Production of Recombinant Proteins

Novel Microbial and Eukaryotic Expression Systems

Edited by
Gerd Gellissen

WILEY-VCH Verlag GmbH & Co. KGaA

Production of Recombinant Proteins

Edited by
Gerd Gellissen

Further Titles of Interest

G. Walsh

Proteins

Biochemistry and Biotechnology

2001
ISBN 0-471-89906-2

G. Walsh

Biopharmaceuticals

Biochemistry and Biotechnology

2003
ISBN 0-470-84326-8

Jörg Knäblein and Rainer H. Müller (Eds.)

Modern Biopharmaceuticals

Design, Development and Optimization

2005
ISBN 3-527-31184-X

M. Schleef (Ed.)

DNA-Pharmaceuticals

Formulation and Delivery in Gene Therapy and DNA Vaccination

2005
ISBN 3-527-31187-4

G. Gellissen (Ed.)

Hansenula polymorpha

Biology and Applications

2002
ISBN 3-527-30341-3

H.J. Rehm, G. Reed, A. Pühler, P. Stadler (Eds.)

Biotechnology

Second, Completely Revised Edition
Volume 2, Genetic Fundamentals and Genetic Engineering
1992
ISBN 3-527-28312-9

H.-J. Rehm, G. Reed, A. Pühler, P. Stadler, A. Mountain, U.M. Ney, D. Schomburg (Eds.)

Biotechnology

Second, Completely Revised Edition
Volume 5a, Recombinant Proteins, Monoclonal Antibodies, and Therapeutic Genes
1998
ISBN 3-527-28315-3

R.D. Schmid, R. Hammelehle

Pocket Guide to Biotechnology and Genetic Engineering

2003
ISBN 3-527-30895-4

Production of Recombinant Proteins

Novel Microbial and Eukaryotic Expression Systems

Edited by
Gerd Gellissen

WILEY-VCH Verlag GmbH & Co. KGaA

Edited by

Prof. Dr.Gerd Gellissen
Ringstrasse 30
42489 Wülfrath
Germany

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data: A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>

Printed in the Federal Republic of Germany
Printed on acid-free paper

Composition ProSatz Unger, Weinheim
Printing Strauss GmbH, Mörlenbach
Bookbinding J. Schäffer GmbH i. G., Grünstadt

ISBN 3-527-31036-3

This book is dedicated to my wife Gabi
and my sons Benedikt, Georg, and Ulrich.

Preface

Gene technology has invaded the production of proteins, and especially production processes for pharmaceuticals. At the beginning of this new technology only a limited number of microorganisms was employed for such processes, namely the bacterium *Escherichia coli*, followed by the baker's yeast *Saccharomyces cerevisiae* as a microbial eukaryote. For both organisms a wealth of information was available which stemmed from a long tradition of safe use in science and, in case of the yeast, also from food manufacturing. However, certain limitations and restrictions urged the search for alternatives that were able to meet the requirements and demands for the expression of an ever-growing number of target genes. As a consequence, a plethora of microbial and cellular expression platforms were developed. Nonetheless, the range of launched products still leans for the most part on production in a restricted set of organisms, with most of the newly identified microbes being applied to research in academia.

Despite superior characteristics of some industrially employed platforms, limitations and restrictions are still encountered in particular process developments. In a publicly funded program, Rhein Biotech has set out with academic partners in the recent past to identify additional microbes with attractive capabilities that could supplement its key system, *Hansenula polymorpha*. As such, the Gram-positive *Staphylococcus carnosus*, the thermo- and osmotolerant dimorphic yeast species *Arxula adeninivorans*, the filamentous fungi *Aspergillus sojae*, and the nonsporulating species *Sordaria macrospora*, were developed. This development was supplemented by tools such as the definition of fermentation conditions and a "universal vector" that can be employed to target a range of fungi for the identification of the most suited platform in particular process developments. The application of these platforms and tools is included in the business concept of a new German biotech start-up company, MedArtis Pharmaceuticals GmbH, Aachen.

The present book is aimed at providing a comprehensive view of these newly identified and defined systems, and comparing them with a range of established and new alternatives. The book includes the description of two Gram-negative organisms (*E. coli* and *Pseudomonas fluorescens*), the Gram-positive *Staphylococcus carnosus*, four yeast species (*Arxula adeninivorans, Hansenula polymorpha, Pichia pastoris* and *Yarrowia lipolytica*), and the two filamentous fungi *Aspergillus sojae* and *Sordaria macrospora*. The description of these microbial platforms is further supplemented by an overview on expression in mammalian and plant cells.

