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Review Article

Molecular detection of mutant bacteria by radiation

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Abstract

Objective: The aim of this article to study molecular detection of mutant bacteria after exposition physical mutagenesis.

Study design: Meta-analysis study design.

Backrgound: Molecular genetics is a sub-agrarian of biology that items how various in the textures or expression of DNA molecules obvious as different among organisms. Molecular genetics often utilized an "investigative approach" to determine the textures and function of genes in an organism's genome utilizing genetic screens. Real-time PCR can be utilized to quantify nucleic acids via two common procedures: comparative quantification and absolute quantification. Ultimate quantification allow the exact numeral of target DNA molecules via comparison with DNA standards utilizing a calibration curve. It is therefore important that the PCR of the sample and the standard have the

similar amplification competence. Bacteria, singular bacterium) are ubiquitous, commonly free-living organisms often consisting of one biological cell. They composed a large domain of prokaryotic microorganisms. Typically a little micrometres in length, bacteria were among the headmost life forms to appear on Earth and are present in most of its environment.

Results: Mutations are enhanced via not only intrinsic factors for example inherent molecular mistakes but else via extrinsic mutagenic agents for example UV radiation. Morever, identifying the mutational features for both factors is essential to accomplish a comprehensive understanding of development processes both in naturlize and in artificial condition. Though there have been extensive Ttreatise on intrinsic factors, the mutational profiles of extrinsic factors are shaky understood on a genomic standardize. Here, the explored the mutation profiles of UV radiation, a ubiquitous mutagen, in *Escherichia coli* on the genomic scale. The accomplished an development experiment beneath periodic UV radiation for 28 days. The accumulation rapidity of the mutations was found to raise so that it transcended that of a typical mutator strain with insufficient mismatch repair modes. The huge contribution of the extrinsic agents to all mutations results raised the risk of the devastation of inherent error rectification systems. The spectrum of the UV-enhanced mutations was wider than that of the spontaneous mutations in the mutator. The wide spectrum and high upper determine of the hesitance of occurrence supposed ubiquitous roles for UV radiance in expedite the development process The analysis of the synonymous substitutions that accumulated through the development trails revealed a unique mutational spectrum. The spectra of the spontaneous

substitutions were previously reconnoitred for a wild type and *mutS*-defective mutator strain· Contrary to the wide distributions for the wild type, the mutator strain prohibited two peaks at AT to GC and GC to AT and very small hesitations for the another substitutions. This transition biased spectrum of the mutators was same to that another mutators strains with deficient mismatch-repair and/or proofreading processes. Else confirmed the same spectra features of the two mutators used in this treatise *ΔS* and *ΔHSB*, even although there were fewer accumulated substitutions than the values recorded in the previous studies. Compared with the exemplary spectrum of mutators, the spectrum of the UV-enhanced synonymous substitutions for all strains inclusive Co was broader. The portion of GC to TA was still high whilst the fractions of AT to TA, AT to GC and GC to TA were at levels comparable to that of the wild type, the UV-enhanced substitutions inclusive not only transitions but else transversions same to the spontaneous substitutions in the wild type. Microorganisms often organize their gene expression at the level of transcription and translation in restraint to solar radiation. The present the use of both transcriptomics and proteomics to progress knowledge in the field of bacterial response to destructive radiation. Those treatise pertain to different application areas for example fundamental microbiology, water treatment, microbial ecology and astrobiology. Even although it has been confirmed that mRNA abundance is not always consistent with the protein regulation.

Conclusion: Various techniques for molecular diagnosis of mutant bacteria via physical mutagenesis. Also, gene expression and conventional molecular

detection techniques are utilized to diagnose mutant bacteria via physical mutagenesis.

Key words: Genotypic detection, Irradiation, Mutagenesis and Microorganisms.

Introduction

Biology's field of molecular genetics examines the ways in which DNA molecule structure and expression vary among organisms . Scientists in the field of molecular genetics employ a process known as "investigative genetics" to determine the structure and/or function of genes in the genome of a particular organism [3]. Cellular, molecular, biochemical, and biotechnology studies are all founded on the merger of numerous biological subfields. Scientists search for gene mutations or change a gene in order to link a gene sequence to a specific phenotype. Many genetic diseases can be treated and even cured using a powerful technology called molecule genetics, which identifies mutations that are linked to certain genetic conditions [4].

For understanding molecular genetics , the Central Dogma of genetics is essential. As stated in the Central Dogma, DNA repeats itself through the process of transcription and translation [5]. The Central Dogma and the genetic code are utilized to understand the translation of RNA into proteins. ribosomes translate RNA into proteins, whereas mitochondria perform DNA replication

and transcription from DNA [6]. Having four base pairs in the genetic code means that the coding is completely redundant (adenine, cytosine, uracil, and guanine) , various combinations of these base pairs can yield the same amino acid (read in triplicate)[1] Proteomics and genomics, two branches of biology, owe their origins to Molecular Genetics and the Central Dogma [7].

Researchers employ reverse genetics to identify the genes or gene variations that are responsible for a specific trait. Mutagens (chemicals or radiation) or transposons are used to produce random mutations in order to screen individuals for a particular trait. When the target phenotype is difficult to see, Mutagenesis can be followed by a selection or other secondary test in organisms such as bacteria or cell cultures. To distinguish between mutants and non-mutants, the cells can be genetically engineered to express an antibiotic resistance gene or a fluorescence reporter reporter [8].

