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ANTIBIOTIC PERSISTENCE OF *ESHERICHIA COLI*IN HIGH AND LOW NUTRIENT CONDITIONS

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ABSTRACT

Aging in unicellular organisms and is presumably due to asymmetric distribution of damaged proteins and other components during cell division. The asymmetry-induced aging is inevitable or an adaptive response is debated. Minimum inhibitory concentration results were expected to show that the low cultures adapted to oligotrophic nutrient medium would be highly sensitive to the different antibiotic concentrations since there is a cost involved in carrying the antibiotic genes, but instead the results showed that the cell cultures grown in low nutrient media were resistant to the antibiotic and had a higher growth rate which indicated that there was no cost involved. The, results suggest that cellular aging due to asymmetric division may shows plasticity as well as evolvability in response to the nutritional environment.

KEYWORDS: **Aging, asymmetric division, self-fabricated slide, oligotrophic nutrient medium**

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INTRODUCTION

Bacteria are always considered as organisms that do not age. This property is attributed to bacteria due to binary fission which is assumed to proceed without equal distribution of damaged and undamaged constituents. Studying aging in cells involves two key aspects: asymmetric division and stage of life cycle which leads to reproduction. Aging is known to occur in bacteria and in yeast that divide with morphological asymmetry [1, 2, 3.] For species that appear to divide symmetrically, aging is demonstrated relatively recently [4] and is thought to arise from aggregation and asymmetric inheritance of oxidatively damaged proteins [5, 6, 11]. In fact, the two cells after division can be viewed as a parent cell and a daughter cell rather than as two sister cells. Most bacteria reproduce by dividing into two seemingly identical cells. It was assumed that both cells are born equally young, and that these organisms do not therefore show any decrease of condition with age[7].

The aging process is driven by a lifelong accumulation of molecular damage, which results in gradual increase in the fraction of cells carrying defects. After some time has passed, the increasing levels of these defects interfere with both the performance and functional reserves of cells, resulting in ageing, disability and disease. Stress, adverse environment and poor nutrition can increase the rate at which molecular damage arises. Intrinsic maintenance mechanisms, such as DNA repair and antioxidants lower the rate of accumulation [8]

Most work on microbial aging has been done with the eukaryotic budding yeast, *Saccharomyces cerevisiae*[9]. Cell division in budding yeast is asymmetric, with the larger mother cell easily distinguishable from the budded daughter cell. A bit later studies on *Escherichia coli* provided substantial evidence, that some bacterial species do really age, which opened up a new era in the aging research on the prokaryotic models. For species that appear to divide symmetrically, aging was demonstrated relatively recently and is thought to arise from aggregation and asymmetric inheritance of oxidatively damaged proteins.[10]

It is assumed for many years that cells that divide symmetrically do not age and are functionally immortal. Eric Stewart and colleagues tested this idea by analyzing repeated cycles of reproduction in *E.coli*, which reproduces without a juvenile phase and with an apparently symmetric division. *E.coli* reproduces by dividing in the middle. Each resultant cell inherits an old pole and a new pole, which is made during the division. The new and the old pole contain slightly different components that look the same but are physiologically asymmetrical. At the next division, one cell inherits the old pole again (plus a brand new pole), while the other inherits a not very old pole and a new pole. Thus, Stewart and co-workers reasoned, that an age in divisions can be assigned to each pole and hence to each cell. They found that the cells inheriting the old poles has a reduced growth rate, decreased rate of offspring formation, and increased risk of dying as compared to the cells inheriting the new pole. Thus, although the cells produced when *E.coli* divide look identical, they are functionally asymmetric and the old pole cell is effectively an aging parent[4].

In an attempt to elucidate the pros and cons of symmetrical and asymmetrical bacterial division, Watve et al. modelled growth and the propagation of growth-limiting components of a unicellular system using a modified Leslie matrix framework. As developed, the model points to asymmetrical division favoring rapid growth, whereas symmetry results in slow growth but higher efficiency; i.e. a higher growth yield.[12]Similarly, using an individual-based simulation approach, Ackermann et al.[10, 12]

found that a differentiation between an aging parental cell and a rejuvenated progeny readily evolves to cope with self-inflicted damage. Also, upon transient external stresses reaching lethal levels, an asymmetrical segregation of irreparable damage may permit survival of the clone at the expense of the "mother-type" cells, in which the damage is retained.[13]

1) Factors involved in aging of bacteria

Protein aggregates are accumulation of misfolded abnormal proteins. Protein misfolding is an inevitable process in all cells that is enhanced by internal and environmental stress. Lindner et.al (2008) in their findings state that, aggregated proteins are linked to cellular degeneracy in many age related consequences[14]. Sam Dukan and his co-workers(2008) demonstrated the presence of protein aggregates overrepresenting abnormal proteins in an exponentially grown *E.coli* culture. Protein aggregates could be considered as damage to the cell and pass from one generation to the next, thus accumulating over time or senescence. Chaperone is a protein that assists the de novo non- covalent folding/unfolding of proteins and also provides an important means of restoring activity to conformationally damaged proteins. Molecular chaperones are protein machines that recognize non-native states of other proteins and, by controlled binding and release, assist these substrate proteins to fold properly. Mutations in these chaperones give rise to the formation of protein aggregates[15].A common molecular characteristic of aging to emerge from such studies is theoccurrence and accumulation of damage to macromolecules and progressive accumulation of damaged macromoleculesinside and outside cells.However, it is generally agreed thatincreased molecular damage and heterogeneity are the fundamental basis of aging and age-associated pathologies.[16]

