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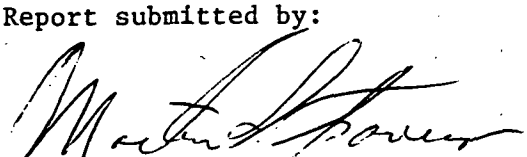

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1. Evaluation of the terminal sterilization process for unmanned lander spacecrafts was continued. An experiment was performed to test the homogeneity of the model terminal sterilization cycle oven by loading the oven in the conventional manner with 70 trays of 29 cups each. The top shelf and bottom shelf were loaded with 24 trays each and the middle shelf with 22 trays. Each cup was inoculated with approximately 10^5 spores of B. subtilis var. niger, and placement of trays was made on the basis of a random number chart. The oven was closed and purged with dry nitrogen (mil. spec.) overnight. The oven was then manually programmed from room temperature to 120 C over an approximate 7 hr period, held for 1 hr, and allowed to cool for 2 hrs. The integrated lethality of the program was equal to 3.5 hrs at 120 C.

Before heating, one cup from each tray was pulled for assay in order to estimate the original concentration. Analysis of these assays indicated that the average number of organisms per cup was 7.1×10^5 and that the distribution around the mean fell well within the requirements to assume no significant differences between cups.

The heat treatment applied reduced the viable population approximately 5 logs, the mean concentration being 21.36 organisms per cup. Examination of the position of the survivors in the oven suggested that the front row of trays on the top shelf did not receive the same thermal experience as the rest of the oven. If this row was excluded, the mean number of survivors was 18.25 per cup. Although there was a small shelf to shelf variation, this is not regarded as a serious problem. The major problem encountered with these data (in terms of meeting the constraints necessary for expressing results in the form of probability statements) relates to the skewness of the distribution of survivors. Accordingly an effort is being made to evaluate these patterns to determine if reasonable probability statements can be generated from data collected in the model terminal sterilization facility.

A new USDA monograph (in the process of being published) by Dr. Ruth Gordon of Rutgers University necessitated re-examination of Apollo identification scheme for Bacillus sp., since the scheme is based primarily on the 1952 USDA monograph No. 16 by Smith, Gordon and Clark. Evaluation of the presently used Bacillus sp. identification scheme version II was undertaken using ATCC and NRS standard Bacillus stock cultures obtained from Dr. Gordon. The new monograph on Bacillus identification revealed the addition of new biochemical tests which provided more consistent results than those employed in the earlier monograph. A version III of the identification scheme was developed to include the new biochemical tests and then challenged by testing with known ATCC and NRS stock cultures. The biochemical tests were done in triplicate for each Bacillus culture when version II and III of the Bacillus identification schemes were compared and evaluated.

Table 1 shows the biochemical test deviations encountered with the version II identification scheme. Data was not available from Dr. Gordon's monograph for anaerobic reduction of nitrate or hydrolysis of gelatin

for any of the stock cultures. When the total number of all test deviations were enumerated, 102 were detected for version II compared to 54 using version III scheme as shown in Table 2.

Version III of the Bacillus scheme was incorporated into the computer Qualitative Identification Program, and Table 3 shows the results when the computer program was challenged by test reactions of 72 standard cultures of Bacillus sp. Correct identifications were made for 68 (94.4%) cultures. Four cultures had more than two deviations and were identified as atypical by the computer.

Version III Bacillus identification scheme (Table 4) was adopted for general use and will be incorporated in the Apollo Identification Scheme. Media and methods employed in the version III scheme will be reported later.

Studies to examine the possibility of shifts in biochemical reaction patterns during storage and subculture of environmental Bacillus isolates were continued. Thirty environmental Bacillus sp. isolates from the MSOB clean room and thirteen standard cultures obtained from Dr. Gordon's collection were subjected to three types of handling prior to identification. Cultures were initially identified after two subcultures on TSA using the version III Bacillus scheme. The three culture handling methods were as follows:

1. Two sequential subcultures on TSA and storage at 4 C for 5 weeks.

2. Ten sequential subcultures on TSA, twice weekly for 5 weeks. (Cultures identified after 5 and 10 subcultures)

3. Cultures from 2. above were subcultured and stored at 4 C for 3 weeks.

Changes in biochemical characters due to subculture and storage are shown in Table 5. Variations were observed in most biochemical tests regardless of the type of handling. The greatest number of deviations occurred in the test for anaerobic growth. Results indicate that Bacillus sp. are capable of extreme variations within a single species or biochemical test. Studies are continuing using different stock cultures and fresh environmental isolates.

