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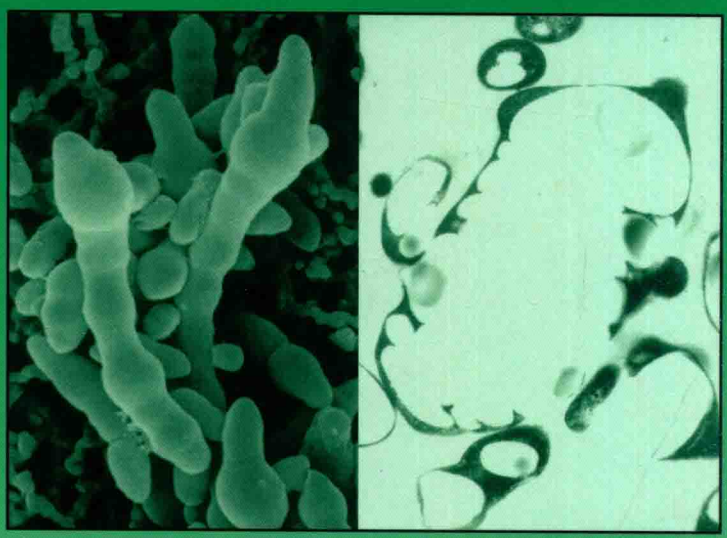
Microbial Processes and Products

Edited by

José Luis Barredo



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José-Luis Barredo

R&D Biology
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Microbial Processes and Products

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Preface

The development of biotechnology over the last 20 years, and particularly the use of recombinant DNA techniques, has rapidly expanded the opportunities for human benefits from living resources. Efforts to reduce pollution, prevent environmental damage, combat microbial infection, improve food production, and so on can each involve fermentation or the environmental release of microorganisms. Many products of fermentation technology, such as alcoholic beverages, bread, antibiotics, amino acids, vitamins, enzymes, and others, have been influenced by the progress of recombinant DNA techniques. The development of new products or the more efficient manufacturing of those already being produced often involve the use of microorganisms as cell factories for many productions and biotransformations.

Microbial Processes and Products is intended to provide practical experimental laboratory procedures for a wide range of processes and products mediated by microorganisms. Although not an exhaustive treatise, it provides a detailed "step-by-step" description of the most recent developments in such applied biotechnological processes. The detailed protocols we provide are cross-referenced in the Notes section, contain critical details, lists of problems and their troubleshooting, as well as safety recommendations that may not normally appear in journal articles and can be particularly useful for those unfamiliar with specific techniques.

The lead chapter of *Microbial Processes and Products* represents an overview on strain improvement programs and strategies to optimize fermentation processes. The remaining chapters detail comprehensive experimental methods for the optimal design of microbial metabolite production and for applying biotechnological processes to the manufacture of products used worldwide for human health, nutrition, and environmental protection, including semisynthetic derivatives of cephalosporins, erythromycin, antitumor compounds, plasmids for gene therapy and DNA vaccination, L-lysine, vitamins B_2 and B_{12}, the sweet-tasting protein thaumatin, the carotenoids β-carotene and astaxanthin, the polysaccharide gellan, and the bacteriocin-producing bacteria for sausage fermentation. Furthermore, the uses of the phenylacetyl-CoA catabolon for the enzymatic synthesis of penicillins, aromatic biotransformations, synthesis of new bioplastics, biosensor design, the synthesis of drug vehicles, and the development of a phosphatase encoding gene as a reporter and monitor gene expression are illustrated.

Additionally, *Microbial Processes and Products* offers techniques for analysis and quantification, including antimicrobial metabolites and carotenoids, volatile sulfur compounds, metabolic pathway fluxes, gene expression arrays, proteome analysis, methods to understand the mechanisms underlying bacterial modulation of the innate immune response, bioleaching activity, and microbial metal sulfide oxidation, and heavy metals remediation. Finally, three overview chapters on the transport of biological material, the deposit of biological material for patent purposes, and protection of biotechnological inventions are included.

Microbial Processes and Products has been written by outstanding experts in the field and provides a highly useful reference source for laboratory and industrial professionals, as well as for graduate students in a number of biological disciplines (biotechnology, microbiology, genetics, molecular biology) because of the uncommonly wide applicability of the procedures across the range of areas covered.

I am indebted to the authors who, in spite of their professional activities, agreed to participate in this book, to Dr. John Walker, Series Editor, for his encouragement and advice in reviewing the manuscripts, and to the rest of the staff of The Humana Press for their assistance in assembling this volume and their efforts in keeping this project on schedule. Last but not least, I warmly acknowledge my wife Natalia and our children Diego, José-Luis, Álvaro, and Gonzalo for their patience and support.

