INIS-PH--015

PH0100007

PUTATIVE RADIORESISTANT BACTERIAL ISOLATE FROM SEWAGE WATER

An Investigatory Project
Submitted to:
Ms. Charisma Coloma
Ms. Maricar Ribo

By:
Group Number 1
Ang, April
Chua, Patricia
Perez, Kristine
Rey, April
Rivor, Kristel
San Pablo, Czarina
Santos, Ernestine

In Partial Fulfillment of the Requirements in Chemistry

Miriam College High School
Katipunan Road, Quezon City
January 29, 2001

32 / 40

TABLE OF CONTENTS

List of Tables and Figures

ABSTRACT

Sewage water was collected from a stagnant body of water in Balara, Quezon City. Approximately 150 ml was aseptically transferred into eight Erlenmeyer flasks. Seven flasks were then subjected to different doses of radiation at the ^{60}Co Irradiation Facility, PNRI which are as follows: 0.01 kGy, 0.1 kGy, 0.5 kGy, 1 kGy, 5 kGy, 10 kGy, and 15 kGy. The remaining flask was used as the control. After irradiation, all the different treatments were subjected to colony count at the Culture Collection Laboratory, NSRI. Results showed that the colonies from sewage water treatments irradiated at 0.01 kGy (Treatment A), 0.10 kGy (Treatment B), and 0.50 kGy (Treatment C) exhibited a decreasing trend with colony counts 4.60×10^3 CFU/ml, and 1.30×10^3 CFU/ml, and 26 CFU/ml, respectively. Contrastingly, at 1 kGy (Treatment D), high colony count of 2.95×10^3 CFU/ml was observed which is even higher compared to the control (1.02×10^3 CFU/ml). Treatment E that was irradiated at 5 kGy manifested low survival rate (25 CFU/ml) indicating the presence of few putative intermediate radioresistant bacteria. Radiation dose treatments higher than 5 kGy (*i.e.*, 10 kGy and 15 kGy) exhibited no bacterial survival.

ACKNOWLEDGEMENTS

First of all, the researchers would like to express their utmost gratitude to Ms. Charisma Coloma and Ms. Maricar Ribo for the guidance and support that they have given to the research group. They would also like to thank the following people at Miriam College High School: Mrs. Lilibeth Pinpin for her encouragement, Mr. and Mrs. Frederick Delfin for their encouragement and for providing us with protocols, Mr. Dante de Leon for his advice, Ms. Carmelita Abadicio for her support and encouragement, and Mrs. Marcy Llose for providing us with some of the materials we needed. The researchers would also like to thank Ms. Julieta Reyes, the high school principal of Miriam College, for being so supportive of our undertaking.

The researchers would like to thank the Philippine Nuclear Research Institute (PNRI), especially Dr. Alumanda M. dela Rosa, for providing us with the materials, facilities, and personnel that we needed for the radiation procedures. The group would also like to appreciation to the following: Mr. Alejandro Nato for helping and guiding us, particularly in our paper and other laboratory procedures, Ms. Carol Coloma for giving us information regarding the paper, and for helping us in coordinating with NSRI and *"Ate Vina"*, Ms. Luvy Lanuza for monitoring the radiation process, Mr. Jun Modera for conducting the radiation process and for explaining the things we couldn't understand, and Mrs. Joy David for handling our paperwork.

Thank you to the Natural Science Research Institute (NSRI) and its personnel, especially Ms. Vina Argayosa, for conducting the colony count.

Thank you to all those who have allowed us to use invade their homes to fix our paper, most especially Mrs. Isabel San Pablo. Thank you to the people who have either allowed us to use their cars and vans as transportation, or drove us to where we needed to go, namely: Mr. Engracio D. Ang, Mrs. Amelia Melody Ang-Gayon, Mr. and Mrs. Andres A. Rey, Mrs. Isabel San Pablo, Mr. and Mrs. Ernesto Santos, Jerry Comoda, Arnel Corpuz, *Mang* Mando, *Mang* Lito, *Kuya Danny, Kuya* Arnold,and *Kuya* Rodel. Thank you, also, to those who have patiently waited or readjusted their schedules in order to accommodate our busy agenda.