Due to unusually high dry heat resistance values of several naturally occurring spore populations collected in the Cape Kennedy area, it was thought that certain environmental conditions or stresses indigenous to that area may have accounted for the high degrees of biochemical instability when freshly isolated Bacillus sp. were subcultured. Accordingly, 14 environmental isolates were collected in the Phoenix area for a preliminary study. Identification scheme, version III, was used and the isolates were subcultured twice weekly on TSA for a period of 10 weeks. Biochemical tests were performed weekly in duplicate for the duration of

the subculture period. The number of cultures showing a change in a particular biochemical character at any point during the 20 subcultures is shown in Table 6. As with the Cape Kennedy isolates, a high degree of instability was noted indicating that this phenomenon is not peculiar to the Cape Kennedy area. The only consistent change noted in the biochemical tests was the gain of ability by 8 isolates to ferment mannitol after two subcultures. The remainder of deviations from the original reactions consisted of an irregular pattern of losses and gains of abilities during the 20 subcultures. Ten additional environmental isolates from the Phoenix area are being examined and results will be reported later.

2. Identifications were made of heat resistant Bacillus sp. isolated from the thermal studies conducted in Phoenix. From 62 isolates, 39 (63%) of these were identified as atypical Bacillus sp. Because of the high percentage of atypical species, the data was studied to determine any common relationships between isolates. Table 7 shows the correlations between the source isolated, heat exposure time, and the positive biochemical test reactions. The data showed that of those isolates which survived prolonged heating intervals above 8 hrs, 90% had the ability to utilize nitrates which could indicate a taxonomical relationship.

3. The study to determine the thermal resistance of naturally occurring airborne spores (Q.R. #35-38) was continued using the heating times of 2, 4, 6, and 8 hrs at 125 C. This set of experiments (31-42) marks the end of experiments at 125 C. Future experiments will be carried out using 113 C and 6, 12, 18, and 24 hrs heating times. Table 8 summarizes the data from experiments 1 through 42. The designations Area I and Area II have been used in place of "old area" and "new area" (Q.R. #37) respectively. Initially there appeared to be a difference in levels of fallout between Area I and Area II (Q.R. #37), but in subsequent experiments, no qualitative or quantitative differences were observed between Area I and II.

   A summary of all teflon experiments conducted at 125 C according to heating time is shown in Table 9. Eight hours was the longest heating time yielding survivors. The D_{125C} values were calculated using the FN-MPN technique of Pflug and Schmidt and ranged from 25 to 126 minutes.

   As previously mentioned (Q.R. #34), isolates including Bacillus firmus and B. sphaericus isolates from Apollo spacecraft samplings, heat resistant isolates from AO Hangar vacuum cleaner dust (Q.R. #33-35) and sporeformers recovered from spacecraft piece-parts using the biodetection grinder (Q.R. #34 and #35) were sporulated on TAM Sporulation Agar and tested for heat resistance at 125 C using the 5-tube FN-MPN technique (Q.R. #26 and #35). In addition, 3 isolates from the initial teflon ribbon fallout experiment by JPL personnel (Q.R. #35) were included in the assays. Table 10 shows the D_{125C} values and identifications (Bacillus identification scheme II) for 59 isolates. Excluding the Apollo isolates (chosen particularly for the firmus or sphaericus species), 54% of the isolates tested were either B. subtilis or atypical Bacillus sp. Of particular interest were the

large number of atypical Bacillus sp. within the piece-part isolate group. Also, the most heat resistant isolate in the series was from the JPL teflon ribbon experiment (isolate no. 7-R-18, D_{125C} = 166 min). As shown in Table 11, no correlation could be made between heat resistances and species with this series of isolates.