2) Phenotypic plasticity

It was also reported that *E.coli* grown in oligotrophic environments had greater morphological and functional symmetry in cell division. Both phenotypic plasticity and genetic selection appeared to shape cell division time asymmetry but plasticity was lost on prolonged selection. Lineages selected on high nutrient concentration showed greater frequency of presumably old or dead cells. The results suggested that cellular aging driven by asymmetric division may not be hardwired but shows substantial plasticity as well as evolvability in response to the nutritional environment.[17]

The basis of studying aging in *E.coli* in our work included 2 aspects of work 1) subbing of previously adapted cultures upto 2600 generations under high and low nutrient conditions so as to create a selective pressure and observe consistency in growth rate.2) To study if cells growing on low nutrient conditions are Persisters and whether any cost is involved during adaption

We carried out subbing of previously cultured two strains of *E. coli* (*E. coli* KL16 and *E.coli*2563 obtained from IISER(Indian Institute of Science Education and Research) continuously under two sets of nutritionally extreme (high and low nutrient concentrations) conditions. Using 1g% (v/v) and 0.01g% (v/v) glucose as the sole sources of carbon and energy, cultures were serially transferred up to an estimated 600 cell generations such that at the end of the prolonged sub-culturing we had six cell lineages, four selected for either of the nutritional environments and two wild types. The cultures were transferred frequently to ensure that they did not experience prolonged stationary phase or starvation any time. The strain selected under high caloric environment as *H* and the one selected under low caloric environment as *L*. After an estimated 2600 generations each of the lineages were exposed to different antibiotics with varying concentrations.

MATERIALS AND METHODS

1) Media composition for commitment of cultures

Composition of Glucose mineral medium: Na_2HPO_4 0.1 gm%, KNO_3 0.3 gm%, $(NH_4)_2SO_4$ 0.05 gm%, NaCl 0.05 gm%. Glucose: For high concentration medium: 10 mg/ml; for dilute medium: 0.1 mg/ml. All media were prepared in distilled water and pH was adjusted to 7 and sterilized by autoclaving at 115°C for 20 minutes.

2) Selection under high and low nutrient concentrations

Continuous sub-culturing of all the cell lineages was done on agar plates with media composition mentioned above. An advantage of using solid medium was that any contaminant could be easily detected and avoided. The sub-culturing was done from 10–15 colonies. The number of generations per sub-culturing was estimated from the estimated mean number of cells per colony. On high nutrient concentration the estimated number of generations was 22 and on low nutrient concentration 19 to 20.

3) Minimum Inhibitory Concentration (MIC)

MIC was carried out for *KL16* and*2563 E.coli*strains which are selected on high and low concentrations of Glucose mineral medium. MIC was also carried out for the wild-type strains. Luria Bertani broth (LB composition- Tryptone 1%, Yeast extract-O.5%, Sodium Chloride-1%, pH- 7) is used as liquid medium for broth dilution tests.MIC was carried out using the antibiotic Tetracycline (Pfizer, Oxytetracycline-50mg/ml) and Streptomycin (Piramal Healthcare, Ambistryn) and its varying concentrations. The test tubes were inoculated with 0.1 ml of suspension, further incubated at 37ºC for 24 hrs. Minimum Inhibitory Concentrations were recorded as the lowest concentration of antibiotic showing no visible growth in the broth as compared to control.

RESULTS

The cell cultures earlier subbed for 2000 generations were continued and subbing was further done for more than 600 generations, by the end of six months all cell lines were 2600 generations old.

The adapted and wild-type strains were exposed to various concentrations of two antibiotics namely Streptomycin and Tetracycline. Concentration of Streptomycin exposed was 1mg/ml and 1.5mg/ml and concentration of tetracycline exposed was 0.05 mg/ml, 0.1 mg/ml. It was expected that a cell line grown on low glucose concentration should not be able to withstand high antibiotic concentration of streptomycin and tetracycline, but it was observed that all three cell lines i.e. wild, high and low show growth in presence of antibiotic. This observation hints that there is no loss of operons and genes which are responsible for the survival in drastic environments. Further it was thus observed that the cell line grown on low nutrient conditions had a good growth rate as compared to high nutrient medium.