If the trait is caused by multiple genes, then a complementation test can be used to determine which of the genes is responsible for the phenotype. These genes can then be divided into three categories: those that have gained or lost their functions, those that have no influence, in addition to the ones that have remained constant (the mutant gene masks the phenotype of another gene). A mutation's exact location and kind can be determined through sequencing. [9]. It is an impartial strategy that often delivers a high number of unexpected outcomes, which is known as forward genetics . despite the fact that it can be costly and lengthy . Researchers have successfully used the nematode worm

Caenorhabditis elegans, the fruit fly Drosophila melanogaster, and the zebrafish Danio rerio to investigate the phenotypes generated by genetic abnormalities [10].

The term "reverse genetics" refers to molecular genetics techniques that are used to determine the phenotype of a gene of interest after an intentional mutation. The phenotypic is utilized to figure out what the gene's function is when it isn't mutated. Mutations in the gene of interest can be unintentional or purposeful [11]. Mutations may be a mis-sense mutation caused by nucleotide substitution, a frameshift mutation caused by a nucleotide addition or deletion, or the entire addition or deletion of a gene or gene section are all examples of mutations. A gene knockout occurs when a gene is deleted, losing its ability to operate as a gene e.g. knockout mice. Misunderstood mutations can result in a whole or partial loss of function, which is called a knockdown. RNA interference can be used to achieve a reduction in mRNA levels (RNAi) [12]. Introducing genes into the genome of a living thing is another possibility. A process known as transgenesis) to generate a gene knock-in, which leads in the host gaining the ability to perform a certain function. Because the gene of interest is already known, these techniques are more rapid than forward genetics in terms of producing results. However, these techniques have some inherent bias when it comes to selecting whether or not to link a phenotype to a specific function [13].

Mutagenesis is the process of altering an organism's genetic information through the occurrence of a mutation. In either case, it can be caused by exposure to mutagens. It is also possible to conduct experiments in the

laboratory. An organism's genetic code can be altered by a chemical or physical agent known as a mutagen, which causes an increase in the rate of mutations. Cancer and other heritable diseases can result from the process of mutagenesis in nature, which is a driving force of evolution. Their discoveries led to the creation of mutagenesis as a scientific discipline. M. Robson was a household name in the first part of the twentieth century [14].

A new mutation is passed down from one parent to their offspring on average every 60 generations in humans. Human males tend to pass on an average of two extra mutations to their offspring for every additional year of age [15,16].

Bacteria (singular bacterium) are widespread, free-living creatures that are composed of only one biological cell. Bacteria (singular bacterium) are the most common type of bacteria. They are a vast class of prokaryotic bacteria that can be found all over the world. It is believed that bacteria, which are normally only a few micrometres in length, were among the first life forms to originate on Earth and can now be found in virtually all of the planet's ecosystems. In addition to soil and water, bacteria can be found in a range of other areas such as acidic hot springs, radioactive waste, and the Earth's deep biosphere. Bacteria play a crucial part in the nutrient cycle, recycling nutrients and fixing nitrogen from the environment. The decomposition of dead bodies is a natural process that occurs as part of the nutrient cycle; microbes are responsible for the putrefaction stage of this process. Extremophile bacteria transform dissolved compounds such as hydrogen sulfide and methane into energy in the biological

communities surrounding hydrothermal vents and cold seeps, thereby supplying the nutrients required to sustain life. Bacteria can survive with plants and animals in symbiotic and parasitic relationships, depending on the situation. It is estimated that the vast majority of bacteria have not yet been identified, and that many species are hard to produce in the laboratory. Bacteriology is a branch of microbiology that is devoted to the study of bacteria [17].

The polymerase chain reaction (PCR) is utilized when executing a real-time polymerase chain reaction (real-time PCR) (PCR). DNA molecule tracking does not occur at the end of the PCR like it does in ordinary PCR, but rather during the PCR itself (in real time). It is possible to perform quantitative and semi-quantitative real-time PCR with real-time PCR (number of DNA molecules above/below a certain threshold) in real-time PCR (semi-quantitative real-time PCR). Detection of real-time PCR products is accomplished using one of two common methods: (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, or (2) sequence-specific DNA probes made up of oligonucleotides labeled with a fluorescent reporter that can only be detected after hybridization with the probe's complementary sequence, or both [18].

The MIQE guidelines recommend using the acronym qPCR for quantitative real-time PCR and RT-qPCR for reverse transcription–Qpcr [3] Although most authors use the abbreviation "RT-PCR" to refer to reverse transcription polymerase chain reaction rather than real-time PCR, this isn't always the case [19].

Quantification of gene expression applications Traditional It is unreliable to measure gene expression using DNA detection technologies. On order to obtain an accurate quantitative result, it is necessary to detect mRNA on a northern or Southern blot, as well as PCR products on gels or in a Southern blot. If you run a conventional PCR with 20–40 cycles, you will see that the amount of DNA product produced does not increase proportionally to the amount of target DNA introduced into the PCR. [20].

