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Review on Compositions, Formation, and Genetic Control of Bacterial Biofilm

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Abstract: *Biofilm is an innate defensive way through which bacterial cells are linked together to be associated with the biotic and abiotic surfaces within a self-produced matrix of extracellular polymeric substance. A biofilm is consisted of attached microbial cells sessile within a matrix of extracellular polymeric secretions (EPS). EPS has been composed of ; proteins arranged from 1-2% including enzymes, DNA and RNA less than 1%, while the polysaccharides was 1-2% and the main remaining component is, water up to 97% which is responsible for the flow of nutrients inside biofilm matrix. which surround and protect bacterial cells. The EPSmatrix is typically composed of polysaccharides, proteins, lipids, and extracellular DNA (eDNA). Biofilm fashioning is commonly considered to occur in four main steps: (1) Microbial attachment to a surface, (2) micro-colony formation, (3) biofilm maturation and (4) detachment of bacteria which may then colonize new regions of interest. Biofilm formation and dispersal are highly controlled proceduresadjusted at the genetic level and by environmental signals. From among the latter, quorum sensing (QS), cyclic diguanosine-5'-monophosphate, and small RNAs are considered as the main regulators of bacterial biofilms. The formation of microbial biofilms is an important causative for failure of antimicrobial treatment. Biofilm-associated infections represent one of the most important threats of modern medicine. According to National Institutes of Health (NIH) about 65% of all microbial infections, and 80% of all chronic infections are associated with biofilms.Gene expression of 30 to 50%of unknown functionoftentimes involvedin biofilm procedure formation.*

Key words: *Bacteria, Biofilm, Composition, Genetic control.*

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I. Brief History

Bacterial cellsshown two kinds of growth modesare planktonic cell and sessile aggregate and the latter represents the biofilm phase.Microorganisms cells are stick to each other on a surfaces encased or covered within matrix of extracellular polymeric substance produced by bacteria themselves called biofilm[1]. For first time in Holland Anthony van Leeuwenhoek (1632–1723) from Delft, observed and described animalcule biofilms by using his primitive microscope on surfaces ofhis tooth as well as he saw aggregated microbes in the "scurf of the teeth" and from "particles scraped off his tongue and this observation was considered as the first microbial biofilm discovery[2]. Later Louis Pasteur (1822–1895) observed and sketched aggregates of bacteria as the cause of wine becoming acetic[2].

Dobell in 1960, redacted the term biofilm and a cautioned the world about the important role of biofilm[3]. The term 'film', which indicate to microbial adhesion, assemblage, and multiplication on surfaces, previously used in marine microbiology to distinguish between adhering (sessile) bacteria and free-swimming 'planktonic' organisms from 1933 to 1935[4,5].

In everywhere we can be find the biofilms in nature, industrial areas, hospitals, bathrooms, laboratories, waste water facilities and commonly occur on hard surfaces submerged in or exposed to an aqueous solution. The famous microbiologist Louis Pasteur (1822–1895) also observed and sketched bacterial aggregates causing wine to become acetic which ultimately led to his discovery of pasteurization[6].

BIOFILM COMPOSITION

Biofilms are accumulate or heapsof one or more than one kinds of microorganisms that can grow on live and non-live surfaces. Actually there are many types of Microorganisms that form biofilms including bacteria, fungi and protists. One of typical example of bacterial biofilm formation is a slimy buildup on dental plaque which produced by some types of bacteria. All these microorganisms present naturally in the oral cavity and are normally harmless such as *Streptococcus mutans*and other anaerobes, though the precise composition varies by location in the mouth. Examples of such anaerobes include fusobacterium and actinobacteria[7,8,9].*Streptococcus. mutans* and other anaerobes are the initial colonisers of the tooth surface.As well as*Streptococcusmutans* play important role in the establishment of the early biofilm community[10,11].

All kinds of biofilms are produced as a extracellular polymeric substances (EPS) consisted of;proteins arranged from 1-2% including enzymes, DNA and RNA less than 1%, while the polysaccharides was 1-2% and the main remaining component is, water up to 97% which is responsible for the flow of nutrients inside biofilm matrix. Common various components of biofilms are listed in (Table1) signify the biofilm integrity and making the biofilm resistant against various environmental factors[12,13].

