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Preliminary Report
October 1, 1972 – December 31, 1972

A STUDY OF PSYCHROPHILIC ORGANISMS ISOLATED FROM THE MANUFACTURE AND ASSEMBLY AREAS OF SPACECRAFT TO BE USED IN THE VIKING MISSION

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A STUDY OF PSYCHROPHILIC ORGANISMS
ISOLATED FROM THE MANUFACTURE AND ASSEMBLY AREAS
OF SPACECRAFT TO BE USED IN THE VIKING MISSION

Preliminary Report of Planetary Quarantine Activities
October 1, 1972 - December 31, 1972

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FOREWORD

This preliminary report summarizes work performed for the National Aeronautics and Space Administration by the Department of Biology at Hardin-Simmons University, supported by NASA Grant NGR 44-095-001, and covers the period from October 1, 1972 - December 31, 1972. The relationship with the NASA Planetary Quarantine Program has been most stimulating to the Division of Science at H-SU, and it is hoped that this project will be a significant contribution to the activities of NASA in its present and future planetary exploration programs.

The report covers the sampling of soils from the manufacture and assembly areas of the Viking spacecraft, the methodology employed in the analysis of these samples for psychrophilic microorganisms, and temperature studies on these organisms. Results showing the major types of organisms and the percentage of obligate psychrophiles in each sample are given and discussed. The report also includes a description of work currently in progress and future work planned for the remainder of the grant period. Emphasis in all areas is toward application of these results to the objectives of the planetary quarantine program.

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A STUDY OF PSYCHROPHILIC ORGANISMS ISOLATED FROM THE MANUFACTURE AND ASSEMBLY AREAS OF SPACECRAFT TO BE USED IN THE VIKING MISSION

Introduction

Since one purpose of the Planetary Quarantine Program is to determine guidelines for the prevention of contamination of Mars with organisms which might grow in the Martian environment, it is essential that all possible groups of microorganisms associated with these planetary vehicles be studied in this respect. The success of the planetary quarantine measures can only be met with extensive investigation of all factors which might contribute to this problem; therefore, the primary objective of this investigation is to make a deliberate attempt to determine the presence and concentration of psychrophilic organisms in various areas associated with the Viking spacecraft and to determine if these will grow in some of the environmental conditions suggested for Mars.

A great deal of previous research has been conducted on organisms isolated from the manufacture and assembly areas of the Viking spacecraft, but most of these has dealt primarily with the heat resistance of mesophilic sporeformers. Although it is generally agreed that the psychrophiles may not be the most heat resistant of the microorganisms, they should not be excluded from investigations related to planetary quarantine because these may include organisms with the physiological characteristics to grow in the hostile environment of Mars. Also, it is known that some sporeformers, aerobic and anaerobic, possess the ability to grow at low temperatures, and this group includes the more heat resistant microorganisms.

Work has also been done on the ability of organisms to grow in simulated Martian environments, and these have demonstrated that many organisms can grow in the conditions used. However, there are many factors which have not been investigated, and most of these investigators suggest that there is a definite need for more work in this area. Some of these have also pointed out that because of the conditions on Mars, some of the most likely organisms to grow on this planet will include those capable of growth at low temperatures. For these reasons, it appears to be of great value to extend these previous studies to include the isolation and study of psychrophilic organisms from areas directly associated with the Viking spacecraft.

It is recognized that there are numerous definitions of psychrophilic organisms including those based on optimum growth temperature and those based on possible growth ranges (maximum and minimum temperatures for growth). The latter usually is defined as the formation of macroscopic colonies within a certain time period. Since the definitions vary tremendously among individual investigators, it was necessary to define the conditions used in this project. In order to include as many potential psychrophiles as possible, primary isolation was performed at 6-7^{o}C for 10-14 days (21 days for fungi). Subsequent temperature studies included growth at 2-3^{o}C (10-14 days), 24^{o}C (3-5 days), and 32^{o}C (48 hrs.). Organisms showing growth at 3^{o}C, but not at 32^{o}C in the required time are defined in this project as obligate psychrophiles. Many of these did show growth at 24^{o}C, and this is shown in the results. Since many investigators prefer a more rigid definition, results also show the percent of organisms which grew at 3°C, but not at the other two temperatures.

It is also pertinent to include a study of psychrophilic sporeformers, and this has been done, or is in progress, for both aerobes and anaerobes. The presence of these organisms will be of considerable interest because of their potential heat resistance and because of their potential ability to grow in the Martian environment. If these can grow under the condition to be used in this study, they may indeed be the most relevant group for intensive investigations.

Non-sporeforming organisms which can grow under the conditions of this project will be relevant if some future interplanetary vehicles are not subjected to terminal heat sterilization, or if recontamination of the spacecraft occurs after heat sterilization. Therefore, the non-sporeforming psychrophiles should not be overlooked as insignificant.

Attempts have been made in this investigation to isolate several types of phototrophic and autotrophic organisms incubated at 6-7°C for 30-60 days. Counts were not made on these two groups, but attempts were made to demonstrate their ability to grow at this temperature.

Procedure

Soil samples were obtained from sites at the manufacture area of the Viking spacecraft in Denver, Colorado and from various areas at Cape Kennedy where the spacecraft might be housed in preparation for launching. These samples were taken from areas around main entrances through which dust contamination might enter the building, and these sites are indicated in Table 1. All samples were surface samples, no deeper than 5-6 inches and included grass if it was present at the site.

The samples were returned to the laboratory and treated as shown in Figure 1. The samples were thoroughly mixed, and ten grams of each was diluted in 0.1% peptone prior to plating. The first bottle in each dilution series contained glass beads for better dispersal of soil particles during mixing. Subsequent dilution tubes were mixed on a Vortex Jr. Mixer (Scientific Industries, Inc., Queens Village, New York) to assure thorough mixing. One-tenth ml. amounts were transferred to the surface of Trypticase Soy Agar (BBL) plates and Mycophil Agar -pH 4.0- (BBL) plates and spread with glass spreading rods.