The two most prevalent methods for quantifying nucleic acids with real-time PCR are relative quantification and absolute quantification [21], by comparing target DNA molecules to DNA standards and utilizing a calibration curve, absolute quantification determines the exact number of target DNA molecules. As a result, it's critical that the sample and reference PCRs have the same amplification efficiency [22]. Internal reference genes are used to evaluate the relative expression of the target gene in comparison to other genes. Complementary DNA changes are produced by the alteration of mRNA expression levels (cDNA, generated by reverse transcription of mRNA). Relative quantification is faster and does not require a calibration curve because the amount of the studied gene is compared to the amount of a control gene. Relative quantification data can be compared across different RTqPCRs because the units used to measure them are irrelevant. Included housekeeping genes are meant to compensate for non-specific variation. such as variations in the RNA amount and quality that might reduce the efficiency of reverse transcription and, therefore, the PCR process in its entirety. In order for the approach to work, the reference gene must remain constant [5].

Because of its great sensitivity and dynamic range in identifying DNA, RT-qPCR can be used to identify genetically engineered species. A less precise option is to do a genomic or protein analysis instead.. Rather than the transgene, the problem lies with amplification of promoter, terminator, or even intermediary sequences employed in vector engineering. It is common practice to count the number of transgene copies used in the creation of a transgenic plant. When a single copy control gene from the treated species is used, its relative abundance can be quantified [6].

Results and Discussions

In *Escherichia coli* mutations accumulate when exposed to Ultraviolet radiation

Mutations are caused by both extrinsic mutagenic stimuli such as UV radiation and intrinsic mutagenic causes such as inherent molecular defects. It is critical to establish the mutational properties of both components in order to acquire a thorough an understanding of how evolution works in the wild as well as in laboratories. For all the work done on intrinsic factors, little is known about how extrinsic factors affect the genetic code . A frequent mutagen, UV radiation, was examined in Escherichia coli genome-wide. During the course of 28 days, we experimented with UV light. Most mutator strains' mismatch repair systems were unable to keep up with the rate of mutation accrual. As a result of the substantial contribution of extrinsic causes to mutations, the probability of innate error-correcting systems being removed increased The range of UV-

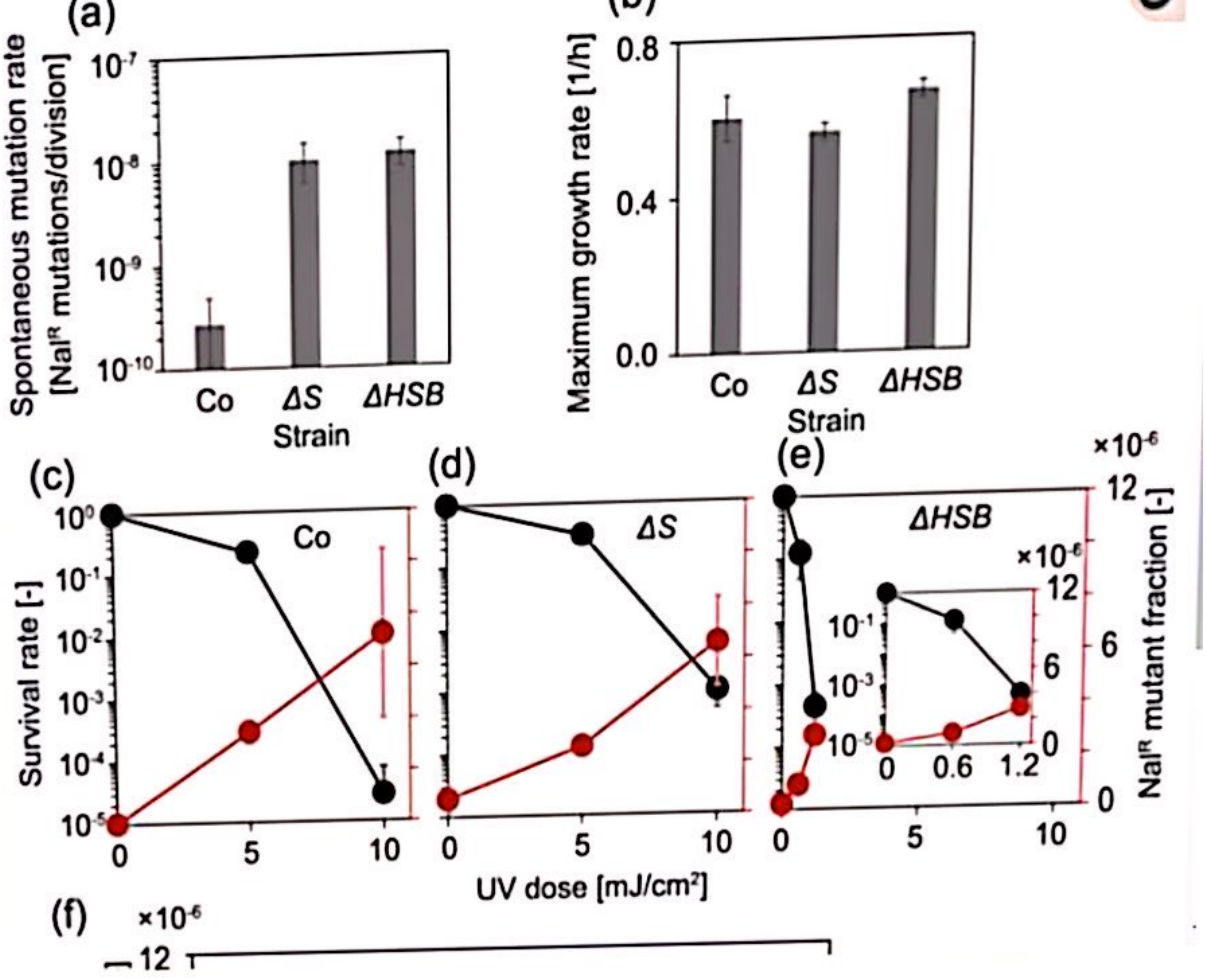
induced mutations in the mutator was greater than the range of spontaneous mutations. UV radiation has a wide spectrum of effects and a high upper limit of occurrence, implying that it has a widespread role in speeding up the evolutionary process [1].