Table no 1: Biofilm chemical composition[14].

S. No	Components	Percentage of matrix	Origin
1	Microbial cells	2-5 %	Extracellular
2	DNA and RNA	< 1-2 %	Cell lysis
3	Polysaccharides	1-2 %	Extracellular
4	Proteins	< 1-2 %	Extracellular and cell lysis
5	Water	Up to 97%	Extracellular
6	Ions	? bound and free	Extracellular

Sothe biofilm's architecture consist of two main components i.e. water channel for nutrients transport and a region of densely packedcells having no prominent pores in itthat is making microbial cells are arranged in way with significant different physiology and physical properties[15,16]. Bacterial biofilms are normally beyond the access of antibiotics and human immune system that have been enhanced potential to bear and neutralize antimicrobial agents and result in prolonged treatment. During bacterial biofilm formation some genes switch on then bywhich activate the expression of stress genes which in turn switch to resistant phenotypes due to certain changes e.g. cell density, nutritional or temperature, pH and osmolarity[13,15].

HOW BIOFILM IS FORMED

Steps of Biofilm fashioning is a highly and very complex process, by which bacterial cells transform from planktonic phase to sessile mode of growth[17]. (Okada M, et al.2005).Alsoseveral studies have been revealed that biofilm formation is dependent on the activation of expression of specific genes that guide the establishment of biofilm foundation[17,18].The procedure of biofilm formation take place through a series of proceedings leading to adaptation under various nutritional and environmental conditions[19,20,21,22].Those a multi-stepsprocedure in which the bacterial cells undergo certain changes after adhering to a surface called biofilm life cycle (Figure no 1).

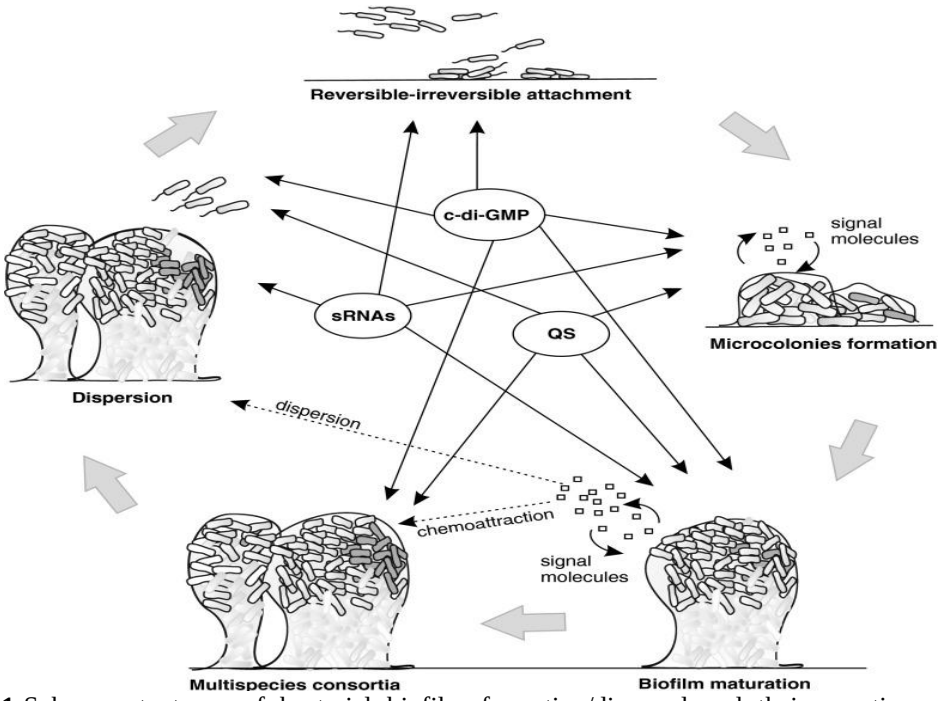

Figure no 1: Subsequent stages of bacterial biofilm formation/dispersal and their genetic regulation. (i) reversible, followed by irreversible, attachment to the surface, (2) formation of microcolonies, (3and 4) biofilm maturation leading to the formation of bacterial consortia, and (v) biofilm dispersal. The regulatory involvement of quorum sensing (QS), bis-(3'-5')-cyclic diguanosine monophosphate (c-di-GMP), and small RNAs (sRNAs) is shown by the arrows.

