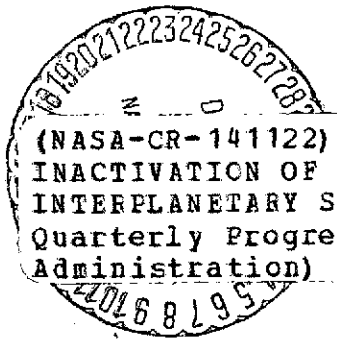
ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Thirty-seventh Quarterly Report of Progress

Order No. W-13411

April 1, 1974 - June 30, 1974



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Introduction

For the past several years, dry heat sterilization studies for spacecraft sterilization were based upon the thermal inactivation characteristics of Bacillus subtilis var. niger spores. However, other workers have demonstrated significantly more heat resistant spores under dry heat. These spores are naturally occurring populations (i.e., mixed populations of spores employed directly without intermediate isolation and subculture on conventional laboratory media).

More recently, results from Cape Kennedy "fallout strips'" experiments (J. R. Puelo, personal communication) showed recovery of microbial survivors from 113 C and 0.134% RH dry-heat cycles. These survivors were subcultured in tubes of supplemented TSA. Thirty-three of these isolates were identified at Cape Kennedy and biochemical test results are shown in Table 1. According to some workers, spores lose much heat resistance when subcultured. Other workers found that spore resistance (heat or radiation) was maintained or gained by applying cultural techniques.

In view of these findings we concentrated our efforts this quarter in developing some means of growing these isolates and then conducting studies to characterize the thermal profile of these isolates.

I. EXPERIMENTAL

A. Microbiological procedures

Thirty-three of these recovered isolates were subcultured in tubes of TSA medium (fortified with 0.1% starch and 0.2% yeast extract) at the Cape Kennedy laboratory and sent to the FDA Cincinnati Food Research Laboratory.

Of the 33 isolates, 4 were selected at random and were subcultured into tubes containing trypticase soy broth plus 0.1% starch and 0.2% yeast extract. These tubes were incubated at 35 C for 1-2 weeks. One of the isolates (8-25) failed to grow, whereas the other 3 isolates had growth after more than one week of incubation.

1. Production of spore crop

Three spore crops from the actively growing cells (4-6, 6-12, and 7-11) were produced by surface inoculating spore growth agar medium (Seitz filtered glucose, 0.25%; Casamino acids (Technical), 0.25%; yeast extract, 0.5%; $MnSO_4 \cdot H_2O$, 0.001%; $FeSO_4 \cdot 7H_2O$, 0.0014%) in three 250-ml Roux culture flasks followed by incubation for 10 days at 35 C. Spores were harvested by flooding approximately 100 ml of double distilled sterile water to the bottle and scraping gently the matted spore surface. The spore suspension was poured in an Erlenmeyer flask containing sterile glass beads and then shaken thoroughly. After shaking, the spore suspension was poured through sterile non-absorbent cotton. Additional

double distilled sterile water was passed through the cotton to make a final volume of 150 ml. The spore suspension was centrifuged at 6,000 rpm for 30 minutes at 5 C.

The sediment was resuspended in fresh double distilled water (100 ml) and placed in a 45 C water bath overnight. Following this heat treatment, the suspension was centrifuged and washed five times in 150 ml of double distilled sterile water at 6,000 rpm for 30 minutes at 5 C. The final clean spore suspension was observed microscopically (Bartholomew and Mittwer's stain) for debris and percentage of spores present. The clean spores were stored in double distilled sterile water at 5 C.

B. Thermal inactivation studies

The experiments were carried out in the conventional manner. Four spore crops (4-6, 6-12, 7-11, and B. subtilis var. niger) were suspended in double distilled sterile water for the working suspension. The suspensions were sonified and after sonication dispensed with a repeating dispenser in 0.01 ml amounts in stainless steel cups to give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 200 x 306 tin cans. Each can contained four shelves (30 cups per shelf) for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 110 minutes at 45 to 50 C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 minutes for the

first 100 minutes, followed by five consecutive purges of nitrogen, with a vacuum cycle between each purge. After drying, the cans, lids, and contents were removed from the oven and cooled to about 30 C in the equilibration hood to give about 1.3 μg H_2O/cm^3 per can.

