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Review Article

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Genetic Interactions and Microbial Fitness

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Abstract Microorganisms can create different genotypes either by mutation or genetic recombination. Transformed or genetically modified bacteria are used in many targets as in the synthesis of large quantities of insulin, in biomining and in bioremediation. Bacterial plasmids carry genes that help the host adaptation and to become antibiotic resistant or metal tolerant. Particularly, plasmid carried genes that encode plasmid-specific functions, such as self-replication, partitioning, and conjugative transfer, however, its continued persistence remain unclear. Allowing the bacterial population to grow to infinite numbers result in the undesirable mutations which require many generations before death is achieved. In spite of plasmid benefit, they may reduce their host fitness. Genetic recombination can be found in many groups of DNA and RNA viruses. Viral mutation happened if an error occurred in its genome. Inserted fragments of viral DNA into the host chromosomes lead to virus-induced gene mutations.

Keywords Natural selection, bacterial transformation, vector shuttle, plasmid paradox, SARS-COVID 19, mucormycosis

Introduction

The genetic evolution of microorganisms depends on mutation, recombination, natural selection, genetic drift and gene flow [1,2]. Stress-induced mutagenesis is a major evolutionary process that allows bacteria to adapt unfavorable environmental conditions, such as nutrient starvation, exposure to antibiotics, osmosis, and acidity.

Evolution results in resuming cellular regulatory and metabolic networks. Mutation rate itself may also be subjected to adaptation through a selection process on the genes related to DNA repair [3,4]. Although the change is usually harmful and deleted by selection, some mutations are beneficial to the microorganism [5,6]. The cell reads the sequence of the gene in groups of three bases on an mRNA molecule which codes for an amino acid. Frame shift mutation alter the grouping of these bases and changes the code for amino acids and the resulting protein is usually not operating. Increased point mutations frequency which are due to DNA polymerase mutations, are common either by a decrease in nucleotide-built accuracy or a deficient 3′-5′ exonuclease activity. In contrast, DNA polymerase alleles that decrease the frequency of point mutations (anti-mutators) are extremely rare. However, these modifications have an energy cost [7], and therefore, they are subjected to decrease the rapidity of DNA chain elongation and thus DNA replication capacity and cell growth (8).

Applying LFEAP mutagenesis strategy, different DNA modifications, such as point mutations, substitutions, deletions, and insertions result in mutational changes in plasmid DNA in a cost-efficient manner with high efficiency and reliability [9].

Gene mutations have varying effects on health, depending on where they occur and whether they alter the function of essential proteins [10]. Alkyl-lesions damage may cause mutations directly, most damage-induced mutations result in induction of the SOS response and transient hyper mutation. In *E. coli*, a pressing system termed the SOS global response to DNA damage, in which the cell cycle is prevented and DNA restore and mutagenesis is encouraged [4,11]. Mutation rates in bacteria can be increased by the general stress, the heat-shock, and the stringent

responses which regulate error-prone polymerases [12,13]. Recombinant DNA technology is employed to produce proteins required for health and nutrition [14]. Improved genetically stable recombinant plasmids are applied in, food, pharmaceutical and in chemical industries [4,15-17]. Lately, expression (transcription and translation] of the inserted section of DNA was well developed and many foreign proteins were produced in *B. subtilis* cells successfully by using different kinds of expression plasmids [18-20].

A study concerning recombination-induced DNA replication, the placement of genes on a conjugal plasmid, and a transient mutator state that produce adaptive mutations in *E. coli* states that chromosomal DNA is frequently broken as a result errors in DNA synthesis during replication or due to exogenous damage agents [3]. The result of exogenous or endogenous agents for adaptive evolution in E. *coli, S. typhimurium, S.cerevisiae and C. albicans* may cause extensive DNA damage in nongrowing cells [21]. The mutations that result will depend on the nature of the damage and the active repair enzymes. Bacterial DNA replication and transcription co-occur and utilize the same DNA template [22]. The environment contains many DNA-damaging agents, both physical (ionizing radiation, UV light) and chemical (alkylating, crosslinking, oxidizing agents, etc.). Moreover, cellular metabolism can produce DNA-damaging byproducts and intermediates, such as reactive oxygen species. However, cells contain a number of proteins and specific DNA repair systems that help maintain its correct structure.