Due to the lower dry heat temperatures being tested for the terminal sterilization cycle for spacecraft, the resistance of the standard spore crop of B. subtilis var. niger (BG-SSM10, Q.R. #25) was examined by plating of survivors after exposure to 113 C in the usual manner employing 5 stainless steel strips per heating interval. The starting number of spores per strip was 5×10^6 and the population was reduced approximately 2 logs in 9 hrs of exposure. The D_{113C} calculated from a best fit line of survivor points was 3.5 hrs.

TABLE 1. DEVIATIONS FROM EXPECTED RESULTS COMPARING ATCC AND NRS STOCK CULTURE REACTIONS TO IDENTIFICATION SCHEME VERSION II

Microorganism	No. of Cultures	Tests										Deviations From Stock Reactions
		50° Growth	An. Glucose	An. Nitrate	P.R. Mannitol	Starch	Gelatin	Citrate	VP	Nitrate	Indol	
B. subtilis	4	1	2	ND			ND	4				7
B. megaterium	5	5	2	ND			ND	3				10
B. licheniformis	5		3	ND			ND	5				8
B. cereus	6	6		ND	4	1	ND	6				17
B. firmus	5	5		ND	2		ND					7
B. pumilus	5	2	5	ND			ND	4				11
B. sphaericus	6	1		ND			ND	5				6
B. laterosporus	5	1		ND			ND		4		2	7
B. coagulans	6		1	ND			ND	1				2
B. brevis	6	2	1	ND	2		ND	1	1	2		9
B. alvei	6		1	ND			ND				4	5

TABLE 1. DEVIATIONS FROM EXPECTED RESULTS COMPARING ATCC AND NRS STOCK CULTURE REACTIONS TO IDENTIFICATION SCHEME VERSION II (Continued)

Microorganism	No. of Cultures	Tests										Deviations From Stock Reactions
		50° Growth	An. Glucose	An. Nitrate	P.R. Mannitol	Starch	Gelatin	Citrate	VP	Nitrate	Indol	
B. macerans	6		1	ND			ND	2	1			4
B. polymyxa	6		6	ND			ND					6
B. circulans	5	3		ND			ND					3
TOTAL	76	26	22	ND	8	1	ND	31	6	2	6	102

ND = no data

TABLE 2. DEVIATIONS FROM EXPECTED RESULTS COMPARING ATCC AND NRS STOCK CULTURE REACTIONS TO IDENTIFICATION SCHEME VERSION III

Microorganism	No. of Cultures	Tests									Deviations From Stock Reactions
		Starch	Casein	P.R. Mannitol	VP	Citrate	Nitrate	Anaerobic Growth	Tyrosine	Phenyl-alanine	
B. subtilis	4										0
B. megaterium	5								2		2
B. licheniformis	5				1						1
B. cereus	6	1		2	1			1			5
B. firmus	5			1	1		1			3	6
B. pumilus	5							1			1
B. sphaericus	6	1	1	1	1	2		2	1	4	13
B. laterosporus	3										0
B. coagulans	5	3	2			2	1	2			10
B. brevis	5			2	1	1	1	1	1		7
B. alvei	6			1	2				2		5

TABLE 2. DEVIATIONS FROM EXPECTED RESULTS COMPARING ATCC AND NRS STOCK CULTURE REACTIONS TO IDENTIFICATION SCHEME VERSION III (Continued)

Microorganism	No. of Cultures	Tests									Deviations From Stock Reactions
		Starch	Casein	P.R. Mannitol	VP	Citrate	Nitrate	Anaerobic Growth	Tyrosine	Phenyl-alanine	
B. macerans	6				1	1					2
B. polymyxa	6										0
B. circulans	5		1					1			2
TOTAL	72	5	4	7	8	6	3	8	6	7	54

TABLE 3. RESULTS OF COMPUTER IDENTIFICATION USING ATCC AND NRS STOCK CULTURES

Microorganism	Number of Cultures	Identification			
		Exact	1 Deviation	2 Deviations	Atypical
B. subtilis	4	4			
B. megaterium	5	5			
B. licheniformis	5	4	1		
B. cereus	6	3	1	2	
B. firmus	5	0	4	1	
B. pumilus	5	4	1		
B. sphaericus	6	1		4	1
B. laterosporus	3	3			
B. coagulans	5	1	1	1	2
B. brevis	5	4			1
B. alvei	6	3	3		
B. macerans	6	5	1		
B. polymyxa	6	6			
B. circulans	5	2	3		
TOTAL	72	45	15	8	4

TABLE 4. CURRENT IDENTIFICATION SCHEME FOR BACILLUS SP.

