Int J Pharm Bio Sci 2013 July; 4(3): (B) 1 - 6

Research Article Microbiology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

BIOMOLECULE QUANTIFICATION OF NUTRIENT SELECTED STRAINS

MRS. BHATIA AMRITA*, MRS. ULFAT BAIG , SHAIKH NAAZNEEN AND CYRIL PRISCILLA.

**Department of Microbiology, Abeda Inamdar Senior College, Azam Campus, Pune- 411001, India*

ABSTRACT

Aging is a complex process and it is obvious in macroscopic organisms. In organisms like yeast, Drosophila, *E coli* etc there is a greater potential to identify the molecular mechanisms involved but identifying and quantifying aging is harder. Molecular damage includes DNA damage, protein damage and free radical attack for which the main targets are the macromolecules. Dietary restriction has shown to lower the rate of production of free radical reaction. Recent work in our laboratory has quantified biomolecules using nutrient selected strains of *E.coli* as it is a simple experimental model for studying aging. *E.coli* was subjected to extreme high and low nutrient conditions in order to determine their adaptability. The biochemical characteristics of the biomolecules and the differences in their cell content under two nutritional conditions were determined.

KEY WORDS: **Aging, Molecular damage, Dietary restriction, Biomolecules, Adaptability**

MRS. BHATIA AMRITA
Department of Microbiology, Abeda Inamdar Senior College,
Azam Campus, Pune- 411001, India

Corresponding author

INTRODUCTION

Aging can be defined as a progressive deterioration of functions and fitness increasing the probability of death overtime [1]. It was generally believed that the first organisms did not age and that aging thus evolved at some point in the history of life. When and why this transition occurred is a fundamental question in evolutionary biology [2]. Aging at the molecular level is characterized by the progressive accumulation of molecular damage [3].The nature of the aging process has been the subject of considerable speculation. Suggested possibilities include (i) encodement of aging in DNA (ii) progressive formation of misfolded proteins during protein synthesis (iii) cross linkage of macromolecules(iv) in higher organisms, "attack" of the immune system on self-antigens and (v) reactive oxygen species (ROS) and other free radicals (FRs) formed by the action of extrinsic inducers of damage and as a consequence of intrinsic cellular metabolism involving oxygen, metals and other metabolites(vi) nutritional glucose and its metabolites, and their biochemical interactions with ROS and FRs [3,4]. Aging is a fundamental characteristic of all living organisms [5]. It has been demonstrated in unicellular organisms and is presumably due to asymmetric distribution of damaged proteins and other components during cell division [6]. The accumulation of damage is currently assumed to be responsible for the ubiquitous progressive decline in the functional capacity of aging cells or organisms [7] .The genetic code contains all the information needed to repair and/or replace any impairments formed with aging [1]. One of the prominent markers of aging is protein aggregation, associated with cellular degeneracy in many age-related diseases [5]. Caloric restriction, also called dietary or food restriction, has been known to extend mean and maximum life span and to postpone the manifestations of aging in rodents [8]. Dietary restriction has been shown to extend life span in *C.elegans* and several other organisms, including yeast, drosophila and mammals. Interestingly, dietary restriction also reduces the incidence of age related diseases such as cancer and neurodegeneration in *C.elegans* and rodent models [9]. Caloric restriction in yeast possesses all of the hallmarks of the mammalian phenomenon [8].In our laboratory we have tried to quantify biomolecules of nutrient selected strains. The precultured strains of *E.coli* (*E.coli* AU1 & AU2) were subjected to two sets of nutritionally extreme (High and Low concentration) conditions using glucose (at a concentration of 1g% and 0.01g %) as a sole source of carbon and energy. Packed cell volume, glucose tolerance and quantification of biomolecules of these nutrient selected strains was done.

MATERIALS AND METHODS

Media Composition

Na_2HPO_4 0.1g%, KNO_3 0.3g%, $(NH_4)_2SO_4$ 0.05g%, NaCl 0.05g%, pH 7 . Glucose: for high concentration medium 1 g%, for low concentration 0.01g%. Media was prepared in distilled water and autoclaved at 115^0C for 20 minutes whereas glucose was autoclaved separately in distilled water at 115^0C for 20 minutes.