All media used throughout this investigation were prepared as directed, cooled, allowed to stand at room temperature for 24-36 hours, and chilled at $7^{o}C$ for at least 24 hrs. prior to use. This was done to prevent possible damage to psychrophiles by addition of melted agar; therefore, the spread-plate technique was employed on all counts. The different media used in this experiment were all prepared from the same "lot" of dehydrated media. These "lot" numbers and other pertinent information concerning preparation of media are maintained in a separate media preparation log. All differential media and test reagents used were tested for reliability using known organisms prior to use. All manipulations were performed in a laminar flow cabinet.

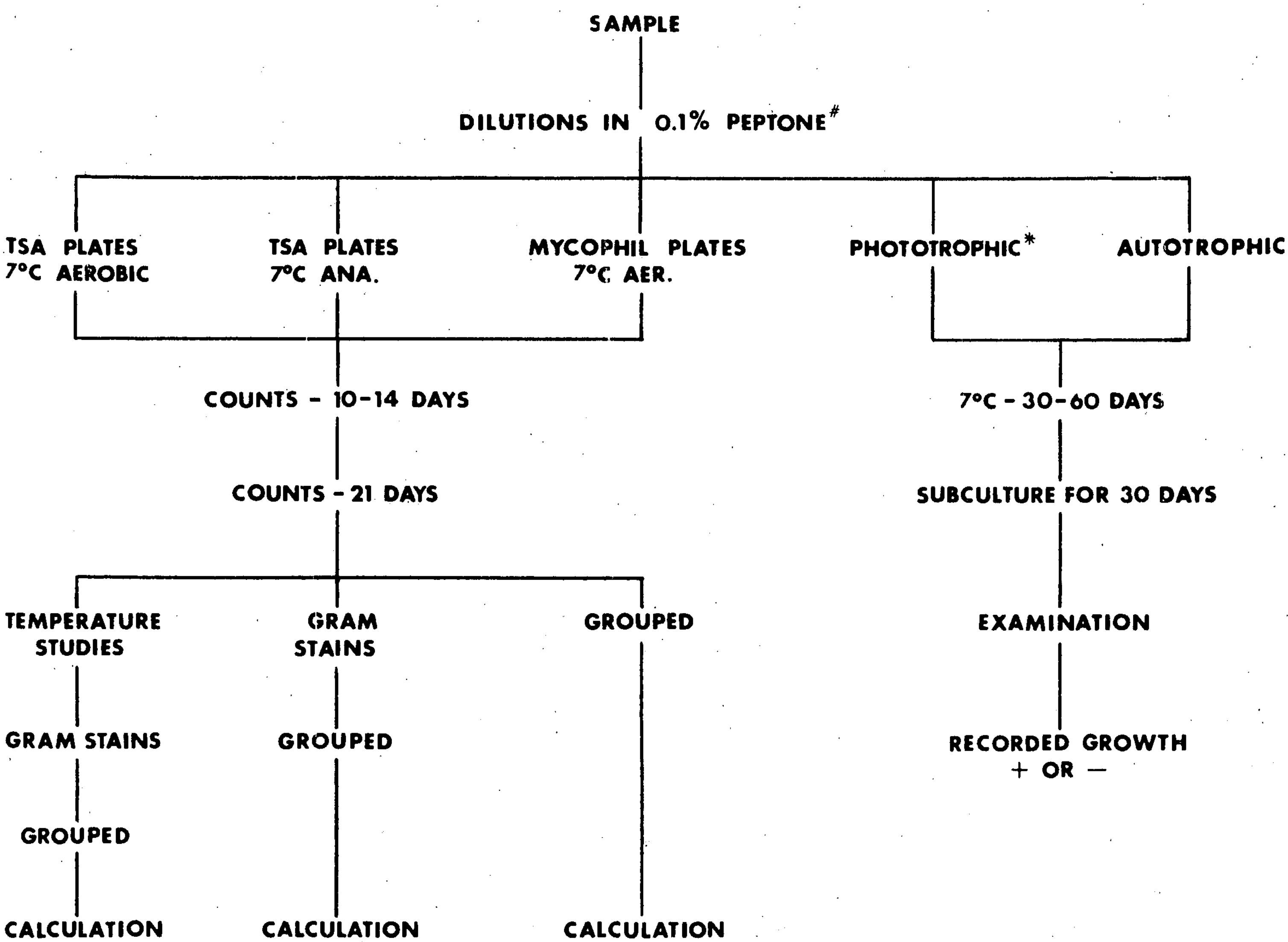
Table 1

SAMPLE SITES

CODE	SOURCE
	From the Manufacture Area in Denver, Colorado
M1	Outside high bay area on cooling tower side
M2	West side of high bay area*
M3	Back of high bay area
	From the Assembly Area at Cape Kennedy
K1	Bldg. M7-1469 East of low bay door on north side of bldg.
K2	Bldg. M7-1469 West of low bay door on north side of bldg.
K3	Bldg. M7-1469 Directly in front of low bay door
K4	Bldg. M7-1469 East of high bay door on south side of bldg.
K5	Bldg. M7-1469 West of high bay door on south side of bldg.
K6	Bldg. M7-1469 Directly in front of high bay door
K7	Bldg. AO Directly in front of high bay (west side) Dark sand
K8	Bldg. AO Directly in front of high bay (west side) Light sand
K9	Bldg. AO From the curb directly in front of high bay
K10	Bldg. AO Main personnel entrance on east side of bldg.
K11	Bldg. AO From vacuum units inside bldg.
K12	Bldg. AE Outside main entrance to the clean room

*From roadbed of fill dirt - not native soil

FIG. 1. SAMPLE TREATMENT



ALL MEDIA STORED AT 7°C FOR AT LEAST 24 HOURS PRIOR TO USE

* LIGHTED INCUBATOR AT 7°C

Duplicate plates were prepared for aerobic, anaerobic, and fungal counts. The plates were placed in the 7°C incubator (Freas model 805) as soon as possible after inoculation, and only the anaerobic plates were allowed to reach room temperature during the manipulations. The anaerobic plates were rechilled after inoculation, placed in Brewer Anaerobe Jars with Gas-Pacs and Anaerobic Indicators (BBL), and placed in the 7°C incubator as soon as anaerobic conditions were achieved as shown by the indicator (approximately 3-4 hrs.). A freshly inoculated TSA slant of Alcaligenes fecalis was placed in each anaerobe jar as a biological indicator of anaerobiosis. All incubators were monitored with maximum-minimum registering thermometers (Taylor - Model No. 5458) which were checked daily. The only deviations from 7°C were no more than 1-2°C, and these were usually lower than 7°C. Increases in temperature occurred during times when samples were being added to or removed from the incubators.