I would like to thank all academic partners who co-operated in the development of these new platforms. I gratefully acknowledge funding by the Ministry of Economy NRW, Germany (TPW-9910v08). I would also like to thank D. Ellens, M. Piontek, and F. Ubags, who inspired me to edit this book.

I also express my gratitude to all authors for their fine efforts and contributions, and thank Dr. Paul Hardy, Düsseldorf, for carefully reading some of the manuscripts. I also acknowledge the continuous support of Dr. A. Pillmann and her staff at Wiley-VCH.

Aachen, October 2004 *Gerd Gellissen*

Foreword

The availability of ever-increasing numbers of eukaryotic, prokaryotic, and viral genomes facilitates the rapid identification, amplification, and cloning of coding sequences for technical enzymes and pharmaceuticals, including vaccines. To take advantage of the treasures of information contained in these sequences, elegant multiplatform expression systems are needed that fulfill the specific requirements demanded by each potential application; for example, economy in the case of technical enzyme production, or safety and authenticity in the case of pharmaceutical production. Therefore, while *Escherichia coli* and other bacteria may be perfectly suited for technical enzyme production or the production of selected pharmaceuticals requiring no special modification, eukaryotic organisms may be advisable for applications where safety (e.g., no endotoxin), contamination, or authenticity (e.g., proper protein modification by glycosylation) are of concern. While the choices of microbial and eukaryotic expression systems for the production of recombinant proteins are many in number, most researchers in academic and industrial settings do not have ready access to pertinent biological and technical information as it is usually scattered in the scientific literature. This book aims to close this gap by providing, in each chapter, information on the general biology of the host organism, a description of the expression platform, a methodological section (with strains, genetic elements, vectors and special methods, where applicable), and finally some examples of proteins expressed with the respective platform. The described systems are well balanced by including three prokaryotes (two Gram-negative and one Gram-positive), four yeasts, two filamentous fungi, and two higher eukaryotic cell systems (mammalian and plant cells). The book is rounded off by providing valuable practical and theoretical information about criteria and schemes for selection of the appropriate expression platform, about the possibility and practicality of a universal expression vector, and about comparative industrial-scale fermentation. The production of a recombinant Hepatitis B vaccine is chosen to illustrate an industrial example. As a whole, this book is a valuable and overdue resource for a varied audience. It is a practical guide for academic and industrial researchers who are confronted with the design of the most suitable expression platform for their favorite protein for technical or pharmaceutical purposes. In addition, the book is also a valuable study resource for professors and students in the fields of applied biology and biotechnology.

Fort Collins, Colorado, U.S.A., June 2004 *Herbert P. Schweizer, Ph.D.*

Contents

List of Contributors

Josef Altenbuchner
Institute for Industrial Genetics
University Stuttgart
Allmandring 31
70569 Stuttgart
Germany

Oliver Bartelsen
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Erik Böer
IPK Gatersleben
Correnstr. 3
06466 Gatersleben
Germany

René Brecht
ProBiogen AG
Goethestr. 54
13086 Berlin
Germany

Pascale Brocke
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Jochen Büchs
Biochemical Engineering
RWTH Aachen University
Worringer Weg 1
52056 Aachen
Germany

Lawrence C Chew
The Dow Chemical Company
Biotechnology, Research and Development
5501 Oberlin Dr.
San Diego, CA 92121
USA

James M Cregg
Professor and Director of Research
Keck Graduate Institute of Applied Sciences
535 Watson Drive
Claremont, CA 91711
USA

Ulrike Dahlems
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Jürgen Drossard
Institute of Molecular Biotechnology
RWTH Aachen University
Worringer Weg 1
52056 Aachen
Germany

Rainer Fischer
Institute of Molecular Biotechnology
RWTH Aachen University
Worringer Weg 1
52056 Aachen
Germany

Roland Freudl
Institut für Biotechnologie 1
Forschungszentrum Jülich GmbH
52425 Jülich
Germany

Claude Gaillardin
Microbiologie et Génétique Moleculaire
UMR1238 INAPG-INRA-CNRS
Institut National Agronomique
Paris-Grignon
78850 Thiverval Grignon
France

Gerd Gellissen
MedArtis Pharmaceuticals GmbH
Pauwelsstr. 19
52047 Aachen
Germany

Margreet Heerikhuisen
TNO Nutrition and Food Research
Department of Microbiology
P.O. Box 360
3700 AJ Zeist
The Netherlands

Stephan Hellwig
Fraunhofer Institute for
Molecular Biology and Ecology (IME)
Worringerweg 1
52074 Aachen
Germany
and
MedArtis Pharmaceuticals GmbH
Pauwelsstr. 19
52047 Aachen
Germany