Culture Number	Starch	Casein	P.R. Mannitol	VP	Citrate	Nitrate	Anaerobic Growth	Tyrosine	Phenyl-alanine	I.D.
B. alvei	+	+	-	+	-	-	+	-	-	
B. cereus	+	+	-	+	+	+	+	+	-	
B. circulans	+	-	+	-	-	-	+	-	-	
B. coagulans	+	-	-	+	-	-	+	-	-	
B. firmus	+	+	+	-	-	+	-	-	+	
B. laterosporus	-	+	+	-	-	+	+	+	-	
B. licheniformis	+	+	+	+	+	+	+	-	-	
B. macerans	+	-	⊕	-	-	+	+	-	-	
B. megaterium	+	+	+	-	+	-	-	+	+	
B. polymyxa	+	+	⊕	+	-	+	+	-	-	
B. pumilus	-	+	+	+	+	-	-	-	-	

TABLE 4. CURRENT IDENTIFICATION SCHEME FOR BACILLUS SP. (Continued)

Culture Number	Starch	Casein	P.R. Mannitol	VP	Citrate	Nitrate	Anaerobic Growth	Tyrosine	Phenyl-alanine	I.D.
B. subtilis	+	+	+	+	+	+	-	-	-	
B. sphaericus	-	V	-	-	+	-	-	-	+	
B. lentus	+	-	+	-	-	-	-	ND	ND	
B. brevis	-	+	V	-	V	V	-	+	-	

(+) - Indicates gas production

ND = no data

V = variable

TABLE 5. CHANGES IN BIOCHEMICAL TESTS OF BACILLUS MICROORGANISMS AFTER FOUR TYPES OF CULTURE HANDLING PRIOR TO IDENTIFICATION

ATCC and NRS Stock Cultures	Number of Test Cultures	Deviations from Initial Reactions[1]								
		Starch	Casein	P.R. Mannitol	VP	Citrate	Nitrate	Anaerobic Growth	Tyrosine	Phenyl-alanine
1a. Transferred 5x	13	3	1	0	3	0	1	2	1	0
1b. Transferred 10x	13	2	1	5	4	3	5	6	1	1
2. Stored 5 weeks	13	2	2	0	3	4	1	5	1	1
3. Transferred 10x & Stored 3 weeks	13	2	3	2	3	4	1	6	3	1
TOTAL DEVIATIONS		9	7	7	13	11	8	19	6	3
Environmental Isolates										
1a. Transferred 5x	30	2	1	7	3	8	4	13	3	2
1b. Transferred 10x	27[2]	4	3	6	10	3	6	13	2	1
2. Stored 5 weeks	30	1	1	5	3	2	4	11	2	3
3. Transferred 10x & Stored 3 weeks	21[3]	3	2	6	3	3	7	9	3	1

TABLE 5. CHANGES IN BIOCHEMICAL TESTS OF BACILLUS MICROORGANISMS AFTER FOUR TYPES OF CULTURE HANDLING PRIOR TO IDENTIFICATION (*Continued*)

ATCC and NRS Stock Cultures	Number of Test Cultures	Deviations from Initial Reactions[1]								
		Starch	Casein	P.R. Mannitol	VP	Citrate	Nitrate	Anaerobic Growth	Tyrosine	Phenyl-alanine
TOTAL DEVIATIONS		10	7	24	19	16	21	46	10	7

[1]Cultures were subcultured twice and biochemical tests were run in triplicate for obtaining initial reactions.

[2]Three cultures lost.

[3]Nine cultures lost.

TABLE 6. CHANGES IN BIOCHEMICAL TESTS DUE TO SEQUENTIAL SUBCULTURE OF ENVIRONMENTAL *BACILLUS* SP. ISOLATES COLLECTED IN PHOENIX[1]

Test	Deviations from Initial Reactions (14 cultures tested)
1. Starch Hydrolysis	4
2. Casein Hydrolysis	4
3. Mannitol Fermentation	8
4. Voges-Proskauer	2
5. Citrate Utilization	2
6. Nitrate Reduction	3
7. Anaerobic Growth	7
8. Tyrosine Decomposition	1
9. Phenylalanine Deamination	0

[1]Isolates were subcultured twice weekly on TSA for a period of 10 weeks. Biochemical reactions were determined weekly using duplicate tests.

