N-63-4-3

Technical Report

R 256

SURVIVAL OF SEWAGE BACTERIA IN ZERO-CENTIGRADE SEA WATER

30 June 1963

U. S. NAVAL CIVIL ENGINEERING LABORATORY
Port Hueneme, California

SURVIVAL OF SEWAGE BACTERIA IN ZERO-CENTIGRADE SEA WATER

Y-R011-01-047

Type C

by

J. E. Halton, W. R. Nehlsen

ABSTRACT

The survival of Escherichia coli, a common indicator organism among sewage bacteria was studied in sea water at 0 C. It was found that from the time of inoculation about 70 percent of the organisms survived for 8 days, 34 percent for 15 days, and 1 percent for 35 days.

INTRODUCTION

The study of the survival of Escherichia coli in sea water at 0 C was made under the Navy Laboratories Research program as an adjunct to the development of a suitable sanitation system for use at advanced bases, notably under conditions encountered in the polar regions.

When raw sewage is discharged into very cold water, the natural processes of purification operate at considerably different rates than in temperate water. Although the need for biochemical oxygen is much less serious because of lowered biological activity, the mortality rate of pathogenic organisms is retarded. To gain further information on the magnitude of this latter effect, the common indicator organism, E. coli, was studied at near-freezing temperatures.

Before this study could be accomplished, it was necessary to select a method whereby the test organism could be recovered quantitatively from natural sea water incubated at 0 C. Various methods were investigated with respect to the preparation of the pure culture (test organisms), type of selective media, and plating procedures. The method developed by A. F. Carlucci and David Pramer[1] was selected. This technique uses Tergitol 7 agar with TTC (Triphenyltetrazolium chloride) added to differentiate E. coli colonies.

TEST PROCEDURES

The basic test procedure was to obtain survival data by inoculating bottles of sea water with known numbers of E. coli organisms and then making periodic checks of the number surviving after various periods of time. Unsterilized sea water was inoculated and then stored at 0 C. Details of the various techniques are given in the following paragraphs.

Preparation of Media and Inocula

Tergitol 7 agar is selective for the development and isolation of coliform organisms.[2] In the technique employed, the addition of 40 ppm TTC to the media greatly facilitated the identification of E. coli without influencing its growth. With TTC, the test organisms produced brown-centered colonies with yellow halos and were readily differentiated from colonies of Aerobacter aerogenes which had dark red centers and blue halos.[3]

The media was prepared by suspending 33 grams of dehydrated Tergitol 7 agar in 1 liter of cold distilled water. The suspension was then boiled for a few minutes until all the medium was dissolved. It was then sterilized in the autoclave for 15 minutes at 15 pounds pressure at 121 C. Final reaction of the medium was a pH of 6.9. When the media was sterile, 40 mg of TTC was added per liter, and completed media was poured into sterile 250-ml Erlenmeyer flasks and stored in a refrigerator.

Approximately 16 ml of Tergitol 7 agar were poured into each plate and allowed to solidify. Disposal plastic petri dishes 100 x 15 mm were used throughout the tests. These plates were poured well in advance so the agar would have sufficient time to solidify and to insure a dry surface.

The agar plates were inoculated by smearing the surface with the appropriate dilutions of the specimen, using a bent glass rod. Pour plates were tried but did not give satisfactory results, and the method was discarded. For these tests, 0.03 ml of inoculum was used per plate and distributed over the surface with the bent glass rod. The plates were incubated for 24 hours at 37 C, and colony counts were made. The incubators were kept well-saturated with moisture.

The bacteria used in these studies consisted of a suspension of washed cells of a pure culture of E. coli prepared by suspending 18- 24-hour-old nutrient lactose slant cultures of E. coli in sterile tap water. The cells were collected by centrifugation and washed three times with sterile tap water. The final cell suspension was adjusted with sterile tap water to a density equal to a cell concentration of approximately 4×10^8 per ml. Then, 1-ml samples of this inocula were used for inoculating the fresh sea water samples prior to treatment.

