Biotechnology & Genetic Engineering Reviews

Volume 4

Edited by
G. E. RUSSELL

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Editor:
GORDON E. RUSSELL
Emeritus Professor of Agricultural Biology,
University of Newcastle upon Tyne

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Preface

Volume 4 of *Biotechnology & Genetic Engineering Reviews* continues the 'pot-pourri approach' (the term used by one reviewer of Volume 3) of earlier volumes, and contains a varied mix of biotechnological subjects. Such a mixture, to encourage workers in different disciplines to be aware of major developments in others, was until recently considered 'unfashionable'. The editorial team of *Biotechnology and Genetic Engineering Reviews* has therefore often felt that it was swimming against the tide. However, we were not alone. For example, Professor J. L. Harley, President of the Institute of Biology, has recently deplored the tendency towards the 'fragmentation' of broadly based subjects (in that case, biology) with the consequent weakening of those subjects. It is particularly gratifying, therefore, that a reviewer of Volume 3 wrote: 'The reviews are clearly written and up to date. This makes reading the chapters which do *not* deal with one's specific area much more palatable'. Another reviewer has commented '. . . this excellent series continues reviewing important areas of biotechnology and genetic engineering with the purpose of keeping specialists aware of new developments in fields of biotechnology other than their own'. This is exactly what we are trying to do and, clearly, the tide of scientific opinion in this matter is beginning to turn.

As in Volume 3, we have listed the main headings of each chapter in the contents list, because we feel sure that this will help readers to pinpoint particular topics quickly and reliably. In addition there is a detailed index, which will show that there are many, often unexpected, cross-references between apparently unrelated chapters. Volume 4, therefore, reflects both the great diversity of scientific, medical and industrial topics—which together constitute biotechnology in the wide sense—and the interrelationships between these topics.

In summary, we hope that this series of review articles will, to some degree, help to break down the barriers between the many different disciplines involved in biotechnology and continue the improvement in communication between all biotechnologists. The reviews should also provide a valuable source of reference for research and development scientists, teachers and students, at both undergraduate and postgraduate levels.

GORDON E. RUSSELL
July 1986

Contributors

JOSEF ALTENBUCHNER, *Lehrstuhl für Genetik, Universität Regensburg, D-8400 Regensburg, Federal Republic of Germany*

J. BARBER, *AFRC Photosynthesis Research Group, Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2BB, UK*

J. BELL, *Department of Biology, The Polytechnic, Sunderland, Tyne and Wear SR1 3SD, UK*

ALAN W. BUNCH, *Biological Laboratory, The University of Kent, Canterbury, Kent CT2 7NJ, UK*

ROBIN W. CARRELL, *Department of Haematology, School of Medicine, University of Cambridge, Cambridge CB2 2QL, UK*

O. G. CHAKHMAKHCHEVA, *Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 11781 Moscow, USSR*

J. COLBY, *Department of Biology, The Polytechnic, Sunderland, Tyne and Wear SR1 3SD, UK*

JOHN CULLUM, *Department of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology, PO Box 88, Manchester M60 1QD, UK*

J. R. DUNCAN, *Agriculture Canada, Animal Diseases Research Institute, Nepean, PO Box 11300, Station H, Nepean, Ontario, Canada K2H 8P9*

V. A. EFIMOV, *Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 11781 Moscow, USSR*

FIONA FLETT, *Department of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology, PO Box 88, Manchester M60 1QD, UK*

M. GOODFELLOW, *Department of Microbiology, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, UK*

MARTIN GRIFFIN, *Department of Life Sciences, Trent Polytechnic, Clifton Lane, Nottingham NG11 8NS, UK*

RALPH E. HARRIS, *Biological Laboratory, The University of Kent, Canterbury, Kent CT2 7NJ, UK*

M. D. HENNING, *Agriculture Canada, Animal Diseases Research Institute, Nepean, PO Box 11300, Station H, Nepean, Ontario, Canada K2H 8P9*

CHING T. HOU, *Exxon Research & Engineering Company, Annandale, New Jersey 08801, USA*

AKIRA KIMURA, *Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan*

C. M. LYONS, *Microbial Technology Group, Department of Microbiology, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, UK*

ANN. M. MAGOR, *Department of Life Sciences, Trent Polytechnic, Clifton Lane, Nottingham NG11 8NS, UK*

J. B. MARDER, *AFRC Photosynthesis Research Group, Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2BB, UK*

K. H. NIELSEN, *Agriculture Canada, Animal Diseases Research Institute, Nepean, PO Box 11300, Station H, Nepean, Ontario, Canada K2H 8P9*

YU. A. OVCHINNIKOV, *Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 11781 Moscow, USSR*

WOLFGANG PIENDL, *Department of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology, PO Box 88, Manchester M60 1QD, UK*

S. L. STURLEY, *Box G, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912, USA*

E. WILLIAMS, *Microbial Technology Group, Department of Microbiology, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, UK*

T. W. YOUNG, *British School of Malting and Brewing, Department of Biochemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK*

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1
Genetic Manipulation of Commercial Yeast Strains

