Biotechnology: employing organism as bioreactors

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Abstract

Biological products, especially proteins, have numerous applications including prevention, diagnosis, and treating diseases. Advances in biotechnology in recent years have opened up many ways to manufacture these products in large scales. To engineer biopharmaceuticals, often pro and/or eukaryotic sustainable resources are used. Selection of the cellular factory depends on the type and application of protein needed. In this review, we explore current resources used to produce biologics, examine these resources critically for their biological output, and finally highlight impact of using sustainable resources in modern medicine.

1. Introduction

Biological materials obtained from sustainable sources have positively impacted life expectancy and lowered morbidly and mortality in numerous ways (1). One important example is insulin as the first human protein which is manufactured through biotechnology and using bacteria or yeast as miniature factories (2). The scope of applications for biologically derived materials is vast, ranges from biopharmaceutical to food industries and even daily household goods (3,4). Sustainable resources are derived from plants, bacteria and fungi (5). We can categorize biologically derived products into three major classes: i) biopharmaceuticals such as antibiotics, antitumor agents, and immunomodulators (6), ii) industrial products (e.g. industrial process of large volume, such as food, fermented products, textiles, detergents, and paper industry (7)), where enzymes must be produced in

bulk (8), and iii) agricultural processes (i.e. it is desirable to develop a suitable low-cost process for efficient production of highly valued enzymes in agriculture. For example, researchers have successfully achieved large amounts of tyrosinase in recombinant *Escherichia coli*. Tyrosinase is a required enzymes in plants for the biosynthesis of phenolic polymers, it also play an important role in plant wound healing (9). Table 1 highlights a few of the products, their sources of production and their usage.

Microorganisms are the most important source of biomaterials. There are diverse and abundant groups of organisms on earth, soil, water and other environmental resources. The potential of microbes could be harnessed to revolutionize the productivity of industrial and biopharmaceutical biotechnology. For instance, in drug discovery, by 2002, microbes were the source for 22,500 bioactive compounds. Of these, 17% were obtained from unicellular bacteria (mainly *Pseudomonas* and *Bacillus*), 45% from *filamentous bacteria* (*Actinomycetes*) and 38% from *fungi* (4). To this date, more than 100 natural products are under investi-

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Table 1. Examples of biologically derived products.

Category	Application	Product	Source
Biopharmaceutical	Antitumor agent	Bleomycin (10)	*Streptomyces verticillus*
		Mitomycin C (11)	*Streptomyces caespitosus*
	Antibiotic	Gentamicin (12)	*Micromonospora purpurea*
	Immunosuppressant	Sirolimus (11)	*Actinomycete*
	Ant diabetic agent	Insulin (2)	*Saccharomyces cerevisiaeas*
Industrial	Food Industry	Alcoholic (13) beverage	*Saccharomyces cerevisiaeas*
		Cacao beans (11)	*Aspergillus* sp.
		Diary (14)	Lactic acid bacteria
Agriculture	Insecticide	Dipel	*Bacillus thuringiensis*
		Thuricide	*Bacillus thuringiensis*

gation in clinical trials, and many more products are at the preclinical stages (3, 15). Looking from another angle, in food industry yeasts and filamentous fungi have been used for centuries in diverse biotechnological processes (16). Fungal fermentation technology is traditionally used in production of bread, bear, cheese, and soy derivatives.

Although we have achieved tremendous success in biotechnology, still there are increasing demands for new medicines and food products (17). What is more important is addressing the limitations of current production techniques, whether the conventional cultivation methods or novel biotechnological process. We are aware that environmental microbes are the main source of new enzymatic activities, owing to their enormous metabolic capability and diversity. However, much of these resources currently remain untapped. Second, the large-scale availability of existing microbial products like enzymes is not always reliable and is often costly. In addition, product slow stability in fermenters is one limiting issue. Finally, the lack of mechanisms to protect enzymes against attack occurring in biological systems is another major hurdle to overcome to achieve optimal activity. This is also true in the therapeutic use of microbial proteins as drugs, which, as foreign proteins, might be destroyed by blood proteases. Additionally, microbial drugs can also illicit im-

munological responses when used as therapeutics.