Preparation of Sea Water

Sea water samples were collected at high tide, approximately 1/2 mile offshore from the Oxnard Small-Boat Harbor. The area is fairly free of pollution and receives little or no fresh water dilution. The samples were collected in sterile ground-glass-stoppered sampling flasks. The temperature of a portion of the water sampled was measured on the spot. The duration of the trip from the sampling site to the Laboratory did not exceed 1/2 hour. A portion of water from each sample was tested promptly on arrival at the Laboratory, to determine pH, salinity, and the number of coliform bacteria, using Tergitol 7 agar. A Beckman pH meter electrometrically determined pH. Salinity was determined by titration with $AgNO_3$, according to the Mohr method.[4] Coliform bacterial counts of the raw sea water were made by inoculating the surface of the plates of Tergitol 7 agar with TTC with 0.02 ml of the water sample or dilutions thereof. Coliforms were counted after 48 hours incubation at 37 C.

Survival Results

When the preliminary tests of the sea water samples were completed, inoculum was prepared for the survival experiment. The inoculum was a pure culture of E. coli cells suspended in sterile tap water. The concentration of the final cell suspension approximated 4×10^8 per ml.

For quantitatively determining survival of the cells, each of two 100-ml portions of the same recently collected sample of sea water, in sterile 150-ml Erlenmeyer flasks, was inoculated to contain approximately 4×10^6 cells of E. coli per ml. The water in the flasks was swirled manually to insure uniform distribution of the inoculum. An aliquot was then taken from each of the test flasks, and appropriate dilutions were plated to determine the initial number of test cells. The flasks were incubated at 0 C without agitation. Survival of the test organisms was determined daily for the first 8 days, and then on various days thereafter until an elapsed incubation period of 35 days had expired (Table I).

A series of plates was inoculated with 0.02 ml of various dilutions of the samples being tested, and the inoculum was distributed over the dry surface of the agar with a bent glass rod. Plates were then incubated at 37 C for 24 hours, and colony counts were made (Figure 1).

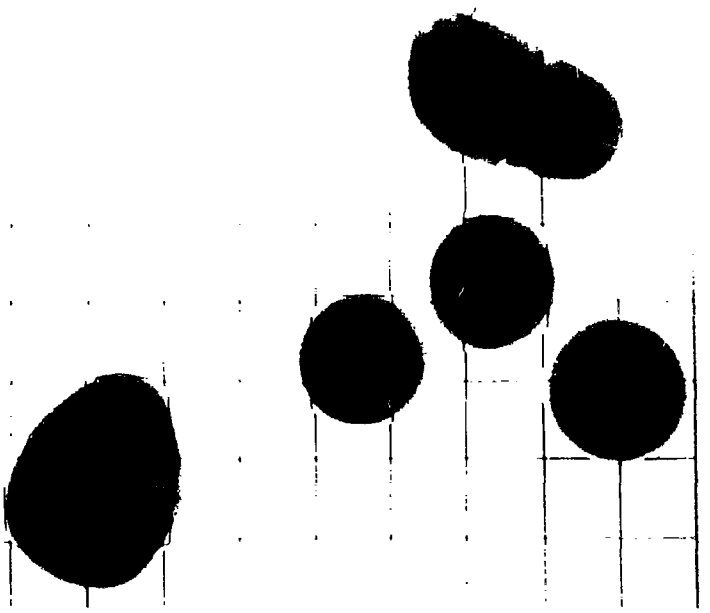

Figure 1. E. coli colonies on Tergitol 7 plates.