Viability and mutation probability increased in E. coli after exposure to Uv radiation, according to the research.

Mutagens such as ultraviolet light (UV radiation) are hazardous . and it was predicted that it would decrease cell viability and increase the likelihood of mutations. Three E. coli strains were tested for UV sensitivity and the rate of natural mutation in the absence of UV exposure to see how these fundamental UV radiation activities influenced the organisms. One was E. coli MDS42, which was given the name Co because it had a good error-correction system. ΔS and HSB are two mutant strains. were constructed from Co by deleting the genes involved in error correction (*ΔmutS* for *ΔS* and *ΔmutH*, *ΔmutS*, and *ΔuvrB* for *ΔHSB*). In the mismatch repair system, loss of the mutS or mutH genes results in an increase in mutation rates, which has already been shown8. Nucleotide excision repair is mediated in part by the uvrB gene. Repairing genomic DNA damages induced by UV radiation is a key role of this activity. UvrB defects lower the native UV resistance. Thus, Δ HSB remained developed to remain a UV-sensitive mutator strain [23, 24].

First complete that the mutators from Co. had a higher rate of spontaneous mutation. A fluctuation test using a mutation that causes an acid called nalidixic acid resistance (NalR) confirmed that the two mutators had around 40 times the

mutation rate of Co. (Figure . 1a). Only a little variation in growth rate was seen between these mutator strains (Figure . 1b , ANOVA, $F(2 , 177) = 109$, $p < 0.05$) . These similar rates matched those found in a previous study, which found that when the mutation rate climbed over 100 times that of the wild type 8, the growth rate reduced significantly. Figure . 1c–e shows that UV radiation as a mutagen has a negative effect on the three strains' abilities to survive and adapt to their environment . For all strains, the survival rate, or the percentage of viable cells, declined as UV dosage rose. The NalR mutant fraction, on the other hand, grew in number. In other words, there was a negative correlation between the properties of viability and adaptability (Fig. 1f). In contrast to the other strains, HSB showed lower viability when exposed to UV light, The lack of uvrB is consistent with this (Figure . 1e, black circles). The NalR mutant percentage of this strain increased dramatically when the UV exposure was lowered (red circles) . Due of HSB's large and notably deadly mutation generation per UV dosage when compared to the two mutator strains' equivalent mutation rates without UV exposure, its UV-sensitive viability can be explained. Furthermore, It has been demonstrated in this study that it is possible to maintain a high level of UV-induced mutation rate simply by maintaining the survival fraction constant, regardless of UV dosages, sensitivity to UV exposure, or any other variable that affects survival fraction or spontaneous mutation rates [9].



: (1): UV radiation influences ancestral strain mutability and survival. (3) ;es of spontaneous mutation in three strains before to the experiment . 95 percent lential intervals estimated by MSS26. (b) Highest growth rates of ancestral strains. bars reflect standard deviations (n = 60). Affected survival rates (black circles) NalR mutant percentages among survivors (c–e) for the ancestral strains (Co, S, HSB) (red circles). Error bars reflect standard deviations (n = 3) . Co (black s), S (grey circles), and HSB (grey circles) (white circles). The data was re-plotted after (c – e).

ıtionary changes in viability and mutability in the presence of UV exposure

Treatments of 5 and 10 mJ/cm2 UV improved the survival rate in some lineages of all strains that had been exposed to UV during the evolution experiment, These results are in line with those that showed an increase in UV exposures during the course of the study (Figure . 3a, purple bars with asterisks). When it came to this growth, UV radiation was not a role (Figure . 3 a, grey bars). Adaptive evolution toward UV tolerance occurred in some lineages in the presence of UV radiation, which the researchers consider to be essential. During the evolution experiment, we discovered that UV light had a significant effect on lineage survival rates, which was consistent with our findings. Some lines of descent, notably S and HSB, did not exhibit a substantial improvement in UV tolerance during our short-term research (see below). The organism's mutability increased as a result of exposure to UV rays during the evolution experiment. It was found that some lineages exhibited this pattern even when they were not exposed to UV radiation (Figure. 3b). Even though viability increased in the evolution studies, individuals selected from populations did not lose their ability to adapt, demonstrating that probable error-correcting systems did not improve across the board (Fig. 3c). When it comes to viability and UV resistance, there is no evidence to suggest that UV-induced mutations are the outcome of the evolution of claimed error-correcting mechanisms in the first place [25].

