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REVIEW ARTICLE

Synthetic Biology Perspectives of Microbial Enzymes and Their Innovative Applications

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Abstract Microbial enzymes are high in demand and there is focus on their efficient, cost effective and eco-friendly production. The relevant microbial enzymes for respective industries needs to be identified but the conventional technologies don't have much edge over it. So, there is more attention towards high throughput methods for production of efficient enzymes. The enzymes produced by microbes need to be modified to bear the extreme conditions of the industries in order to get prolific outcomes and here the synthetic biology tools may be augmented to modify such microbes and enzymes. These tools are applied to synthesize novel and efficient enzymes. Use of computational tools for enzyme modification has provided new avenues for faster and specific modification of enzymes in a shorter time period. This review focuses on few important enzymes and their modification through synthetic biology tools including genetic modification, nanotechnology, post translational modification.

Keywords Microbial enzymes · Nanotechnology · Synthetic biology · Enzyme modification

Introduction

Enzymes are produced biologically by organisms for their cellular functioning. They act as a catalyst for conversion of molecules to carry out necessary biological functions. Enzyme binds non-covalently to the substrate and forms an enzyme–substrate complex, which further changes to form product, and the enzyme reverts to its native configuration. Enzymes are used widely for an industrial purposes like pulp and paperboard industry, textiles, bio-bleaching, food industry and biofuel industry [1, 2].

Enzymes are obtained from various plant, animal, and microbial sources. Among all these sources, microbes are the most efficient and explored source for enzyme production as microbes can be easily cultured and enzymes are easily obtained in a little span of time due to their shorter life cycle [3]. They are also easy to manipulate by synthetic biology tools. The normal metabolic pathway of microbes is regulated by different metabolic engineering tools to get the desired characteristics of enzymes [4]. Some examples of enzymes used in industry are xylanases, amylases, laccases, inulinase, nitrilase, lipases, and proteases [5]. Most of the naturally produced microbial enzymes have many limitations for industrial use such as low catalytic efficiency, activity, and stability at high temperature and variable pH. These enzymes need to be modified according to the needs of industries. Substrate specificity, enzyme stability, and cost of enzymes are the major problems faced in industries [4, 6]. Synthetic biology provides a platform to engineer microorganisms to produce thermostable and specific enzymes for the industrial purpose [1].

A Recap: Some Important Enzymes and Their Notable Microbial Sources

Enzymes are of immense importance for increasing cost effective production in industries [1]. They are used from basic food and feed industry to the advanced dye removing industry. They are superior to chemical modification

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methods as they don't produce toxic substances and hence decrease the environment pollution as well [7].

Xylanases

Xylan is the second most abundant hemi cellulosic polysaccharide. Xylanases are hydrolytic enzymes which break down xylan with the help of other glycosidases. Xylanase is produced from a number of microbes which include bacteria, filamentous fungi, yeast, and actinomycetes. Xylanase is used in the pulp and paperboard industry for the transformation of lignocellulosic biomass into fermentation products. Biofuel is also produced during this lignocellulosic conversion [1, 2]. The xylan hydrolysis by xylanase generates xylooligosaccharides (XOS) that can be used in functional foods for production of prebiotics. Some potential thermostable xylanase producing micro organism are—*Chaetomium thermophilum*, *Caldicellulosiruptor* sp., *Rhodothermus marinus*, *Thermotoga* sp., *Nonomuraea flexuosa*, *Thermoascus aurantiacus*, *Malbranchea cinnamomea* strain S168, *Paecilomyces varioti*, *Caldicoprobacter algeriensis* strain TH7C1 and *Thermomyces lanuginosus* [1, 2].

Laccases

Enzymes belonging to laccase family are multi copper oxidases that are used in the lignin degradation. Laccases are used in a number of industries which include the textile and dye industry, waste matter treatment and bioremediation, beverage processing and baking industry, pulp and paper industry [8]. Laccases are exploited from various microbial and fungal sources, such as *Alternaria tenuissima* KM651985 [9], *Peroneutypa scoparia* [10], *Coprinopsis cinerea* [11], *Trichoderma harzianum* [12] and *Panus strigellus* [13].