An unrepaired DNA damage presents a very serious trouble to a cell because it may lead to harmful mutations or cell death [11]. Therefore, cells have developed five major repair pathways where different kinds of DNA damage can be detected and repaired: homologous recombination, nonhomologous end joining, nucleotide excision repair, base excision repair, and mismatch repair in which disruption or deregulation of DNA repair pathways may result in genome instability [23-25].

Numbers of mismatch repair proteins inherited by the offspring, proportions of lethal mutations and mortality rates are among the main parameters that influence the constant composition of a population [25-27].Organisms developed many different mechanisms to deal with DNA damage in an error-prone and error-free post replication repair [28].

Gene regulation and variability in bacteria

Bacteria can acquire and incorporate DNA from other microorganisms or their environment into their genome. The bacterial genes exist on one circular chromosome and other genetic elements [10,29]. High levels of multidrug resistance are usually associated with mobile genetic elements that encode specific resistance genes. The mobile genetic elements found in bacteria–insertion elements in terms of size, structure, and transmission; transposons, integrating bacteriophage, integrating conjugative elements, self-splicing group II introns, retrons, integrons which constitute a flexible gene pool that can be exchanged between species and contribute recombination by horizontal gene transfer [30]. This horizontal collection of DNAs contains either a site-specific recombination system as in gene cassettes or other shorter sequences selfish element [31-33]. Obtaining plasmids influences bacterial community structure, and evolutionary adaptation [34,35]. Given the selfish nature of the elements, it is not surprising that their mobility would be linked to bacterial stress responses and can induce DNA breaks and rearrangement. Hosts would attempt to regulate the movements of their inhabitant elements.

Plasmids and gene dynamics

Plasmids are extrachromosomal DNA elements that can be found in bacteria and in other forms of life. Plasmids mediate horizontal gene transfer because their molecules have reasonable sequence lengths with carrying patch sequences that can be used to study a wide variety of DNA-related processes. DNA segregation ensures the passage of the complete genetic information from parent to daughter cells, is a critical process in cell division [36]. Bacterial circular chromosome segregation can be accomplished in only a few minutes with the other major chromosomal processes of replication and gene expression. The complex functions and massive size of the chromosome present a major barrier to understand the underlying physical mechanisms that determine DNA segregation. Plasmids are vector of DNA self-replicating pieces that can help circulation of non-essential genes [37], and are utilized to explore DNA segregation which is transferred in nature by bacterial conjugation, natural transformation or transduction [38,39]. Transfer of antibiotic resistance genes plays an important role in the development of multidrug resistance in bacteria [40]. Conjugative and nonconjugative plasmids in recipient bacteria while conferring them advantageous traits as antimicrobial resistance, antibiotic resistance, virulence, tolerance to heavy metal in detoxication or the catabolism of unique beneficial nutrient sources and ecological interactions cause a significant cost to their host [41,42]. Plasmid persistence in a population depends on stable plasmid inheritance mediate the horizontal transmission of genetic information between bacteria. Mutations in copy number regulatory circuits and defective systems in partitioning, have been described as factors of instability.

A population of bacteria growing in a nonlimiting medium includes mutator bacteria and transient mutators defined as wild-type bacteria which, due to occasional transcription or translation errors, present a mutator phenotype [26]. [43-46] studied the chromosomal adaptive mutations generated by plasmid transfer, the influence of a lost native plasmid or the gain of a new plasmid, the control of plasmid-encoded regulators on chromosomal gene expression and the plasmid-specific regulators of chromosomal gene expression. The involvement of regulators homologous to chromosome-encoded proteins is clarified by the H-NS-like proteins, and by the Rap-Phr.H-NS and Hha belong to the nucleoid-associated family of proteins and modulate gene expression in response to environmental stimuli. Genes coding for these proteins can be either chromosomally or plasmid-encoded. Although plasmid regulation by chromosomal regulators is generally well known, chromosome regulation by plasmid reports is reduced considerably [45].