S. L. STURLEY* AND T. W. YOUNG**

British School of Malting and Brewing, Department of Biochemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK

Introduction

Yeasts of the genus *Saccharomyces* are among the most cultivated micro-organisms exploited by man. A graphic record of baking and brewing was made on the wall of a Fifth Dynasty Egyptian tomb dating from about 2400 BC. This brewing process used malted barley to provide fermentable substrates, but the yeast must have originated from contamination of raw materials (and eventually brewing equipment) and the air. Beer brewing and consumption was a well-established practice in Western Europe in 55 BC when Pliny noted the production of intoxicating beverages from barley. Strains of *Saccharomyces* are also used in baking, distilling and the production of fermented foods such as soy sauce. Yeasts from different genera are used in the treatment of spent sulphite liquor from paper manufacture (*Candida utilis* and *C. tropicalis*), the treatment of whey from cheese manufacture (*Kluyveromyces fragilis* and *K. marxianus*) and the production of single-cell protein from alkanes (*Yarrowia lipolytica*). Many of the commercially employed yeasts have no sexual phase to their life cycles (e.g. *Candida* spp.) or produce very few viable sexual spores (e.g. brewing strains of *Saccharomyces cerevisiae*). In consequence, these strains may be genetically modified only by the techniques of mutation, transformation, cell hybridization (independent of sexual fusion) and protoplast fusion. Those strains of *Saccharomyces* yielding good spore viabilities, and other ascosporogenous yeasts, are also amenable to genetical manipulation by more conventional sexual processes.

This review will concentrate on the manipulation of commercial yeasts with particular reference to brewing strains. Many of the problems en-

Abbreviations: ARS, autonomously replicating sequence; CEN, yeast centromeric sequence; ER, endoplasmic reticulum; GOI, gene of interest.

* Present address: Box G, Division of Biology & Medicine, Brown University, Providence, RI 02912, USA.
** To whom correspondence should be addressed.

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countered are in common with those found when biotechnologists attempt to manipulate genetically defined (laboratory) strains of *S. cerevisiae* and to obtain expression of foreign DNA and secretion of foreign proteins. A review of these procedures has been given by Kingsman *et al.* (1985).

From the microbiological point of view, the fundamentals of the practice of brewery fermentation have evolved little since the demonstration by Pasteur in 1876 that fermentation required the participation of living organisms, and that of Hansen in 1888, that yeast could be isolated and propagated in pure culture. However, the technology of brewing and, in particular, the use of procedures and processing aids to improve product quality and production efficiency have evolved very significantly over the same period. From the point of view of the genetic engineer, the most interesting developments have been in the use of enzymes as processing aids to compensate for the inability of brewing yeasts to perform certain tasks. For example, amyloglucosidase is used in some products to convert carbohydrate, not fermentable by brewing yeast, to a fermentable form and the plant protease papain is used to hydrolyse protein, which is not attacked by brewing yeast, and to prevent the formation of haze. In addition, since the time of Pasteur and Hansen, the biochemistry of fermentation has been elucidated and yeast has been developed as an organism for genetic studies. This latter development owes much to the pioneering work of Winge (1935) on the life-cycle of *S. cerevisiae*, and of Lindegren and Lindegren (1943) on mating types. A very comprehensive genetic map of *S. cerevisiae* (an indispensable tool for the genetic engineer) is available (Mortimer and Schild, 1980). The demonstration of the suitability of yeast for use with recombinant DNA techniques (Hinnen, Hicks and Fink, 1978) originally developed in *Escherichia coli*, has led to renewed interest in the genetical manipulation of commercial yeast strains. These advances are seen as increasing the opportunities for modifying existing commercial strains to introduce novel characteristics. It is not surprising that, given its susceptibility to both classic and new genetical manipulation procedures and its acceptability for commercial fermentation processes using well-established technologies, *S. cerevisiae* is rapidly becoming a host of choice for use in the pharmaceutical and medical fields to produce non-yeast products.

The situation with regard to the brewing industry is rather different. The industry produces a well-characterized product by an essentially traditional process (*Figure 1*). The nature of the brewing process embodies certain unique constraints when compared with the use of, for example, yeast in baking, distilling or wine production. Thus:

1. The raw material used is principally an enzymic digest of malted barley produced by mashing and the source of the enzymes is the barley malt, although in some instances enzymes of fungal or bacterial origin may be used as process aids;
2. In most modern brewing processes, specialized brewing yeasts are maintained in a laboratory, cultured and transferred to specialized propagation equipment to produce the amount needed to inoculate a small fermenter.