After inoculation of plates for the previous counts, one ml. amounts were transferred from the 1:10 dilution to triplicate tubes of synthetic media designed for isolation of blue-green algae, green algae, green sulfur bacteria, purple sulfur bacteria, purple non-sulfur bacteria, Nitrobacter, Nitrosomonas, Ferrobacillus, and Thiobacillus. These media were prepared according to formulations given by Stanier[1] and Frobisher[2]. These were then incubated at 7°C for 30-60 days and

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[1]Stanier, R. Y., M. Doudoroff, and E. A. Adelberg, The Microbial World, 2nd ed., 1963, Prentice-Hall, Inc., Englewood Cliffs, N. J., 456-458.

[2]Frobisher, M., Fundamentals of Microbiology, 8th ed., 1968, W. B. Saunders Co., Philadelphia, 544-545.

examined macroscopically and microscopically (phase-contrast and staining). Media inoculated for isolation of phototrophic organisms were placed in a lighted incubator. Cultures showing positive growth were subcultured into fresh media, incubated for another 30 days and again examined. Tentative identification of most cultures showing positive results on subculture have been performed.

As can be seen from Figure 1, the aerobic psychrophiles have received the most attention. This is because they are the predominant type isolated. Further studies on anaerobes and fungi are in progress, and at present the fungi have been grouped on the basis of macroscopic observation only. The anaerobes have been observed with the gram and spore stains and by colonial morphology.

Aerobic plates showing countable (30-300 colonies) results were selected, and all colonies were transferred from these to TSA slants for incubation at $3^{o}C$ (10-14 days), $24^{o}C$ (3-5 days), and $32^{o}C$ (48 hrs.). Plates with higher counts were examined for low populations of organisms which did not appear on the countable plates. After growth had occurred, the results were recorded and organisms showing growth at $3^{o}C$, but not at $32^{o}C$, were classified as obligate psychrophiles. The organisms were then reincubated for 7-10 more days for better growth and pigment production.

All isolates from the manufacture area were examined individually by staining and limited biochemical tests. From these results plus the temperature studies and colonial characteristics, the organisms were grouped and are presently being identified. From these detailed procedures it became apparent that the organisms could be accurately grouped on the basis of temperature studies and careful examination of colonial characteristics. Since the psychrophiles require so long for determination of biochemical tests, and due to the large number of samples involved, it was decided to alter the procedures for the Cape Kennedy samples. These samples were treated like the samples from the manufacture area to the point of staining and biochemical tests; however, instead of studying each individual isolate, the organisms were grouped on the basis of temperature studies and careful examination of colonial characteristics at all temperatures at which they grew. Any differences in colonial characteristics caused the organisms to be placed into different groups. Random tubes were then picked from each group for further testing, the number of tubes picked depending upon the size of the group. These isolates were then examined by staining and biochemical tests. The results from these procedures have allowed the grouping of organisms for determination of distribution of the different types. Specific identification of these organisms has not been completed on most samples, but they are currently being tested.

Results

Results of total counts isolated at 7°C in 10-14 days are presented in Table 2 and Figures 2, 2A, and 2B. As can be seen, the total counts from the manufacture area of the Viking spacecraft are higher than those from the Cape Kennedy samples. This should be expected due to the seasonally warmer temperatures in Florida. Counts of samples from the manufacture area are in close agreement with one another, and counts from the Cape Kennedy samples are also in close agreement. A comparison of distribution of the major groups of isolated organisms (aerobic, anaerobic, and fungi) also appears in these tables

Table 2

NUMBER OF ORGANISMS ISOLATED FROM EACH SAMPLE*
(% OF TOTAL)

SAMPLE	TOTAL	AEROBIC	ANAEROBIC	FUNGI
M1	1.74×10^6	1.7×10^6 (97.7)	2.7×10^4 (1.6)	1.3×10^4 (0.7)
M2	3.94×10^5	3.5×10^5 (88.8)	4.4×10^4 (11.1)	4.5×10^2 (0.1)
M3	9.10×10^6	7.7×10^6 (84.6)	3.0×10^3 (0.03)	1.4×10^6 (15.4)
K1	6.04×10^4	4.8×10^4 (79.5)	9.6×10^3 (15.9)	2.8×10^3 (4.6)
K2	8.18×10^4	5.1×10^4 (62.3)	2.8×10^4 (34.2)	2.8×10^3 (3.5)
K3	1.60×10^4	1.3×10^4 (78.1)	2.1×10^3 (13.1)	1.4×10^3 (8.8)
K4	3.60×10^4	6.7×10^3 (18.6)	2.4×10^4 (66.7)	5.3×10^3 (14.7)
K5	2.73×10^4	2.3×10^4 (84.2)	3.9×10^3 (14.3)	4.1×10^2 (1.5)
K6	1.02×10^5	9.4×10^4 (92.2)	4.1×10^3 (4.1)	3.7×10^3 (3.7)
K7	1.89×10^4	1.3×10^4 (69.3)	1.7×10^3 (9.0)	4.1×10^3 (21.7)
K8	7.14×10^3	6.5×10^3 (91.0)	2.5×10^2 (3.5)	3.9×10^2 (5.5)
K9	5.64×10^3	4.2×10^3 (74.0)	4.5×10^2 (8.0)	9.9×10^2 (18.0)
K10	2.45×10^4	1.9×10^4 (77.6)	2.2×10^3 (9.0)	3.3×10^3 (13.4)
K11	8.38×10^3	6.1×10^3 (73.0)	1.9×10^3 (23.0)	3.8×10^2 (4.0)
K12	1.47×10^5	1.4×10^5 (95.1)	6.9×10^3 (4.7)	2.3×10^2 (0.2)