Table I. Survival of Escherichia coli in Sea Water at 0 C

Days	Sample Number 1		Sample Number 2		Average (%)
	Cells/ml	Percent	Cells/ml	Percent	
0	3,610,000		4,435,000		
1	3,780,000		4,490,000		
2	3,755,000		2,115,000		
3	3,900,000		3,380,000		
4	3,400,000		3,290,000		
5	3,700,000		2,940,000		
6	2,915,000		3,750,000		
7	3,615,000	100	3,600,000	100	100
8	2,875,000	80	2,765,000	62	71
10	2,475,000		2,225,000		
13	1,865,000		1,565,000		
15	1,450,000	40	1,250,000	28	34
20	460,000		385,000		
29	193,800		95,000		
35	67,000	1.9	12,600	0.3	1
44	40,250		9,500		

TEST RESULTS

The average temperature of the sea water was 12 C at the time of sampling. The pH averaged 8.2 at 17 C. Average salinity was 18,240 parts per million chloride. There was no E. coli in any of the fresh sea water samples plated, nor were any Aerobacter aerogenes detected in any of the samples tested.

The results of the quantitative determination of E. coli in sea water samples stored at 0 C confirm previous works[5,6] that low temperatures, comparable to those encountered in polar areas, favor survival of bacteria in sea water. Table I shows the percent of E. coli which survived at 0 C over a period of time (in days). The table also shows that the numbers of E. coli tend to fluctuate up and down during the initial week of incubation, with the cell concentration a little less than 4×10^6 per ml. This is comparable to the initial cell concentration prior to incubation at 0 C. After 7 days, the cell counts decreased daily. Results were confirmed with a duplicate test. The E. coli surviving after 35 days at 0 C averaged in excess of 1 percent or approximately 36,000 per ml. These survival times are much greater than those reported in Reference 1 for warmer waters. That data shows that at 20 C only 11 percent of E. coli survived for 48 hours.

The eventual decline in numbers is probably due to the diminishing source of food supply. This was especially evident in a supplementary test when a fresh sample (100 ml) of sea water (free of coliform organisms) was inoculated with 1-ml of suspension containing only 9500 cells of E. coli taken from an old test sample. After a 1-day incubation at 0 C, plate counts of this fresh inoculated sample showed that cells had increased to 510,000 per ml.

This large increase confirms the belief that the nutrient supply was an important factor in governing the survival of organisms; it also indicates the absence of antagonistic organisms in the sea water used.

When sewage is discharged in polar seas, reduction of numbers of sewage bacteria would be mainly a result of dilution and sedimentation. The presence of antagonistic organisms would further reduce the numbers. A need for disinfection of sewage discharges in polar regions is indicated if there is any possibility that sewage effluents will disperse to inhabited coastal areas.

CONCLUSION

Low sea water temperatures favor the survival of large numbers of sewage bacteria.

REFERENCES

1. Carlucci, A. F., and D. Pramer, "An Evaluation of Factors Affecting the Survival of Escherichia coli in Sea Water. Part I - Experimental Procedures." Applied Microbiology, Vol. 8, No. 4 (July 1960), pp 243-247.

2. Chapman, G. S. "A Superior Culture Medium for the Enumeration and Differentiation of Coliforms." Bacteriol. Proc. (1947), p 504.

3. Chapman, G. S. "A Culture Medium for Detecting and Confirming Escherichia coli in Ten Hours." American Journal of Public Health, Vol. 41, (1951), p 1381.

4. Standard Methods for the Examination of Water and Wastewater by APHA, AWWA and WPCF, Eleventh Edition. New York, 1962.

5. Nusbaum, I. and R. M. Garver. "Survival of Coliform Organisms in Pacific Coastal Waters." Sewage and Industrial Wastes, Vol. 27 (1955), pp 1383-1390.

