

## **The Effect of Metabolic Processes on Bacterial Richness and Diversity**

The term biodiversity was broadly defined as “life on earth” in 1986. However, a more specific definition of biodiversity is variation in species based on multiple factors in genetic, taxonomic, and ecological levels. In the taxonomic level biodiversity is defined as richness (11). Scientists have not been able to determine the cause of change in biodiversity and richness in animals, plants, and most importantly bacteria until recently. The Statistical examination of species richness based on latitude formed in 1950's and became more developed as time passed. According to Fuhrman, J. A. who conducted a recent research on diversity gradient in planktonic marine bacteria, planktonic diversity and richness increase from poles towards tropics due to increase in temperature (3). The pattern of increase in biodiversity has been approved by many researchers; however, there is discrepancy in terms of what causes these changes in biodiversity and richness. This paper explains different hypotheses about the patterns of biodiversity and richness in micro species including planktons which led to Fuhrman’s discovery, explains that increase in temperature boosts molecular evolution and metabolic rates of species and increases biodiversity, suggests light as an important factor for the growth and richness of photosynthetic prokaryotes, suggests improvements to Fuhrman’s methodology, ARISA (automated ribosomal intergenic spacer analysis) in calculating bacterial diversity. Although the work done by Fuhrman provides a good explanation for planktonic change in diversity from poles toward the equator, I think temperature alone does not provide the sole explanation for the species diversity and other factors, such as, nutrients has to be considered.

In 1966, Pianka provided a number of hypotheses such as area and time hypotheses which became the bases for the further explorations in species biodiversity and richness and their relations to geographical regions (11). Area hypothesis has been approved through many studies and shows that the relationship between the number of observed species and the area of the sampling is universal and true for all organisms (3). The time hypothesis is divided to ecological and evolutionary components. The first one, states that species exist in particular habitats but they did not have enough time to spread within these regions, and the later states that in some regions species have not have enough time to evolve yet, but as the time goes, new species will evolve and become more diverse. These hypotheses can explain regional but not latitudinal gradient in diversity since rich diversity still can be found in glacier regions (9). Later, these hypotheses gave rise to the newer hypotheses about species richness. Willing et al. provided more recent hypotheses for latitudinal gradients: ambient energy hypotheses, and evolutionary speed hypotheses. Ambient energy hypothesis is based on solar energy effects on physical environment through temperature. This hypothesis concludes that polar regions, despite tropical regions, have less favorable condition ideal for the growth of most organisms (11). In addition, there is more variation in season and living conditions in higher latitudes in contrast to tropical regions which are more stable and less variable. In comparison to lower latitudes, higher latitudes are associated with colder more extreme winters, lower annual average temperature, and shorter seasonal growth which create more unfavorable environments for organisms. On the other hand, for the organisms, it is more physiologically costly to live in higher latitudes compared to lower latitudes since "the ambient temperature in polar regions is outside the thermal neutral zone of many

organisms for the majority of the year” (11). According to another hypothesis, at the certain depth, the diversity between communities’ increase as the distance between them increase. For instance, a study for deep sea bacteria shows that the community similarity decreases over a 1000-km distance at 1000m depth in Atlantic ocean and over 3500-km distance in 3000m depth in the pacific ocean which clearly shows that bacterial communities are not similar at ocean depth. These differences may be due to the difference in regional temperature, since different depths and distances of the ocean have different temperature due to factors like upwelling and their latitudinal location (2). These hypotheses are led to more recent hypotheses, namely, evolutionary speed hypothesis, which states the richness of species closer to the tropical area is higher since higher temperature increases evolutionary rate. In tropical regions, this increase in evolutionary rate is due to faster speciation rate, which in turn, is due to higher mutation rates, shorter generation times, and higher selection pressure (11). According to Mittelbach et al. based on 'evolutionary speed' hypotheses, environmental temperature affects the kinetic rate of biological processes and the effect is strongest in tropical area where the temperature is relatively higher. The relevance between the temperature and energy can be explained through Boltzmann--Arrhenius formula, which explains the relation between the energy flux and evolutionary rate and defines mass-specific metabolic rate,  $B$  (Jsec<sup>-1</sup>g<sup>-1</sup>) as  $B = B_0 e^{(-E/kT)}$ , in which,  $E$  represents energy and  $T$ , the absolute temperature (K). Therefore, the higher the temperature, the higher the metabolic rate will be (1). Mittelbach further states that the increase in metabolic rate leads to higher mutation rate and considered to be an important factor to cause molecular evolution of new taxa (6).