*Cells/gm. of soil

FIG. 2 COUNTS OBTAINED FROM CAPE KENNEDY SAMPLES

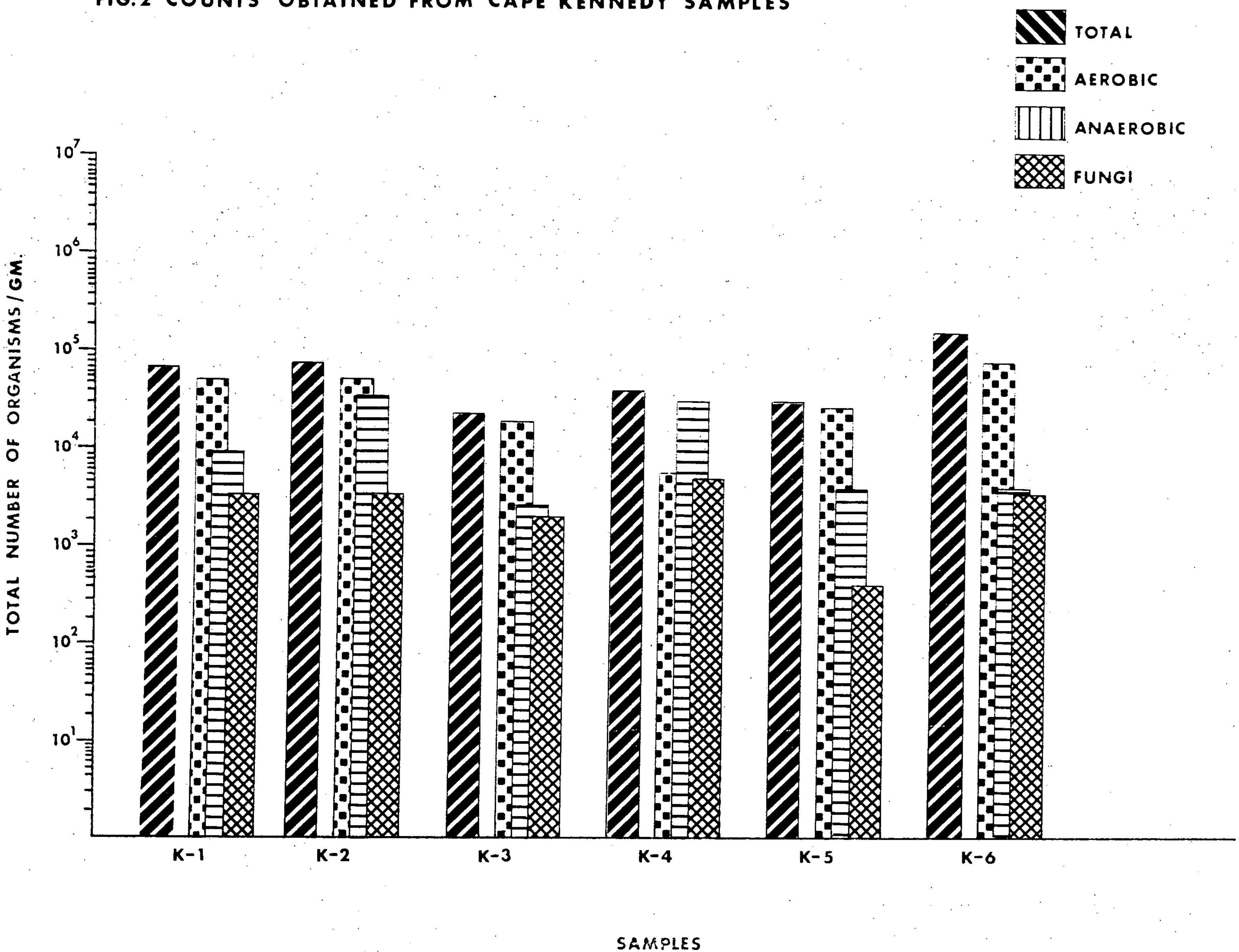

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FIG. 2-A COUNTS OBTAINED FROM CAPE KENNEDY SAMPLES

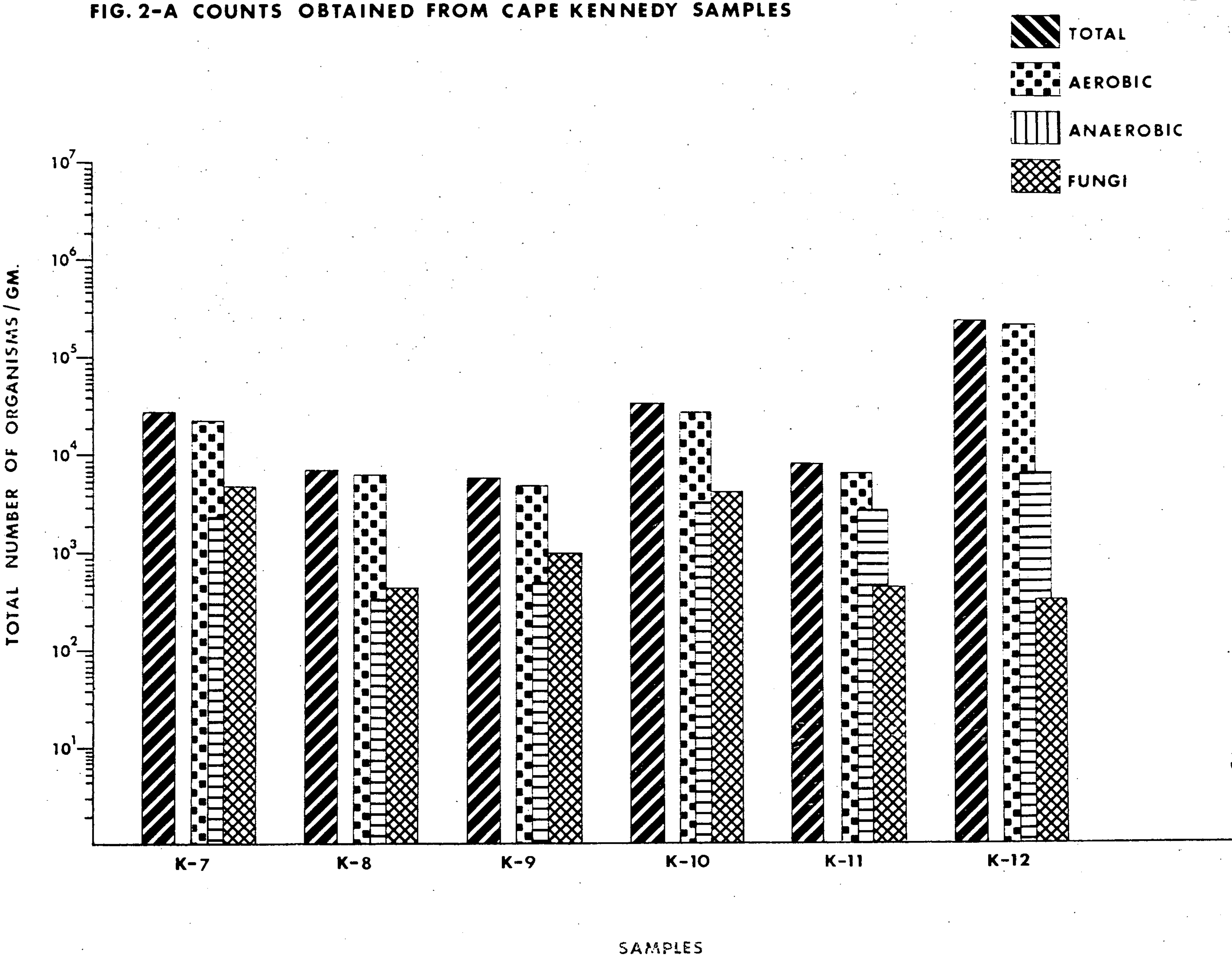

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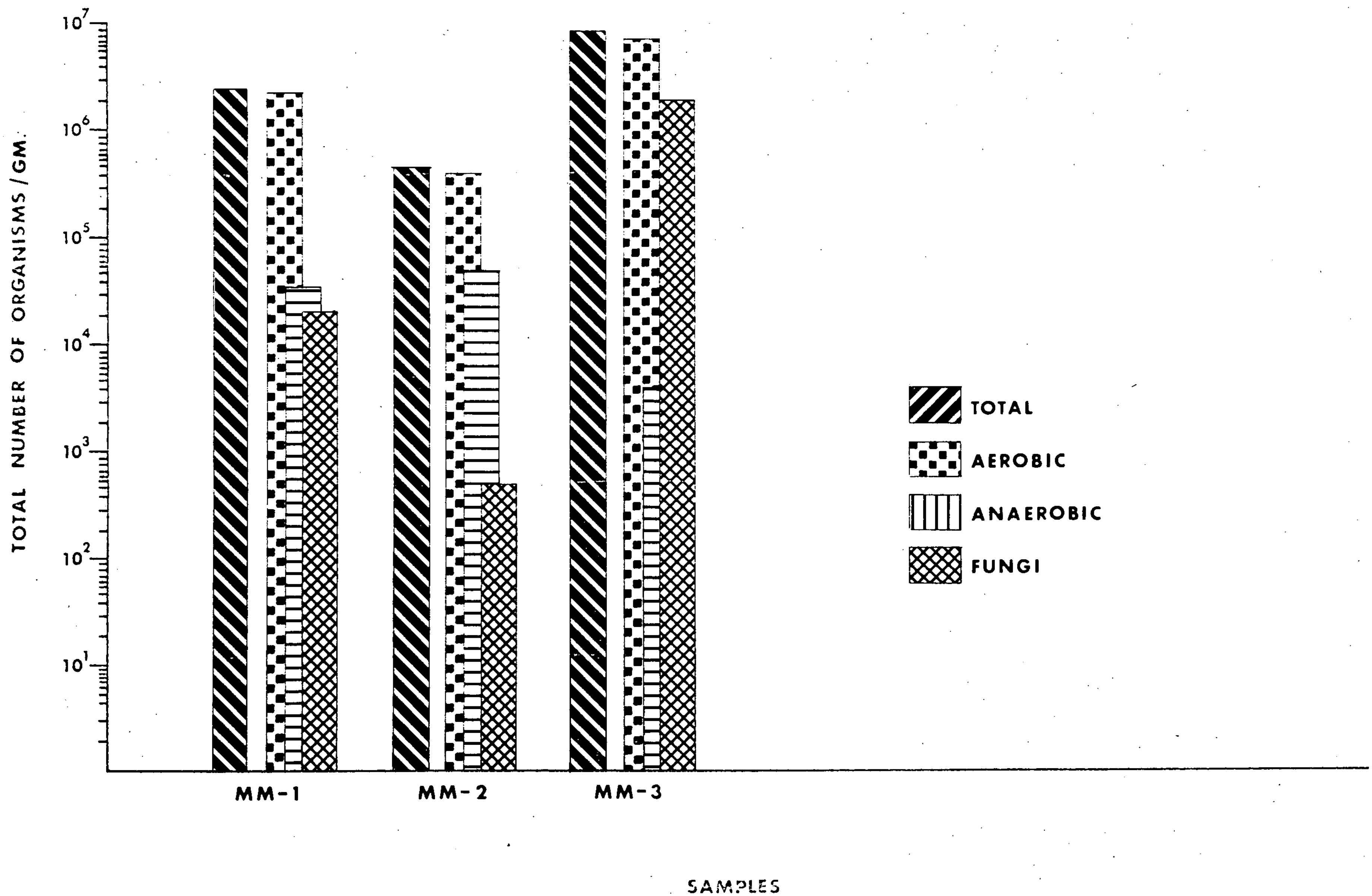
FIG. 2-B COUNTS OBTAINED FROM MANUFACTURE AREA SAMPLES



/3

and figures. In all but one case the aerobic counts were the highest, with the anaerobic and fungal counts comprising a smaller percentage. In ten of the fifteen samples the anaerobic count was higher than the fungal count.

Attempts to isolate phototrophic and autotrophic organisms showed that only blue-green algae, euglenoids, diatoms, and green algae grew at these temperatures, and these were isolated in only a few samples, as shown in Table 3. Tentative identification for most of the isolates is given. On primary isolation, several samples showed growth of various types of bacteria, usually in small concentrations, but upon subculturing, these bacteria would not grow. It was assumed that these bacteria had been obtaining nutrients from the small amounts of soil added to the initial tubes and could not grow on subculture. No photosynthetic bacteria were isolated at any time.

The fungal isolates have been grouped on the basis of macroscopic appearance only, and these results are shown in abbreviated form in Table 4. This is a brief observation of the fungi, but more specific identification and temperature studies will be performed and will be available for future reports. However, this information is included to give an idea of the distribution of fungi isolated from these samples.