Last, but definitely not least, thank you to GOD, for granting us the patience and strength to go on in the times we felt like quitting, and for giving us all the things we needed, material or otherwise... For your greater glory, Lord.

Chapter I

INTRODUCTION

A. Background of the Study

Radiation causes many bad effects. To us humans, it causes radiation sickness, cancer, genetic mutations and to some cases, death. It has three effects on living cells. First, cells repair themselves like having no damage at all. Secondly, cells die and are being replaced by normal processes. Lastly, cells change their reproductive structure. To the environment, it is known as a pollutant. It pollutes our natural resources which when people inhale or ingest will cause them the sicknesses mentioned above.

There are two types of effects of radiation. The first type of effect is the direct effect of radiation. Direct effects cause rupture on the plasma membrane. When the nucleus is directly impacted by radiation, the production of DNA is delayed in a period of time indefinitely in which the cell dies in a given range of doses. Another outcome is the chromosomal aberration. This is the change in normal shape of chromosome and this happens when the object is exposed to 35 rem. The last effect is the release of digestive enzymes from lysosomes will digest important cell components. The second type of effect is the indirect effect of radiation in cells. This causes the free radical production of cells This effect is the most common to happen because it does not require high doses of radiation. Doses for direct effects are unusually high for general public exposure. This is why indirect effects are more possible to happen.

Gamma radiation is the one that is used in this investigatory project. It is one of the three types of ionizing radiation. It has a very short wavelength but it has the most energetic form of electromagnetic radiation. This type of radiation is emitted by excited nuclei. The nucleus also releases alpha and beta rays along with gamma because the nucleus is left excited after giving off alpha and beta radiation. Gamma rays have various applications, which include treatment of cancer, diagnostics, sterilization of medical supplies like sanitary napkins, gloves, syringes, and capsules used to make tablets. This certain radiation is also used in the industry in inspecting of castings and welding, as well as, in food processing to kill microorganisms that cause spoilage.

Radioactive contamination in water is due to radioisotopes, which reach the underground water, and consequently pollute it. Since it eventually causes several human diseases, scientists have aimed to find an environment-friendly solution, wherein an organism would be able to withstand extreme amount of radiation and break down radioactive materials into simpler forms (DeWitt, 2000). The search for such radioactive-resistant (or radioresistant) organisms has been rendered difficult by the fact that only a few could truly withstand radiation. Fortunately, *Deinococcus radiodurans*, a gram-positive bacterium belonging to the family *Deinocae* that has these desired characteristics was discovered (Boomer, 2000).

The bacterium, *D. radiodurans*, is a pink colored bacteria that can survive 1.5 million rads of gamma irradiation. Though that dose of radiation shreds the bacteria's genome into hundreds of pieces, this organism has the remarkable ability to repair its DNA damages in one day, replicate without any gene defect and survive. (http://www.mailbase.ac.uk/lists/radiobiology/1999-11/0001.html) Also, according to other papers, it has been found out that *D. radiodurans* can be found in the following sources: sewage water, rich soil, manure, and other nuclear power plants.

However, since *D. radiodurans* is not yet available in the Philippines, this study aims to determine whether *D. radiodurans* or bacteria having the same main characteristics of the latter can be obtained from sewage water.

Sewage water was the source used in this project. Through our knowledge, we know that different kinds of bacteria thrive in sewage water. This is one of the reasons why the researchers want to use sewage water as the source. Another reason is because of its availability. Since sewage water can be obtained almost everywhere, it was easy for the researchers to find a good source. There are also some diseases that can be caused by sewage water. Some of these are typhoid, bacterial dysentery, cholera, poliomyelitis, meningitis, gastroenteritis, hepatitis A, Amoebic dysentery, and diarrhea.