Cornelis P Hollenberg
Institut für Mikrobiologie
Heinrich-Heine-Universität
Universitätsstr. 1
40225 Düsseldorf
Germany

Christine Ilgen
Keck Graduate Institute of Applied
Sciences
535 Watson Drive
Claremont, CA 91711
USA

Zbigniew A Janowicz
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Volker Jenzelewski
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Hyun Ah Kang
Korea Research Institute
of Bioscience and Biotechnology (KRIBB)
52 Eoen-dong
Yusong-gu, Daejeon 305–333
Korea

Jens Klabunde
Institut für Mikrobiologie
Heinrich-Heine-Universität
Universitätsstr. 1
40225 Düsseldorf
Germany

Ulrich Kück
Lehrstuhl für
Allgemeine und Molekulare Botanik
Ruhr Universität
44780 Bochum
Germany

Gotthard Kunze
IPK Gatersleben
Corrensstr. 3
06466 Gatersleben
Germany

Joan Lin-Cereghino
Department of Biological Sciences
University of the Pacific
Stockton, CA 95211
USA

Catherine Madzak
Microbiologie et Génétique Moleculaire
UMR1238 INAPG-INRA-CNRS
Institut National Agronomique
Paris-Grignon
78850 Thiverval Grignon
France

Ralf Mattes
Institute for Industrial Genetics
University Stuttgart
Allmandring 31
70569 Stuttgart
Germany

Karl Melber
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Georg Melmer
MedArtis Pharmaceuticals
Pauwelsstr. 19
52047 Aachen
Germany

Frank Müller
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Jean-Marc Nicaud
Microbiologie et Génétique Moleculaire
UMR1238 INAPG-INRA-CNRS
Institut National Agronomique
Paris-Grignon
78850 Thiverval Grignon
France

Kyung-Nam Park
227–3, Kuga-li, Giheung-Eup
Yongin-Shi, Kyunggi-do
Korea

Stefanie Poeggeler
Lehrstuhl für Allgemeine und
Molekulare Botanik
Ruhr Universität
44780 Bochum
Germany

Peter J Punt
TNO Nutrition and Food Research
Department of Microbiology
P.O. Box 360
3700 AJ Zeist
The Netherlands

Tom M. Ramseier
The Dow Chemical Company
Biotechnology, Research and Development
5501 Oberlin Dr.
San Diego, CA 92121
USA

Diane M. Retallack
The Dow Chemical Company
Biotechnology, Research and Development
5501 Oberlin Dr.
San Diego, CA 92121
USA

Thomas Rose
ProBiogen AG
Goethestr. 54
13086 Berlin
Germany

Volker Sandig
ProBiogen AG
Goethestr. 54
13086 Berlin
Germany

Stephan Schaefer
University Rostock
Department of Medical Microbiology and Virology
Schillingallee 70
18055 Rostock
Germany

Stefan Schillberg
Fraunhofer Institute for Molecular Biology and Applied Ecology (IME)
Worringerweg 1
52074 Aachen
Germany

Jane C. Schneider
The Dow Chemical Company
Biotechnology, Research and Development
5501 Oberlin Dr.
San Diego, CA 92121
USA

Charles H. Squires
The Dow Chemical Company
Biotechnology, Research and Development
5501 Oberlin Dr.
San Diego, CA 92121
USA

Christoph Stöckmann
Biochemical Engineering
RWTH Aachen University
Worringer Weg 1
52056 Aachen
Germany

Alexander WM Strasser
Ringelsweide 16
40223 Düsseldorf
Germany

Manfred Suckow
Rhein Biotech GmbH
Eichsfelder Str. 11
40595 Düsseldorf
Germany

Henry W Talbot
The Dow Chemical Company
Biotechnology, Research and Development
5501 Oberlin Dr.
San Diego, CA 92121
USA

Richard M Twyman
Department of Biological Sciences
University of York
Heslington
York
YO10 5DD, UK
England

Cees AMJJ van den Hondel
TNO Nutrition and Food Research
Department of Microbiology
3700 AJ Zeist
The Netherlands