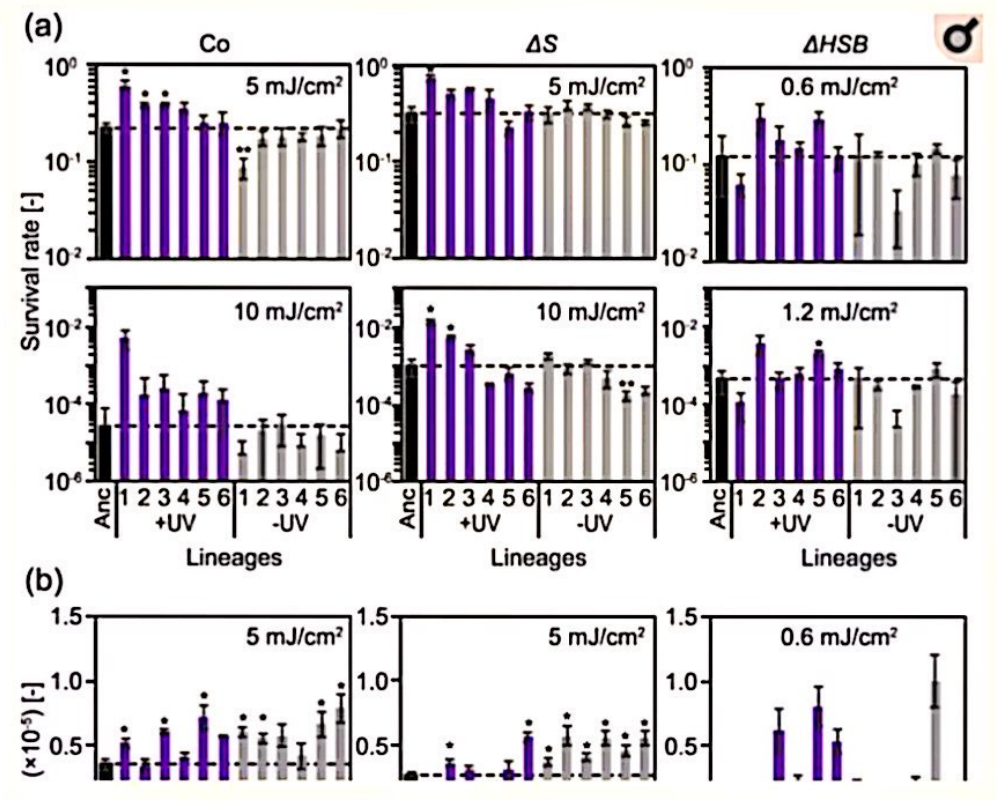


Figure (2): UV radiation impacts established strain viability and mutability. Finally, the evolution experiment shows the final populations' survival rates and their ancestral strains' fraction of NalR mutants. These were 5 and 10 mJ/cm2 for Co, S, and HSB respectively. 6 lineages of each produced strain were averaged. Error bars show standard deviation. A single asterisk denotes an increase in survival rate or mutation fraction, while two indicate a decrease (t or Mann-Whitney U tests, FDR less than 0.05; see Methods for more information about these tests). Ancestral strain survival rates and NalR mutations are shown in black circles, while lineages exposed to UV are shown in purple/grey circles. (a) and (b) redrawing the graphs in this manner (b). The ancestral strain levels are depicted by dashed lines on a vertical/horizontal axis.

There is growth selection after exposure to UV radiation in evolution studies, therefore growth charges can be used as a selecting characteristic. We compared

the maximal growth rates of evolved lineages to those of their ancestors who were not exposed to UV radiation in order to verify this hypothesis (Figure . 4). When exposed to UV radiation, all strains generated higher maximum growth rates than predicted following the evolution trials (Mann-Whitney U test, 0.05). The maximal growth rates of two mutator strains increased statistically significantly (Mann-Whitney U test, 0.05) level in the absence of UV exposure. Growth adaptability was aided by a higher mutation rate, as demonstrated by this experiment, which found that only the lineages of Co that were not exposed to UV during the evolution experiment exhibited no significant rise. If UV-induced mutations were not responsible for the matador lineage's greater maximum growth rates, they were just a minor factor. The matadors were not exposed to UV radiation [26].

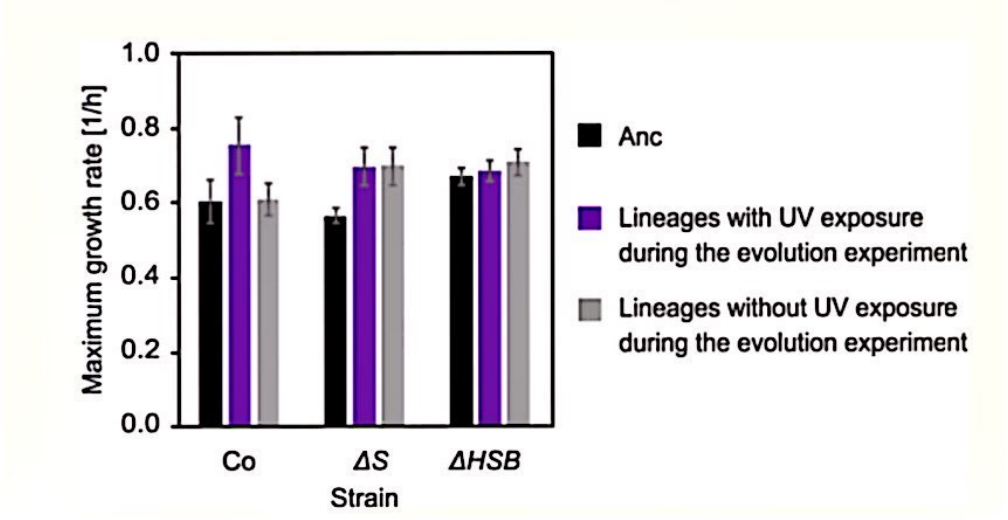


Figure (3): Traits of strain evolution Following UV exposure on 28th day , the final populations were compared to their parental strains. Refer to Fig. 1b when plotting

Anc. Each developed strain had six mean lineages. The error bars show SD. In all cases, maximal growth rates increased compared to their predecessors (t-test, $p < 0.05$). The accumulation of mutations during the UV exposure experiment in evolution.