Amylases

Amylase was the first, discovered, and isolated enzyme [14]. Amylases are starch hydrolyzing enzymes which are classified as endo-amylase and exo-amylase that act on α-1,4- and α-1,6-glucosidic bonds of starch and glycogen. Amylases are particularly used in the dairy industry, where they reduce processing time during manufacture and increase the safety and shelf life of products [15]. Amylases are also used in the detergent industry due to their stability at alkaline pH [16]. Microbial source of amylases is *Thermococcus hydrothermalis* [17], *B. subtilis* strain AS01a [18].

Proteases

Proteases are a group of most important commercial enzymes which are used for hydrolysis of peptides and proteins [19, 20]. Proteases can be obtained from various sources, but due to easy genetic manipulation and technical advantage, microbes are the best potential source for protease production [21, 22]. They can be classified on the basis of reaction catalyzed, site of their action or their active site. Proteases are used actively in the food industry (bakery and cheese making), for production of antimicrobial bioactive peptides, cleaning industry and leather industry [23]. Proteases are potentially produced from diverse microbial sources which include *Exiguobacterium profundam* sp. MM1 [22], *Bacillus cereus* strain S8 [21], *Lysinibacillus fusiformis* C250R [24], *Aspergillus* sp. [25–27].

Lipases

Lipases are also known as triacylglycerol acyl hydrolases which hydrolyze fats and oils into free fatty acid and glycerol. They are omnipresent in environment; occurring in animals, plants, bacteria, and fungi [28]. Microbial lipases display a broad array of industrial relevance as they show higher stability, high conversion rate of the substrate into the product, highly adaptable to ecological circumstances and the ease in genetic modification and growth situations [29]. Bacterial species producing lipases come under the genus *Pseudomonas*, *Staphylococcus* and *Chromobacterium*; while that of fungi are present mainly in the genus *Geotrichum*, *Penicillium*, *Mucor*, *Aspergillus*, *Rhizopus* [28–30].

Nitrilases

Nitrilases are used to hydrolyze nitriles to their respective carboxylic acid and ammonia group in a single step [31, 32]. Nitrilases are useful in the industry, but due to their less stability and inactivation at a higher temperature, they are not the potential candidate to use in the industry [32]. Synthetic biology tools have been applied to produce thermostable nitrilases [33]. Nitrilases are used in chemical industries for the production of plastic, fibre, paper. They are also helpful in the production of herbicides for the agriculture sector and pharmaceutical drugs for health benefits [31]. Nitrilases are produced by a number of microorganisms which include bacteria from genera *Rhodococcous*, *Nocardia*, *Pseudomonas*, *Arthrobacter*, *Bacillus*, *Klebsiella* etc.; yeasts belonging to genera *Candida*, *Pichia*, *Aureobasidium*, *Debaryomyces*, *Geotrichum*, *Hanseniaspora*, *Gibberella*, *Williopsis*, *Torulopsis*, *Kluyveromyces*, *Saccharomyces*, *Exophiala*, *Cryptococcus* and Rhodotorula, and fungi of genus *Aspergillus*, *Fusarium*, *Penicillum* [31].

Inulinases

Inulinases are inulin hydrolyzing enzymes which produce fructose and fructooligosaccharides upon the breakdown of inulin [34, 35]. They are potentially produced by bacteria,