Fuhrman supports Mittelbach's hypothesis based on temperature, since based on his studies, the marine environments that are rich in biodiversity are mostly the ones with higher temperature (3). In his research, Fuhrman mostly considers near-surface planktonic marine bacteria since these organisms are most closely associated with solar energy. He states that his discovery on increase in richness and diversity from poles toward equator is due to elevated level of metabolic processes such as dispersal, rate of reproduction, species interaction, adaptive evolution, speciation, and mutation due to increase in temperature. Although a specific amount of genetic variation causes the same amount of morphological and ecological changes, these changes happen with exponentially higher rates in the warmer tropical regions because of higher mutation rates and shorter generation time (1). A study on foraminifera shows that to produce a certain level of evolutionary change,  $1.8 \times 10^{13}$  J/g of energy must be fluxed to cause a substitution per nucleotide, and this energy is substantially higher in tropical regions relative to temperate and polar regions(1). Furthermore, fossil records also indicate that speciation rates increase as we go from poles toward the tropics. Based on these fossil records, the speciation rates are significantly lower in temperate regions relative to the habitats in tropical latitudes. Based on Another study on foraminifera, in which, data was collected from sea-surface and depth of 200m based on small ribosomal rRNA-encoding DNA from arctic to tropical waters, temperature is linearly related to the logarithm of the rate of neutral molecular evolution. The depth analysis on foraminifera's population shows that foraminifera in deeper depth has less molecular evolution rates since the temperature is lower in higher depths. Therefore, the thermal state of habitats affects DNA evolutionary rates of foraminifera (1). Foraminifera's diversity can be used as a

reliable source to study biodiversity since there is a broad fossil record of foraminifera's communities. The broad distribution of Foraminifera from poles to tropics and its close grazing relation to bacteria enables us to understand bacterial diversity and richness (1). Therefore, the kinetic metabolism of organisms has strong influence on their biodiversity (3). In my opinion, although evolutionary speed hypotheses provides an in depth explanation for different patterns of biodiversity and richness in different latitudes of oceans, the area, time hypothesis and the effect of light also have to be considered. Different areas of the ocean have different bacterial richness and biodiversity in different seasons and climatic conditions. Furthermore, the effect of light itself has to be considered as an important factor to affect photosynthetic bacteria's metabolic activities. Cyanobacteria bacteria rely mostly on light for their metabolic activities. Since different latitudes in the ocean receive different intensities and wavelengths of light, it is expected to observe different patterns of biodiversity among photosynthetic planktons. According to a study by Hewson et al. light affects gene expression. The study compares light and dark phases in bacterial activity and states that light level affects transcription of genes that are involved in nutrient transport and acquisition, cellular growth and metabolism. For example, after exposing to light during the day and dark phase at night, it became apparent that transcripts from *Prochlorococcus* were involved in photosynthesis (psa and psb photosystem genes) and their level of activity was affected by light exposure (6). Moreover, this study shows that photosystems I and II metabolic rate vary geotropically and based on light level. A later study also expressed that the metabolic rate of C1, oxidative phosphorylation and photosynthesis genes are more highly expressed during the day compared to the night, which can inform us that light has an important effect in the