It was determined through whole-genome sequencing that the populations had a set number of genetic alterations that were detected (Tables 1 and S1). For the evolution experiments, we used the number of synonymous substitutions to calculate the accumulation rate of base pair substitutions (BPSs) (Figure . 5). Accumulation and spontaneous mutation rates increased even if UV radiation was absent (Fig. 1a). Co mutations were reduced when UV radiation was not present, but the rate of change was still comparable to the wild-type mutation rate from a previous study [26]. In the absence of UV radiation, the accumulation rates of the mutators were higher than those of Co6. HSB exhibited a higher accumulation rate than S in fluctuation testing, which varied (Figure. 1a). This variation may be attributable to the fact that different techniques employ varied numbers and locations of marker genes [27]. There were much more mutations detected by genomic sequencing than were detected by the fluctuation test. It is also possible that the two mutators have a somewhat different set of mutational capabilities. Resistant strains may have variable mutation rates because of the presence of unique mutations in their mutants. Due to this, the evolution experiment without UV light produced different accumulation rates and spontaneous mutation assessments for different genetic backgrounds, as predicted. Afterwards, we estimated the accumulation rates in the presence of ultraviolet light (UV). The evolution experiment was a success , we found no significant correlation between the number of synonymous

substitutions and advantageous traits such as survival rate in response to UV (ρ= 0.04 and 0.27 for 5 mJ/cm2 and 10 mJ/cm2, respectively, $p > 0.05$) or maximal growth rate (ρ = 0.04, $p > 0.05$), indicating that few of the accumulated mutations were beneficial. UV-induced mutations, on the other hand, are expected to occur only infrequently, in comparison to spontaneous mutations. While under the influence of ultraviolet radiation, all pressures accumulated at approximately the same rate (ANOVA, $F(2, 14) = 0.15$, $p = 0.86$). These rates were much higher than those found in the absence of UV exposure (a 26-fold and a 3.4-fold increase in the accumulation rates of Δ HSB andΔ S, respectively). UV-induced mutagenesis was found to predominate over spontaneous mutation rates, and accumulation rates could be equal (Figure 5) independent of changes in UV dosage exposed (Figure 2c), viability in response to UV (Figure 1c–e), and/or spontaneous mutation rates (Figure 1d–e) (Figure 1). (Figure. 1a) [28].

The UV-induced mutations' mutational spectrum and local sequence context

During evolution studies, synonymous substitutions were analyzed to reveal a separate mutational spectrum (Fig. 6a and Table S2). MutS-deficient mutator strain6 and wild type3 have previously been studied for the frequency of spontaneous substitutions (Fig. 6a, top and middle). We found noticeable spikes in the frequency of GC/AT mutations in Mutator strain in comparison to the wild type (Figure . 6a, bottom). Mutator strains with poor mismatch repair or proofreading mechanisms were similarly shown to have a transition-biased spectrum. This study employed S and an HSB as mutators since their spectrum

properties were similar, and this was expected given the low number of substitutions reported in previous investigations (Table 1). Conventional mutators produced more and more diversified conjugate mutations than ultraviolet light (UV). GC to TA percentages in the wild type were identical to those in the AT to TA, AT to GC, and AT to TA fractions in the wild type. To put it another way, UV radiation had no effect on replacements in the wild [1].

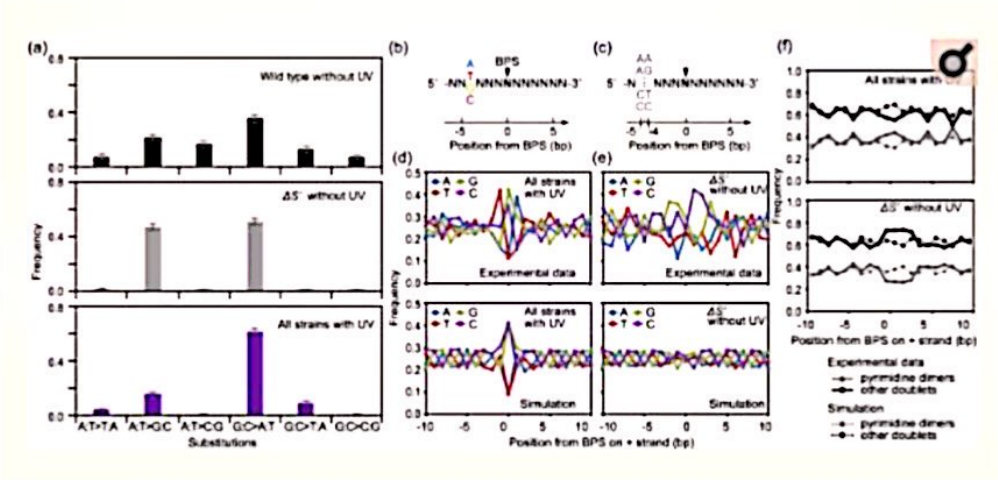


Figure (4): BPS spectra and context. (a) The frequency of each synonymy type. The Δ S' spectrum for the wild kind (top) and mutS-defective strain (center) were derived from prior investigations. 3,6. For the UV-induced mutation spectrum, all strains were combined (bottom). A 1,000 Monte Carlo simulation produces a standard deviation. These distributions were not comparable (Kolmogorov – Smirnov test , $p < 0.05$). The frequency of BPS was determined for each singlet (b,d,e) or doublet (c,f) nucleotide (10 bp to 10 bp). The frequency of BPS was computed for each nucleotide (A, T, G, and C) 4 bp from the BPS in (b) or for each doublet 4.5 bp from the BPS in (c) (c). The Monte Carlo simulations used the mutational spectra found for each dataset.