The results of the investigations on anaerobic isolates is only partially completed, but thus far, they show that the samples from the manufacture area contain 70-80% sporeformers and 20-30% gram positive, non-sporeforming rods. The samples from Cape Kennedy have shown all sporeformers with the exceptions of K9 and K10 which showed 71.4% and 40.9% sporeformers, respectively, with the remainder being small, gram

Table 3

PHOTOTROPHIC ISOLATES*
(Tentative Identification)

TYPE OF MEDIUM	SAMPLE				
	M1	K1	K2	K6	K7
Blue-green Algae	Unicellular (Chrococcus)	Euglenoid + Unicellular (Chrococcus)	--	Biflagellate, Unicellular with large chloroplast (Chlamydomonas)	--
Green Algae	Masses of long chains of irregular cells - branched filaments	Euglenoid + Unicellular (Chlorella) + few diatoms	Euglenoid + Unicellular (Chlorella)	Long, filamentous algae (Ulothrix)	Long, filamentous algae (Ulothrix)

*No autotrophic organisms showed positive growth on subculture
All samples not listed showed negative results
No photosynthetic bacteria were isolated

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Table 4

PERCENTAGE OF TYPES OF FUNGI AND ACTINOMYCETES

BASED ON MACROSCOPIC EXAMINATION*

TYPE	SAMPLE														
	M1	M2	M3	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12
Yeasts White	30.8	0.9	60.0	-	2.9	2.2	11.0	4.6	15.3	-	-	30.5	3.0	6.6	5.4
Pink	-	-	-	-	3.7	5.1	-	3.5	6.9	-	-	18.1	-	21.3	5.4
Actinomycetes	3.1	0.9	5.0	-	0.7	-	-	-	-	-	-	-	6.1	-	-
Black Molds	27.7	2.8	10.0	-	8.1	8.7	80.7	75.9	58.3	26.8	5.2	23.2	-	9.9	33.8
White Molds	10.8	-	5.0	90.0	53.0	33.3	7.4	14.9	18.1	73.2	92.2	20.3	54.6	27.9	27.0
Green Molds	-	36.1	5.0	10.0	-	-	-	-	-	-	-	-	-	-	-
Gray Molds	16.9	50.8	12.5	-	31.6	44.2	-	1.1	-	-	2.6	-	36.3	34.3	28.4
Red Molds	6.1	-	2.5	-	-	4.3	0.9	-	1.4	-	-	0.7	-	-	-
Tan Molds	4.6	8.5	-	-	-	2.2	-	-	-	-	-	7.2	-	-	-

*Identifications in progress

positive rods in which no spores could be detected. Investigations are currently being conducted on these isolates to determine if they are obligate anaerobes, and temperature studies will be performed to determine if they are obligate psychrophiles.

The aerobic bacteria which have been isolated have been investigated in more detail since they are the predominant types present. These were subjected to temperatures of $3^{o}C$, $24^{o}C$, and $32^{o}C$ as described earlier, and the results of these temperature studies are shown in Table 5 and Figures 3 and 3A. The organisms showing growth at $3^{o}c$ and no growth at $32^{o}C$ are considered as obligate psychrophiles, although the figures further subdivide this group into those that will grow at $24^{o}C$ and those not growing at $24^{o}C$. Also shown are the organisms which grew at all three temperatures, and this largest group contains the facultative psychrophiles. As can be seen, all samples contain obligate psychrophilic organisms according to the definition in this report, and 12 of the 15 samples contain organisms which can grow at $3^{o}C$, but not at $32^{o}C$, nor $24^{o}C$. The distribution of these latter organisms range from 0.0% of the aerobic count in three samples to 16% in K2. If the percentages of psychrophiles from the figures appear high, it must be remembered that the original isolation of samples was done at $7^{o}C$.

Although only a few of the organisms from the aerobic TSA isolates have been identified at least to genera, results in Table 6 show the distribution of major types of organisms isolated from these samples. These are grouped on the basis of staining and colonial characteristics, but more detailed identification is in progress on those samples not yet completed. The organisms so far identified include _Micrococcus_, _Corynebacterium_, _Brevibacterium_, _Flavobacterium_,

Table 5

TEMPERATURE STUDIES FROM AEROBIC TSA SAMPLES
(GIVEN IN %)

Sample	+3°C,* -24°C, -32°C	+3°C, +24°C, -32°C	Total Obligate Psychrophiles
M1	2.0	30.0	32.0
M2	2.0	6.0	8.0
M3	3.7	8.3	12.0
K1	10.0	8.0	18.0
K2	16.0	12.0	28.0
K3	-	3.7	3.7
K4	1.5	-	1.5
K5	1.7	-	1.7
K6	6.8	4.6	11.4
K7	7.1	10.0	17.1
K8	9.9	43.7	53.6
K9	2.1	25.0	27.1
K10	10.0	10.5	20.5
K11	-	23.1	23.1
K12	-	2.0	2.0