This paper i) explores the currently established resources for biologically derived macromers (with heavy emphasis on biopharmaceuticals) both conventional and genetically engineered, ii) introduces methods of production, and extraction, iii) discusses some of the obstacles of current production and isolation techniques for proteins from cultures and recombinant protein bioprocesses, and iv) highlights the impacts of techniques in transforming the standard sources of today protein production into higher efficiency in future.

2. Currently employed microbial resources
2.1. Products with conventional microbial origin

One of the most important applications of biologically derived products is pharmaceutical/medical. Pharmaceutical products include i) antibiotics, ii) antitumor agents, iii) enzyme inhibitors, iv) hormones, v) antibodies, and vi) growth factors that some examples are gathered in Table 2.

To engineer pharmaceuticals, often bacterial and fungal resources are used. Antibiotics are microbial metabolites that have been isolated from culture broth of microorganism. Antibiotics have saved countless lives since the discovery of the sulfonamides and β-lactams in 1930s. These breakthrough discoveries initiated a ''golden era'' of antibiotic research that lasted about 40 years.

Table 2. Examples of biopharmaceutical products.

Products	Source	Usage
Tetracycline(18)	Streptomyces aureofaciens	Antibiotic (tetracyclines)
Oxytetracycline	Streptomyces rimosus	Antibiotic (tetracyclines)
chlortetracycline(19)	Streptomyces aureofaciens	Antibiotic (tetracyclines)
Erythromycin(11)	Saccharopolyspora erythraea	Antibiotic (tetracyclines)
Amphotericin B(20)	Streptomyces nodosus	Antifungal (polyene)
Ticoplanin(21)	Actinoplanes teichomyceticus	Antibiotic (Glycopeptides)
Bleomycin	Streptomyces verticillus	Antitumor agent
Mitomycin C	Streptomyces caespitosus	Antitumor agent
Streptozotocin(22)	Streptomyces achromogenes	Antitumor agent
Pentostatin(11)	Streptomyces antibioticus	Antitumor agent

During this time, most current classes of antibiotics were discovered. An analysis by Berdy revealed that by 2002, about 16,500 antibiotics were known (23). Since 2000, the situation has improved, with five more new classes of antibiotics launched (1) (Table 3): linezolid (approved 2000), daptomycin (approved 2003), retapamulin (approved 2007), fidaxomicin (approved 2010) and bedaquiline (approved 2012). Majority of the therapeutically relevant antibiotics are produced in bacteria and fungi. Many antibiotics were produced by bacteria and especially by Actinomycetes (30). Some of the new released antibiotics and their origins are provided in Table 3 (1).

  Actinomycetes produces modified β-lactams such as cephamycins and thienamycin that are routinely prescribed in clinic to treat bacterial infections. In addition, thienamycin, one of the last commercial antibiotics discovered, made by Streptomyces cattleya (24). Another important category of antibiotics is aminoglycosides, extracted from Streptomyces and Micromonspora species. Specifically, streptomycin is produced from Streptomyces griseus, and gentamicin, from Micromonospora purpurea (11,25). One of the most important antibiotics that heavily impacted infectious disease therapy and is currently used in addressing drug resistant bacteria in clinic is vancomycin, extracted from Amycolatopsis orientalis culture media (26). Table 2 summarizes other examples of clinically applicable antibiotics and the bacterial sources used for their mass production.

  Cancer chemotherapeutics are other pharmaceutical materials produced by microorgan-

Table 3. List of recently approved antibiotics from biological origin.

Year approved	Drug name	Class	Source organism
2001	Telithromycin	Macrolide	Actinomycete
2001	Biapenem	Carbapenem	Actinomycete
2002	Ertapanem	Carbapenem	Actinomycete
2003	Daptomycin	Lipopeptide	Actinomycete
2005	Tigecycline	Tetracycline	Actinomycete
2007	Retapamulin	Pleuromutilin	Fungus
2008	Ceftobiprole medocaril	Cephalosporin	Fungus
2009	Tebipenem pivoxil	Carbapenem	Actinomycete
2009	Telavancin	Glycopeptide	Actinomycete
2010	Ceftaroline fosamil	Cephalosporin	Fungus
2011	Fidaxomicin	Tiacumicin	Actinomycete

isms. Most of these agents are products of Strep-tomycetes, and were discovered testing for their activity against bacterial infections. Since, the molecules, detected in the screening, were too toxic to be used as anti-infective drugs clinical application was not considered for years. Later, the same molecules attracted much attention after their antitumor activity in animals was demonstrated. In general, the cytotoxic mechanisms of these bacterial products is by causing permanent damage to the DNA of growing cells resulting in a rapid decline in cell viability. Specifically, actinomycins are of historic importance, being the first antibiotics isolated from Actinomyces antibioticus. Although important, since actinomycins toxicity was too high, it is primarily used as investigating tools in molecular biology. The only actinomycin derivative with clinical application is actinomycin D (dactinomycin), in use from 1964 for the treatment of Wilms' tumor in children.