The cans were sealed and removed from the equilibration hood. The seams on each can were soldered and wiped to prevent leakage of water vapor during the heating cycle. The cans were heated at 113 C for varying times and cooled in an ice bath. The cans were opened with an automatic can opener, and sterile microbeads were added in each sample cup (heat treated and non-heat treated) and placed in 10 ml of sterile peptone water and sonified for 24 minutes. Spore assays were made by the conventional plate count method using trypticase soy agar fortified with 0.1% starch and 0.2% yeast extract. The plates were incubated for 3-5 days at 35 C and the results were plotted on semilog paper.

C. Heat shock treatment

For spore crop (7-11) non-heated samples and samples exposed at 113 C for 5 and 10 hr were heat shocked for 5 minutes at 100 C in peptone water before plating.

II. RESULTS AND DISCUSSION

The survival curves for spores of B. subtilis var. niger, B. brevis (4-6), B. lentus (6-12), and B. coagulans (7-11) treated at 113 C and 0.134% RH are presented in Fig. 1. In all cases, the heat resistance of the three Cape Kennedy spore isolates were significantly higher than the heat resistance for B. subtilis var. niger

spores. About 6 log kill was achieved in 10 hr for B. subtilis var. niger spores, whereas for one of the isolates (B. brevis or 4-6) less than a log kill resulted in 60 hr exposure.

It is evident from the results obtained in this study that the three spores produced from isolates (4-6, 6-12, and 7-11) exhibited a high dry-heat resistance compared to spores of B. subtilis var. niger.

It appears that if the right cultural conditions were applied, the inherent resistance of spores from a parent cell could be maintained.

Table 1. Biochemical test results on heat survivors from Cape Kennedy experiments of fallout strips

Culture number	Starch	Casein	Mannito	VP	Citrate	Nitrate	Anaerobic growth	Tyrosine	Phenylalanine	Identification
1-12	+	+	-	-	-	+	-	-	-	Atypical B.
1-29	-	-	-	-	-	-	-	-	-	Atypical B.
2-18	+	-	-	-	-	-	-	-	-	B. lentus
5-19	-	-	-	-	-	-	-	-	-	Atypical B.
*4-6	-	-	-	-	-	+	-	-	-	B. brevis
*6-11	-	+	-	-	-	+	-	-	-	B. brevis
*6-12	+	-	-	-	-	-	-	-	-	B. lentus
6-25	+	+	-	-	-	-	-	-	-	Atypical B.
6-28	+	-	-	-	-	-	-	-	-	B. lentus
6-32	+	-	-	-	-	-	-	-	-	B. lentus
*7-11	+	-	-	+	-	+	+	-	-	B. coagulans
8-14	-	+	-	-	-	-	-	+	-	Atypical B.
*8-25	+	+	-	-	-	-	-	-	-	Atypical B.
*8-28	+	+	-	-	-	+	-	-	-	Atypical B.
9-12	+	+	+	-	-	-	-	-	-	B. lentus
9-13	+	-	-	-	-	-	-	-	-	B. lentus
10-2	+	+	-	-	-	+	-	-	-	Atypical B.
10-20	+	-	+	-	-	-	-	--	-	B. lentus
10-30	+	-	-	-	-	+	-	--	+	Atypical B.
10-17	+	-	-	-	-	-	-	-	-	Atypical B.
13-20	+	-	-	-	-	-	-	-	-	B. lentus
16-16	+	-	-	-	-	-	-	-	-	B. lentus
16-23	+	-	-	-	-	-	-	-	-	Atypical B.