B. Statement of the Problem

The researchers' aim in this project is to detect the presence of radioresistant bacteria from sewage water.

Specifically, the researchers want to answer these questions:

1. Can bacteria, found in sewage water, survive high radiation dose?
2. Until what radiation dose can bacteria found in sewage water survive?

C. Significance of the Study

The significance of this paper is to give preliminary information to other researchers. This entails other interested people to have ideas of the background of a topic that can be relative to this investigatory project. It would then stimulate the interest of all researchers to perform more studies on radioresistant bacteria in the country, which could lead to breakthroughs on health researchers. Also, in regards for biosafety guidelines and economic considerations, it would be best to have, at the ultimate, bacteria directly isolated in the country.

D. Scope and Limitations

Sewage water obtained from one source located in Balara, Quezon City are irradiated at seven different doses which are: 0.01 kGy, 0.1 kGy, 0.5 kGy, 1 kGy, 5 kGy, 10 kGy and 15 kGy. The researchers have discovered that the experiment was limited by the time. Time was the biggest factor that affected the process of doing the experiment. The group did not conduct the procedure on colony count because NSRI, the lab that was used, did not allow the researchers to do the work. Consequently, the results were limited to the report that NSRI gave. The method of irradiating was also not conducted by the group because the assigned people at the Philippine Nuclear Research Institute (PNRI) said that only trained workers can undergo the procedures to secure the safety of the researchers. When the samples of sewage water were brought to PNRI, the samples were not irradiated with gamma radiation all at the same time because of lack of space in the chamber where it was exposed to ^{60}Co. Some of the samples waited for a few days before it was exposed to radiation. The group was limited to only eight samples because of money restrictions.

Chapter II

REVIEW OF RELATED LITERATURE

A. Radioresistant Bacterium

In 1956, cans of ground beef sterilized by gamma radiation started mysteriously to swell. Inside the cans, a microorganism with a reddish pigment somehow survived the radiation. It was later dubbed as *Deinococcus radiodurans*, the toughest bug in the world (United States News and World Report, 1998).

Researchers have tried to explain in the DNA-repair mechanism undergone by *D. radiodurans* when irradiated and found out that irradiation causes the double-stranded DNA molecules to scatter into several fragments, only for *D. radiodurans*. A double-stranded break is the most difficult kind of DNA damage to repair (Internet: http://sites.netscape.net/skennedysd/generepairdresistance. October 2000). In fact, the well-studied *Escherichia coli* would not survive after two or three double-stranded breaks unlike *D. radiodurans* that can repair itself within hours, mutation-free (Internet: http://sites.netscape.net/skennedysd/generepairdresistance. October 2000). Studies show that *D. radiodurans* can withstand up to 120 double-stranded breaks and still survive. Within eight to ten hours after irradiation, the genes of *D. radiodurans* are restored. During gene repair, cellular replication is stopped. When radiation hits cells, it creates reactive forms of oxygen rendering important cellular compounds including the nucleotide building blocks of DNA oxidized and possibly causes faulty DNA replication.

An enzyme, MutT, which depletes the oxidized nucleotides of the cell, prevents this. Most bacteria have only one *MutT* gene but *D. radiodurans* has 20 *MutT* genes.

Another repair mechanism of *D. radiodurans* involves RecA, a protein found in all bacteria which has the ability to compare broken DNA strands with other copies of the molecule that are broken at a different place. Once the broken strands are matched with other copies in the molecules, RecA repairs the molecules through homologous recombination. Another important feature of this repair process is the ability to stop the cell building activities while the DNA is being repaired. Polymerase takes elements from the environment of the cell and put them together according to the DNA instruction. However, if the DNA strand is broken, the cell building process will stop and lead to the death of the cell. *D. radiodurans* actually ceases the action of the polymerases first, then goes on with DNA repair once the DNA is repaired, the polymerase are allowed to continue their cell-building process.