Subculturing

Continuous subculturing of all the strains was done on selective media plates. (Media composition mentioned above). The strains that were grown in high media were labeled as AUr1, AUr2 and that in Low media as AUp1, AUp2 .Cultures were maintained in log phase by frequently transferring them into fresh medium.

Glucose Tolerance

Glucose tolerance test for both the strains was done on mineral media. Glucose concentrations

for high media: 1.1 -1.8 g% was used and for low media: 0.001- 0.004g% was used.

Experimental design
Sample preparation

Strains were subjected to different extreme conditions. The current medium conditions for the experiment are denoted by AUp1and AUp2 for low and AUr1 and AUr2 for high.

Packed cell volume

Cell density was determined by PCV by filling the glass Wintrobe tubes (TT3 Toptech) with the suspension avoiding air bubble .The tubes were centrifuged (Centrifuge: Elteck RC 4100D) at 3000rpm (775 x g) for 20 minutes. Bacterial culture 0.5 O.D (660nm) was used.

Quantification of Biomolecules

Biomolecules like proteins, carbohydrates and lipids were estimated. Total Proteins were estimated using Folin Lowry method. [15] Total carbohydrates were estimated using Phenol Sulphuric acid method. For Total Proteins and Carbohydrates cells were lysed. All the cultures of E.coli were enriched in their respective media and incubated at 37^0C for 16-18 hours. Cells were lysed using SDS (Sigma 10g %) and lysozyme (10mg/ml) at a final concentration of 0.4mg/ml each.

Total lipids

Total lipids were estimated using Sulpho-Phospho-Vanilliin method [10]. 50ml of bacterial culture of respective strains at an optical density of 0.5 at 660nm were centrifuged at 13000 rpm (14567 x g) for 1 minute. The cells were washed twice by resuspension of 10mM sodium phosphate at pH 7.2 and centrifuged at 13000 rpm for 1 minute. The pellet was suspended in buffer at 1/60th of starting volume [11]. The cell suspension was sonicated 5 times for 20 seconds with 2 minutes between each burst. The sonicated lysate was centrifuged at 10,000 rpm.The methanol - chloroform extraction was performed after the sonication step. 95% methanol, chloroform and sample were added in the ratio of 2:1:0.8. After mixing and 10 minutes incubation at 4^0 C, the lipids were separated from the water - soluble material by dilution of the extraction mixture with one volume of chloroform followed by one volume of water. The sample was then centrifuged for 15 minutes at 3000 rpm. The chloroform layer was removed completely by gently inserting a glass pipette through the water-methanol phase and the material that collects at the interphase. The chloroform extract was used immediately after removal [11].Quantification by Sulpho-Phospho-Vanilliin method: 5ml concentrated H_2SO_4 was added to a test tube containing 0.1 ml of total lipids. The tube was heated for 10 min in a boiling water bath, cooled, and 0.4ml aliquot was placed in a clean, dry tube. Blank contained 0.4ml of concentrated H_2SO_4. To each tube 6ml of phospho- vanillin reagent was added. Vanillin reagent was prepared by dissolving 0.6g of vanillin (Sigma), in 8-10ml of absolute ethanol before diluting to 100ml with distilled water. This solution was mixed with 400ml of concentrated phosphoric acid with constant stirring. It was stored in a dark bottle at room temperature. The mixture was set in dark for 45 mins and the final color was measured at 525 nm [12].

RESULTS

Sub culturing

The strains were cultured on agar plates up to approximately 1100 generations with media composition as mentioned above.

Glucose tolerance

In order to determine the glucose tolerance, the strains were grown in different glucose concentration. AUr1and AUr2 could tolerate glucose in the range of 1- 1.4g% whereas AUp1 and AUp2 could not grow in decreased concentration of glucose (range 0.001g%-0.004g %) thus unable to adapt to the drastic conditions. The experiment was done in triplicates to avoid errors.

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Packed cell volume

Cell density of each strain was determined by PCV using glass Wintrobe tube. All strains showed 0.1µl/ml of cell density.

Total Protein Estimation

Concentration of Total protein was determined using Folin Lowry method. Bovine Serum Albumin (200µg/ml) was used as a standard.