José-Luis Barredo

Contributors

María-José Amaya • *Grupo Uriach, Barcelona, Spain*

Sagrario Arias • *Departamento de Bioquímica y Biología Molecular, Universidad de León, Spain*

José Arnau • *Department of Fungal Biotechnology, Biotechnological Institute, Hørsholm, Denmark*

Elsa Arias-Barrau • *Departamento de Bioquímica y Biología Molecular, Universidad de León, Spain*

Heiko Barg • *Institut für Mikrobiologie, der Carolo-Wilhelmina-Universität, Braunschweig, Germany*

José-Luis Barredo • *R&D Biology, Antibióticos S. A., León, Spain*

Francisco Bermejo • *Departamento de Química Orgánica, Universidad de Salamanca, Spain*

Linda F. Bisson • *Department of Viticulture and Enology, University of California, Davis, CA*

Stephanie Blum • *Nestlé Research Centre, Lausanne, Switzerland*

Klaus Bosecker • *Section Geomicrobiology, Federal Institute for Geosciences and Natural Resources, Hannover, Germany*

Peter M. Bramley • *School of Biological Sciences, Royal Holloway, University of London, UK*

Jesper Breum • *Department of Fungal Biotechnology, Biotechnological Institute, Hørsholm, Denmark*

Lan-Szu Chou • *Center for Microbe Detection and Physiology, Utah State University, Logan, UT*

Stefania Cresti • *Dipartimento di Biologia Molecolare, Università degli Studi di Siena, Italy*

Adele Cutler • *Department of Mathematics and Statistics, Utah State University, Logan, UT*

Juan-Luis de la Fuente • *R&D Biology, Antibióticos S. A., León, Spain*

Luc De Vuyst • *Research Group of Industrial Microbiology, Fermentation Technology, and Downstream Processing, Department of Applied Biological Sciences, Vrije Universiteit Brussel, Brussels, Belgium*

José-Luis Del Río • *Grupo Uriach, Barcelona, Spain*

Ben Dias • *Kraft Foods Inc., Glenview, IL*

Maria Margarida Diogo • *Center for Biological and Chemical Engineering, Instituto Superior Técnico, Lisbon, Portugal*

ARTURO P. ESLAVA • *Centro Hispano-Luso de Investigaciones Agrarias, Universidad de Salamanca, Spain*
IGNACIO FAUS • *Grupo Uriach, Barcelona, Spain*
UWE FITZNER • *Dres. Fitzner, Münch und Kluin, Ratingen, Germany*
PAUL D. FRASER • *School of Biological Sciences, Royal Holloway, University of London, UK*
CESIRA L. GALEOTTI • *I.R.I.S. Chiron s.r.l., Siena, Italy*
BELÉN GARCÍA • *Departamento de Bioquímica y Biología Molecular, Universidad de León, Spain*
LEI HAN • *Dow AgroSciences LLC, Indianapolis, IN*
ELMAR HEINZLE • *Biochemical Engineering Institute, Saarland University, Saarbrücken, Germany*
NABILA IBNOU-ZEKRI • *Nestlé Research Centre, Lausanne, Switzerland*
MASATO IKEDA • *Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, Nagano, Japan*
ENRIQUE A. ITURRIAGA • *Area de Genética, Departmento de Microbiología y Genética, Universidad de Salamanca, Spain*
DIETER JAHN • *Institut für Mikrobiologie, Carolo-Wilhelmina-Universität, Braunschweig, Germany*
MARTINA JAHN • *Institut für Mikrobiologie, Carolo-Wilhelmina-Universität, Braunschweig, Germany*
FRÉDÉRIC LEROY • *Research Group of Industrial Microbiology, Fermentation Technology, and Downstream Processing, Department of Applied Biological Sciences, Vrije Universiteit Brussel, Brussels, Belgium*
PALOMA LIRAS • *Área de Microbiología, Departmento de Ecologia, Genética, y Microbiologia, Facultad de Ciencias Biológicas y Ambientales, Universidad de León, Spain*
JOSÉ M. LUENGO • *Departamento de Bioquímica y Biología Molecular, Universidad de León, Spain*
LYNNE E. MACASKIE • *School of Biosciences, The University of Birmingham, UK*
MARCO MALTEN • *Institut für Mikrobiologie, Carolo-Wilhelmina-Universität, Braunschweig, Germany*
PAULA A. MARA • *Department of Viticulture and Enology, University of California, Davis, CA*
JUAN F. MARTÍN • *Instituto de Biotecnología, León, Spain*
LAURA MATEOS • *Departamento de Microbiologia y Genetica, CSIC/ Universidad de Salamanca, Spain*