6. Orlob, G. T. "Viability of Sewage Bacteria in Sea Water." Sewage and Industrial Wastes, Vol. 28 (1956), pp 1147-1167.

DISTRIBUTION LIST

SNDL Code	
	Chief, Bureau of Yards and Docks (Code 70)
23A	Naval Forces Commanders (Taiwan Only)
39B	Construction Battalions
39D	Mobile Construction Battalions
39E	Amphibious Construction Battalions
39F	Construction Battalion Base Units
A2A	Chief of Naval Research - Only
A3	Chief of Naval Operation (OP-07, OP-04)
A5	Bureaus
B3	Colleges
E4	Laboratory ONR (Washington, D. C. only)
E5	Research Office ONR (Pasadena only)
E16	Training Device Center
F9	Station - CNO (Boston; Key West; San Juan; Long Beach; San Diego; Treasure Island; and Rodman, C. Z. only)
F17	Communication Station (San Juan; San Francisco; Pearl Harbor; Adak, Alaska; and Guam only)
F41	Security Station
F42	Radio Station (Oso and Cheltanham only)
F48	Security Group Activities (Winter Harbor only)
H3	Hospital (Chelsea; St. Albans, Portsmouth, Va; Beaufort; Great Lakes; San Diego; Oakland; and Camp Pendleton only)
H6	Medical Center
J1	Administration Command and Unit - BuPers (Great Lakes and San Diego only)
J3	U. S. Fleet Anti-Air Warfare Training Center (Virginia Beach only)
J4	Amphibious Bases
J19	Receiving Station (Brooklyn only)
J34	Station - BuPers (Washington, D. C. only)
J37	Training Center (Bainbridge only)
J46	Personnel Center
J48	Construction Training Unit
J60	School Academy
J65	School CEC Officers
J84	School Postgraduate
J90	School Supply Corps
J95	School War College
J99	Communication Training Center
L1	Shipyards

Distribution List (Cont'd)

SNDL Code	
L7	Laboratory - BuShips (New London; Panama City; Carderock; and Annapolis only)
L26	Naval Facilities - BuShips (Antigua; Turks Island; Barbados; San Salvador; and Eleuthera only)
L30	Submarine Base (Groton, Conn. only)
L32	Naval Support Activities (London & Naples only)
L42	Fleet Activities - BuShips
M27	Supply Center
M28	Supply Depot (Except Guantanamo Bay; Subic Bay; and Yokosuka)
M61	Aviation Supply Office
N1	BuDocks Director, Overseas Division
N2	Public Works Offices
N5	Construction Battalion Center
N6	Construction Officer-in-Charge
N7	Construction Resident-Officer-in-Charge
N9	Public Works Center
N14	Housing Activity
R9	Recruit Depots
R10	Supply Installations (Albany and Barstow only)
R20	Marine Corps Schools, Quantico
R64	Marine Corps Base
R66	Marine Corps Camp Detachment (Tengan only)
W1A1	Air Station
W1A2	Air Station
W1B	Air Station Auxiliary
W1C	Air Facility (Phoenix; Monterey; Oppama; Naha; and Naples only)
W1E	Marine Corps Air Station (Except Quantico)
W1H	Station - BuWeps (Except Rota)
	Deputy Chief of Staff, Research and Development, Headquarters, U. S. Marine Corps, Washington, D. C.
	President, Marine Corps Equipment Board, Marine Corps School, Quantico, Va.
	Chief of Staff, U. S. Army, Chief of Research and Development, Department of the Army, Washington, D. C.
	Office of the Chief of Engineers, Assistant Chief of Engineering for Civil Works, Department of the Army, Washington, D. C.
	Chief of Engineers, Department of the Army, Attn: Engineering R & D Division, Washington, D. C.
	Chief of Engineers, Department of the Army, Attn: ENGCW-OE, Washington, D. C.
	Library of Congress, Washington, D. C.
	Director, Office of Technical Services, Department of Commerce, Washington, D. C.

Distribution List (Cont'd)

Director, U. S. Army Engineer Research and Development Laboratories, Attn: Information Resources Branch, Fort Belvoir, Va.

Headquarters, Wright Air Development Division, (WWAD-Library), Wright-Patterson Air Force Base, Ohio

Headquarters, U. S. Air Force, Directorate of Civil Engineering, Attn: AFOCE-ES, Washington, D. C.