* + = Growth
  - = No growth

FIG.3 TEMPERATURE STUDIES ON CAPE KENNEDY AEROBIC ISOLATES

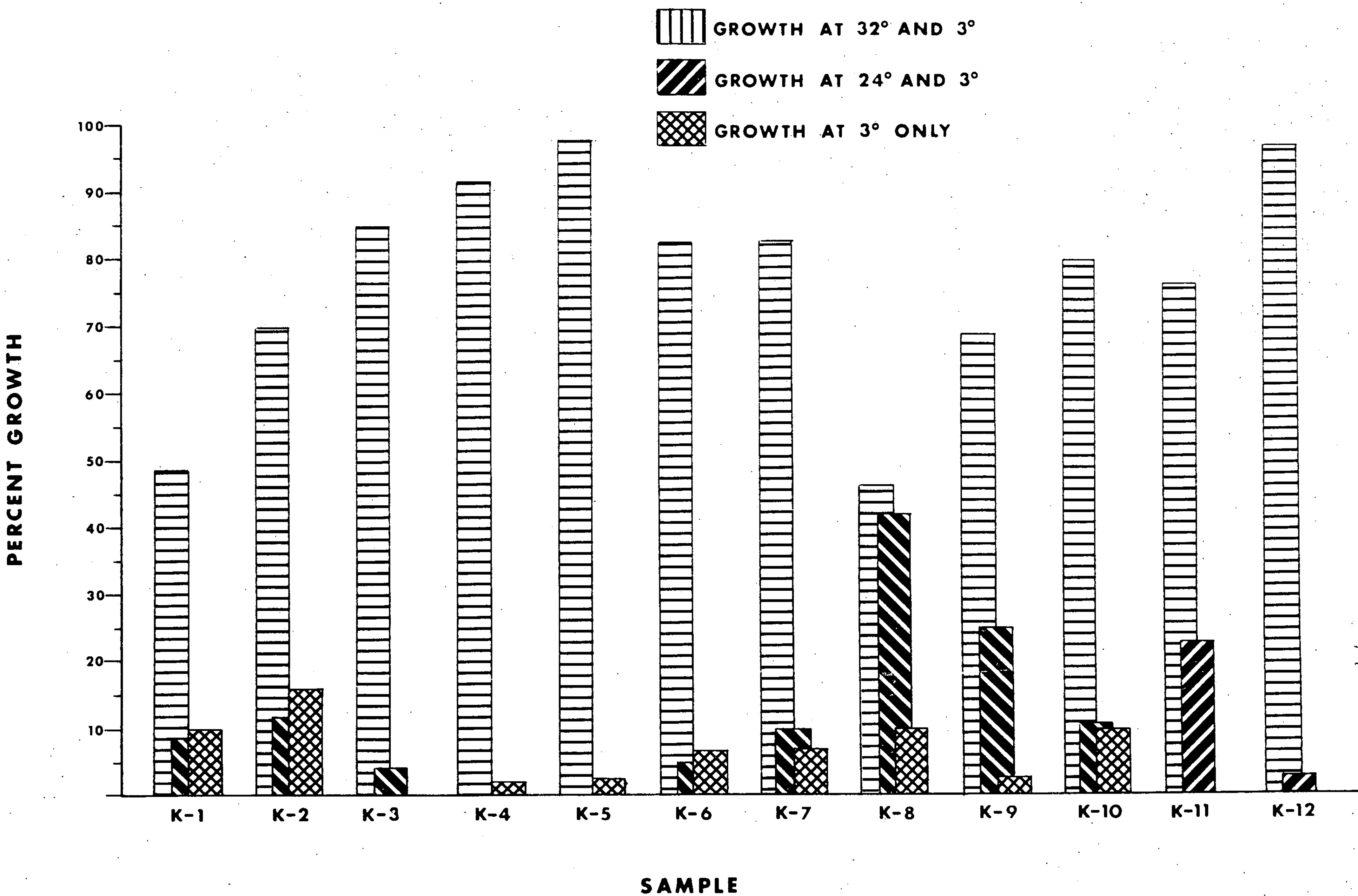

FIG. 3-A TEMPERATURE STUDIES ON MANUFACTURE AREA AEROBIC ISOLATES

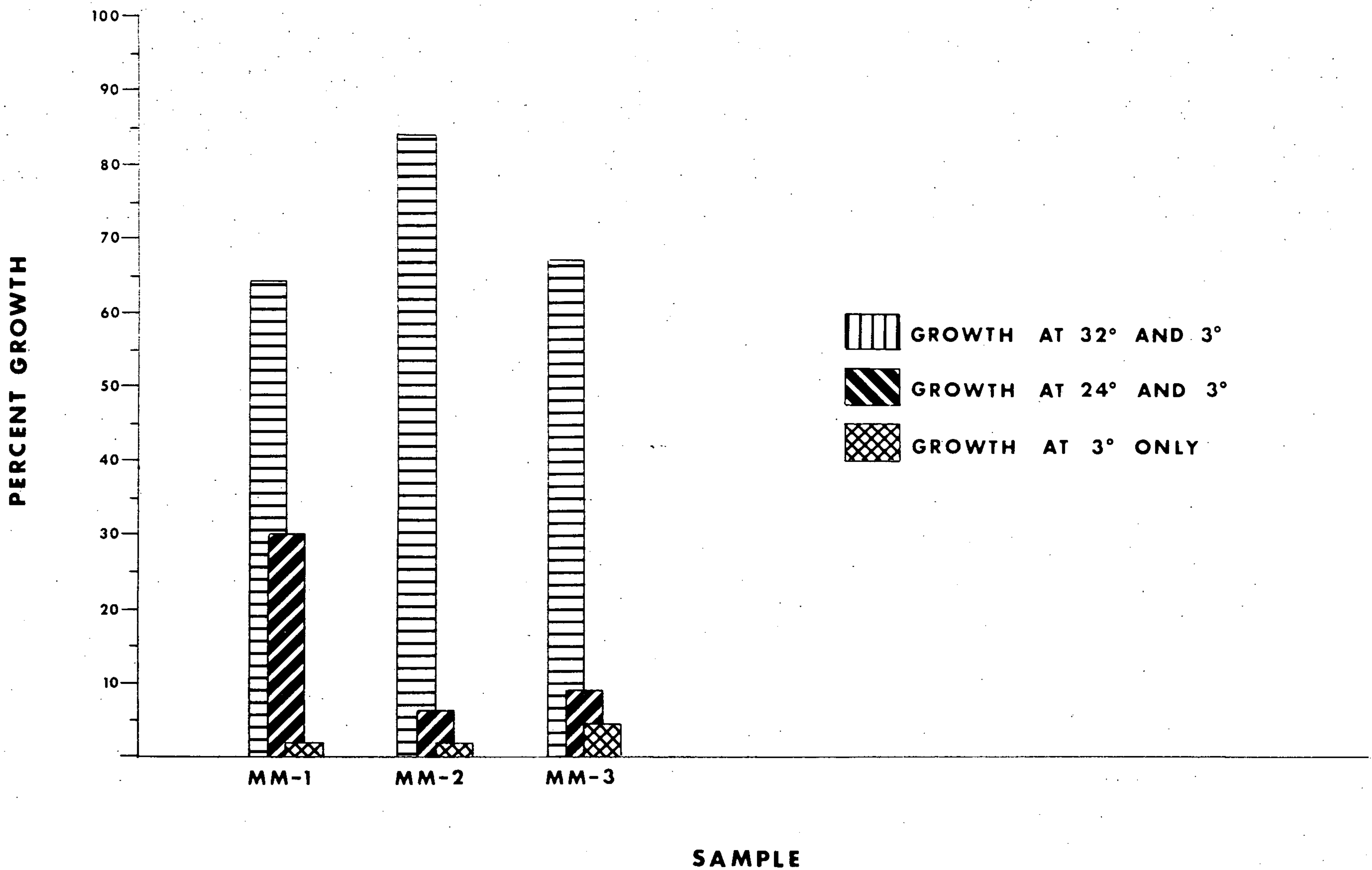

Table 6

PERCENTAGE OF TYPES OF ORGANISMS ISOLATED FROM AEROBIC TSA SAMPLES

TYPE	SAMPLE														
	M1	M2	M3	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12
G+ cocci															
White	12.0	12.0	-	-	2.0	-	1.5	-	33.0	-	1.4	-	-	-	40.0
Pigmented	20.0	6.0	21.1	4.0	-	3.0	-	10.0	11.3	2.3	-	50.0	2.1	9.3	4.0
G+ rods, small, non-sporeforming															
White	38.0	64.0	56.0	26.0	32.0	2.2	3.8	5.0	34.0	60.5	45.1	4.2	29.0	13.8	42.0
Pigmented	22.0	4.0	13.8	2.0	16.0	32.9	39.7	-	6.9	25.7	-	20.7	8.4	13.8	1.3
G+ rods, large, no spores detected															
White	-	-	-	8.0	8.0	13.5	30.9	-	-	9.3	-	-	22.6	29.9	5.3
Pigmented	-	-	-	20.0	26.0	24.7	-	3.3	3.4	-	11.2	-	27.4	15.4	2.7
G+ rods, sporeforming															
White	-	-	-	2.0	4.0	8.9	6.7	8.3	9.1	-	5.6	4.2	7.4	5.4	-
Pigmented	-	-	1.8	10.0	-	3.0	1.5	1.7	-	0.7	2.8	4.2	1.6	-	4.0
G- rods															
White	4.0	10.0	-	2.0	6.0	0.7	5.3	35.0	-	1.5	25.5	-	0.5	3.1	0.7
Pigmented	-	-	5.5	4.0	4.0	8.9	3.8	36.7	2.3	-	8.4	10.4	0.5	6.2	-
Yeast	-	-	1.8	-	-	-	3.8	-	-	-	-	2.1	0.5	0.8	-
Mold	-	-	-	18.0	2.0	-	-	-	-	-	-	-	-	2.3	-
No growth on subculture	4.0	4.0	-	4.0	-	2.2	3.0	-	-	-	-	4.2	-	-	-

Achromobacterium, and *Bacillus*.

One of the most obvious results from Table 6 is that the samples from the manufacture area contained essentially no aerobic sporeformers, while all of the Cape Kennedy samples showed a relatively high percentage of these organisms. Temperature studies on these organisms as compared to non-sporeformers show that the sporeformers apparently are not obligate psychrophiles. As mentioned earlier these organisms have been investigated by staining procedures to demonstrate the presence of spores. Since this investigation was designed to isolate psychrophilic organisms, heat-shocking experiments were not performed. However, organisms reported as sporeformers showed the presence of numerous spores after incubation at low temperatures for 21 days.

Discussion and Conclusion

From the foregoing, it is apparent that the samples tested contain psychrophilic microorganisms of various types. Depending upon how the term obligate psychrophile is defined, various concentrations of their presence has been demonstrated. If, as in this report, the definition includes organisms growing at $3^{o}C$, but not at $32^{o}C$ in the required times, the concentration of obligate psychrophiles varies from 1.5% in K4 to 53.6% in K8. On the other hand, if a more rigid definition such as growth at $3^{o}C$ and not at $24^{o}C$ or $32^{o}C$ is used, their concentration ranges from 0.0% in three of the samples to 16% in K2. Again, it is pointed out that these figures are based on percentages of aerobic organisms which were originally isolated at $7^{o}C$. The intent of this report is not to debate the definitions of psychrophilic organisms, but to demonstrate the concentration of psychrophiles in the areas associated with the Viking spacecraft, and this has been clearly demonstrated. Other investigators have demonstrated that organisms which are obligate psychrophiles on primary isolation may lose this characteristic after culturing in an artificial environment; therefore, the important conclusion is that these are at least psychrophilic organisms because of their ability to grow at low temperatures. Obviously, 100% of these are psychrophiles, but the majority are facultative.