TABLE 7. BIOCHEMICAL REACTIONS OF HEAT STRESSED ATYPICAL BACILLUS SP.

Number of Isolates	Source	Time at 125 C	Positive Test Reactions
17	ESF Ster. & Assembly	8 - 16 hrs	Anaerobic nitrate; Nitrate
5	MSOB High Bay	10 - 11 hrs	Anaerobic nitrate; Nitrate
5	ESF Ster. & Assembly	8 - 16 hrs	Nitrate
6	1:100 Dil. Cape Soil	1 - 3 hrs	Starch; Gelatin
1	1:100 Dil. Cape Soil	2 hrs	Starch; Gelatin; Phenol Red Mannitol
1	Hangar AO	9 hrs	Starch; Gelatin; Phenol Red Mannitol
2	MSOB High Bay	13 hrs	Starch; Gelatin; Phenol Red Mannitol

ESF = Explosive Safe Facility

MSOB = Manned Space Operations Building Class 100,000 Cleanroom

Hangar AO = Class 100,000 Cleanroom

TABLE 8. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY - 125 C

EXPERIMENT NUMBER	AREA	N_0 TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
1	MSOB - I	5.4×10^3	2.4×10^2	2.4×10^2	3 hr. - 3/6	B. circulans-2, Atypical Bacillus
					6 hr. - 0/6	
					9 hr. - 0/6	
					12 hr. - 0/6	
2	"	4.0×10^3	1.2×10^2	8.0×10^1	3 hr. - 0/6	
					6 hr. - 0/6	
					9 hr. - 0/6	
					12 hr. - 0/6	
3	"	3.1×10^3	1.4×10^2	1.6×10^2	3 hr. - 0/6	
					6 hr. - 0/6	
					9 hr. - 0/6	
					12 hr. - 0/6	
4	"	4.1×10^3	4.3×10^2	1.6×10^2	3 hr. - 0/6	
					6 hr. - 1/6	Atypical Bacillus
					9 hr. - 0/6	
					12 hr. - 0/6	
5	MSOB - II	4.9×10^3	2.7×10^2	3.6×10^3	1 hr. - 5/6	B. sphaericus-2, B. lentus, B. firmus, Atypical Bacillus
					2 hr. - 3/6	B. cereus, B. firmus, Atypical Bacillus
					3 hr. - 1/6	B. polymyxa
					4 hr. - 1/6	B. subtilis
6	"	3.8×10^3	2.6×10^2	2.6×10^3	1 hr. - 4/6	B. lentus-2, B. subtilis, Atypical Bacillus
					2 hr. - 3/6	B. polymyxa, B. pantothenticus, Atypical Bacillus
					3 hr. - 0/6	
					4 hr. - 2/6	B. lentus-2

TABLE 8.THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY - 125 C (Continued)

EXPERIMENT NUMBER	AREA	N_0 TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
7	MSOB - II	1.4×10^3	1.4×10^2	4.8×10^2	1 hr. - 1/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	B. sphaericus
8	"	4.0×10^2	4.0×10^1	2.4×10^2	1 hr. - 0/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	
9	"	4.8×10^2	5.6×10^1	8.0×10^1	1 hr. - 0/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	
10	"	5.6×10^2	1.5×10^2	1.6×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
11	"	1.3×10^3	2.1×10^2	4.0×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
12	"	6.4×10^2	8.8×10^1	2.4×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
13	"	1.4×10^3	1.4×10^2	9.6×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	

TABLE 8. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY - 125 C (Continued)