Gene transition and plasmid stability

Plasmid encode genes for beneficial traits to their host, in return they are coasty to their host. Fitness cost plasmids will be lost over time in the lack of purifying selection or the beneficial plasmid genes will be integrated into the host genome. Plasmid's persistence even in the absence of positive selection, for long time may be due to host-plasmid co-adaptation, plasmid hitchhiking, or cross-ecotype transfer [42]. The fitness effects of plasmids have a critical influence on their ability to mix with new bacterial hosts and so, on the evolution of plasmid-mediated antibiotic resistance [47].

Horizontal gene transfer is an important mode of adaptation, evolution and diversification of prokaryotes and eukaryotes and a major event in the emergence of bacterial pathogens and mutualist [48,49]. Accessory replicons such as plasmids and megaplasmids are characterized by the lack of essential genes and GC content different from the GC content of the chromosome [50]. Numerous genes on plasmids or megaplasmids were acquired through horizontal gene transfer.

A shuttle vector is a plasmid constructed so that it can propagate in two different host species [51]. Different insertion sites led to different properties of the shuttle plasmids [52]. PWB980 is a promising expression vector in Bacillus for its high copy number and high stability. However, the low transformation rate of recombinant plasmids to the wild cells limits its application [53].

High copy number plasmids require a system to ensure that replication is inhibited once the number of plasmids in the cell reaches a certain threshold. And are generally regulated through antisense RNA or iteron binding groups mechanisms [54]. A high copy number does not necessary guarantee stability unless the plasmid has an effective partitioning system.

There are two active partition strategies common to low-copy plasmids: par systems type I and II [55]. Both involve three components: an adaptor protein, a motor protein, and a centromere, which is a sequence area in the plasmid that is recognized by the adaptor protein. The centromere-bound adaptor nucleates polymerization of the motor, leading to filament formation [37] which can pull plasmids apart (par I) or push them towards opposite poles of the cell (par II). No such active partition mechanisms are known to occur in high copy number plasmids Among the many different types of plasmids, high-copy-number plasmids, which lack an active motor-protein-driven partitioning system. In this case, vertical transmission is generally considered accidental due to the unplanned distribution of plasmids in the cytoplasm.

Fitness and plasmid paradox

Fitness cost result in plasmid loss in bacterial populations over the long period of time with the lack of purifying selection for plasmid-encoded genes. Moreover, any beneficial gene carried by the plasmid could finally move to the chromosome. Under increased mutation pressure, multiple clones within a population may gain new mutations, and then compete with each other for fixation [4]. Thus, an individual with a higher mutation rate may accumulate more deleterious mutations overall, which can result in lower fitness. For this reason, selection has been predicted to reduce mutation rates. High mutation rates limit evolutionary adaptation in *E. coli* [4].

Each new mutation in an individual can increase, decrease, or have no effect on its fitness traits like fertility and survivorship [56]. The fitness effect of mutations is key in determining their future and their frequency distribution in natural populations, which greatly affects the level of genetic variation. Alleles almost surely do not enjoy constant fitness through time. Instead, the fitness, either absolute or relative, fluctuates through time in response to physical and biological changes in the environment. In practice, mutagenesis can require many generations before death is achieved, the bacterial organized cell death in a population is presented to award fitness to the surviving relatives in the form of sporulation, nutrition, and infection-inclusion [57].

A decrease in fitness as a result of toxic, insoluble protein is an example of collateral fitness effect. Collateral fitness effects aren't obtained from changing the ability of the gene to perform its physiological function. Destructive collateral fitness can be reduced by a decrease in protein mass. Systematic study of mutations in a bacterial protein finds extensive collateral fitness effects that were connected with protein cluster, irregular protein processing, deficient protein transport across membranes, incorrect disulfide-bond formation, induction of stress-response pathways, and unexpected changes in cell properties [56].

Undesirable protein mutations are commonly expected of how they damage the protein's ability to perform its physiological function. However, mutations might also be harmful if they cause negative effects on one of the numerous other cellular processes [56]. The frequency and significance of such collateral or secondary fitness effects of a given unintended action or inaction effects are unknown.