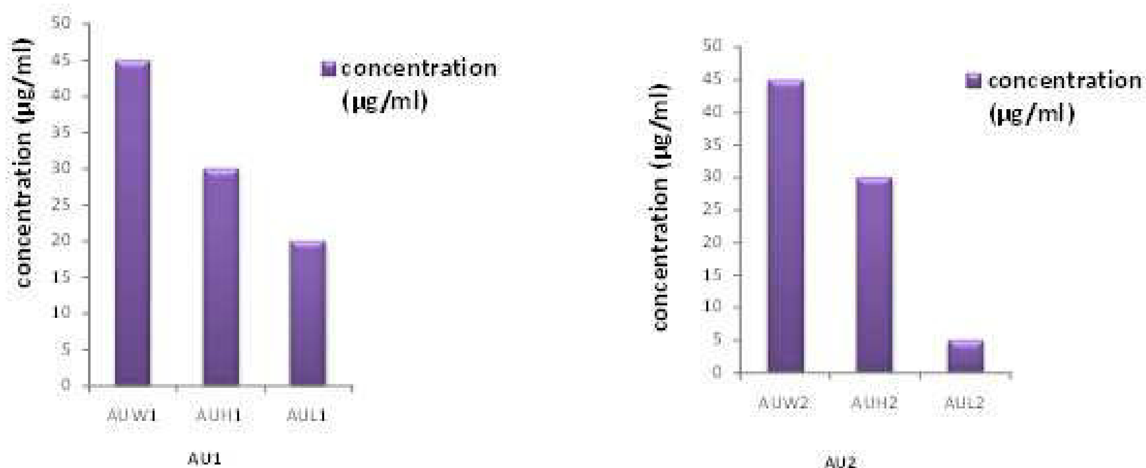

Figure Total Protein Estimation by Folin Lowry method

Total Carbohydrate Estimation

Concentration of Total Carbohydrate was determined using Phenol Sulphuric acid method. Glucose (100µg/ml) was used as a standard.

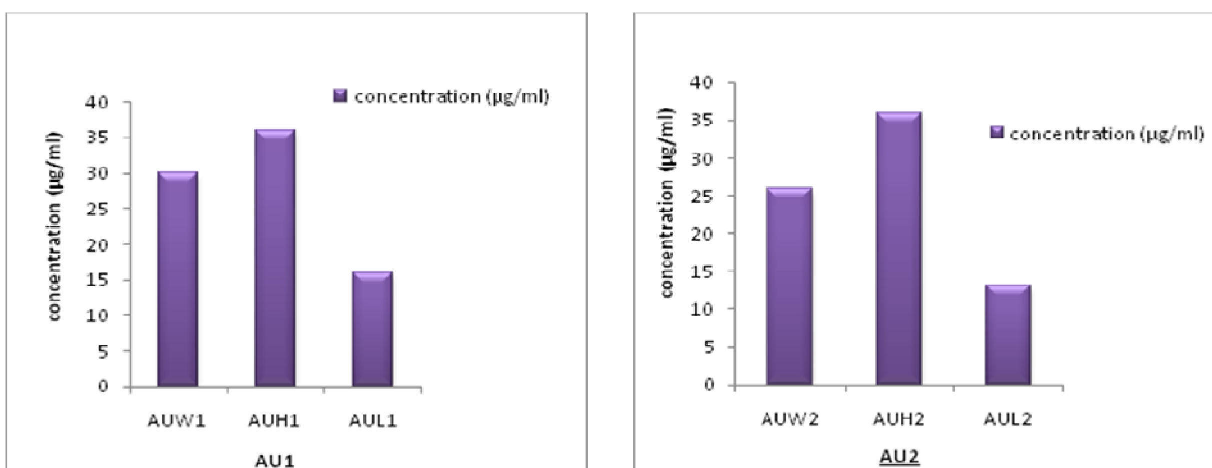

Figure Total Carbohydrate Estimation by Phenol Sulphuric acid method

Total Lipid Estimation (Sulfo-Phospho-Vanillin method)

Total Lipids extracts was used for preparing calibration graph. The total Lipid concentration for AUr1, AUr2, AUp1, AUp2, AUw1 and AUw2 was same.

