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Mutation accumulation in bacteria exposed to UV radiation

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Abstract

Reducing native complexity from living organisms has significance in several aspects such as academic application as model organism and industrial application as factory of useful material. In particular, reduced complexity is also interesting in the field investigating the minimal form of "life-as-we-know-it." However, subtracting or inactivating genes from the genome without growth defects is difficult due to the complexity of gene network and error proofing functions. In this study, using model bacteria *Escherichia coli* (*E. coli*), we designed a culture system using ultraviolet as a mutagen in order to select possible mutants growing rapidly with genomic inactivation. Here, we demonstrated that the culture system could accumulation of many mutations and preservation of growth ability. These results suggest that our method is effective to obtain functionally reduced genome of *E. coli.*

Introduction

E. coli is broadly used as a representative model microorganism in many biological fields such as genetics and genetic engineering. In some growing industrial fields such as pharmacy and energy production, *E. coli* or other microorganisms have been used as factories of useful chemical compounds. These microorganisms have intricate gene networks acquired through their evolutionary processes. Such inherent complexity often poses an obstacle to arbitrary genetic operation. Therefore, a reduction of such complexity through genomic inactivation would be helpful in several applications both academic and industrial. In addition, the attempt of functional reduction of the microorganism's genome itself has importance in investigation of the minimal form of "life-as-we-know-it."

An *E. coli* stain with 20% reduced genome has been engineered (Pósfai, et al. 2006), with the manual approach focusing on the genes which are considered as non-essential and attempting to remove them from the genome. But this approach is difficult to perform repeatedly because it is required to choose the set of genes that does not reduce the growth rate in every single operation of gene replacement. On the other hand, in nature, large genome reduction with gene inactivation is observed in endosymbionts (Mira, et al. 2001). This phenomenon is considered as the result of mutation accumulations under weak selection pressure due to the compensatory support from their hosts for the lost functions of the endosymbionts. Most mutations extinguish the function of the gene and are neutral or deleterious for the bacterial growth (Eyre-Walker and Keightley, 2007). In order to apply that in-nature genome reduction process to evolutionary engineering, necessity of long term caused by low mutation rate and degradation of bacterial growth need to be resolved.

In this research, ultraviolet (UV) exposure was used as the mutagen which raises mutation rate as the dosage increases, and the subculture experiment was achieved with growth selection to maintain bacterial growth ability. In that way, the problems of long term and degradation of growth are supposed to be solved. This novel method allow high speed mutation fixation maintaining growth rate aiming at reduction of bacterial genome in term of the number of its functions.

Materials and Methods

We performed daily transfers of the bacterial cultures for 28 days using *E. coli* MDS42 strain whose genome was manually reduced from the progenitor. The bacteria were incubated in M63 minimal medium. Standard bactericidal lamps were used as the UV source. The UV dosages were measured and arranged by changing exposure time, distance from the lamp and usage of filters. The experiment was replicated six times and achieved simultaneously.

At the first day of the subculture experiment, a saturated pre-culture of MDS42 was diluted to 100 times with fresh M63 medium and applied 100 µl each into 5 wells on the 96-well microplate. These 5 wells were irradiated with different dosages of UV respectively. After sealing, the plates were incubated at 37 ^{o}C with shaking at 550 rpm for 24 h.

As the daily operation of the subculture (Fig.1), at first, optical density (OD) of 595 nm were measured for the five wells. The wells whose UV dosage was the largest among the survived wells (OD>0.1) were sampled and diluted to 100 times with fresh M63 medium, and poured 100 µl each into 5 wells on a new 96-well microplate.

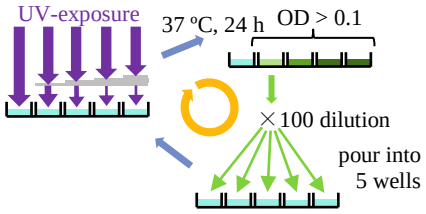

Figure 1: Cycle of the culture system with UV-exposure.

The coordination of the UV dosage was conducted with the filters, which had each a different numbers, and all wells were exposed to UV simultaneously. One filter dims UV to 0.6 times, and each well has 0, 2, 4, 6 or 8 filters, respectively. The exposure was raised as the linages obtained UV-resistance and the number of filter of the selected wells decreased. The dosage on the well without filter was tracked on a daily basis and defined as the basic dosage. The basic dosage values of 6 lineages were identical for each day through the experimentation.

