INDUSTRIAL RECOMBINANT PROTEIN PRODUCTION

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Contents

Summary

This article describes the state of the art of industrial r-protein production with emphasis on integral bioprocess design. First, the market of recombinant proteins is analyzed. Next, the main issues associated with this technology are identified, and strategies for their solution are discussed. Finally, regulatory and legal aspects relevant for the commercialization of recombinant products are presented, and future perspectives are discussed. All these aspects are illustrated with specific industrial applications to provide a practical perspective to the reader. To date the major impact of recombinant products has been in the pharmaceutical sector, thus, this contribution will be mostly emphasized in such an area.

1. Introduction

It has been more than twenty years since the first recombinant (r-) protein was obtained and the industrial production of r-products became possible (see also - *Mammalian Cell Culture*; *Genetic engineering of bacterial cells*; *-Genetic engineering of mammalian cells*). Since then, hundreds of proteins from very different origins (from viral to human) have been produced by genetically modified organisms. Recombinant protein production has opened a whole new era for mankind. Many proteins that were very scarce and difficult to obtain can now be readily produced. Also, health concerns associated to the use of proteins isolated from human or animal tissues have now been dissipated. For example, during the 1980's 60 per cent of hemophilics were infected by HIV from the utilization of plasma-purified Factor VIII, but now the use of r-organisms has provided a safe and reliable source of this clotting factor. Moreover, several diseases can now be prevented by the use of safe new-generation recombinant vaccines. The new biotechnology industry based on r-products includes the production of therapeutics, prophylactics, and diagnostics, for both human and veterinary medicine applications[see also - *Medical biotechnology*]. The use of r-proteins has also transformed food processing, enzyme production, agriculture, and other areas. Even when industrial r-protein production is nowadays a reality, costly processes have restricted its benefits to only a relatively small fraction of humankind. To revert such a tendency it is necessary to develop improved productive processes for reducing costs and maintaining high quality products. Moreover, new emerging products will require further developments in bioprocess engineering.

The production of r-proteins can be broadly divided into four general steps: Cloning the DNA [see also - *DNA as genetic material*] of interest in a suitable vector under an adequate promoter; transforming and stabilizing the host cells; biosynthesis of the desired protein under controlled conditions; and recovery and purification of the r-product and comparison with its native counterpart. For an adequate industrial production, all four steps have to incorporate safety and regulatory issues into an integral process. Moreover, bioprocess design should also consider the market and application of each r-product. All these aspects are discussed in detail in the following sections.

2. Markets and Products

Modern biotechnology is science-based and requires highly skilled personnel which can strongly interact with pairs of various disciplines. Furthermore, the gap between

developments in basic science and their industrial application is small and diminishing. Therefore, to maintain the lead and technological independence in the area of industrial r-protein production, countries must pursue active research and development. This occurs in European countries which on average invest 2 per cent of their GDP in research or in the U.S., which is leader in new product development with 81 per cent of the biotechnology patents issued worldwide. Accordingly, almost all r-products and the technology for their production are owned by U.S. or European companies. In contrast, non-developed countries only invest, in the best of cases, 0.5 per cent of their GDP in research. This has caused that benefits derived from modern biotechnology still remain accessible to relatively few countries. For example, U.S. has two thirds of the market of recombinant pharmaceuticals. Furthermore, approximately half of all modern biotechnology companies (roughly 2 000) are based in the U.S., which represent a fifteen-billion dollar market in the year 2000 (Table 1). In comparison, Japan has less than 10 independent biotechnology companies, even when it has the second largest pharmaceutical market (19 percent of the world market of ethical drugs and sales for more than 37 billion dollars).

Sector	Base Year 2000	Forecast Annual 2003	Forecast Annual 2008	Forecast Annual 2025	Forecast Annual 2050	Average Annual Growth Rates 2000-2050 (ppa)
Medical						
Human Therapeutics	11 700	16 100	27 000	63 500	215 000	6
Human Diagnostics	2 500	3 100	4 300	8 400	18 000	4
Subtotal	14 200	19 200	31 300	71 900	233 000	
Nonmedical						
Agriculture	780	1 000	2 300	8 600	58 000	9
Speciality Chemicals	550	900	2 000	4 750	26 700	8
Nonmedical Diagnostics	320	400	600	1 050	2 300	4
Subtotal	1 650	2 300	4 900	14 400	87 000	
Total	15 850	21 500	36 200	86 300	320 000	6

ppa: Percent per annum.

Adapted from: R.E. Shamel and A. Udis-Kessler. (1999) Biotechnology in the 21st Century. An Imaginary Journey into the Future. Gen. Engr. News. 19 (21): 19.