CARMEN MÉNDEZ • *Departamento de Biología Funcional e Instituto Universitario de Oncología del Principado de Asturias, Universidad de Oviedo, Spain*
WOLFGANG MINAS • *Anbics Management-Services AG, Zurich, Switzerland*
SATOSHI MITSUHASHI • *Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan*
JUNKO OHNISHI • *Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan*
TAMMI L. OLINEKA • *Department of Viticulture and Enology, University of California, Davis, CA*
ELÍAS R. OLIVERA • *Departamento de Bioquímica y Biología Molecular, Universidad de León, Spain*
JEAN M. OVIGNE • *Nestlé Research Centre, Lausanne, Switzerland*
TAMÁS PAPP • *Area de Genética, Departmento de Microbiología y Genética, Universidad de Salamanca, Spain*
SARAD PAREKH • *Dow AgroSciences LLC, Indianapolis, IN*
MARION PATERSON-BEEDLE • *School of Biosciences, The University of Birmingham, UK*
DUARTE MIGUEL F. PRAZERES • *Center for Biological and Chemical Engineering, Instituto Superior Técnico, Lisbon, Portugal*
JOÃO ANTÓNIO QUEIROZ • *Department of Chemistry, Universidade da Beira Interior, Covilhã, Portugal*
JOSÉ-LUIS REVUELTA • *Departamento de Microbiologia y Genetica, CSIC/ Universidad de Salamanca, Spain*
MARTA RODRÍGUEZ-SÁIZ • *R&D Biology, Antibióticos S. A., León, Spain*
CHRISTINE ROHDE • *DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany*
GIAN MARIA ROSSOLINI • *Dipartimento di Biologia Molecolare, Università degli Studi di Siena, Italy*
JOSÉ A. SALAS • *Departamento de Biología Funcional e Instituto Universitario de Oncología del Principado de Asturias, Universidad de Oviedo, Spain*
GERHARD SANDMANN • *Botanical Institute, J.W. Goethe University, Frankfurt, Germany*
ÁNGEL SANDOVAL • *Departamento de Bioquímica y Biología Molecular, Universidad de León, Spain*
MARÍA A. SANTOS • *Departamento de Microbiologia y Genetica, CSIC/ Universidad de Salamanca, Spain*

EDUARDO J. SCHIFFRIN • *Nestlé Research Centre, Lausanne, Switzerland*
SERENA SCHIPPA • *Dipartimento di Scienze e di Sanità Pubblica, Università La Sapienza, Rome, Italy*
AXEL SCHIPPERS • *Section Geomicrobiology, Federal Institute for Geosciences and Natural Resources, Hannover, Germany*
MICHELLE SERGENT • *Laboratoire de Méthodologie de la Recherche Expérimentale, Faculté des Sciences et Techniques de Saint Jérôme, Marseille, France*
HEIDI SISNIEGA • *Grupo Uriach, Barcelona, Spain*
APOSTOLOS SPIROPOULOS • *Department of Viticulture and Enology, University of California, Davis, CA*
KARL-PETER STAHMANN • *Technische Mikrobiologie. FB Bio-, Chemie- und Verfahrenstechnik. Fachhochschule Lausitz, Senftenberg. Germany*
MARIA C. THALLER • *Dipartimento di Biologia, Università di Roma Tor Vergata, Roma, Italy*
GUILLAUME E. VANOT • *Faculté des Sciences et Techniques de Saint Jérôme, Marseille, France*
JAN C. VERDOES • *Dyadic Nederland BV, Zeist, The Netherlands*
HANS VISSER • *Section of Fungal Genomics, Wageningen University, The Netherlands*
THIERRY VON DER WEID • *Nestlé Research Centre, Lausanne, Switzerland*
VERA WEIHS • *DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany*
BART WEIMER • *Center for Integrated BioSystems, Department of Nutrition and Food Sciences and Center for Microbe Detection and Physiology, Utah State University, Logan, UT*
THOMAS P. WEST • *Olson Biochemistry Laboratories, Department of Chemistry and Biochemistry, South Dakota State University, Brookings, SD*
CHRISTOPH WITTMANN • *Biochemical Engineering Institute, Saarland University, Saarbrücken, Germany*
YI XIE • *Center for Microbe Detection & Physiology, Department of Nutrition and Food Science, Utah State University, Logan, UT*

Contents

1

Development of Improved Strains and Optimization of Fermentation Processes

Lei Han and Sarad R. Parekh

Summary

Microbial strains overproducing commercially important metabolites are routinely obtained through mutagenesis and random screening and/or selection. Advances in recombinant DNA technology have made it possible for engineering improved microbial strains by specific addition or deletion of certain genes. The key to the genetic engineering approach, however, is the identification of genes controlling metabolite production. In recent years, innovative technologies have been developed to allow researchers to investigate the genetics and physiology of a microorganism on a global scale. Knowledge gained from these studies is beginning to modernize strain development processes. Fermentation processes must be constantly optimized in order to maximize the potential of each improved strain. This chapter reviews the various methods of developing improved strains and addresses the specific issues concerning each method. In addition, strategies commonly employed to optimize fermentation processes will be analyzed. Finally, new technologies and how they can help strain development and fermentation process optimization will be discussed.