The anthracyclines are among the most effective antitumor antibiotics, being active against many types of cancer than any other chemotherapy agent. The first anthracycline discovered was daunorubicin, a product of Streptomyces peucetius, discovered in1966 (27). A year later, doxorubicin was reported, the product of a variant of the same microorganism. Doxorubicin has been routinely used in clinic ever since, as one of the most utilized chemotherapeutics in treating lymphomas, leukemias, and breast, uterine, ovarian, and lung cancers (27).

Bacteria have important roles in realization of organ transplant, as one of the major achievement in medicine. During organ transplant; host immune system would detect the guest organ as a foreign body. Thus, suppressing host immune system allows the guest organ to survive and function in host body. Tacrolimus and sirolimus are most important and heavily used immunosuppressive agents in clinic which are originated from bacteria, Actinomycetes. Currently, they are used in heart, liver and kidney transplants.

Enzyme inhibitors are used as therapeutics to suppress pathological pathways. Many of these enzyme inhibitors are produced by bacteria. Among these, acarbose, a pseudo-polysaccharide produced by an Actinoplanes strain is used in treating diabetes type II (28). Acarbose inhibits

intestinal α-glucosidase thus reducing the conversion of starch into glucose. Lipstatin is also a pancreatic lipase inhibitor produced by Streptomyces toxytricini that interferes with intestinal absorption of fats and lowers fat absorption (29). Orlistat, which is used clinically worldwide as anti-obesity drug, is a chemically synthesized hydrogenated derivative of natural product lipstatin.

Besides bacteria, fungi have also been used as a source for pharmaceutical productions. Fungi produce the classical subfamilies of antibiotics like penicillins and cephalosporines. From 1941 that the first penicillin was isolated from Penicillium chrysogenum, a lot of different species of Penicillium have been studied for production of antibiotics. The only broadly useful antifungal agent from fungi is griseogulvin (30). It is used for treatment of mycotic diseases of human, veterinary and plant systems. It is synthesized by many species of Penicillium. A known immunosuppressant, cyclosporin A, is also from fungal origin. It was originally discovered as a narrow-spectrum antifungal peptide produced by a mold, Trichoderma polysporum (31). It has been proven to be a powerful immunosuppressant in mammals, being widely used during and after bone marrow and organ transplant in humans.

Microorganisms also play important roles in other industries beside pharmaceutical industry. For instance, in food and beverage industry, microbes like S. cerevisiae are used to make bread and alcoholic beverages (32). Other species such as Aspergillusare used to ferment cacao beans (33) and Penicillium species to produce various sausages and cheeses (16). Even in ancient times, vinegar was made by filtering alcohol through wood shavings, allowing microbes growing on the surfaces of the wood pieces to convert alcohol to vinegar. Likewise, in production of alcohols, S. cerevisiae is used to convert sugars to alcohol. Still the same organisms are used in highly mechanized settings for large scale production of beverages.

Bacteria are used in the production of many food products, including yogurt and many types of cheese. Lactic acid bacteria are among the most important groups of microorganisms used in food industry and are largely included in the genera *Carnobacterium*, *Enterococcus*, *Lacto-*

bacillus, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. These bacteria can degrade a variety of carbohydrates, with lactic acid being the predominant end product. A major application of lactic acid bacteria is in production of probiotics products. Many beneficial health effects for probiotics have been reported and include protection against enteric pathogens, improved digestion by means of enzymes to metabolize otherwise indigestible food nutrients (e.g., lactase to hydrolyze lactose in lactose intolerant consumers), stimulation of the intestinal immune system, and improvement of intestinal peristaltic activity (14).

In addition, lactic acid bacteria can produce bacteriocins that have antimicrobial activity (34). Interestingly, such antimicrobial activity is antagonistic to other bacteria, especially toward bacteria closely related to the bacteriocin-producing strain. Bactericins lead to enhancement of the quality and safety of foods. With the discovery of bacteriocins and the development of more efficient approaches to deliver them to foods, the importance of lactic acid bacteria in preserving and providing enhanced safety of food will continue to increase for the foreseeable future.