These temperature studies were performed to emphasize the point that many organisms in natural soils in the manufacture and assembly areas of the Viking spacecraft are capable of growth at low temperatures. This same adaptability may enable them to survive and to grow in a more hostile environment, such as the Martian environment.

Although specific characterization of these isolates has not been completed at this early report, sufficient information has been given to give a general idea of the distribution of the major types of psychrophilic organisms present in the samples tested. It is apparent that sporeforming organisms are present in these samples and can exhibit growth at low temperatures. Some of these produced abundant growth at 3°C, but the majority of them showed only moderate to slight growth after 10-14 days. This group will be particularly important to investigate because of their potential heat resistance. It is obvious that one important point for investigation is to determine if the suggested sterilization cycle for the Viking spacecraft will destroy these spore-forming organisms. There are a number of NASA-sponsored investigators who have the proper equipment for such studies.

Some investigators feel that the phototrophic or autotrophic organisms will be among those most likely to grow in the Martian environment. From the results given here, the sites which were examined appear not to contain certain phototrophic and autotrophic bacteria which can grow at low temperatures, but a few of the samples do contain various types of algae which can grow under psychrophilic conditions. These results indicate agreement with those who feel that the phototrophic and autotrophic bacteria do not possess the adaptibility demonstrated by the heterotrophs.

In conclusion, it has been shown that areas associated with the Viking spacecraft do contain diverse populations of psychrophiles in fairly high concentration, and that nearly all of them contain sporeforming organisms capable of growth at low temperatures.

Plans for Future Work

More specific identifications will be performed on all isolates, and a sample of each type will be lypholized for future reference or use. Temperature studies will be completed on the anaerobes and fungi.

Isolates will soon be subjected to experimental procedures employing some of the environmental conditions suggested for Mars to determine if any of them can grow under these conditions. In his report to the Committee on Space Research in Madrid in 1972, A. A. Imshenetsky described a simplified system for experiments concerning simulated Martian environments. Attempts will be made to use a similar design in this present investigation.