Key Words: Strain improvement; fermentation; mutation; genetic engineering, scale-up.

1. Introduction

Microbial strain improvement involves the application of one or a combination of strategies that result in the development of new strains with desired phenotypes. The most commonly sought phenotype is increased metabolite production. Other desired phenotypes include reduced production of side products or ease of scalability at operation. Improving microbial strains for desired phenotypes has been the cornerstone of all commercial fermentation processes. Even today, much of the emphasis placed on improving microbial strains is the result of the diversity of the metabolites produced by microorganisms that have

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found novel applications in the food, chemical, agricultural, health care, and pharmaceutical industries.

Biologically active molecules synthesized by microorganisms isolated from nature are usually produced in extremely low quantities. The reason is that those molecules are either nonessential for the survival of the microorganisms or present in sufficient quantities to satisfy their primary needs. Low productivity of the metabolite of interest translates into high manufacturing cost per unit of the product *(1)*. Therefore, manufacturing of commercially important metabolites directly from microorganisms isolated from nature is often economically unfavorable. However, there are ways of enhancing productivity to make the production process economically viable. Lower fermentation manufacturing and capital costs can be achieved through improvements in the design of fermenters *(2)*. The greatest opportunity to lower manufacturing cost without significant capital investment, however, comes from microbial strains with increased productivity or the ability to utilize low-cost raw materials or some other beneficial traits *(3)*. Improved microbial strains can be obtained by using microbes isolated from nature as a starting point and by employing one or combinations of strategies.

Conventionally, strain improvement is achieved through random mutation and screening or selection, the so-called classical approach. This empirical method has been practiced for more than 50 yr and has a long history of success *(4)*. The best known example is the titer improvement achieved for penicillin *(5)*. In view of the long practice of the classical approach, it continues to be the primary strain improvement strategy of any newly established strain improvement program. One reason for continued interest in the classical approach is that this approach does not require prior knowledge of the metabolite biochemical pathway, regulation, or transport. Another reason is that the advancement made over the years in the precision and sensitivity of analytical instruments dramatically increases the reliability and sensitivity of detection. Furthermore, automation and miniaturization of screening processes have significantly reduced system variability and increased screening throughput.

In addition to the classical approach, there are targeted approaches for developing improved strains, including enrichment methods and genetic engineering. One significant advantage of the targeted approaches is that only a small number of strains have to be screened and evaluated when the rationale behind screening and genetic engineering is sound. Therefore, the key to the targeted approaches has been to identify the genes controlling metabolite production. Previously target gene identification was a time-consuming and painstaking task. Researchers were able to work only on a few

leads at a time because of the complexity and difficulty involved in identifying the right gene target. However, with the aid of genomic sequencing, expression analysis, protein analysis, and metabolic flux analysis that offer the understanding of microbial physiology on a global scale, researchers are better equipped to identify potential targets. Ideally, the classical approach and targeted approaches are integrated to create a synergistic effect for rapid strain development.

Finally, because titer increments seen in small-scale fermentation might not scale to production fermenters, improved strains must be validated at a pilot scale designed to mimic fermenter conditions for production. In addition, this intermediate step allows engineers to discover potential problems associated with improved strains and affords the opportunity for further enhancing strain performance.

This chapter reviews the various methods used to improve microbial strains and fermentation processes and addresses the issues associated with each method. New technologies and their potential applications in strain development and process improvement will be discussed. The reader is also directed to the recent reviews concerning the classical approach, enrichment methods, and fermentation process improvement ***(1,6–8)***.

2. Classical Approach

2.1. Process

The process of obtaining improved strains involves repetition of three steps: (1) mutation of a parent strain to introduce new genetic alteration in the genome, (2) random screening and assessment of mutagenesis survivors in small-scale fermentation vessels, and (3) quantification of the metabolite and identification of potentially improved strains. Potentially improved strains are evaluated again in multiple repetitions to confirm statistically an improvement over the parent strain. Each time an improved strain is identified and confirmed, it is used as a new parent strain in a new round of mutation and screening and/or selection (*see* **Fig. 1**).

2.1.1. Mutagenesis

The first key step of the classical approach is the generation of a mutant population. Alterations at the gene level in a microorganism are typically achieved by subjecting the organism to a mutagen. There are a variety of mutagens, which include X-ray, ultraviolet (UV) light, hydroxylamine, and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine ***(7)***. Different mutagens have different mechanisms of action, including base transitions, base deletions, or base additions. It is essential at the