In addition, applications of some microbial enzymes like proteasas, lipase, transglutaminase, phospholipase, xylanase and proteases in food industry are immense (8,35), ranging from texturizing to flavoring. Recently, much work has been carried out on the application of transglutaminase from *Streptoverticillium* sp. as a texturing agent in the processing of sausages, noodles and yoghurt (36). Also, within the baking industry there is an increasing focus on lipolytic enzymes. Recent findings suggest that lipases can be used to substitute or supplement traditional emulsifier, as the enzyme degrade polar wheat lipids to produce emulsifying lipids in situ. In addition, efforts are made towards understanding further the bread staling and the mechanisms behind the enzymatic prevention of staling when using alpha-amylases and xylanase (8).

2.2. Products from modern technology

Modern biotechnology revolutionized utility of microorganisms as producers of pharmaceutical products. Genetic engineering provided methods for obtaining new bioactive compounds or to improve metabolite production. One of the routine approaches is by altering biosynthetic genes or inserting selected genes into the DNA of an antibiotic-producing strain.

The technique of altering genes can be categorized in following classes: (a) over-expression of a heterologous promoter gene, used in production of cephamycin from Nocardia lactamdurans. It has been done via over-expression of the latgene (encoding lysine-6-aminotransferase) from strong heterologous promoters (37); (b) over-expression of the positive regulatory gene, like over-expression of ccaR, a gene resulting in enhancement of cephamycin C and clavulanic acid production in *Streptomyces clavuligerus* (38); and (c) transposition mutagenesis, that is has been successfully used to improve productivity of daptomycin from Streptomyces roseosporus. In fact, transposition mutants were found producing 50% more daptomycin than the original strain.

Even more important, the progress of modern biotechnology modernized the industrial production of mammalian proteins and peptides by bacteria and other hosts. Modern biotechnology impacted: i) biosynthesis of known clinically useful proteins such as insulin and human

Table 4. Examples of recombinant proteins and their applications.

Protein Category	Bacterial Source for recombinant protein production	Application in medicine
Human interferon alpha(39)	*E. coli*	hepatitis and cancer treatments
Human Beta Interferon(40)	*E. coli*	hepatitis
Human growth hormone(41)	*E. coli*	treatment of dwarfism, bone fractures, skin burns, bleeding ulcers and AIDS
Vaccine(42)	*Mycobacterium tuberculosis*	Tuberclosis treatment

growth hormone and the generation of monoclonal antibodies, ii) identification and cloning of genes encoding clinically relevant proteins such as interferons, interleukins, colony stimulation factors, cytokines, thrombolytic agents, and vaccines, and iii) finally mass production of macromolecules (e.g., receptors, ligands, enzymes, cytoskeleton proteins, adhesion molecules, signaling proteins, and regulatory elements) to be used in screening for modulators. Some of the mentioned examples are gathered in Table 4.

Genetic engineering combined with modern microbiology enables production of proteins from selective genes. In fact, with the use of biotechnological industry, the production of recombinant enzymes and biopharmaceutical proteins is of major importance (15). The development of recombinant DNA technology enabled the expression of heterologous genes into pro-or eukaryotic hosts which do not naturally harbor these pieces of DNA. This method is used with the aim of gene therapy to engineer bacteria like Listeria monocytogenes, *E. coli,* and *Salmonella* sp. One specific example is ampicillin-sensitive strain of L. monocytogenes as a good vector for delivery of DNA to target cells (43). One study demon¬strated that L. monocytogenes can deliver DNA to tumor cells *in vitro.* This system utilized a novel approach whereby the host strain expressed a phage lysin from the Listerial actA promoter when the strain entered the host cell cytoplasm. Thus the vector was autolysed in target cell cytoplasm followed by release of DNA, entry of DNA into the nucleus, and expression by the eukaryotic machinery. Recent experiments provided the first evidence of DNA delivered by L. monocytogenes to tumors *in vivo* (in MF1-nu/nu athymic mice) and also *ex vivo* in resected human breast tumor tissue. In a related study, a highly ampicillin sensitive host strain of *L. monocytogenes* grew in mice solid tumor grand later was lysed in situ following admin¬istration of ampicillin. Consequently, upon bacterial lysis therapeutic DNA was released in tumor microenvironment.