EXPERIMENT NUMBER	AREA	N_o TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
14	MSOB - II	1.1×10^3	1.3×10^2	2.4×10^2	2 hr. - 3/6	B. circulans, Actinomycete, Atypical Bacillus
					4 hr. - 1/6	Actinomycete
					6 hr. - 0/6	
					8 hr. - 0/6	
15	"	4.0×10^2	5.6×10^1	1.6×10^2	2 hr. - 1/6	B. sphaericus
					4 hr. - 1/6	B. sphaericus
					6 hr. - 0/6	
					8 hr. - 0/6	
16	"	4.0×10^2	7.2×10^1	1.6×10^2	2 hr. - 0/6	
					4 hr. - 0/6	
					6 hr. - 0/6	
					8 hr. - 0/6	
17	"	6.4×10^2	6.4×10^1	3.2×10^2	2 hr. - 0/6	
					4 hr. - 0/6	
					6 hr. - 0/6	
					8 hr. - 0/6	
18	"	1.5×10^3	2.1×10^2	4.0×10^2	2 hr. - 1/6	B. sphaericus
					4 hr. - 0/6	
					6 hr. - 2/6	B. circulans, Atypical Bacillus
					8 hr. - 0/6	
19	"	4.8×10^2	4.8×10^1	8.0×10^1	2 hr. - 0/6	
					4 hr. - 0/6	
					6 hr. - 0/6	
					8 hr. - 0/6	
20	"	1.9×10^3	2.2×10^2	4.0×10^2	2 hr. - 2/6	Actinomycete, B. lentus
					4 hr. - 0/6	
					6 hr. - 1/6	B. lentus
					8 hr. - 1/6	B. sphaericus

TABLE 8. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY - 125 C (Continued)

EXPERIMENT NUMBER	AREA	N_O TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
21	MSOB - II	2.4×10^2	5.6×10^1	8.0×10^1	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
22	"	5.6×10^2	8.0×10^1	8.0×10^1	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
23	"	7.2×10^2	8.0×10^1	2.4×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
24	"	9.6×10^2	1.1×10^2	2.4×10^2	2 hr. - 1/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	B. lentus
25	"	2.9×10^3	5.2×10^2	8.8×10^2	2 hr. - 1/6 4 hr. - 1/6 6 hr. - 0/6 8 hr. - 0/6	B. sphaericus B. sphaericus
26	"	2.5×10^3	8.2×10^2	3.2×10^2	2 hr. - 3/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 3/6	B. subtilis, B. lentus-2 B. sphaericus-2, Lost in process
27	"	2.0×10^3	6.6×10^2	4.0×10^2	2 hr. - 1/6 4 hr. - 1/6 6 hr. - 0/6 8 hr. - 1/6	Lost in process Lost in process Actinomycete

TABLE 8. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY - 125 C (Continued)

EXPERIMENT NUMBER	AREA	N_O TOTAL COUNT	N_O SPORES	N_O MOLDS	SURVIVORS	IDENTIFICATION
28	MSOB - II	3.2×10^3	2.3×10^2	3.2×10^2	2 hr. - 3/6	B. firmus, B. subtilis, B. polymyxa
					4 hr. - 1/6	Actinomycete
					6 hr. - 1/6	B. sphaericus
					8 hr. - 0/6	
29	"	1.8×10^3	2.1×10^2	7.2×10^2	2 hr. - 2/6	Actinomycete, B. polymyxa
					4 hr. - 1/6	Atypical Bacillus
					6 hr. - 0/6	
					8 hr. - 0/6	
30	"	1.0×10^3	1.7×10^2	1.6×10^2	2 hr. - 3/6	Actinomycete-2, Atypical Bacillus
					4 hr. - 1/6	B. firmus, B. sphaericus, Atypical Bacillus
					6 hr. - 0/6	
					8 hr. - 0/6	
31	"	9.6×10^2	1.2×10^2	2.4×10^2	2 hr. - 1/6	Actinomycete
					4 hr. - 0/6	
					6 hr. - 0/6	
					8 hr. - 0/6	
32	"	5.9×10^3	2.6×10^2	4.4×10^3	2 hr. - 3/6	B. lentus-2, Atypical Bacillus
					4 hr. - 1/6	B. lentus
					6 hr. - 0/6	
					8 hr. - 1/6	B. subtilis
33	"	2.8×10^3	4.0×10^2	1.4×10^3	2 hr. - 5/6	B. megaterium-3, B. pumilus, Atypical Bacillus
					4 hr. - 2/6	Actinomycete, B. lentus
					6 hr. - 2/6	Actinomycete, Atypical Bacillus
					8 hr. - 0/6	

TABLE 8. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY - 125 C (Continued)