Karsten Winkler
ProBiogen AG
Goethestr. 54
13086 Berlin
Germany

1
Key and Criteria to the Selection of an Expression Platform

Gerd Gellissen, Alexander W.M. Strasser, and Manfred Suckow

The production of recombinant proteins has to follow an economic and qualitative rationale, which is dictated by the characteristics and the anticipated application of the compound produced. For the production of technical enzymes or food additives, gene technology must provide an approach which has to compete with the mass production of such compounds from traditional sources. As a consequence, production procedures have to be developed that employ highly efficient platforms and that lean on the use of inexpensive media components in fermentation processes. For the production of pharmaceuticals and other compounds that are considered for administration to humans, the rationale is dominated by safety aspects and a focus on the generation of authentic products. The demand for suitable expression systems is increasing as the emerging systematic genomics result in an increasing number of gene targets for the various industrial branches (for pharmaceuticals, see Chapter 16). So far, the production of approved pharmaceuticals is restricted to *Escherichia coli*, several yeasts, and mammalian cells. In the present book, a variety of expression platforms is described ranging from Gram-negative and Gram-positive prokaryotes, over several yeasts and filamentous fungi to mammalian and plants cells, thus including greatly divergent cell types and organisms. Some of the systems presented are distinguished by an impressive track record as producers of valuable proteins that have already reached the market, while others are newly defined systems that have yet to establish themselves but demonstrate a great potential for industrial applications. All of them have special favorable characteristics, but also limitations and drawbacks – as is the case with all known systems applied to the production of recombinant proteins. As there is clearly no single system that is optimal for all possible proteins, predictions for a successful development can only be made to a certain extent, and as a consequence misjudgments leading to costly time- and resource-consuming failures cannot be excluded. It is therefore advisable to assess several selected organisms or cells in parallel for their capability to produce a particular protein in desired amounts and quality (see also Chapter 13).

The competitive environment of the considered platforms is depicted in Table 1.1. A cursory correlation exists between the complexity of a particular protein and the complexity and capabilities of an expression platform. Single-subunit proteins can easily be produced in bacterial hosts, whereas proteins that require an authentic complex mammalian glycosylation or the presence of several disulfide bonds neces-

Table 1.1 Some key parameters for the choice of a particular expression system. The column "Expression system" provides the list of the systems described in the various chapters of this book. The column "Classification" provides a rough classification of these organisms. The coloring of the fields indicates the complexity of the respective organism, increasing in the order light gray, medium gray, dark gray. In the following columns, positive and negative aspects are distinguished by the coloring of the fields. Light gray indicates negative, and dark gray positive features. Fields in medium gray indicate an intermediate grading. The column "Development of system" distinguishes between "early stages" and "completely developed". The latter indicates that the full spectrum of methods and elements for genetic manipulations, target gene expression, and handling is available. "Early stages" shall indicate a yet incomplete development. In "Disulfide bonds" and "Glycosylation", two examples of post-translational modification are addressed which may be especially important for heterologous protein production. Prokaryotes have, in general, a strongly limited capability of forming disulfide bonds. If one or more disulfide bonds is necessary for the target protein's activity, a eukaryotic system would be the better choice. If the target protein requires *N*- or *O*-glycosylation for proper function, prokaryotic systems are also disqualified. The production of a glycoprotein for the administration to humans requires special care. So far, only mammalian cells are capable of producing human-compatible glycoproteins. Glycoproteins produced by two methylotrophic yeasts, *Hansenula polymorpha* and *Pichia pastoris*, have been shown not to contain terminal α1,3-linked mannose, which are suspected to be allergenic. For the other yeasts and fungi listed, the particular composition of the glycosylation has yet not been determined, which here is valued as a negative feature. "Secretion" of target protein can be achieved with all systems shown in the list. However, in case of the two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas fluorescens*, "Secretion" means that the product typically accumulates in the periplasm; the complete release requires the degradation of the outer membrane. The following three columns, "Costs of fermentation", "Use of antibiotics", and "Safety costs" refer to a subset of practical aspects for production of a target protein. In general, the "Costs of fermentation" in mammalian cells are much higher than in plant cells, fungi, yeasts, or prokaryotes, due mainly to the costs of the media. However, the use of isopropyl-thiogalactopyranoside (IPTG)-inducible promoters can increase the costs of target protein production in *E. coli* and *P. fluorescens*, as indicated by the medium gray fields. The use of antibiotics in fermentation processes is becoming increasingly undesired. If a therapeutic protein is to be produced in *E. coli* or *Staphylococcus carnosus*, a plasmid/host system should be chosen that allows plasmid maintenance without the use of antibiotics. "Safety costs" refers to the capability of the production system of carrying human pathogenic agents. In this regard, the mammalian-derived cell systems display the highest risks, for example as carriers of retroviruses. "Processes developed" indicates whether processes based on a particular system have already entered the pilot or even the industrial scale, associated with the respective knowledge. "Products on market" indicates which systems have already passed this final barrier.

▶