3. Role of genetic engineering in modern biotechnology

3.1. Advent of genetic engineering

The introduction of genetic engineering by Cohen and Boyer in 1973 laid the foundation for the current biotech industry, which is based on using microorganisms or cell cultures for production of proteins that can serve as pharmaceuticals, often referred to as biopharmaceuticals. A few years later, researchers at Genentech (leader in biopharma industry in United States) cloned the genes for human insulin and growth hormone, and expressed them in *Escherichia coli.* Genetech researches demonstrated the utility and applicability of genetic engineering in creating genetically engineered bacteria that produce these two human proteins. In 1982 this led to marketing of the first biopharmaceutical, human insulin. In 1985 Genentech received FDA approval to market protropin, human growth hormone used in children with growth hormone deficiency, as their first product. In 1987 this was followed by the tissue-plasminogen activator (t-PA, Activase), another Genentech product, an enzyme that can resolve blood clots in patients with acute myocardial infarction. Also in 1987 Novo (now Novo Nordisk), a major insulin producing company based in Denmark, launched human insulin produced by the yeast Saccharomyces cerevisiaeas, a replacement for their human insulin enzymatically derived from porcine insulin (6). In 10 years (1982-1991), seven recombinant pharmaceutical products were approved by FDA and marketed (11).

3.2. Frequently used hosts for recombinant protein production

Production and extraction of recombinant protein must be done in a controlled fashion and with minimum number of steps. To produce recombinant protein various hosts ranging from simple bacteria, to more advanced eukaryotes and very complex insects and animal cells are used. Hosts are often selected based on the suitability of the host for producing certain protein. At the beginning of 2009, among the 151 pharmaceutical recombinant proteins marketed, 45 were produced by E. coli, 28 by S. cerevisia, and 59 by mammalian cells (44).

One of the commonly used hosts to produce various proteins is *E. coli*. It is commercially used since early 1980s as a source of rapid and economical protein production (4). *E. coli* propagates rapidly on inexpensive media, and is easy to genetically modify (45). To this date, *E.coli* is used as cell factory to produce approximately 30% of the biopharmaceuticals.

In contrast, using *E. coli* as host has drawbacks related to necessary protein post translational modifications (5). In general, most proteins undergo some form of modification following translation. These modifications result in mass changes that are detected during protein analysis. Post-translational modifications such as glycosylation, phosphorylation, and sulfation, to name a few, serve many functions. Post-translational modification of amino acids extends the range of functions of the protein by attaching it to other biochemical functional groups, changing the chemical nature of an amino acid, or making structural changes. For instance, phosphorylation plays critical roles in regulation of many cellular processes including: cell cycle, growth, apoptosis and differentiation. Thus, the identification and characterization of phosphorylation sites is crucial for the understanding of various signaling events. Glycosylation is another important post-translational modification which plays crucial roles in cellular processes such as protein sorting, immune recognition, receptor binding, inflammation, and pathogenicity.

Recently, *Bacillus strains* are given higher attention in academia and industry to address a few challenges associated *E. coli* (46). *Bacillus strains* do not have lipopolysaccharide-containing outer-membranes as do Gram-negative bacteria. Therefore, they can secrete the recombinant protein into the culture medium in high amounts. The species generally used for protein expression are *Bacillus megaterium*, *B. subtilis*, *B. licheniformis* and *B. brevis* (5). In industry, *B. subtilis* is often used due to its ability to secrete proteins in high quantity. The genomes of *B. subtilis* and *B. licheniformis* have been sequenced, and there is no production of harmful exotoxins or endotoxins. The secretion of the desired proteins into the fermentation medium results in easy downstream processing, eliminating the need for cell disruption or chemical pro-

cessing techniques. This makes recovery relatively efficient and cost-effective. One drawback associated with *B. subtilis* is the production of proteases, which sometimes destroy the recombinant proteins.