The -80 ^{O}C frozen stocks of the ancestor and 6 evolved lineages (28th day) were analyzed as follows. The maximum growth rates in the absence of UV exposure were measured from the increase rate of OD values in exponential growth phase. The purified genomic DNA samples of the lineages were sequenced by Illumina whole genome sequencer, Miseq. We identified a list of base pair substitutions (BPSs) and short insertions/deletions (indels) for each samples using the standard software SAMtools.

Results

The average UV-dosage of the selected wells among the 6 lineages was increased over the experimentation (Fig.2). This increase was not only caused by the operation which raised the basic dosage, since the average increased even while the level of basic dosage was steady. This result means that the lineages obtained UV resistance through the experimentation.

The maximum growth rates in the absence of UV exposure of the ancestor and the evolved strains are 0.60±0.1 [h^{-1}] and 0.75±0.1 [h^{-1}] (mean±SD), respectively. Therefore, the growth rates were maintained without drop through the experiment.

Genomic resequencing revealed the number of BPSs and short indels as shown in Table 1. The Ka/Ks, the ratio of non-synonymous substitutions to synonymous substitutions, was 0.53±0.14 lower than 1, suggesting a strong negative selection. That is, the mutations altering protein sequence were basically disfavored through the evolution. In addition, the mutation rate was about 6.0×10^{-8} [$bp^{-1}generation^{-1}$] which is about 100 times higher than reported value of *E. coli* mutation rate (5.4×10^{-10} [$bp^{-1}generation^{-1}$], (Drake, et al. 1998)).

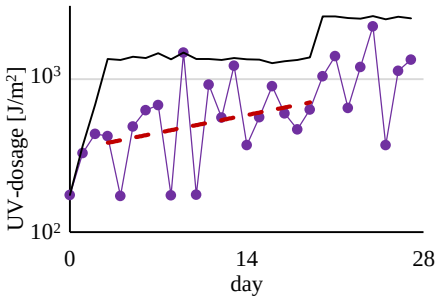

Figure 2: Acceptable UV-dosage increased through the subculture experiment. Daily records of mean UV-dosage on 6 lineages (purple circle plots) oscillated, but the exponential curve fitting (red dashed line) raised even while the basic exposed value (black solid line) was not increased.

BPSs		indels	Ka/Ks	mutation rate [$bp^{-1}generation^{-1}$]
syn.	non-syn. (stop codon)			
36.8 ± 7.4	64.2 ± 22.5 (5.8 ± 3.0)	3.0 ± 2.7	0.53 ± 0.14	$7.9 \pm 1.6 \times10^{-8}$

Table 1: Number of detected mutations suggest the existence of selection pressure and high mutation rate. The numbers of BPSs and indels are represented as per genome per lineage [mean±SD, n=6.] Ka/Ks value is adjusted with the ratio of synonymous sites and non-synonymous sites (1:3.24). Mutation rate was calculated considering that the fraction of synonymous mutations in the genome is 21.0 %, and that lineages passed 560 generations through the subculture.

Discussion

UV-dosage of the selected wells were increased even in the period of that the amount of UV-exposure was steady. Therefore it is thought that the lineages obtained UV resistance through the subculture. This consideration suggests that the evolutionary process during the subculture was influenced by UV. Consequently, the subculture environment had about the 100 times higher mutation rates than usual, according to the numbers of BPSs detected on the evolved lineages. In addition, whole genome resequencing revealed the strong negative selection that purges a part of deleterious mutations from the population. The existence of this selective effect is consistent with the fact that the growth rate of the evolved lineages were maintained.

Despite such negative selections purging about 30 non-synonymous mutations estimated from synonymous substitutions, more than 60 non-synonymous BPSs per lineage were detected. Therefore, most mutations were fixed on the *E. coli* genome through the experimentation. Interestingly, the total number of the mutations into stop codon and short indels was 8.8 per lineage (5.8 mutation into stop codon and 3.0 short indels.) It is expected that mutated genes will lose their function, even if part of the sequence keeps its integrity. Therefore, it is suggested that this culture system may be used as an effective tool to accumulate inactivation mutation on the *E. coli* genome, causing genome reduction.

According to the above, we suggest that the fixation and accumulation of many mutations on the *E. coli* genome could be proceeded without growth defects through the growth selection with high mutation rate.

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