Synonymous BPSs were analyzed in greater depth to determine which single nucleotide in the immediate sequence context (10 bp to 10 bp) is most likely to appear with all BPS (Fig. 6b–e). Using Monte Carlo simulations, BPSs and their associated mutational spectra were generated at random in the genomes as a null hypothesis based on the absence of a biased context. In terms of UV's mutagenesis spectrum, BPSs are more likely to be induced at G or C. (Figure . 6a, and green and purple lines at 0 bp in Figure . 6d). According to Fig. 6, BPSs were more likely to occur on the fifth or third side of A or T on a DNA sequence (red line at 1bp and blue line at +1bp) as shown. Those arrangements "5-TC-3" and "5-TG-3" were vulnerable to UV radiation alterations as a result There is evidence that dipyrimidine sites can be a hotspot for mutations because they frequently produce DNA lesions (pyrimidine dimers) in response to UV exposure and are more likely to introduce BPSs, notably C through T at the previously damaged sites19. A prior study6 employed mutS-deficient strain, which did not have these basic patterns for spontaneous substitutions. We found that +2 bp side of G, +2 bp side of C, 3 side of G, or 5 side of C may be an error-prone motif after running the simulation [29].

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Refrences

1-Atsushi Shibai,[1] Yusuke Takahashi,[1] Yuka Ishizawa,[1] Daisuke Motooka,[2] Shota Nakamura,[2] Bei-Wen Ying,[3] and Saburo Tsuru[4]. Mutation accumulation under UV radiation in *Escherichia coli* . Sci Rep. 2017; 7: 14531. Published online 2017 Nov 6. doi: 10.1038/s41598-017-15008-1.

2-Sabine Matallana-Surget[1,2,*] and Ruddy Wattiez[3]. Impact of Solar Radiation on Gene Expression in Bacteria (2013). Proteomes. 2013 Sep; 1(2): 70–86. Published online 2013 Jul 16. doi: 10.3390/proteomes1020070.

3-Waters, Ken (2013), "Molecular Genetics", in Zalta, Edward N. (ed.), The Stanford Encyclopedia of Philosophy (Fall 2013 ed.), Metaphysics Research

Lab, Stanford University, retrieved 2019-10-07.

4-Alberts, Bruce (2014-11-18). Molecular biology of the cell (Sixth ed.). New York, NY. ISBN 9780815344322. OCLC 887605755.

5-Brunner, AM; Yakovlev, IA; Strauss, SH (2004). "Validating internal controls for quantitative plant gene expression studies". BMC Plant Biol. **4**: 14. doi:10.1186/1471-2229-4-14. PMC 515301. PMID 15317655. Archived from the original on 2013-08-02.

6-Holst-Jensen, Arne; Rønning, Sissel B.; Løvseth, Astrid; Berdal, Knut G. (2003). "PCR technology for screening and quantification of genetically modified organisms (GMOs)". Analytical and Bioanalytical Chemistry. **375** (8): 985–993. doi:10.1007/s00216-003-1767-7. PMID 12733008. S2CID 43681211.

7-"A Brief Guide to Genomics". Genome.gov. Retrieved 2020-12-04.

8- Schneeberger, Korbinian (August 20, 2014). "Using next-generation sequencing to isolate mutant genes from forward genetic screens". Nature Reviews Genetics. **15** (10): 662 676. doi:10.1038/nrg3745. hdl:11858/00-001M-0000-0024-CF80-4. ISSN 1471 0056. PMID 25139187. S2CID 1822657.

9- Sung W, Ackerman MS, Miller SF, Doak TG, Lynch M. Drift-barrier hypothesis and mutation-rate evolution. Proc Natl Acad Sci

USA. 2012;109:18488–18492.
doi: 10.1073/pnas.1216223109.

10- Lawson, Nathan D.; Wolfe, Scot A. (2011-07-19). "Forward and Reverse Genetic Approaches for the Analysis of Vertebrate Development in the Zebrafish". Developmental Cell. **21** (1): 48–64. doi:10.1016/j.devcel.2011.06.007. ISSN 1534-5807. PMID 21763608.

11- Kutscher, Lena M. (2014). "Forward and reverse mutagenesis in C. elegans". WormBook: 1–26. doi:10.1895/wormbook.1.167.1. PMC 4078664. PMID 24449699.

12-Hardy, Serge; Legagneux, Vincent; Audic, Yann; Paillard, Luc (October 2010). "Reverse genetics in eukaryotes". Biology of the Cell. **102** (10): 561–580. doi:10.1042/BC20100038. PMC 3017359. PMID 20812916.