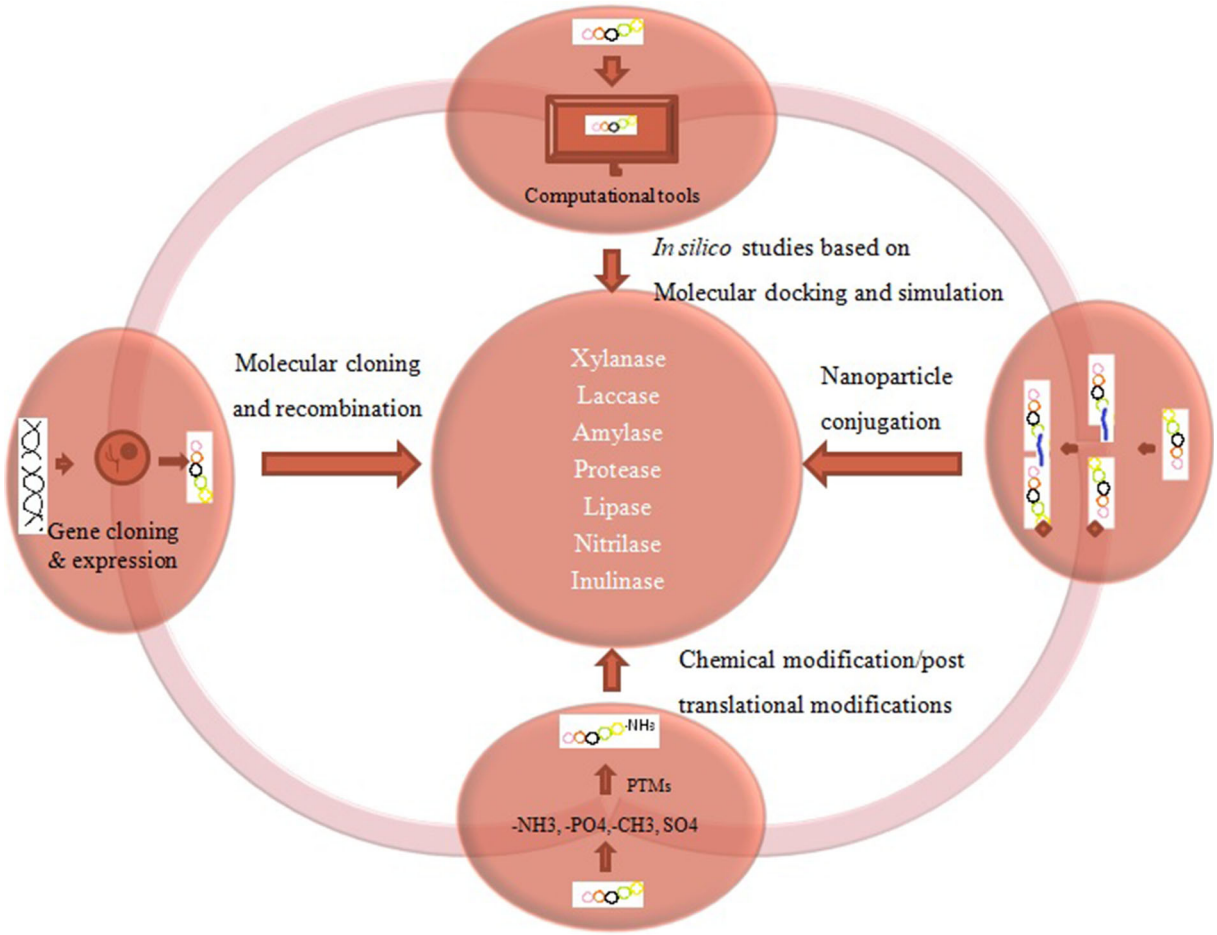

Fig. 1 A snapshot of synthetic biology tools used for modification of microbial enzymes

fungi, and yeasts. Free inulinase has restricted applications at the industrial level because of its limited functional and storage stability, difficulty in recovery from the reaction mixture and its inactivation due to aggregation, which is irreversible. Nanoparticle-based immobilization technique is the most suitable synthetic biology tool for such enzymes [35, 36]. Potential inulinase producing microbes are *Kluyveromyces marxianus*, *Penicillium oxalicum*, *Aspergillus* sp., *Streptomyces* sp., *and Xanthomonas* sp. [35–37].

Synthetic Biology Tools for Enzyme Modification

Synthetic biology is an emerging field which uses various molecular engineering tools and computational tools to manipulate the biochemical pathway of organisms. Synthetic biology tools are used to exploit the full potential of organisms [38, 39]. Figure 1 gives an insight of the use of synthetic biology tools for modification of enzymes and their selection for use in different industries. In Silico synthetic biology tools help in cost effective, less laborious, and less time-consuming production of microbial enzymes, which is the need of the hour to fulfill the demand of the market [40]. Synthetic biology is not only used to alter the biochemical pathway of organisms but also for the modification of proteins and enzymes produced by microorganisms. Proteins and enzymes used in the industry require much more sophisticated parameters than their natural occurrence. So there is always a need to detect or form novel enzymes and proteins for such uses [41]. Synthetic biology tools like molecular engineering and post-translational modification help to design and develop such novel enzymes [42, 43]. It is a bit difficult to analyze a wide array of enzymes for substrate specificity and their different optimum conditions by doing lab work, so synthetic biology tools like molecular simulation, molecular docking, In Silico studies and artificial intelligence has made it possible to study such parameters in a shorter time span by using computational tools [44, 45].