Table 1. Biochemical test results on heat survivors from Cape Kennedy experiments of fallout strips (continued)

Culture number	Starch	Casein	Mannito	VP	Citrate	Nitrate	Anaerobic growth	Tyrosine	Phenylalanine	Identification
18-10	+	-	-	-	-	+	-	-	+	Atypical B.
18-16	+	-	-	-	-	-	-	-	-	B. lentus
18-31	+	-	-	-	-	-	-	-	-	B. lentus
18-29c	+	-	-	-	-	-	-	-	-	B. lentus
18-29w	+	-	-	-	-	-	-	-	-	B. lentus
20-27	+	+	-	-	-	-	-	-	-	Atypical B.
30-6	+	+	-	-	-	+	-	-	-	Atypical B.
32-15					In process					
33-22										
34-11										

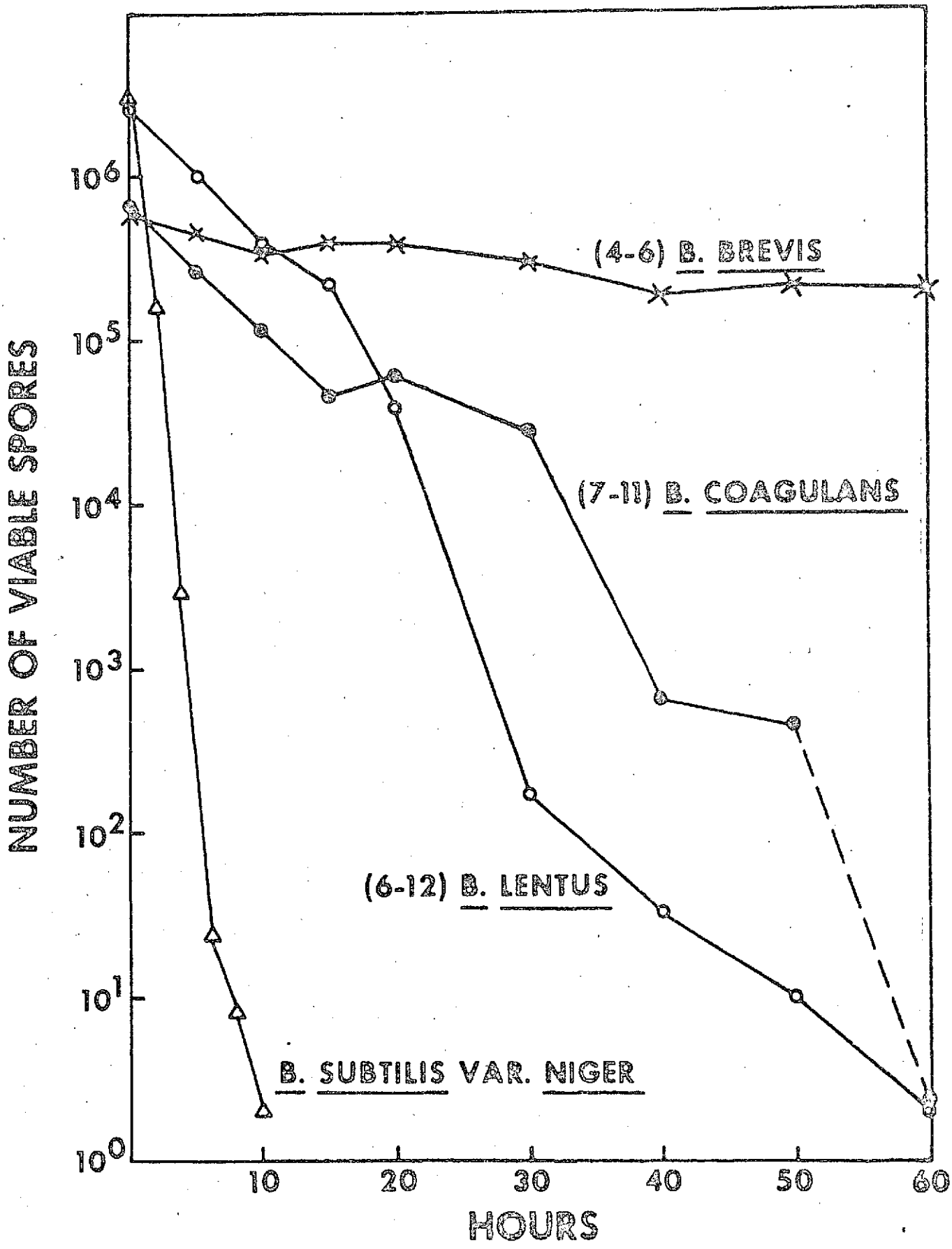

FIG. 1.-- THERMAL INACTIVATION OF SPORES 113° C AND 0.134% R.H (1.3 ug H_2O/CM3)