solid could be viable for weeks. It appears that S. marcescens would remain viable longer in Eccofoam FP than in diatomaceous earth.

Eccofoam is a polyurethane foam that probably is only slowly permeable to water vapor. The rate dry S. marcescens dies is dependent on the relative humidity. Previous studies [27] showed that lyopholized S. marcescens on glass can survive at least several days at high, medium and low RH and for several months at high and low RH's at 25 C but that it dies off at intermediate RH values. It is recognized that a variable RH can be much more lethal than a constant RH, to such organisms.

When about a million S. marcescens cells in aqueous suspension were dried on glass at room temperature for 24 hours only one organism was recovered. However, some S. marcescens survived over a week in diatomaceous earth. The reason for this is probably due to the good survival rate of moist or wet S. marcescens but once the organism dries at normally encountered RH's it dies rapidly. The rate of drying is a highly important factor in the survival rate of vegetative microorganisms. A rapid desiccation as in lyopholization or freeze-drying yields a much greater survival

rate than the relatively slow drying from an aqueous suspension at ambient conditions. It is apparent that the diatomaceous earth dried at a slower rate and was still moist after one day as evidenced by the lack of kill in the one day sample.

Although the test procedure used in this study of the survival of S. marcescens in Eccofoam FP was qualitative, an approximate D value can be calculated since the method of sampling was constant. In other words, the amount of surface exposed by cutting up the sample for assay each time was approximately the same. The death rate is logarithmic and the D value about 4.5 days. Without considerably more data the death rate for S. marcescens in diatomaceous earth cannot be determined.

The ability of vegetative bacteria to survive in an electronic component undoubtedly depends upon the microorganism and the material involved. However, on the basis of the data presented here it must be assumed that vegetative bacteria entrapped in a component can remain viable over an extended period of time.

References

1. Protection Branch Report of Test No. 19-60: "Investigation of Bacterial Contamination Inside Electronic Components. Test I.", dated 14 April 60.

2. Protection Branch Report of Test No. 13-67: "Quantitative Spore Recoveries from Diatomaceous Earth Pellets Used as Protective Material in Dry Heat Sterilization Studies", dated 23 February 1967.

3. Hoffman, R.K. Unpublished data.

Table I.

Recovery of S. marcescens from Eccofoam FP and Diatomaceous Earth

Time (Days)	Number Organisms Recovered From Eccofoam FP*	Number Organisms Recovered From Diatomaceous Earth**
1	6,000,000	35,000,000
7	1,500,000	2,200
14	6,000	-
21	1,500	-

* Each entry is based on the total number organisms recovered from the three samples assayed.
The estimated control = 250,000,000 S. marcescens cells.

** Each entry is based on the total number organisms recovered from the four pellets assayed.
The estimated control = 24,000,000 S. marcescens cells.

\- Not assayed.