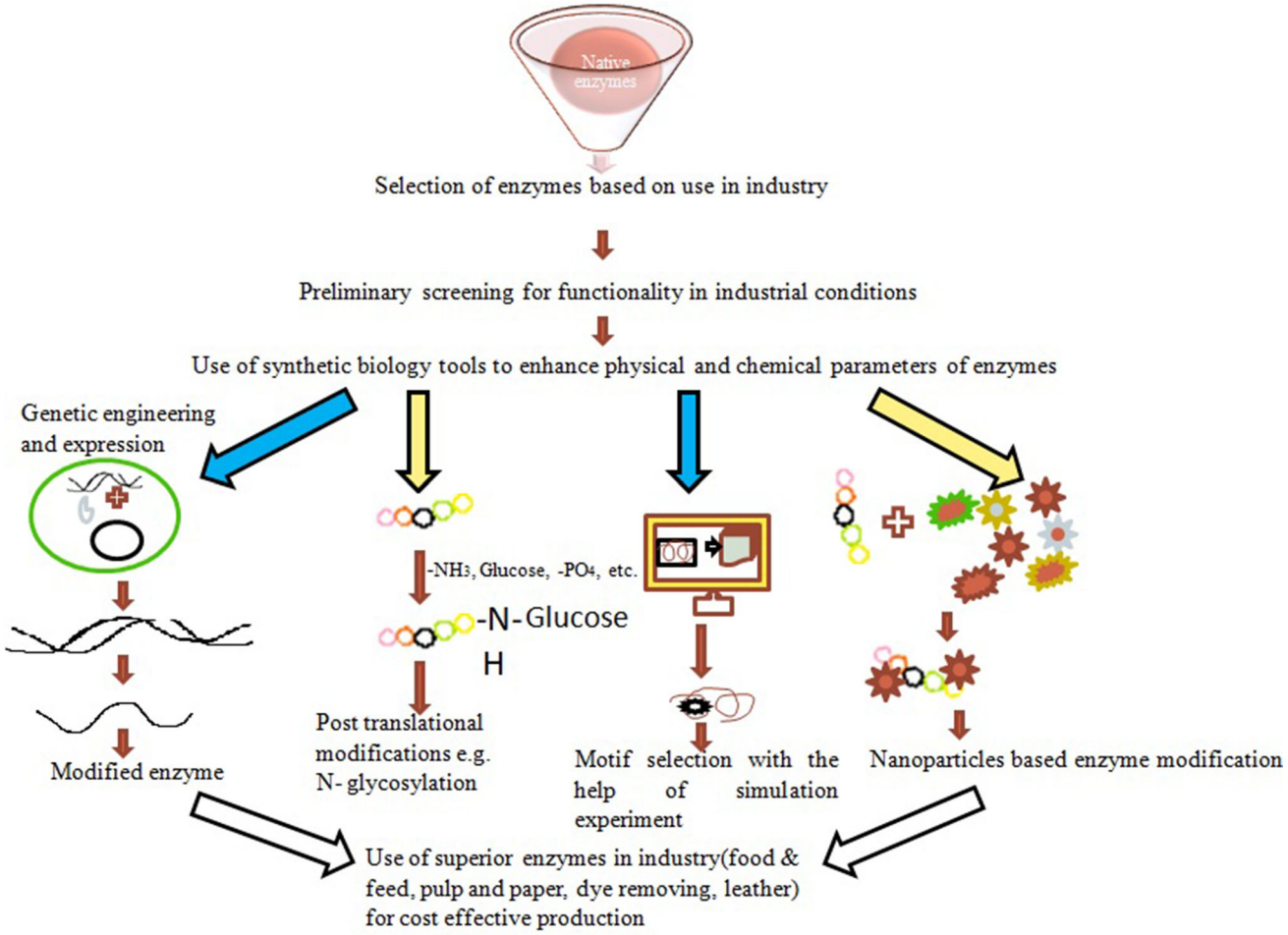

Fig. 2 A preview of enzyme screening and modification tools for their applications in adverse industrial conditions