Microbes which are able to form biofilms are shown to elicit/educe specific mechanisms. After Biofilm formation steps, there are additional supplementary important steps:

1- Attachment initially to a solid surface
2- formation of micro-colony
3- Three dimensional structure formation
4- Biofilm formation, maturation and detachment /dispersal[2].(see figure 2).

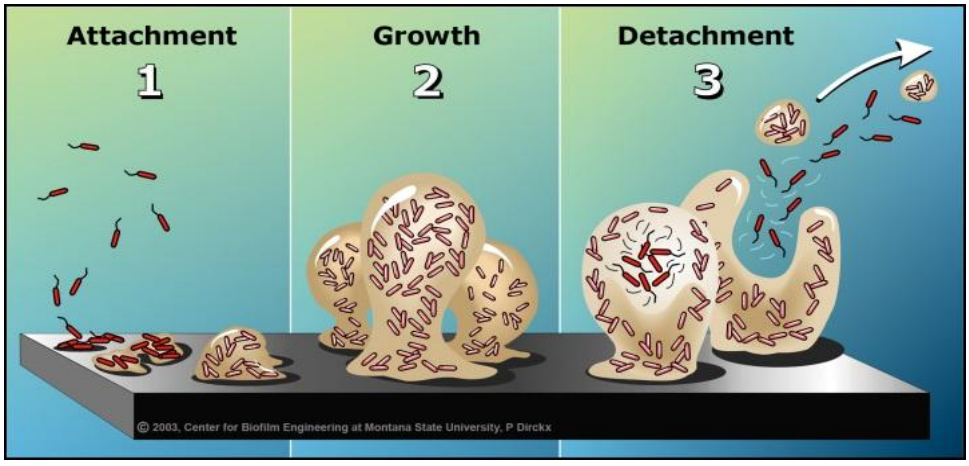

Figure no 2:an introduction to the biofilm life cycle: (1) Free-floating, or planktonic, bacteria encounter a submerged surface and within minutes can become attached. They begin to produce slimy extracellular polymeric substances (EPS) and to colonize the surface. (2) EPS production allows the emerging biofilm community to develop a complex, three-dimensional structure that is influenced by a variety of environmental factors. Biofilm communities can develop within hours. (3) Biofilms can propagate through detachment of small or large clumps of cells, or by a type of "seeding dispersal" that releases individual cells. Either type of detachment allows bacteria to attach to a surface or to a biofilm downstream of the original community.

Formation of a biofilm steps begun with the attachment of freefloating microbes to a surface[23,24].Then first colonist bacteria of a biofilm is adhere to the surface initially by the Weak van der Waals forcesand hydrophobic effects[25,26]. If the colonists are not immediately separated from the surface, they can set themselves more permanently using cell adhesionstructures such as pilus[26].Hydrophobicity phenomena can also affect the ability of bacteria to form biofilms. Microbes with increased hydrophobicity have reduced repulsion between the substratum and the bacterium[13,27].Some of types bacteria species are not able to attach to a surface on their own successfully due to their limited motility but are instead able to anchor themselves to the matrix or directly to other, earlier microbes colonistsNon-motile bacteria cannot recognize surfaces or aggregate together as easily as motile bacteria[13,27].

During surface colonization process, bacterial cells are able to communicate using quorum sensing(QS) products such as N-acyl homoserine lactase (AHL). Once colonization has started, the biofilm grows by a combination of cell division and recruitment. polysaccharidematrices typically enclose bacterial biofilms. In addition to the polysaccharides, these matrices may also include material from the surrounding environment, containing but not limited to minerals, soil particles, and blood components, such as erythrocytes and fibrin[27].The final step of biofilm formation is known as dispersion, and is the stage in which the biofilm is established and may only change in size and shape[13,28].

The nature of biofilm development may allow for an aggregate cell colony (or colonies) to be increasingly resistance to antibiotics.Cell-cell communication or quorum sensing has been studded to be involved in the formation of biofilm in some of bacterial species[27, 28].