EXPERIMENT NUMBER	AREA	N_0 TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
34	MSOB - II	3.4×10^3	4.6×10^2	1.5×10^3	2 hr. - 6/6	B. brevis-2, B. licheniformis-2, B. lentus, Atypical Bacillus
					4 hr. - 0/6	
					6 hr. - 1/6	B. lentus
					8 hr. - 0/6	
35	"	2.2×10^3	2.0×10^2	1.4×10^3	2 hr. - 1/6	Atypical Bacillus
					4 hr. - 1/6	Atypical Bacillus
					6 hr. - 1/6	B. lentus
					8 hr. - 0/6	
36	"	1.2×10^3	2.0×10^2	4.0×10^2	2 hr. - 4/6	B. licheniformis-2, B. subtilis, B. lentus
					4 hr. - 1/6	B. lentus
					6 hr. - 1/6	B. laterosporus
					8 hr. - 0/6	
37	"	4.2×10^3	3.5×10^2	2.6×10^3	2 hr. - 2/6	Atypical Bacillus-2
					4 hr. - 3/6	B. brevis, Atypical Bacillus-2
					6 hr. - 1/6	Atypical Bacillus
					8 hr. - 1/6	B. coagulans
38	"	2.8×10^3	3.0×10^2	6.4×10^2	2 hr. - 5/6	Atypical Bacillus-2, B. brevis-2, Actinomycete
					4 hr. - 2/6	Atypical Bacillus, B. brevis
					6 hr. - 1/6	B. lentus
					8 hr. - 0/6	
39	MSOB - I	2.4×10^3	1.8×10^2	6.4×10^2	2 hr. - 3/6	B. subtilis, B. lentus, Atypical Bacillus
					4 hr. - 1/6	B. brevis
					6 hr. - 0/6	
					8 hr. - 0/6	

TABLE 8. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY - 125 C (Continued)

EXPERIMENT NUMBER	AREA	N_0 TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
40	MSOB - I	2.4×10^3	2.8×10^2	7.2×10^2	2 hr. - 5/6	B. licheniformis-2, B. subtilis-2 Atypical Bacillus
					4 hr. - 2/6	Atypical Bacillus, In process
					6 hr. - 2/6	Actinomycete, In process
					8 hr. - 0/6	
41	"	3.5×10^3	1.8×10^2	2.2×10^3	2 hr. - 3/6	B. subtilis, B. licheniformis-2
					4 hr. - 1/6	B. licheniformis
					6 hr. - 0/6	
					8 hr. - 0/6	
42	"	2.1×10^3	1.9×10^2	6.4×10^2	2 hr. - 3/6	In process-3
					4 hr. - 1/6	In process
					6 hr. - 2/6	In process-2
					8 hr. - 0/6	

TABLE 9. SUMMATION OF RESULTS FROM TEFLON RIBBON EXPERIMENTS AT 125 C - CAPE KENNEDY

Heating Time (hr)	No. of Experiments	Total	N_o Spores	No. Survivors Per Total No. Flasks	D Value (min)	Identification
1	5	2.7×10^3	1.0×10^2	10/30	25	B. lentus-3, B. sphaericus-3, B. subtilis, Atypical Bacillus-2
2	38	1.8×10^3	2.2×10^2	69/228*	43	Atypical Bacillus-13, B. cereus, B. firmus-2, B. polymyxa-3, B. pumilus, B. pantothenticus, B. circulans, B. lentus-9, B. sphaericus-3, B. brevis-4, B. subtilis-7, B. megaterium-3, Actinomycete-8, Lost-2, B. licheniformis-8, In process-3
3	9	2.6×10^3	2.2×10^2	4/54	52	B. polymyxa, B. circulans-2, Atypical Bacillus
4	38	1.8×10^3	2.2×10^2	26/228*	74	B. firmus, B. subtilis, B. lentus-5, B. sphaericus-3, B. brevis-3, B. licheniformis, Actinomycete-3, Lost-1, Atypical Bacillus-8, In process-2
6	37	2.0×10^3	2.2×10^2	16/222*	104	Atypical Bacillus-4, B. circulans, B. lentus-4, B. sphaericus, B. laterosporus, Actinomycete-2, In process-3
8	33	2.1×10^3	2.3×10^2	7/198*	126	B. sphaericus-3, B. subtilis, B. coagulans, Lost-1, Actinomycete
9	4	4.0×10^3	2.0×10^2	0/24	---	----------
12	4	4.6×10^3	2.6×10^2	0/24	---	----------

*Includes experiments still incubating.