Synthetic biology tools are also used to develop microbial consortia, which help in better utilization of substrate and division of labor among different microbes [5, 46]. Synthetic biology tools have been used to engineer several microbial enzymes such as inulinase, laccases, xylanases, lipases, amylases, etc. In this review, the industrial application of these enzymes and the synthetic biology tools applied to modify the microorganism and proteins for their large scale production and efficient use in industry has been described. Figure 2 gives brief information of the enzymatic selection for industries and different synthetic biology tools used for enzyme modification as per the requirement of industries. Synthetic biology uses tools like genetic engineering, in silico studies, molecular docking, artificial intelligence technique, nanotechnology and post-translational modification for better and efficient use of enzymes. A list of bacterial and fungal enzymes modified by different synthetic biology tools is presented in Table 1.

Genetic Engineering

This is the most frequently used technique to enhance the physical and chemical parameters of enzymes for their use in industries [66]. Genetic engineering includes several new approaches like metabolic engineering of microbes to regulate the production of enzymes, use of strong promoters for enhanced production, vector elements for expression of genes like inducers and enhancers, protein tags for isolation, high-performance tools for cloning, process screening and fermentation technologies [67]. Further, the cloning followed by functional appearance of a xylanase gene from *T. lanuginosus* was successfully reported [68]. Several other genetic engineering tools have been successfully applied to increase the thermostability of xylanase enzyme for their industrial use [56–59]. Inulinase was engineered by protease site mutation, which resulted in its thermostability and made possible the large scale production in the bioreactor [69]. Laccase, amylase, protease

Table 1 Synthetic biology tools used for modification of some key enzymes from bacteria and fungi

Sr. No.	Microorganism	Enzyme	Synthetic biology tool applied	References
1.	*Lactobacillus casei* IAM 1045	Inulinase	Genetic engineering of gene *levH1*	[47]
2.	*Aspergillus pumiga*-U4	Inulinase	Immobilization	[48]
3.	*Photobacterium panuliri* strainLBS5T	β-Endoglucanase	Cloning and overexpression	[43]
4.	*Bacillus subtilis* DR8806	α-Amylase	Recombination	[49]
5.	*Staphylothermus marinus*	α-Amylase	Recombination	[50]
6.	*Bacillus acidocaldarius*	α-Amylase	Conjugation to oxidized polysaccharides	[18]
7.	*Aspergillus niger*	α-Amylase	Molecular cloning	[17]
8.	*Aspergillus awamori*	α-Amylase	Immobilization on alginate carriers	[51]
9.	*Rhizopus oryzae*	α-Amylase	Site saturation mutagenesis of H286	[52]
10.	*Pseudoalteromonas phenolica*	Halophilic Protease	Cloning and recombination	[53]
11.	*Acidovorax facilis* ZJB09122	Nitrilase	Codon optimization, random mutagenesis, site saturation mutagenesis	[5, 32]
12.	*Pseudomonas psychrotolerans* L19	Nitrilase	Random mutagenesis and site directed mutagenesis	[54]
13.	*Pyrococcus abyssi*	Nitrilase	Cloning and overexpression	[55]
14.	*Psychrobacter* sp. *strain 2–17*	Xylanases	Ep-PCR and recombinant expression	[56]
15.	*Aspergillus umigates RT-1*	Xylanases	Ep-PCR	[57]
16.	*Trichoderma reesei*	Xylanases	Site-directed mutagenesis by incorporating disulfide bonds	[58]
17.	*Thermoascus aurantiacus CBMAI 756*	Xylanases	Site-directed mutagenesis and overexpression	[59]
18.	*Fusarium incarnatum KU377454*	Lipase	Immobilization on nanoparticle	[60]
19.	*Aspergillus niger*	Lipase	Immobilization by anion-macroporous resin	[61]
20.	*Candida Antarctica* and *Humicola lanuginose*	Lipase	Chemical modification	[62]
21.	*Pleurotus ostreatus*	Laccase	Gold nanoparticle formation	[63]
22.	*Bacillus amyloliquefaciens*	Laccase	Recombination	[42]
23.	*Yersinia enterocolitica* strain 7	Laccase	Molecuar simulation	[40]
24.	*Alternaria tenuissima* KM651985	Laccase	Covalent coupling to polysaccharides	[9]
25.	*Pichia pastoris*	Laccase	Recombination of Lac9 obtained from *Coprinopsis cinerea*	[11]
26.	*Trametes versicolor*	Laccase	Immobilization	[64]
27.	*Pycnoporus sanguineus*	Laccase	N-Glycosylation	[65]

have also been engineered by mutagenesis and cloning to improve their functionality and stability [11, 17, 20, 70].