Stewart et.al studied replicative senescence of *E.coli* and found that bacteria divide asymmetrically. They observed a rate of decline of growth by 1% in cells that inherited old poles. The cells growing slower were the ones that have more often inherited old poles. Since Persisters are not mutants and compromise many subgroups that can be isolated from all the cultures used and show gradual reduction in growth rate and colony sizes which cannot be reverted back.[18]

DISCUSSION

In this work, we carried out subbing of previously adapted *E.coli* strains 2563, KL16 and wild-type to different nutrient concentration cultures obtained from IISER Pune, which were already subbed upto 2000 generations. We further sub-cultured it for

another 600 generations, resulting in cultures adaptedupto 2600 generations. The cultures were adapted under two nutritional conditions i.e. high glucose minimal media (*H*) and low glucose minimal media *(L*). Glucose served as the sole source of carbon and energy for the growth of the organisms. The *E.coli* strains subjected to low glucose concentration were seen to grow continuously even under restricted nutritional environment. It was further seen that the organism growing on low media had a better growth rate as compared to organism growing on high media. This was demonstrated by consistent growth of culture on low media than on high media. This consistency in growth is expected due to higher repair ratesunder low environment conditions.

Minimum Inhibitory Concentration using tetracycline and streptomycinwas carried out for the above mentioned adapted *E.coli* strains.It is hypothesized that a small population of cells called persisters, are bacteria at different stages of aging which become tolerant to antibiotics due to reduced uptake of the antibiotic resulting from a lower expression of electron transport chain pathway.

Our experiments demonstrate that the adapted strains of *E.coli* (2563 and KL16) were resistant to the subjected antibiotic concentrations of tetracycline and streptomycin. The concentrations of tetracycline used in the experiment were in the range of 0.05 mg/ml and 0.1 mg/ml. The wild type cultures were sensitive to 10µg/ml of tetracycline concentration. It was seen that the adapted strains were resistant to the tetracycline concentration of 0.05 mg/ml and 0.1 mg/ml which was observed as turbidity in the tubes. The concentrations of streptomycin used were 1.0mg/ml and 1.5mg/ml. The wild-type strain was sensitive to this concentration, whereas the adapted strains showed resistance at these concentrations. Thus this indicated that adapted organism were able to grow in drastic environmental conditions without the deletion in genes or operons which code for antibiotic resistance genes. Therefore there is no cost involved in living at low nutrient concentrations and organisms are still able to maintain their lifestyle.

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REFERENCES

1. Müller I, Zimmermann M, Becker D, Flömer M, Calendar life span versus budding life span of *Saccharomyces cerevisiae.* Mech Ageing Dev, 12:47-52,(1980)
2. Barker MG, Walmsley RM. Replicative ageing in the fission yeast Schizosaccharomycespombe. Yeast 15: 1511-1518, (1999)
3. Ackermann M, Sterns S, Jenal U. Senescence in a bacterium with asymmetric division.Science300:1920, (2003)
4. Stewart EJ, Madden R, Gregory P, Taddei F., Aging and death in an organism that reproduces by morphologically symmetric division.PLoSBiol3:295-300, (2005)
5. Winkler J, Seybert A, König L, Pruggnaller S, Haselmann U, et al, Quantitative and spatio-temporal features of protein aggregation in *Escherichia coli* and consequences on protein quality control

and cellular ageing. The EMBO Jour29(5):910-923, (2010)
6. Aguilaniu H, Gustafsson L, Rigoulet M, Nyström T, Asymmetric inheritance of oxidative damaged proteins during cytokinesis.Science299:1751-1753, (2003)
7. Martin Ackermann, Bacteria a new model system For Aging studies: Investigations Using Light Microscopy. Biotechniques,Vol 44, No.4: 564-567 (2008)
8. T. B. L. Kirkwood, Understanding ageing from an evolutionary perspective. Journal of Internal Medicine 1365-2796, (2007)
9. Craig Stephens Senescence: Even Bacteria Get Old. Current Biology 15-8, (2005)
10. Martin Ackermann, Lin Chao, Carl T. Bergstrom and Michael Doebeli, On the evolutionary origin of aging. Aging Cell235 –244, (2007)
11. Maisonneuve E, Fraysse L, Lignon S, Capron L, Dukan S,Carbonylated proteins are detected only in degradation-resistant aggregate state in *Escherichia coli*.Jour of Bact190(20):6609-6614, (2008)
12. Watve M, Parab S, Jogdand P, Keni S,Aging may be a conditional strategic choice and not an inevitable outcome for bacteria.ProcNatlAcadSci U S A103(40):14831-14835(2006)
13. Thomas Nyström,A Bacterial Kind of Aging.PLoS Genet3(12): e224, (2007)
14. Linder AB, Madden R, Demarez A, Stewart EJ, Taddei F. Asymmetric segregation of proteins aggregates associated with cellular aging and rejuvination.ProcNatlAcadSci USA105(8):3076-3081(2008)
15. Maisonneuve E, Ezraty B, Dukan S,Protein aggregates: an aging factor involved in cell death.Jour of Bact190(18):6070-6075, (2008)
16. Suresh I.S. Rattan, Increased molecular damage and heterogeneity as the basisof aging. Biol. Chem389, 267–272, (2008)
17. Uttara N. Lele,Ulfat I. Baig, Milind G. Watve, Phenotypic Plasticity and Effects of Selection on Cell Division Symmetry in *Escherichia coli.*PLoS ONE 6(1): e14516, (2010)
18. Jaison Jacob, Persisters show heritable phenotype and generate bacterial heterogeneity and noise in protein expression. Nature Precedings1411.2, (2009)