Table 1. U.S. Biotechnology Product Sales Forecast (in millions of U.S. dollars)

Recombinant products have reached the market in diverse areas, including pharmaceutical, veterinary, food, pesticides, and detergents (see Table 2 for some examples). Noteworthy, approximately 30 r-products in the pharmaceutical sector account for more than 90 per cent of all r-product sales, and only seven proteins produced by nine companies have approximately 70 per cent of the total market: erythropoietin, alpha interferon, hepatitis-B vaccine, granulocyte colony stimulating factor, insulin, human growth hormone and tissue plasminogen activator (tPA). Efforts for developing new recombinant drugs have been mainly directed towards the treatment of cancer [see also - *Molecular approaches to cancer prevention; - P53 and cancer*

treatment], AIDS, vaccines and other health problems affecting mainly U.S. and Europe. Unfortunately, many serious diseases prevalent in developing countries have not received enough attention.

Product	**Year First Approved or Commercialized**	**Main Indication/Application**
Pharmaceuticals (terapeutic/diagnostics)		
Denileukin diftitox (Ontak)	1999	Cutaneous t-cell lymphoma
Hepatitis C virus antigen	1999	Immunoblot assay to detect antibodies to virus
Lepirudin (hirudin)	1998	Anticoagulation in patients with thrombocytomania or tromboembolic disease
Trastuzumab (Herceptin)	1998	Metastasic breast cancer
Salmon calcitonin	1998	Paget's disease, hypercalcaemia of malignancy
Murine-human chimeric antibody (Basiliximab)	1998	Prophylaxis of acute organ rejection
Lime disease vaccine	1998	Active immunization against Lyme disease
Factor IX	1997	Hemophilia B
Interleukin-11	1997	Treatment following high dose chemotherapy
Platelet derived growth factor	1997	Diabetic foot and leg ulcers
Desirudin	1997	Prevention of deep venous thrombosis during surgery
Genetically engineered monoclonal antibody	1997	Crohn's disease
Glucagon	1996	Treatment of hypoglycemia, diagnostic aid
Factor VIIa	1996	Bleeding episodes in hemophilia A or B
Interferon Beta 1a	1996	Relapsing-remitting multiple sclerosis
HIV type 1 protein	1996	*In vitro* diagnostic test kit
Follitropin beta	1995	Treatment of infertility in women
Insulin-like growth factor	1994	Treatment of post-poliomyelitis syndrome
Glucocerebrosidase	1994	Gaucher's disease
Beta interferon	1993	Multiple sclerosis
Dornase alfa inhalation solution (pulmozyme)	1993	Cystic fibrosis
Factor VIII	1992	Hemophilia A
Interleukin 2	1992	Kidney cancer, graft versus host disease
Granulocyte colony-stimulating factor	1991	Adjuvant to chemotherapy, neutropenia (1994), bone marrow transplants (1994)
Granulocyte-macrophage colony-stimulating factor	1991	Infection related to autologous bone marrow transplants (1994)
Gamma interferon	1990	Chronic granulomatous disease
Erythropoietin	1989	Anemia associated with kidney disease, AIDS-related anemia (1991)
Haemophilus B conjugated vaccine	1988	*Haemophilus influenza* type B
Tissue plasminogen activator	1987	Acute myocardial infarction, acute pulmonary embolism (1990)
Interferon alpha	1986	Hairy-cell leukemia, Kaposi's sarcoma (1988), venereal warts (1988), hepatitis B (1992).

HBsAg	1986	Hepatitis B vaccine
Human growth hormone	1985	Dwarfism, short stature associated with renal insufficiencies, growth hormone deficiency
Insulin	1982	Diabetes
Veterinary Pharmaceuticals		
Vaccine	1998	Neonatal enterotoxicosis
Somatosalm	1997	Osmoregulation in immature salmons
Feline vaccine	1996	Feline leukaemia virus
Bovine somatotropin	1993	Enhancement of bovine milk production
Food and Feed		
Phytase	*	Increase bioavailability of phosphorous in animal diets
Pullulanase	1999	Saccharifying and debranching enzymes
Pectin esterase	1999	Processing aid for food and vegetable products
Beta glucosidase	1995	Saccharifying
Endoxylanase	*	Poultry feeding
Aspartic protease	1997	Cheese production
Thaumatin B	*	Sweetener
Pectinase	<1994	Fruit and vegetable juice production, cofee processing
Chymosin	1990	Cheese production
Alpha-amylase	1988	Starch modification
Pesticides		
Bacillus thuringiensis endotoxin	1994	Biopesticide
Detergents		
Lipase	1994	Dairy industry, detergent fat splitting
Subtilisin	<1994	Detergent formulation
Other		
Cellulase	*	Whole grain feedstock and biomass processing
Transgenic mice	*	Medical research
Luciferase	*	Luminescent agent used for diagnostics.
Restriction enzymes	<1994	rDNA techniques

* Data not available.