Nanotechnology

This technique involves the immobilization of enzymes on nanoparticle beads to increase their activity and thermostability [1]. Nanoparticles have special characteristics of acting as immobilization support and conjugating material. Metal coated magnetic nanoparticles were used for xylanases immobilization obtained from *A. niger* [7]. Amino-functionalized magnetic nanoparticles (APTES) of Iron (II, III) oxide were made to immobilize lipase by their covalent linking with the help of glutaraldehyde (as a coupling reagent). The advantage associated with metallic nanoparticle linked enzyme is that they can be easily obtained after reaction by a magnetic field [71]. Protease obtained from *Penaeus vanamei* showed high thermal stability and pH stability when immobilized on ZnO nanoparticles. Also, FTIR, TEM, UV Vis spectroscopy showed that it can be used for the long term in such a form without losing its activity [72].

In Silico Studies, Molecular Docking and Artificial Intelligence Strategy

Computer simulation studies save a lot of time, which is otherwise used in performing lab-scale experimentation. In silico studies computes the data statically and give inference in a short span of time, which is cost effective and less laborious. In silico studies are generally used for knowing interactions of enzyme with different substrates or different

concentration of same substrate at the molecular level, for understanding the effect of alteration at genetic level on substrate detection of enzyme, for detection of homology of enzyme obtained from other sources and increased production with the help of statistical analysis [73–76]. Inulinase enzyme and its substrate-binding efficiency have been analyzed by molecular docking and molecular dynamic simulations [40, 73]. An immobilizing matrix can also be developed by using in silico strategy as is the case of Inulinase [77]. Hybrid statistic tools like GA-ANN and GA-ANFIS also helps in improvement in the functionality of enzymes [66].

Post-translational Modifications

Post-translational modification refers to the enzymatic or non-enzymatic alterations in proteins after their translation process. Enzymatic alteration includes glycosylation, phosphorylation, ubiquitylation, sumoylation, and pegylation while non enzymatic alterations include oxidation and nitration of proteins [78, 79]. Post translational modifications not only increase the stability of proteins but also adapt the bacteria and fungi to tolerate the extreme conditions [80]. Janusz et al. [81] in 2015 have done the proteolytic modification of laccase enzyme of *Cerrena unicolor* FCL139 which increased its efficiency up to 140% for decolorization of dyes. Effect of PEGylation and glycosylation on laccase protein from *Trametes versicolor* was studied which gave a contemplation of different modifications on the same protein [82].

Conclusion and Future Perspective

Although a large extent of research has done for production of enzymes suitable for industrial use including their technological perspectives, functional improvements, optimization, but a vast amount of microflora still remains untouched for exploration [83–85]. Detection of novel microorganisms for the production of enzymes suitable for industrial use or modifying the existing databases based on our understanding of synthetic biology tools are the only way forward. Synthetic biology tools help in the faster and more efficient production of enzymes from microorganisms than traditional tools. Computational tools help in developing microbial enzymes and development of consortia of microorganisms, which can utilize the substrate in a better and efficient manner. By knowing the possible interaction among microorganisms in such consortia, we will be able to design better molecular engineering tools for microorganisms. Computational protein designing adds up to the synthetic biology in technical advantages for de novo protein design and study of their functional aspects [86–90].

Synthetic biology predicts the pathway alteration, design of pathways for the production of different enzymes. But it is not always necessary that the predicted pathway works according to the designed strategy so their always a possibility for the development of new computational tools for designing pathways to get more favorable outcomes so that solution to important biological problems faced in industries can be given. Use of synthetic biology will open new avenues for genetic engineering of microbes for enzyme production and their modification.

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