metabolic rate of bacteria (6). According to Schwalbach et. al, who conducted three experiments on the effects of light on bacterial communities using ARISA ( automated ribosomal intergenic spacer analysis) technique to measure bacterial richness, plastid-containing organisms and cyanobacteria are very sensitive to light and the removal or decreasing the intensity of light reduce their survival since they can not withstand viral lyses or protist grazing (10). However, some groups of phototrophic bacteria (Anoxygenic Proteobacterias such as Roseobacter, SAR11 and SAR86) which contain bacteriochlorophyll pigments show also mixotrophic behavior and can fix Carbon dioxide to sustain up to 20% of their energy demand. Therefore, the effect of light on bacterial richness can only be considered valid if we consider cyanobacteria and photosynthetic bacteria in which, light is the only source of energy (10). It is crucial though to imply appropriate techniques to count bacterial richness and diversity based proposed hypothesis.

Operational taxonomic units (OUT's) are defined based on different 16S-23S rRNA spacer sequence lengths and differ 98% from each other (3). These taxonomic units are used in ARISA technique which is used by Fuhrman in order to estimate bacterial richness. According to a recent research, ARISA technique is a reliable technique that can be used to count bacterial diversity, however, there are some corrections required to use ARISA in some cases (5). This study which considered 722 complete genomes revealed that about 8 percent of bacterial strains do not give a PCR fragment using the primers of ARISA which leads to over estimation of bacterial richness. Although the overall relation between the number of bacteria and the number of fragments stays linear, in higher richness of bacteria this linear relationship disappears because of saturation of

bind fragments. This study suggests where the number of the bind fragment lengths is between 10 and 116, a correction with the formula “[correctedspecies-level OTU richness]<sup>1/4</sup> 0.8\_ [lengthbased OTU richness]<sup>1/6.7</sup>” should be used (5). Xavier et. al has came up with a more recent test to measure bacterial and phytoplankton richness in the ocean based on small ribosomal DNA subunit sequences. This technique is based on liquid bead array technology in which taxon-specific oligonucleotide probes are joined to specific beads and then get fluorescent labels. These fluorescent-labeled beads then are complimentary based pared to the end PCR products (7). The beads-PCR combinations are measured through flow cytometry. This method is very efficient since it can measure hundreds of samples of bacteria as well as eukaryotes in a short time period, and enables us to rapidly alter the bead and probe types. It has been used to detect bacterial pathogens and diversity of phytoplankton types and also bacterial richness in coastal areas. Since this method measures both bacterial and eukaryotes richness, it can also be used to study the interactions and dynamics between different organisms in the ocean and also the factors that affect these dynamics (7). Fuhrman’s results show that bacterial richness was negatively correlated with latitude and positively with temperature. The comparison between his data from the poles and equator shows clear difference in richness, however, between the two, the correlation with richness was not very strong (3). Therefore, through the use of newer techniques, more accurate results can be achieved.

To conclude, many hypotheses based on the biodiversity of marine planktons and bacteria state that the biodiversity and richness increase from poles towards the equator. Although there is not a single explanation for the change in biodiversity, according to Fuhrman, temperature can be assumed as one of the main causes of these changes from

polar to tropical regions since it affects the metabolic processes and the level of speciation. In the case of phototrophic bacteria, in addition to the effects temperature, light appears to play the most important role since it causes the expression of the genes involved in metabolic processes and induce them. Furthermore, efficient techniques are critical to achieve accurate estimations of bacterial diversity and richness. One of the most reliable methods after ARISA is the liquid bead array technology which enables scientists to measure high bacterial diversity in a short time. In my opinion although Fuhrman's explanation on bacterial diversity based on temperature is valid, however, other factors should not be neglected. Gilbert, J. A. et al. believes that three factors: phosphate, silicate and temperature are all responsible for bacterial diversity (4). Temperature and light can increase the metabolic processes and the rate of speciation, however, without enough nutrients and appropriate environmental conditions new species will not be able to survive and reproduce.

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