MOLECULAR MECHANISM OF BIOFILM FORMATION

Molecular mechanisms and proceedings which are regulate the biofilm formation extremely heterogeneous among different species, and even vary between different strains of the same species. However, some features are recognized as general attributes of biofilm formation[29].For example, all biofilms contain an extra-cellular matrix that catch cells together. This matrix is often consisted of a polysaccharide biopolymer along with other components such as proteins or DNA[30].The nature of the matrix exo-polysaccharide greatly varies depending on medium, substrates and growth conditions. *Pseudomonas aeruginosa*is a gram-negative pathogenic bacteria that forms biofilms by producing three distinguished exo-polysaccharides are Alginate,

PEL, and PSL. The importance and contribution of each exopolysaccharide to the matrix varies depending on the strain studied[31,32].For example, Alginate is a produced by mucoid strains of *Pseudomonaaeruginosa*; that are often isolated from lungs of cystic fibrosis patients. Some genes like *pel* gene cluster, encoding a glucose-rich polymer termed PEL, is found in most of the strains analyzed to date, yet its expression strongly varies among strainsDNA[30].The reference strain PA14 used in many laboratories harbors a partial deletion of the *psl* locus, which prevents the PSL mannose-rich polysaccharide from being made[33].

On the other hand Gram-positive bacterium like *Bacillussubtilis* is other example as a model organism for biofilm formation. Different strains *ofBacillussubtilis* are able to secrete two types of distinct polymers: the polysaccharide EPS and poly-δ-glutamate (PGA). Both of them have been described to participate in the process of biofilm formation[34].Yet, they contribute differently depending on the strain and conditions studied. For example, in colony biofilms the undomesticated strain NCIB3610 requires exo-polysaccharide EPS for biofilm formation (Figure. 3). However, no colony biofilm defect is observed in a mutant strain lacking the ability to produce PGA[34].Instead, cells that overproduced PGA formed structureless, mucoid colonies. Another undomesticated strain of *B. subtilis*, RO-FF-1 naturally produces PGA and forms mucoid colonies. PGA production is important for surface-adhered biofilm formation in both RO-FF-1 and the laboratory strain JH642[35].In contrast, the strain NCIB3610 is unable to form robust surface-adhered biofilm[34].

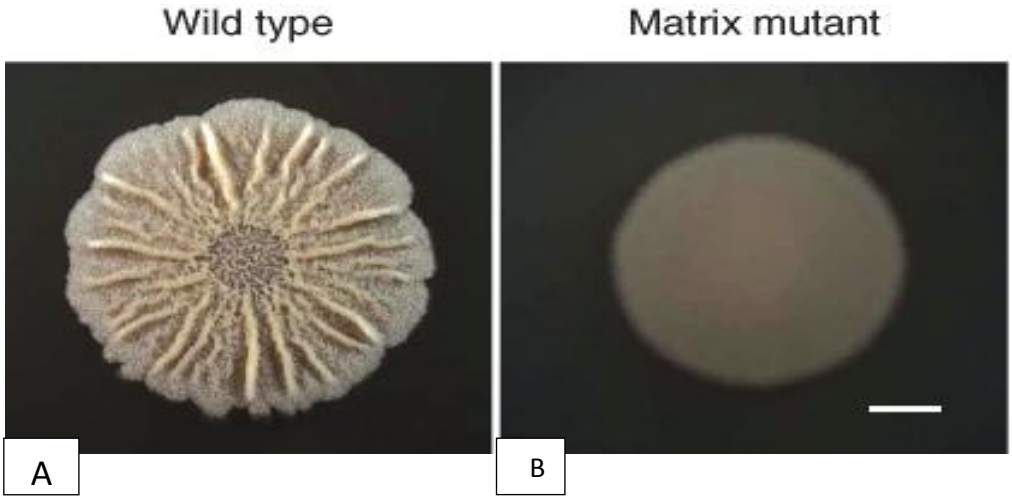

Figure no3: Colony morphology of B. subtilis strain 3610 (A) wild type and B(B) matrix mutant (eps). Top view of cells after 3 days of growth on 1.5% agar MSgg medium. Bar is 5 mm.