Furthermore, another repair mechanism involves the bacterium keeping the DNA intact so that the fragments will not be scattered and disoriented. Researchers speculate that *D. radiodurans* has some kind of scaffolding which provides a structure for the DNA to wrap around a structure and, consequently, give stability to hold fragments of DNA intact and in the right orientation (Internet: http://sites.netscap.net/skennedysd/generepair. October 2000). In addition, this structure makes it easy for RecA to find the broken DNA (Daly *et al.*, 1996; Ballista *et al.*, 1990).

In another study, *D. radiodurans* is modified so that it can partly break down toxic organic compounds such as toluene, a solvent present at many nuclear waste sites. Several genes from *Pseudomonas* were inserted into *D. radiodurans* to enable it to transform ionic mercury, a dangerous heavy metal, to a toxic state (United States News and World Report, 1998).

Researchers have found another radioresistant bacteria which can withstand radiation a log lower than *D. radiodurans*. This bacteria, *Kineococcus*-like, was recovered from a radioactive work area at Savannah River Site in Aiken, S.C. (Internet: http://www.srs.gov/general/sci-tech/fulltext/ms2000220/ms2000220.html)

Chapter III

METHODOLOGY

A. Sampling Site

The sample of sewage water is taken from a considerably polluted creek in Balara. The body of water that the researchers found is stagnant. Its color is dark brown containing large amount of garbage, *i.e.*, plastic cups, bottles, and styrofoam containers. The creek emits a very foul odor, which attracts mosquitoes and flies around the area.

B. Sample Collection

In eight sterile Erlenmeyer flasks, the researchers poured 20 ml of sewage water in each flask. They covered the flasks with cotton balls to prevent outside contamination. Afterwards, they had the flasks irradiated in the following doses: 0.01 kGy, 0.10 kGy, 0.50 kGy, 1 kGy, 5 kGy, 10 kGy and 15 kGy having irradiation times of 0:4:43, 0:47:08, 3:55:40, 7:51:20, 39:16:38, 78:33:17, and 117:49:55, respectively.

C. Colony Counting

The samples were given to the Natural Sciences Research Institute (NSRI), and their personnel were the ones who conducted the colony counting. They first serially diluted the samples to 10^{-2}. One ml aliquots of the undiluted sample and the dilutions were pour-plated in duplicates using Plate Count Agar. The plates were incubated and kept at room temperature for four days and the colonies were observed under a Quebec colony counter. The weighted mean count (WMC) is expressed as colony forming units (CFU) per gram sample and is calculated following the formula:

$$WMC = \frac{n}{(fa*1)+(fb*0.1)+(fc*0.01)...}*df$$

where n = total number of colonies in all plates counted

fa = number of plates with the first countable dilution

fb, fc = number of plates in the succeeding dilutions

df = dilution factor = reciprocal of fa

Chapter IV

RESULTS

Colony count of the control is 1.02×10^3 colony forming units per ml (CFU/ml). For Treatments A, B, C, D, and E, colony counts obtained are 4.6×10^3 CFU/ml, 1.3×10^2 CFU/ml, 2.6×10^1 CFU/ml, 2.95×10^3 CFU/ml, and 2.5×10^1 CFU/ml, respectively. In Treatments F and G, no bacterial count is detected. There is a constant decrease of number of colonies in the samples but treatment D has a different result. Instead of decreasing, high level of bacterial survival, which is even higher than the control, is observed.

Chapter V

DISCUSSION

In the results, there was a general decrease in the number of colony forming units per sample. However, it was also noted that there was a significant increase in the amount of colony forming units per ml (CFU/ml) in both Treatments A (0.01 kGy) and D (1 kGy). The increase in CFU/ml from the control set-up to treatment A is due to the fact that low radiation doses result to higher density of living cells and bacteria and a significant increase in nutrient transfer, waste transport, and reproduction. The number of colonies found in treatment D was almost as many as the number of those found in the control. This could be because 1 kGy is part of an excitatory phase for bacteria. A bacterium, when excited, activates its DNA repair mechanisms and causes it to be more active, accounting for such a large increase in the number of colonies.