13- Doyle, Alfred; McGarry, Michael P.; Lee, Nancy A.; Lee, James J. (April 2012). "The construction of transgenic and gene knockout/knockin mouse models of human disease". Transgenic Research. **21** (2): 327–349. doi:10.1007/s11248-011-9537-3. ISSN 0962-8819. PMC 3516403. PMID 21800101.

14-Beale, G. (1993). "The Discovery of Mustard Gas Mutagenesis by Auerbach and Robson in 1941". Genetics. **134** (2): 393–

399. doi:10.1093/genetics/134.2.393. PMC 1205483. PMID 8325476.

15- Jha, Alok (22 August 2012). "Older fathers pass on more genetic mutations, study shows". The Guardian.

16-Kong, A.; Frigge, M. L.; Masson, G.; Besenbacher, S.; Sulem, P.; Magnusson, G.; Gudjonsson, S. A.; Sigurdsson, A.; Jonasdottir, A.; Jonasdottir, A.; Wong, W. S.; Sigurdsson, G.; Walters, G. B.; Steinberg, S.; Helgason, H.; Thorleifsson, G.; Gudbjartsson, D. F.; Helgason, A.; Magnusson, O. T.; Thorsteinsdottir, U.; Stefansson, K. (2012). "Rate of de novo mutations and the importance of father's age to disease risk". Nature. 488 (7412): 471–475. Bibcode:2012Natur.488..471K. doi:10.1038/nature11396. PMC 3548427. PMID 22914163.

17-Pavan ME, et al. (May 2018). "Proposal for a new classification of a deep branching bacterial phylogenetic lineage: transfer of Coprothermobacter proteolyticus and Coprothermobacter platensis to Coprothermobacteraceae fam. nov., within Coprothermobacterales ord. nov., Coprothermobacteria classis nov. and Coprothermobacterota phyl. nov. and emended description of the family Thermodesulfobiaceae". Int. J. Syst. Evol. Microbiol. **68** (5): 1627–32. doi:10.1099/ijsem.0.002720. PMID 29595416. S2CID 4470260.

18-Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009). "The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments". Clinical Chemistry. **55** (4): 611–622. doi:10.1373/clinchem.2008.112797. PMID 19246619.

19-Logan, Julie; Edwards, Kirstin & Saunders, Nick, eds. (2009). Real-Time PCR: Current Technology and Applications. Caister Academic Press. ISBN 978-1-904455-39-4.

20-Overbergh, L.; Giulietti, A.; Valckx, D.; Decallonne, R.; Bouillon, R.; Mathieu, C. (2003). "The use of real-time reverse transcriptase PCR for the quantification of cytokine gene expression". Journal of Biomolecular Techniques. **14** (1): 33–43. PMC 2279895. PMID 12901609.

21-S. Dhanasekaran; T. Mark Doherty; John Kenneth; TB Trials Study Group (March 2010). "Comparison of different standards for real-time PCR-based absolute quantification". Journal of Immunological Methods. **354** (1–2): 34–39. doi:10.1016/j.jim.2010.01.004. PMID 20109462.

22-Seeley TW, Grossman L. Mutations in the Escherichia coli UvrB ATPase motif compromise excision repair capacity. *Proc Natl Acad Sci USA*. 1989; 86:6577–6581. doi: 10.1073/pnas.86.17.6577.

22- Bar, Tzachi; Kubista, Mikael; Tichopad, Ales (2011-10-19). "Validation of kinetics similarity in qPCR". Nucleic Acids Research. 40 (4): 1395–1406. doi:10.1093/nar/gkr778. ISSN 0305-1048. PMC 3287174. PMID 22013160.

23-Sancar A, Rupp WD. A novel repair enzyme: UVRABC excision nuclease of Escherichia coli cuts a DNA strand on both sides of the damaged region. *Cell.* 1983;33:249–260. doi: 10.1016/0092-8674(83)90354-9.

24- Bar, Tzachi; Kubista, Mikael; Tichopad, Ales (2011-10-19). "Validation of kinetics similarity in qPCR". Nucleic Acids Research. **40** (4): 1395–1406. doi:10.1093/nar/gkr778. ISSN 0305-1048. PMC 3287174. PMID 22013160.

25- Wielgoss S, et al. Mutation rate dynamics in a bacterial population reflect tension between adaptation and genetic load. Proc Natl Acad Sci USA. 2013;110:222–227. doi: 10.1073/pnas.1219574110.

26- Lee H, Popodi E, Tang H, Foster PL. Rate and molecular spectrum of spontaneous mutations in the bacterium Escherichia coli as determined by whole-genome sequencing. Proc Natl Acad Sci USA. 2012;109:E2774–2783. doi: 10.1073/pnas.1210309109.

27- Tsuru S, et al. Genomic confirmation of nutrient-dependent mutability of mutators in Escherichia coli. Genes Cells. 2015;20:972–981. doi: 10.1111/gtc.12300.

28-Kishimoto T, et al. Molecular Clock of Neutral Mutations in a Fitness-Increasing Evolutionary Process. PLoS Genet. 2015;11:e1005392. doi: 10.1371/journal.pgen.1005392.

29- Tillich UM, Lehmann S, Schulze K, Duhring U, Frohme M. The optimal mutagen dosage to induce point-mutations in Synechocystis sp. PCC6803 and its application to promote temperature tolerance. PLoS One. 2012;7:e49467. doi: 10.1371/journal.pone.0049467.