Viruses' quasi-species

Estimating virus mutation rates are important in their evolution and help in combating them [58]. Mutation occurs when an error is incorporated in the viral genome. Virus-induced gene mutations are probably due to insertions of fragments of viral DNA (cDNA) into the host chromosomes [59]. Viral quasi-species refers to a population structure that consists of extremely large numbers of different genomes, termed mutant spectra, mutant swarms or mutant clouds fueled by high mutation rates as viral replication proceeds [60]. RNA viruses have a great potential for genetic variation, rapid evolution and adaptation [61]. Recombination in RNA viruses involves the exchange of genetic information between two non-segmented RNA genomes, as distinct from the reassortment of RNA noticed in viruses containing segmented genomes [62,63]. In the case of RNA viruses both homologous and nonhomologous recombination events were observed.

Episomes integrate into the chromosome of their host upon infection, where they can reside as inertprophages until conditions favor their reactivation [64]. A general inverse correlation between genome size and mutation rate is realized in DNA-based microorganisms including viruses, bacteria and unicellular eukaryotes [65]. RNA viruses mutate faster than DNA viruses, and single-stranded viruses mutate faster than double-strand virus [66]. Viruses undergo minute genetic changes through mutation and major genetic changes through recombination. Some mutations are capable of transpositions and reversions. The long-accepted genetic stability and slow evolution of DNA viruses have been suggested in many reports showing that DNA viruses can exhibit levels of genetic diversity approaching those of some RNA viruses [67]. Virus itself categorize DDR (DNA Damage Response) whether it is a beneficial cellular response to infection or not. However, the effects of DDR on viral genomic stability and DNA genetic diversity remain unclear. In spite of being potent antiviral response, APOBEC3-mediated hypermutation of DNA virus genomes, can assist the appearance of immune escape or drug-resistance mutations [68]. This antiviral activity is associated with the ability to cause hypermutation of retroviral cDNA. Another realization, is that although large DNA viruses show a higher average genomic stability than small DNA viruses and RNA viruses, mutational hot spots can be found at specific genome regions involved in the dynamic virus–host interactions, and virus diversity occurs by the shortly lived genome-wide mutators in DNA viruses. Diversity-generating retro elements [DGRs] create unparalleled levels of protein sequence variation through mutagenic retro homing DGRs provide a clear basis for the ability of some DNA bacteriophages to target mutations in specific genome regions to direct evolution [69]. As a virus replicates, its genes undergo random copying errors. Over time, these genetic copying errors with other changes to the virus, lead to conversion in the virus' surface proteins or antigens. Human immune system uses these antigens to identify and fight the virus. Rates of spontaneous mutation vary enough among viruses. Virus mutators and anti-mutators were discovered in bacteriophage T4 over three decades ago [70].

Evolution of SARS-CoV-2

Severe acute respiratory syndrome (SARS) is a viral respiratory disease caused by a coronavirus [71]. Coronaviruses are single-stranded positive-sense RNA viruses, with the capacity for rapid mutation and recombination [72]. The CoV spike [S] protein plays the most important roles in viral attachment, fusion and entry, and serves as a target for development of antibodies, entry inhibitors and vaccines. The receptor-binding domain (RBD) in SARS-COV-2 S-protein suggest the potential to develop SARS-CoV RBD-based vaccines for prevention of SARS-CoV-2 and SARS-CoV infection [73]. As SARS-CoV-2 spreads worldwide, it is mutating. The new variant of SARS-CoV-2, spreading in the UK is characterized by multiple mutations in the spike protein. A mutant, N501Y is of major concern because it involves one of the six key amino acid residues resulting in a tight interaction of the SARS-CoV-2 receptor-binding domain (RBD) with its cellular receptor angiotensin-converting enzyme 2 (ACE2) [74]. The prolonged SARS-CoV-2 variant infection in a single patient with reduced immunocompetence, explained the

adaptation processes in a virus that occur in a different susceptible animal species and is then transmitted back to humans from the animal hosts. The emergence of a variant with multiple spike protein mutations including RBD mutation Y453F and deletion [69-70] appeared in Denmark during transmission among mink [75]. The variant has aroused through circulation in countries with low sequencing coverage. [76] suggested the possible mechanism of using Indian river phages, especially those of the river Ganga, for treatment of COVID-19 pandemic. In India COVID-19 patients recover from the corona virus struggle against black fungus infection caused by mucormycetes, referring to the fungi that cause mucormycosis [77].

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