Another bacterial model used to study the molecular mechanism of biofilm formation is the Gram-positive pathogen *Staphylococcusaureus*. Most strains of *Staphylococcusaureus* used a polymer of N-acetyl glucosamine (PNAG) also referred to as polysaccharide intercellular adhesion (PIA), to form biofilm[36].The *ica* operon encodes the machinery that synthesizes this polymer, yet not all *S. aureus*strains carry this operon. Even in some of those strains that carry the *ica* operon, deletion of the operon does not impair their ability to make biofilm via an *ica*-independent pathway[36,37].This alternative mechanism relies on the ability of *S. aureus* to express a variety of adhesin proteins that allow cells to attach and colonize a large number of different surfaces[38].

As alluded to above the extracellular matrix of biofilms also ports adhesive proteins. For example *Staphylo0coccus aureus* strain matrix harbors biofilm-associated proteins (termed Bap) that are required for biofilm formationsurfaces[39].These proteins are found anchored to the cell wall of *S. aureus* and serve to hold cells together within the biofilm, probably by interacting with other proteins on the surface of neighboring cells. In certain strains, the expression of Bap proteins eliminates the requirement for exo-polysaccharides for biofilm formation[40].

GENEIC CONTROL OF BACTERAIL BIOFILM

Biofilm is a microbial lifestyle that is believed to require or involve a differential geneexpression compared to that of planktonic bacteria. Recently, we have witnessed a change of focus from the simple hunt for hypothetical essential biofilm genes to the identification of late and more complex biofilm functions. However, finding common bacterial biofilm gene expression patterns through global expression analysis is still difficult. Owing to the apparently minimal overlap between functions involved in biofilm formation by different bacteria,

exploring the biofilm lifestyle could prove to be a case-by-case task for which global approaches show their limits[41].

Genetic analyses have beenshowed the diversity of genetic principles participating in biofilm formation and there are undoubtedly multiple pathways to form a biofilm[2,13].These factors, especially when they are involved in the early steps of biofilm formation, can often be functionally replaced or overridden by others, depending on the media and environment conditions. Therefore, although the study of initial attachment probably still holds some surprises, the quest for an essential adhesion step may be in vain. Recently we witnessed a change of focus from the simple hunt for genes involved in the initial step of adhesion toward the identification, through global analysis, of late biofilm[2,13,40].

Evidence for differential gene expression in biofilms

Early evidence of differential gene expression during a bacterial biofilm formation came fromgene fusion studies which demonstrated that the expression of up to 38% of the *E. coli* bacterial genome may be affected by biofilm formation[42].However, it is likely that the extent of gene expression required to induce the formation of a biofilm may not be of that large of a magnitude, nor require genetic re-programming, as the most recent DNA array analyses performed with different bacterial biofilm models show that only a small proportion of the genome (1 to 15%) undergoes a significant change in expression compared with a non-biofilm mode of growth[43,44]. These studies have created the hope that it may be possible to identify a common universal gene expression pattern within bacterial biofilms. This postulate has received a lot of attention because the identification of such a pattern could allow one to monitor, or control, this lifestyle in situations of both economic or clinical relevance.

Through studies and research, an understanding of gene expression of the biofilm has been achieved. But, due to the absence of experimental gold standards, extracting a biofilm gene expression pattern from the available data is still difficult. Below is briefly presentation what constitute, in our point of view, the strongest trends, or the very smallest important denominator between all the studies that have been done on bacterial biofilms[42,43].

The converting from a planktonic phase to an attached lifestyle

Whereas the requirement of flagellar motility in the early stages of biofilm formation remains controversial[45,46].Different studies shown that flagella might not be required within a mature biofilm. Several studies revealed that genes encoding components of the flagellum are subdued soon after the bacteria touching the surface[42,47,48]. Therefore, the subduing of flagellar gene expression may be one of the first and important documented examples of genetic "reprogramming" leading to the sessile lifestyle.

Expressing genes for polysaccharide production

Rich in water, the matrix is a complex milieu implicated in air-liquid pellicle formation, as well as solid surface-associated biofilm formation. Many biofilm matrix polysaccharide components have been identified recently. In addition of the PIA/PNAG polymer encoded by the *icaABCD*locus in *Staphylococcus aureus*and *epidermidis*, Gr^{-ve} bacteria components such as colanic acid (*E. coli*), alginate, mannose and glucose rich Pel and Pls matrix components (*Pseudomonas . aeruginosa*), cellulose and β-1,6-GlcNac polymer (*Salmonella* and *E.coli*) have been reported to play important roles for biofilm formation[49,50]. These extracellular polysaccharides are the key elements that shape and provide structural support for five bacterial biofilms. However, most of the questions regarding the temporal and spatial regulation of exo-polysaccharide production are still unanswered[50].