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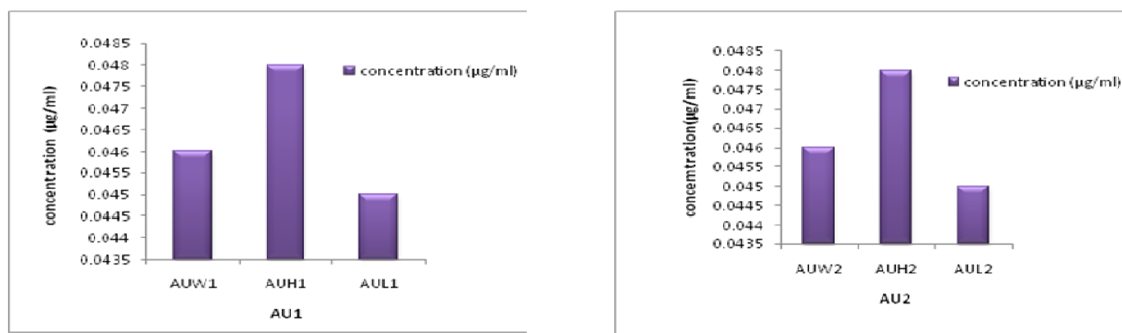

Figure Total Lipid Estimation by Sulfo-Phospho-Vanillin method

DISCUSSION

In order to determine adaptability of the cells to extreme conditions, the strains were grown on different concentrations of glucose, as glucose serves to be the sole source of carbon and energy to the organisms. *E. coli* is thought to be adapted to a feast-and famine existence [13]. When grown in increasing concentrations of glucose ranging from 1.1-1.8g%, *E.coli* AUr1 & *E.coli* AUr2 could tolerate only up to 1.4g% glucose whereas no growth was found when *E.coli* AUp1 & *E.coli* AUp2 were grown in low substrate concentrations ranging from 0.001 - 0.004g% of glucose. These results indicate that the organism could tolerate glucose only up to a certain range, beyond which they were unable to grow. Sub culturing of *E.coli* AU1& *E.coli* AU2 on mineral base agar medium under two different nutritional conditions i.e. High glucose (1g %) & Low glucose (0.01g %). It was done at an interval of 16-18 hours to avoid starvation, as starvation provokes aging. Cultures were regularly maintained on nutrient agar medium. They were sub cultured daily in order to make them adaptable to extreme conditions. Due to upregulation of several high-affinity transport pathways regulated by endogenous inducer synthesis, *E. coli* is extremely adapt in competing in an intermediate, nutrient-limited state [13].Two types of observations suggest that the rates at which *E. coli* grows on a variety of carbohydrates are limited by the rates at which these substrates are taken up by the cells [14]. Bacterial membranes are complex, involved in wide variety of functions, and are known to contain a wide variety of lipids. The nature of different components of membrane can change as a function of physiological and environmental changes. Total lipid content of different bacterial samples was quantitated using Sulpho–phospho–vanillin method. The sulpho–phospho–vanillin method detects each lipid with different yields and also total lipids present in a sample [11]. Total lipid concentration was found same for all the strains.Cell contents were also checked by growing the cultures under varying concentrations of glucose. Cell volume of each strain was determined by PCV. The adapted strains could grow in extreme environmental conditions without incurring much cost. Quantification of biomolecules like proteins, carbohydrates and lipids were carried out in order to determine cell content under different environmental conditions. Further studies can be done by studying gene expression using high-end sequencing techniques.

CONCLUSION

Aging can be defined as a multicausal process leading to a gradual decay of self-defensive mechanisms and an exponential accumulation of damage at the molecular, cellular and organismal level [15]. Proteins have been a prominent marker in aging [5]. However other macromolecules like carbohydrates, lipids and DNA are also responsible for aging. These macromolecules are the main target for free radicals attack. However, it has been observed

that caloric restriction have shown to lower aging in many organisms like *C.elegans, S. cerevisae.* Dietary manipulation is expected to lower the rate of production of free radicals reaction damage [4]. *E coli* can serve as an excellent model for studies related to aging.

ACKNOWLEDGEMENT

We are thankful to Dr. E.M Khan Principal, and the Management of Abeda Inamdar Senior College, Azam Campus, Pune, for their constant support and motivation

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