Yeasts, the single-celled eukaryotic fungal organisms, have been extensively applied as hosts both for the production of biopharmaceuticals and industrial enzymes. They are often used to produce recombinant proteins that are not produced effectively in *E. coli* due to folding or post translational mismatches (5). The customarily used strain is *Saccharomyces cerevisia*, which like *E. coli* is routinely used in many laboratories. It is easy to cultivate and has well-characterized genetics and metabolism. Also, because it is a eukaryote it can perform post-translational modification in contrast to *E. coli*. For this reason S. cerevisiae is used in producing about 20% of biopharmaceuticals. *S. cerevisiae* has (i) a long history of use in industrial fermentation, (ii) secretes heterologous proteins into the extracellular broth when proper signal sequences have been attached to the structural genes, and (iii) performs glycosylation of proteins. However, at times glycosylation by *S. cerevisiae* is not used for mammalian proteins because the O-linked oligosaccharides contain only mannose whereas higher eukaryotic proteins have sialylated O-linked chains. Also, the yeast over-glycosylates N-linked sites results in reduction of activity and receptor-binding, and may cause immunological problems. Major productions of *S.cerevisiae* are insulin (and insulin analogs), human serum albumin, hepatitis vaccines and virus like particles, e.g., for vaccination against human papillomavirus.

Fungi are important mainly as hosts for the production of industrial enzymes and they are rarely applied for the production of biopharmaceuticals. Many filamentous fungi species naturally secrete large amounts of enzymes into the growth medium, explaining their wide use in enzyme production. Furthermore, filamentous fungi grow relatively fast on inexpensive substrates and are recognized as safe organisms. The most important species are Aspergillus, Trichoderma, and Penicillium. Although yeasts and filamentous fungi can grow fast on inexpensive substrates, their inability to execute human-like glycosylation patterns hamper their wide application in the biopharma-

ceutical industry. There have also been attempts to use higher eukaryotes, such as insects and plants.

Chinese hamster ovary (CHO) cells constitute the preferred system for producing monoclonal antibodies or recombinant proteins (47). Chinese hamster ovary cells are by far the most commonly used higher eukaryotes for production of biopharmaceuticals. The use of mammalian cell culture, chiefly immortalized CHO cells, began because of the need for erythropoietin and tissue plasminogen activator production in the early days of the biopharmaceutical effort, i.e., in the 1980s. These glycosylated proteins could not be produced in *E. coli* at that time. Mammalian cell cultures are particularly useful because the proteins are often made in a properly folded and glycosylated form, thus eliminating the need to refold them. Eukaryotic cells are also useful for addition of fatty acid chains and for phosphorylating tyrosine, threonine and serine hydroxyl groups. Mammalian cells also have some drawbacks including poor secretion in media and high cost of production. To this date, 40% of the biopharmaceuticals are produced by mammalian cell cultures, mainly using CHO cells, as these allow for production of proteins with very similar glycosylation patterns as human proteins. Table 5 summarizes some of the advantages and disadvantages of frequently used hosts.

The choice of organism selection as host is highly dependent on the characteristics of the recombinant product. Often higher molecular weight proteins are produced in eukaryotic organisms such as yeast and lower molecular weight proteins are expressed in prokaryotic systems. For proteins that require glycosylation, mammalian cells, fungi or the baculovirus system are chosen.

3.3. Current limitations of protein production in hosts

Microbial products revolutionized industrial and pharmaceutical biotechnologies. However, still there is a need to overcome some of the practical problems associated with large-scale use of enzymes in biocatalysis and as therapeutic agents (48). Three major challenges need to be addressed. First, many proteins are extremely large and re-

Table 5. Advantages and disadvantages of hosts.

Host	Advantages	Disadvantages
E. coli	Rapid growth Ease of fermentation Ease of formation of intracellular disulfide bonds High product yields inexpensive	Inactive proteins Require refolding Proteins may contain endotoxins Lack of post-translational modifications Proteins with nonnative disulfide bonds
Bacillus strains	Strong secretion without endotoxins Manipulated easily Genetically well -characterized system Generally recognized as safe Inexpensive	Production of proteases Inactive recombinant protein due to extracellular enzyme activities
Yeast	High yield Produced glycosylated proteins Highly stable High productivity Inexpensive	Different pattern of post- translational modification from mammalian cells
Mammalian cell	Low risk of contaminated proteins Same post-translational modifications as human cells Suitable for large therapeutic protein production	Poor secretion Expensive

quire specific and complex post-translational modifications, that cannot be provided in conventional microbial hosts. Second, the large-scale availability of existing proteins is not always reliable and is often costly. Finally, the lack of mechanisms to protect microbial products, such as enzymes, against attack occurring in biological systems is a hurdle to overcome to achieve optimal activity.