The stationary phase-like character of the biofilm

Biochemical and genetic evidence support the hypothesis that bacteria probably face different conditions within a biofilm as compared to during planktonic growth[42,51,52]. Most of the biofilm population that is not in direct contact with the nutrient fluids will likely be subjected to progressive micro-aerobic conditions, increased osmotic pressure, pH variation and decreased nutrient accessibility. In *E. coli*, a significant part of the biofilm response involves stationary phase induced genes[47,53].In wild type *B. subtilis*, among the 121 biofilm induced genes that have a known function, 60% of them are activated during sporulation, a phenomenon that is induced by starvation conditions encountered in stationary phase[54]. However, depending on the experimental conditions, the expression of the stationary phase sigma factor, *rpoS*, has been shown to be either repressed by 2-3 fold, or slightly activated, in biofilms in *P. aeruginosa*[43,55]. and the role of *E. coli rpoS*in biofilms remains much debated[47,56,57]. Nevertheless, biofilm conditions often have strong similarities with conditions that prevail in stationary phase (planktonic) cultures.

Activation of stress-induced pathways within biofilms

In contrast to the notion that biofilms may represent a protection against environmental stresses, there is now ample evidence that bacteria develop stress responses within biofilms (seeTable no 2). While this could suggest that living in a biofilm has a cost, it also constitutes one of the major genetic signatures of the biofilm lifestyle. The activation of some stress pathways, like the *cpx*or *rcs*pathways, has been associated with functions such as surfacesensing through the perception of membrane perturbation[53,58,59]. Membrane stress, triggered by bacteria-surface and bacteria-bacteria interactions, could therefore constitute a 6 natural signal for the activation of several regulatory pathways that would promote stabilization and/or maturation of the biofilm. However, the exact role of these stressresponses in the formation and physiology of mature biofilms remains an open question[59].

Table no2: Example of stress responses induced within biofilms.

Function	Genes/Proteins	Organism
Prophages	PF1	*P. aeruginosa*
Prophages	PBSX	*B. subtilis*
Proteases	Clp proteins	*L. monocytogenes*
DNA repair	RecO	*L. monocytogenes*
SOS response	RecA, DinI, SulA	*E. coli*
Chaperons	DnaK, DnaJ	*E. coli*
Heat shock	HtpX, HtpG	*E. coli*
Oxydation stress	Sod proteins, CysK	*L. monocytogenes*
Envelope stress	*cpx*and *rpoE*pathways	*E.coli* and *S. typhimurium*
Sigma factor	W-mediated response	*B. subtilis*

The prevalence of genes of unknown function in biofilm differentially expressed genes

Biofilms are considered to be environments where new, or previously unrecognized,biological properties could be expressed. Thus, it was initially expected that many genes withunknown function could play a role in this lifestyle. Many studies of gene expressionconfirmed that genes of unknown function often represent the largest group of genesdifferentially expressed in biofilms (30 to 50%). However, this proportion is notoverwhelming and, more often than not, even slightly lower than the overall percentage ofsuch genes in the corresponding bacterial genome. Therefore, although it is likely that newaspects of bacterial biology are expressed during biofilm formation, so far, the harvest oftotally new biofilm-related functions has been relatively meager[43,53].

Is each biofilm unique?

Why is it so difficult to find a trend among all the studies that have been performed,even with the same bacteria (*P. aeruginosa*, *E. coli)* in reasonably similar experimental models? It was to be expected that a biofilm formed in a stream would be different from one formed on a medical implant. However, it comes as quite a surprise that three recent transcriptome analyses on genes overexpressed in *E. coli* biofilms share only 2 genes incommon.Hence, not only is what is true for *P. aeruginosa*not true for *E. coli* but what is true for *E. coli* K-12 in one experimental model may not be true for *E. coli* K-12 in another experimental model. If this is confirmed by further studies, one has to seriously consider the possibility that each biofilm may be a world of its own[43,44,55,58].

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