Adapted from: O.T. Ramírez, E. Flores and E. Galindo. (1995) Products and Bioprocesses Based on Genetically Modified Organismms: Review of Bioengineering Issues and Trends in the Literature. Asia Pacific Journal Molecular Biology and Biotechnology. 3, 165-197.

Table 2. Selected Recombinant Products on the Market.

The demand for r-products has increased exponentially during the last fifteen years and the trend should continue in the present decade. In 1998 the market for medicines derived from r-DNA technologies was worth over $13 billion and had a growth rate of 14 per cent per year, whereas the average growth rate of the whole pharmaceutical market was only 6 per cent. Today almost 1 200 new biopharmaceutical products are in clinical trials and many of them are expected to reach the market within this decade. The total market of r-products is expected to increase 12 per cent annually during the next 8 years and reach sales for over $300 x 10^9 by year 2050 (Table 1). In this expanding scenario, the markets of speciality chemicals and agricultural products

derived from biotechnology are expected to grow at the fastest rates (Table 1). The speciality chemicals sector is very active in new r-product development, and due to less regulatory burdens, more r-products are expected to be approved in the near future. In terms of number of products the speciality chemical sector is the most important since it produces for industrial applications more than 50 enzymes, many genetically engineered. Companies producing these proteins have the largest average sales per biotechnological company, with almost \$180 x 10^6 per year in 1997, while the pharmaceutical sector only had \$21 x 10^6 per year. Nonetheless, the speciality chemical sector only represents 3 per cent of the total r-proteins market due to the low added value of its products. Furthermore, industrial applications of r-products usually require very large quantities of protein, therefore very large-scale operations must be performed. Accordingly, highly productive processes are particularly required in this sector for maintaining economic viability.

3. The First Step: Selection of an Expression System.

Application of molecular biology techniques [see also - *Methods in genetical engineering*] can have an important impact on yield and productivity of recombinant bioprocesses. Introduction of a foreign gene whose product is not utilized by the host can perturb cell function at many levels: DNA replication [see also - *DNA replication*], regulation of transcription [see also - *Gene expression and regulation*], ribosome functions, RNA turnover, activities of regulatory proteins, chaperone and protease levels, membrane energetics, postranslational processing, and energy and intermediary metabolism. Thus, r-protein production processes must be carefully designed to reduce negative effects of host-vector interactions. Recombinant bioprocesses are determined in many ways by the selection of the host and vector. For instance, a prokaryotic host requires totally different production and purification schemes than a mammalian expression system. Several issues must be considered upon vector and host selection, such as intrinsic r-product characteristics (size, postranslational modifications), product performance (stability, activity, authenticity) and even financial considerations (final use, quantity required, cost/added value, time for development, market). Additionally, many production parameters (cultivation mode, medium composition, environmental conditions, and others) have an important relationship with gene expression, plasmid copy number, plasmid stability, etc. In the next two sections a general description of different protein expression systems is presented. Such information is necessary for properly selecting an expression system for industrial r-protein production.

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Biographical Sketches

Laura A. Palomares is a Researcher at the Institute of Biotechnology of the National University of Mexico (UNAM). She received her B.S. in Biochemical Engineering at the Monterrey Institute of Technology in Mexico in 1990, and in 1999 a Ph.D. degree from UNAM. Her research interests include the production of recombinant proteins by animal cells. Specifically, her efforts have focused in the design or rational production strategies of rotavirus-like particles using the insect cell-baculovirus expression vector system. She has a wide experience in production of biotechnology products for the food industry.

Francisco Kuri Breña has over 12 years of experience in research and production in the areas of organic chemistry and biotechnology. He received his B.S. and M.S. from the National University of Mexico, and his Ph.D. in organic chemistry from the University of British Columbia (Canada). He worked for almost

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Octavio T. Ramírez received his B.S. in Chemical Engineering from the National University of Mexico (UNAM). He pursued his graduate studies in Drexel University (USA) where he received a Ph.D. degree in biochemical engineering. Presently, he is Professor at the Bioengineering Department of the Institute of Biotechnology at UNAM. Dr. Ramírez has worked the last 15 years in the areas of cell culture engineering and recombinant protein production, with special emphasis in reactor design and in the establishment of operation, monitoring and control strategies. His work has resulted in more than 45 publications, which include work with hybridomas, the insect-cell baculovirus expression vector system, human hematopoietic cells, and recombinant yeast and *E. coli*. He is also a consultant for various companies producing biologics and recombinant products.