Commanding Officer, U. S. Naval Construction Battalion Center, Port Hueneme, Calif., Attn: Materiel Dept., Code 140

Deputy Chief of Staff, Development, Director of Research and Development, Department of the Air Force, Washington, D. C.

Director, National Bureau of Standards, Department of Commerce, Connecticut Avenue, Washington, D. C.

Office of the Director, U S. Coast and Geodetic Survey, Washington, D. C.

Armed Services Technical Information Agency, Arlington Hall Station, Arlington, Va.

Director of Defense Research and Engineering, Department of Defense, Washington, D. C.

Director, Division of Plans and Policies, Headquarters, U. S. Marine Corps, Washington, D. C.

Director, Bureau of Reclamation, Washington, D. C.

Commanding Officer, U. S. Navy Yards and Docks Supply Office. U. S. Naval Construction Battalion Center, Port Hueneme, Calif.

Facilities Officer (Code 108), Office of Naval Research, Washington, D. C.

Federal Aviation Agency, Office of Management Services, Administrative Services Division, Washington, D. C., Attn: Library Branch

Officer in Charge, U. S. Naval Biological Laboratory, Naval Supply Center, Oakland, Calif.

Chief, Bureau of Medicine and Surgery, Attn: Research Division, Navy Department, Washington, D. C.

U. S. Army Cold Regions Research and Engineering Laboratory, Corps of Engineers, P. O. Box 282, Hanover, N. H.

Taft Sanitary Engineering Center, USPHS, Cincinnati, Ohio

Arctic Health Research Center, P. O. Box 960, Anchorage, Alas.

Arctic Aeromedical Laboratory, United States Air Force. APO 731, Seattle, Washi

Library, University of Alaska, Fairbanks, Alas.

Library, Engineering Department, University of California, 405 Hilgard Avenue, Los Angeles

Director, The Technological Institution, Northwestern University, Evanston, Ill.

U. S. Naval Civil Engineering Laboratory
Technical Report R-256
SURVIVAL OF SEWAGE BACTERIA IN ZERO-CENTIGRADE SEA WATER, by J. E. Halton and W. R. Nehlsen
9 p. illus 30 June 63 UNCLASSIFIED

A study of the survival of a sewage bacteria (Escherichia coli) in sea water at 0 C.

1. Sewage Bacteria – Survival at 0 C
I. Halton, J. E.
II. Nehlsen, W. R.
III. Y-R011-01-047

U. S. Naval Civil Engineering Laboratory
Technical Report R-256
SURVIVAL OF SEWAGE BACTERIA IN ZERO-CENTIGRADE SEA WATER, by J. E. Halton and W. R. Nehlsen
9 p. illus 30 June 63 UNCLASSIFIED

A study of the survival of a sewage bacteria (Escherichia coli) in sea water at 0 C.

1. Sewage Bacteria – Survival at 0 C
I. Halton, J. E.
II. Nehlsen, W. R.
III. Y-R011-01-047

U. S. Naval Civil Engineering Laboratory
Technical Report R-256
SURVIVAL OF SEWAGE BACTERIA IN ZERO-CENTIGRADE SEA WATER, by J. E. Halton and W. R. Nehlsen
9 p. illus 30 June 63 UNCLASSIFIED

A study of the survival of a sewage bacteria (Escherichia coli) in sea water at 0 C.

1. Sewage Bacteria – Survival at 0 C
I. Halton, J. E.
II. Nehlsen, W. R.
III. Y-R011-01-047

U. S. Naval Civil Engineering Laboratory
Technical Report R-256
SURVIVAL OF SEWAGE BACTERIA IN ZERO-CENTIGRADE SEA WATER, by J. E. Halton and W. R. Nehlsen
9 p. illus 30 June 63 UNCLASSIFIED

A study of the survival of a sewage bacteria (Escherichia coli) in sea water at 0 C.

1. Sewage Bacteria – Survival at 0 C
I. Halton, J. E.
II. Nehlsen, W. R.
III. Y-R011-01-047