The bacteria left in treatment E (5 kGy) can be considered as intermediate radioresistant bacteria. Intermediate radioresistant bacteria is defined as bacteria, which can survive between 1.5-5.0 kGy. It is not the highest existing radioresistant bacteria, but it does slightly exhibit some qualities of radioresistant organisms.

Chapter VI

CONCLUSION

It is, therefore, concluded that bacteria in sewage water from a polluted creek in Balara can survive high radiation dose of 5 kGy indicating the presence of a putative intermediate radioresistant isolate. Although some bacteria are known to survive radiation doses higher than 5 kGy, there is no isolate present from the samples used in this study.

Chapter VII

RECOMMENDATIONS

It is, thus, recommended that this project be given more time to be able to verify the results obtained. Money should also be considered because the irradiation and colony counting, which are required in this type of project, are expensive. It is also suggested that further research and verification be done on the result of treatment D (1 kGy). Sewage water should be taken from other sources or changed into other media where *D. radiodurans* and other radioresistant bacteria thrive.

BIBLIOGRAPHY

Battista, J.R. Against All Odds: the survival strategies of *Deinococcus radiodurans* Ann. Rev. of Micro. 51.

Madigan, M., J. Martiniko, J. Parker,. Brock Biology of Microorganisms. 9th Ed. New Jersey, Upper Saddle River: Prentice-Hall.2000

Murray, R.G.E. 1981. The Prokaryotes: a handbook on the biology of bacteria. Springer-Verlag. New York.

Pennisi, E..1998. DNA Sequences Provide Grist for Microbiologists. Science. 283.

Sylvia, D., Furhman, J., Hartel, P., Zuberer, D. Principles and Applications of soil Microbiology. Upper Saddle River, New Jersey. Prentice Hall.

Tangley, Laura. .1998. World

Whitman, D..2000." Micheal Daly (research into the use of the Deinococcus radiodurans bacterium in breaking down nuclear waste)." U.S. News and World Report. No.128.2000, 53

Websites:

www.bartleby.com/65/ga/gammarad.html

www.bartleby.com/65/ra/radiosick.html

www.beyond2000.com/news/story_376.html

www.beyond2000.com/news/Dec_99/story_377.html

www.chrsl.chem.isu.edu/~biosci/faculty_listings/fac_pages/jbattista.html, John Battista

www.ebi.ac.uk/proteome/d_radiodurans/1299.html

www.findarticles.com/cf_0/m1218/n25_v125/21212234/pl/article.jhtml, Laura Tangley

www.home.osti.gov/em52/old_abstracts/59786.html

http://lsda.jsc.nasa.gov/scripts/cf/exper.cfm?exp_index=183

www.mailbase.ac.uk/lists/radiobiology/1999_11/0001.html

www.nsf.gov/od/lpa/news/publicat/frontier/10-96/10bugs.htm www.ornl.gov/hgmis/publicat/hgn/vionl/12deino.html

www.ornl.gov/hgmis/publicat/microbial/image3.html

www.ornl.gov/hgmis/publicat/99santa/154.html

www.path.usuf2.usuhs.mil/pathology/minton.html, Kenneth W. Minton

www.research.ucla.edu/tech

www.sciencenews.org/sn_arc98/12_12_98/bob1.#html

www.tigr.org/tdb/cmr/gdr/htmls/background.html

www.tigr.org/tdb/cmr/gdr/htmls/splashpage.html

THIS PAGE IS MISSING
IN THE
ORIGINAL DOCUMENT

APPENDIX

Table 1. Irradiation of sewage water.

