A preliminary assessment of the effects of EM radiation on eubacterial genomes

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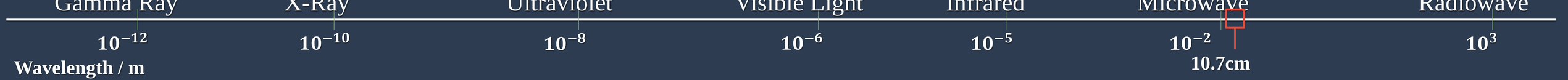


Problem Definition

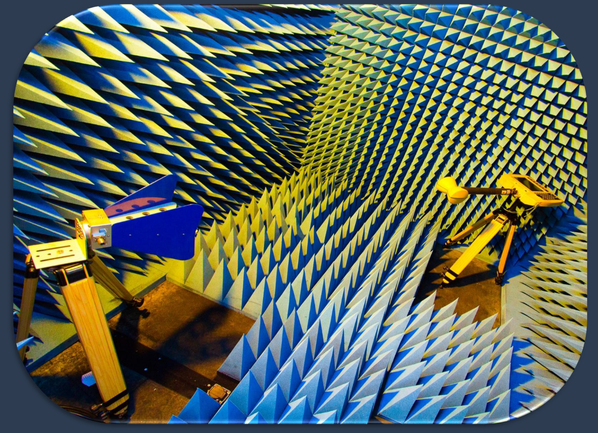
How does radiation at 10.7cm wavelength affect the genetic material of DH5 alpha samples?

Introduction

The effect of EM radiation on living organisms is well characterised and is a principal cause of genetic mutation. Nonetheless, several species are able to tolerate relatively high levels of short-wavelength radiation without undergoing apparent harm. The investigation of such extremophiles provides deeper insight into the characteristics which allow them to tolerate such abiotic conditions.

The primary aim of our project was to investigate the impact of 2.8 GHz radiation on changes in DNA sequences in bacteria. This microwave frequency was chosen since it is a preferred one for monitoring of solar activity. The main focus was specifically on a genetically-modified sample of DH5 alpha (*E. coli*). The procedure consisted of irradiating monoclonal samples of DH5 alpha with microwaves at a frequency of 2.8 GHz for periods of 24 and 48 hours inside an anechoic chamber. The irradiated samples and untreated controls were transferred into a nutrient broth and left overnight within a shaking chamber prior to DNA extraction. Whole genome sequencing of these samples was carried out and the data were used for comparative genomics to obtain a list of variant DNA sequences between control and irradiated microorganisms.

Bioinformatic analysis is currently ongoing, and data presented are still preliminary. Further work in this regard would involve using different frequencies of EM radiation and different prokaryotes.



The Anechoic Chamber at the EMRG lab at the Department of Physics, Faculty of Science, University of Malta, Msida (Malta)

Future Work

Using different wavelengths to irradiate DH5 alpha and other microorganisms

Materials and Methods

- Preparation of samples of genetically modified DH5 Alpha
- Some were placed in the anechoic chamber and irradiated with 2.8GHz microwaves
- A sample was left as a control
- Samples were taken out and irradiated after 24hrs 48hrs respectively
- 50mL tubes were then filled with 25mL of nutrient broth and the samples were transferred into it
- These were left overnight in the shaking chamber to promote bacterial growth
- A bacterial extraction kit was used and the bacterial DNA was checked for purity using a spectrophotometer
- A centrifuge was then used to separate the broth from the pellet prior to DNA extraction
- Pellets containing DNA were sent to EMBL Heidelberg, Germany for analysis where bacterial DNA was fragmented, labelled and run through a sequencing machine

Preliminary Results

- The analysis comprised 1 control sample and 12 test samples; 6 of which were irradiated for 24 hrs and the rest for 48 hrs.
- Full genome analysis gave back data on 2242 genes and showed which individual genes mutated and which were unchanged.
- Preliminary analysis showed that only 14% of the genes in the control sample had remained unchanged; the rest had mutated.
- Comparing test samples themselves, most genes were found to have mutated in 5 out of 6 samples after 24hrs and in all samples after 48hrs.
- 2% of DNA variants mutated in 4 or fewer samples after 48hrs.
- Further analysis will match the genes to their function.

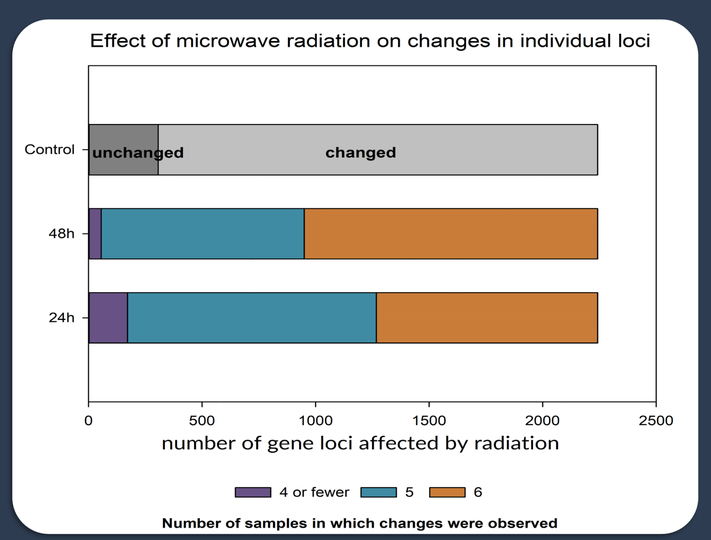


Figure 1: The graph shows a comparison between the samples which were irradiated for 24hrs, 48hrs and the control sample respectively. Gene analysis on 2242 genes was made on all 6 samples for both time exposures. The cumulative bars show the number of samples (out of 6), which have exhibited mutations and in how many genes from the total genome these mutations have occurred.
Although the single control sample appears to show a considerable number of unchanged gene loci when compared to the irradiated samples, the irradiated samples individually showcase a similar percentage of unchanged loci. These however average out, over 6 samples such that every locus is mutated in at least 3 samples.
Given the natural mutagenesis rate in control bacteria this was used to normalize the background mutagenesis in the test samples. further comparative analysis are envisaged to obtain a full list of Gene Ontology functions in the variant results.

Preliminary Conclusions

- As expected, minimal changes were observed when comparing mutations of test samples to those in the control.
- Microwaves have non-ionizing properties hence much less variation is expected to be observed when compared to the usage of higher frequencies.
- With an increase in exposure time, an increase in the number of samples where the genes had varied was observed. This is most likely due to the fact that more time was allowed for random mutations to occur.
- Genes might have also varied due to other factors and not necessarily due to irradiation, such as mutations which may occur during reproduction or due to other abiotic factors.
- Comparison of test samples with respect to control shows that there are still a few genes where mutations occurred in test samples but not in the control.
- These genes are still being investigated with regard to their products and function.

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