3.4. Possible approaches to overcome recombinant protein obstacles

Through the use of recombinant DNA, important genes, especially mammalian genes, could be amplified and cloned in foreign organisms. This provided a possible solution to complex biological problems. Many of the protein-based biopharmaceuticals are produced using technologically advanced microbial and mammalian cellular systems. These cell-based, protein manufacturing technologies offer many advantages, producing recombinant pharmaceutically important proteins that are safe and available in abundant supply. Generally, proteins that are larger than 100 kD are expressed in a eukaryotic system while those smaller than 30 kD are expressed in a prokaryotic system (5). For proteins that require glycosylation, mammalian cells or fungi systems are chosen. So, the problem of lacking the convenient host can be solved through implementing recombinant DNA technology and selecting the suitable host due to specific proteins' characteristics.

Metagenomic approach is another viable method to exploit the largely untapped reservoir of uncultured microbial genomes from natural environments (49). This technology can be used for drug discovery (48). Metagenomics provides a method to extract protein from environmental microbes without the need to culture them in the laboratory. The total genetic material from all organisms present in an environmental sample is obtained directly and transferred into surrogate organisms to generate a metagenome clone library. Metagenomics provides two complimentary approaches in the search for biological products; (i) mining of the genetic information by sequencing and PCR; and (ii) functional screening of clones (50). In the first approach, when the metagenomic composition is known, the search for a particular function or pro-

tein is done by mining the metagenomic sequence data. Once the specific sequence is found by PCR amplification, it can be expressed in surrogate organisms. This approach has been successfully used in the discovery of several new enzymes, including chitinases, carboxypeptidases and lipases (49). The second approach, a functional screening of clones, constitutes a function-based assay, in which surrogate organisms are tested for a particular activity, such as reactions catalysed by particular enzymes, or properties attributed to a particular metabolite, for example antibiotics and anti-tumor agents.

Obtaining proteins from microbial hosts, whether cultivable or uncultivable microbes, is only a first step. One of the main obstacles of using enzymes for industrial or therapeutic purposes stems from their reduced stability relating to solubility issues, mechanical stress and enzyme attack in fermenters and also in biological systems. Microbial proteins need to be formulated in a carrier that provides a protective environment for the enzyme while remaining permeable to target molecules for treatment, avoiding immunological reactions.

Nanotechnology has emerged as a promising tool to address some of e these problems. Generally, it is highlighted that nanotechnology may help diagnosis, drug delivery and tissue regeneration in health-care industries, as well as the field of biocatalysis in the industrial sector. Biotechnological applications of nanotechnology include entrapping and/or immobilizing of proteins with the aim of enhancing their activity and function. Several drugs with nanotechnology formulation are already in use, such as rapamune1, an immunosuppressant and emend1, a medicine for emesis. Other drug, such as semapimod1, an anti-cancer has been approved for clinical trials (48).

Cancer immunotherapy is currently gaining momentum as an invaluable therapeutic strategy for cancers refractory to traditional treatments or those with no effective therapeutic options. In modern immunotherapy, recombinant bacteria expressing tumor-associated antigens are used for the purpose of activating tumor-specific cytolytic *T lymphocytes*. In fact, in this mechanism we use some bacteria to induce immune responses against tumor cells. The recent FDA-approval of the first antigen specific tumor im-

munotherapy, sipleucel-T for prostate cancer (also known as Provenge®), is part of a new wave of immunotherapies that bring the promise of improved efficacy and reduced adverse events in comparison to chemo and radiation therapy (51).

To conclude, the biopharmaceutical industry is multifaceted, dealing with ribozymes, antisense molecules, monoclonal antibodies, genomics, proteomics metabolomics, pharmacogenomics, combinatorial chemistry and biosynthesis, high throughput screening, bioinformatics, nanobiotechnology, gene therapy, tissue engineering and many other matters. Major impacts in

the world have been made by genetic engineering that has alteredmany disciplines including pharmacology, medicine and industrial engineering. In future, biotechnology may increasingly be used in: (i) treatment of chronic and complex acute diseases by development of new drugs and vaccines, (ii) application of recombinant microbes to decrease the effects of environmental pollution, and (iii) development of recombinant bioprocesses to solve worldwide energy.

Conflict of Interest

None declared.

4.References

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