TABLE 10. HEAT RESISTANCE AND IDENTIFICATION OF BACILLUS SP. ISOLATES FROM PERTINENT ENVIRONMENTAL SOURCES

Source and Isolate No.		D_{125C} Value (min)[1]	Identification
APOLLO	42	12	B. firmus
	342	<5	B. firmus
	659	<5	B. sphaericus
	954	12	B. sphaericus
	1026	9	B. sphaericus
	1043	<5	B. sphaericus
	1075	40	B. sphaericus
AO HANGAR	20-1b	6	B. subtilis
	20-2	15	B. badius
	20-4	22	B. subtilis
	20-5	7	B. polymyxa
	20-6	24	B. lentus
	20-8	6	A[2]
	20-9	12	B. polymyxa
	20-13	18	B. licheniformis
	40-1	10	B. macerans
	40-2	7	A
	40-4	22	B. subtilis
	40-5	<5	B. macerans
	40-6	21	B. firmus
	40-7	8	B. badius
	40-9	20	B. coagulans
	40-11	16	B. coagulans

TABLE 10. HEAT RESISTANCE AND IDENTIFICATION OF BACILLUS SP. ISOLATES FROM PERTINENT ENVIRONMENTAL SOURCES (Continued)

Source and Isolate No.		D_{125C} Value (min)[1]	Identification
AO HANGAR	60-2	25	B. cereus
	60-4	27	A[2]
	60-9	32	B. polymyxa
	80-1	15	B. subtilis
	80-2	30	B. subtilis
	80-3	28	B. subtilis
	80-3A	11	A
	80-4	28	B. brevis
	80-4A	21	B. coagulans
	80-5	32	A
	80-5A	28	B. circulans
	90-1	30	B. subtilis
	100-1	25	B. subtilis
	100-2	29	B. subtilis
	120-1	16	B. subtilis
	120-2	30	B. polymyxa
	120-3	7	B. macerans
	4-1	39	B. circulans
	4-2	39	B. subtilis
PIECE-PARTS	A-11-1	43	A
	A-11-1T	23	B. polymyxa
	A-11-2T	26	B. cereus
	A-11-3a	9	A

TABLE 10. HEAT RESISTANCE AND IDENTIFICATION OF BACILLUS SP. ISOLATES FROM PERTINENT ENVIRONMENTAL SOURCES (Continued)

Source and Isolate No.		D_{125C} Value (min)[1]	Identification
PIECE-PARTS	A-11-3b	12	A[2]
	A-11-3T	24	B. subtilis
	A-12-2	9	A
	A-15-1	7	A
	A-15-1T	10	B. polymyxa
	A-16-3a	25	A
	A-16-3b	24	A
	A-17-1	9	A
	B-1-2	24	B. circulans
	C-5-1	36	A
TEFLON-JPL	6-R-18	30	A
	7-R-3	8	B. lentus
	7-R-18	166	A

[1] 5-tube FN-MPN data.
[2] Atypical Bacillus sp.

TABLE 11. DISTRIBUTION OF D_{125C} VALUES IN 59 *BACILLUS* SP. ISOLATES TESTED

Species	No.	D_{125C} Values (min)
A[1]	16	6, 7, 7, 9, 9, 9, 11, 12, 24, 25, 27, 30, 32, 36, 43, 166
B. badius	2	8, 15
B. brevis	1	28
B. cereus	2	25, 26
B. circulans	3	24, 28, 39
B. coagulans	3	16, 20, 21
B. firmus	3	<5, 12, 21
B. lentus	2	8, 24
B. licheniformis	1	18
B. macerans	3	<5, 7, 10
B. polymyxa	6	7, 10, 12, 23, 30, 32
B. sphaericus	5	<5, <5, 9, 12, 40
B. subtilis	12	6, 15, 16, 22, 22, 24, 25, 28, 29, 30, 30, 39
TOTAL	59	

[1]Atypical *Bacillus* sp.