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CR 73431
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This work was performed under NASA Contract NAS-2-1900 under the direction of the Ames Research Center.

IDENTIFICATION OF A RECURRING BACTERIAL CONTAMINANT IN A SPACECRAFT WATER SYSTEM

N70-34398

A Biosatellite spacecraft was successfully launched from Cape Kennedy in June, 1969. The experiment payload was a male Macaque nemestrina. As the prime spacecraft contractor, the General Electric Company, Re-Entry Systems Division, had the responsibility for applying and maintaining biological and chemical contamination control in the various vehicle life support subsystems.

Drinking water for the primate was derived from the hydrogen-oxygen fuel cell. The water was evolved as a by-product of power generation. The vehicle contained a discrete water collection, purification and dispensing system. A portion of the water was also used in the spacecraft thermal control subsystem by means of an evaporative boiler mechanism. Stringent chemical and biological constraints were applied to the potable water supply system to insure its compatibility with the primate experiment objectives. The chemical purification of the water was accomplished by treatment with a series of ion exchange columns. A bacterial filter was used to remove bacteria and fungi originating from the fuel cell and resin beds respectively. Chemical purification of the fuel cell effluent was accomplished with few problems. Effective biological treatment, however, was much more difficult to accomplish. A maximum allowable viable organism count of 200 colonies per milliliter of potable water was specified. The maximum allowable coliform count was 2.2 colonies per 100 milliliters of potable water. Rigid hardware fabrication and test procedures, personnel training and supervision schedules were applied. Bioassays were performed at strategic stages during the various manufacturing and test phases. The complete cooperation of all manufacturing and test personnel was obtained. However, in spite of all precautionary measures, random bacterial contamination repeatedly occurred.

At the first occurrence a mixture of coliforms and other organisms was found. Corrective measures were employed and coliforms ceased to appear in the assays. However, a second unidentified organism seemed to recur as a contaminant in spite of all precautionary measures. The control measures instituted consisted of the following:

1. All water system hardware was solvent cleaned and disinfected or sterilized during the final manufacturing step.
2. All assembled system hardware was disinfected with 70% ethanol prior to each test.
3. System components were touched with disinfected, gloved hands only.
4. The Aerospace Ground Equipment (AGE) used to service the water system was disinfected and bioassayed at regular intervals.
5. Disposable, sterilized transfer and test equipment was used wherever practicable.

Bacterial populations in the range of 10^4 to 10^6 colonies per ml continued to be found in the system at apparently random intervals.

Laboratory testing of the contaminating organism showed that it was capable of growth on a nutrient supply that was undetectable by our standard laboratory analytical procedures. Inocula of the organisms were incubated in flasks containing high purity deionized water, (the same water used to prime and test the spacecraft water system). Additional flasks were incubated using water in which several non-metallic components of the water system had been placed. The organisms were capable of growth in several of these media. (Table I).

An attempt was made to identify the organism (Table II) following the test scheme in Bergey's Manual of Determinative Bacteriology (7th Ed.) for the Pseudomonas family. The organism was tentatively identified as Pseudomonas boreopolis a ubiquitous, saprophytic soil bacteria. Due to the resistance of

this organism to the approved disinfecting agent (70% ethyl alcohol), and its ability to support itself on trace nutrients, it was determined that its growth could not be controlled outside of a completely sterile environment. A bioassay of the primate pre-flight experiment test facility, including the water dispensers and an assay of the primate's mouth indicated the presence of the same organisms. Parallel cultures of the organisms found in the test facility, the primate and the spacecraft water system were tested for similarity by the Pan American Environmental Health Laboratory at Cape Kennedy. The tests showed that a common contaminating organism was implicated in all cases. A search of the primate test history showed that a similar organism had been previously isolated, but not identified, from healthy test animals. Since the organism was not a known pathogen and was also found to be indigenous to the primate, the specification was relaxed to accept a viable count of 10^5 colonies per ml.

CONCLUSION

The recurring bacterial contaminant found in the Biosatellite water system was identified as Ps. boreopolis. The nature of the organism precluded its control without the employment of strict sterile procedures or chemical agents throughout the spacecraft manufacture and test cycle. Additional treatment of the water system by halogenation or other means would be required to control the contaminating organism.

Presented at the 9th Annual Technical Meeting of the American Association for Contamination Control, Anaheim, California - April, 1970.

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TABLE I

P. BOREOPOLIS GROWTH ON VARIOUS NUTRIENTS

25°C Incubation Time (Days)	COLONIES/ML					
	Silicore Rubber	Buna-N "O" Rings	Silastic	Deionized H_2O	Polished H_2O **	Nutrient Broth
1	1.3×10^4	*TNTC	1.0×10^4	1.1×10^4	1.0×10^4	4.0×10^8
2	1.5×10^6	3.8×10^6	3.1×10^6	2.1×10^5	3.0×10^5	6.4×10^8
5	3.3×10^5	3.3×10^6	9.1×10^6	3.3×10^4	7.6×10^4	1.8×10^8
8	7.9×10^4	2.1×10^6	5.2×10^6	4.1×10^4	6.4×10^4	3.1×10^8
12	2.2×10^4	2.3×10^6	5.4×10^6	3.7×10^4	6.8×10^4	3.9×10^8
16	8.0×10^4	2.4×10^5	5.3×10^6	7.3×10^4	1.5×10^5	8.9×10^7
19	2.1×10^4	2.6×10^6	6.2×10^6	3.4×10^3	1.8×10^5	$<10^5$
23	1.6×10^4	2.6×10^6	4.5×10^6	3.9×10^4	3.7×10^5	$<10^4$
30	2.9×10^4	2.8×10^6	4.8×10^6	6.6×10^4	1.5×10^5	$<10^3$

* Too Numerous to Count

** Deionized water that had been processed through ion exchange resin IRA-68 to remove low molecular weight organics.

Starting Inoculum = 1×10^4 Colonies/ml

TABLE II

PSEUDOMONAS SPECIES IDENTIFICATION ON ORGANISM ISOLATED FROM VARIOUS SYSTEMS OF THE BIOSATELLITE

TESTS:

1) Gram (-) motile rods (hanging drop method)

2) Oxidase positive (n, n-dimethyl-p-phenylenediamine oxalate)

3) Liquifies gelatin - 5 days incubation

4) Grows best at 37°C

5) Grows poorly at 40°C

6) Kligler Iron Agar - Slant Butt H_2S

NC NC

7) Simmons Citrate Agar - changes from original color (green) to deep blue after 48 hrs. incubation.

8) Urea not utilized

9) Lactose not fermented; no gas produced

10) Cellulose not utilized after 10 days incubation

11) Colonies - white on nutrient agar

Organism: Ps. boreopolis (tentative)

ACKNOWLEDGEMENT

I would like to acknowledge the technical contributions of Dr. George Webster and Mr. Robert Sanford of the Pan American Environmental Health Laboratories at Cape Kennedy and Miss Jean Negrey of the General Electric Experimental Biology and Biochemistry Laboratory.