Labeled Petri Dishes	Amount of Radiation (kGy)	Irradiation Time	Plate Count CFU/mL
Control	0	0	1.02×10^3
A	0.01	00:04:43	4.6×10^3
B	0.10	00:47:08	1.3×10^2
C	0.50	03:55:40	2.6×10^1
D	1.00	07:51:20	2.95×10^3
E	5.00	39:16:38	2.5×10^1
F	10.00	78:33:17	0
G	15.00	117:49:55	0

Figure 1. Irradiation Chamber

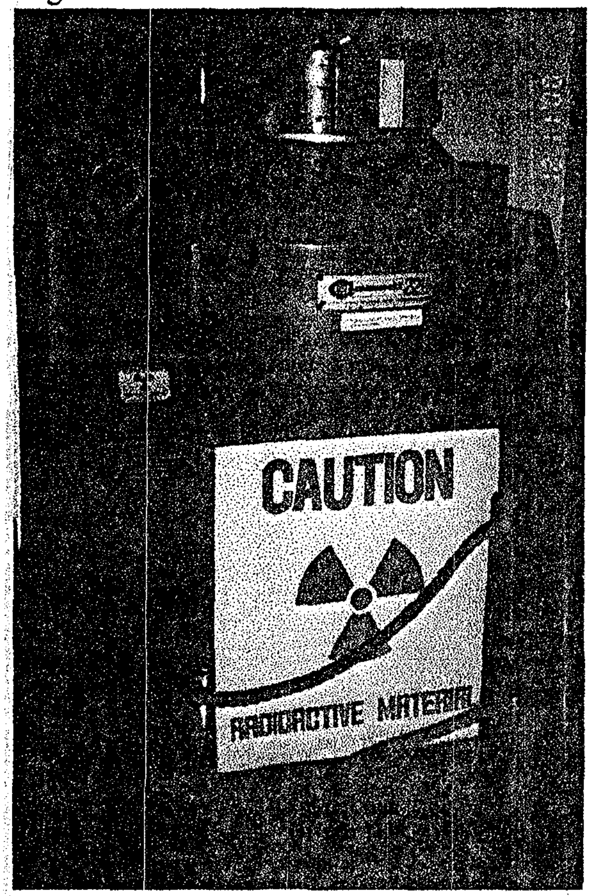

Figure 2. Irradiation Chamber

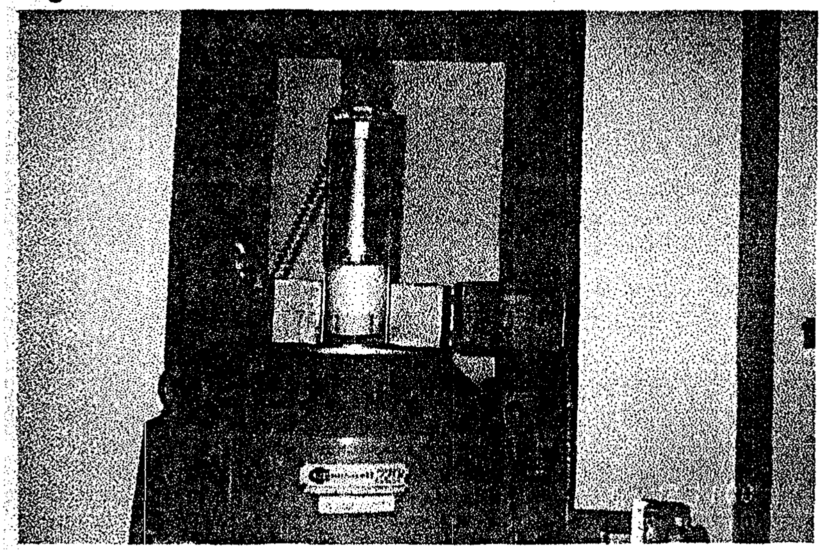

Figure 3. Bar Graph