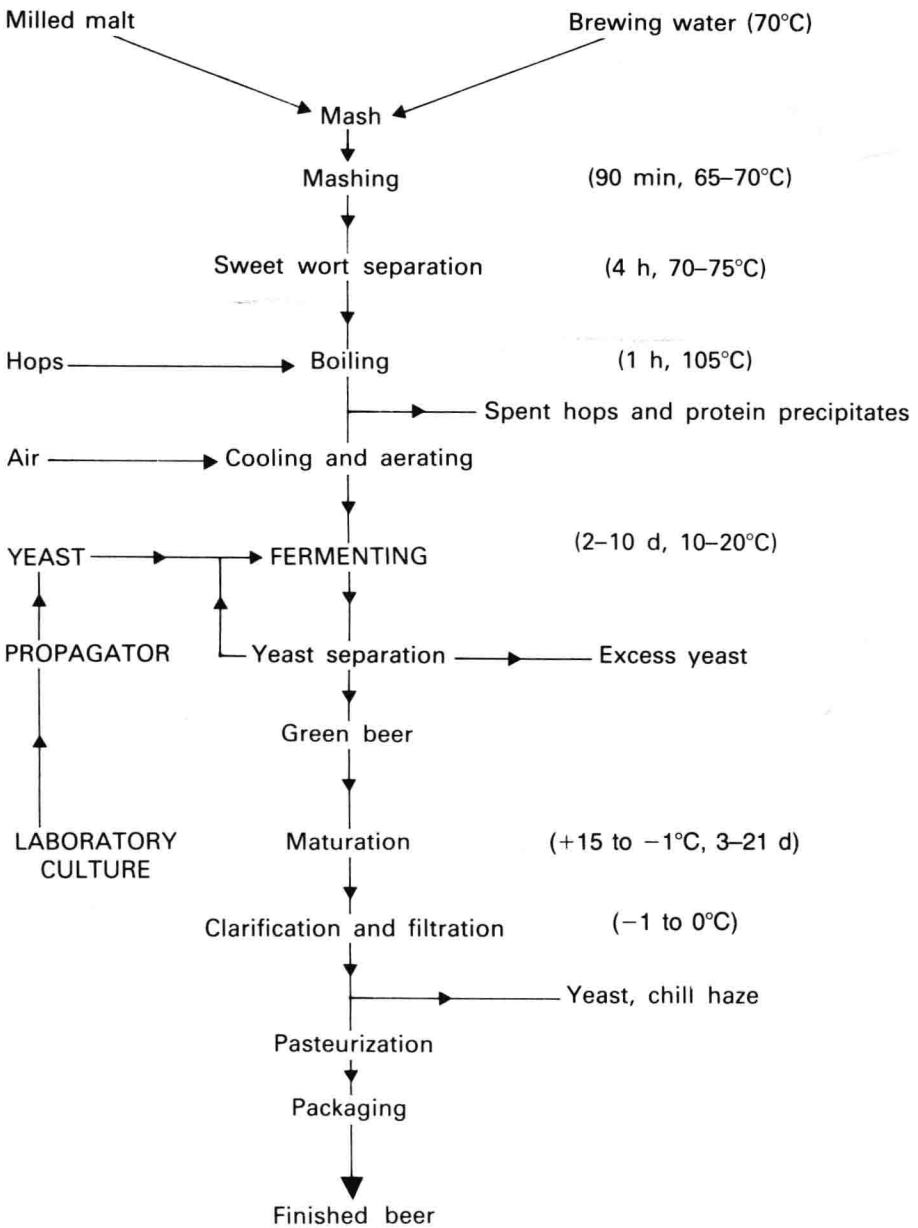

Figure 1. Flow diagram of the brewing process and associated yeast management. Typical times and temperatures for the key operations are indicated in parentheses.

The frequency of this process varies but is kept to a minimum;

3. A proportion of the yeast generated during fermentation is conserved and used to inoculate (pitch) subsequent fermentations. A brewery fermentation of 1000 hectolitres (611 UK barrels) of wort requires some 300 kg (660 lb) wet weight of yeast;
4. Maturation, embodying flavour changes, and stabilization (precipitation of chill haze) are needed.

Genetic manipulation of brewing yeasts may be considered as producing process improvements, more flexibility in choice of raw materials or new products (*Table 1*). In the first two categories the primary concern will be to achieve the objectives without changing the flavour and aroma of the final product. This in itself may prove extremely difficult because the flavour and aroma of beer depend upon the nature and concentration of a large number of minor metabolites of yeast metabolism (e.g. esters, higher alcohols). In addition to flavour, other desirable attributes of brewing yeast must be retained: the yeast must, for example, show adequate utilization of amino acids and sugars with the concomitant production of, and tolerance to, alcohol; cell growth, although inevitable, should be minimized; the flocculent nature of the strain must be retained to expedite the removal of yeast at the end of fermentation, and the strain must be genetically stable, thus ensuring consistent product quality. Commenting in 1938 on genetic change to brewing yeast, Winge stated 'undefined demands cannot be answered to order' (Stewart, 1978).

With these various constraints in mind, *Table 1* indicates those possible improvements to brewing strains which have been, or still are, the subject of active research.

Improvements to the process include lowering the time taken for fermentation, by obtaining yeasts which ferment faster or will withstand higher temperatures; yeasts with a high tolerance to osmotic pressure and alcohol would be capable of fermenting more concentrated substrate, thus increasing productivity (the final product would be diluted to the correct composition); yeast showing a smaller increase in yeast mass (growth rate) during fer-

Table 1. Possible improvements to brewing yeast.

	Improvements producing	
Process improvements	Flexible use of raw materials	New products
Rate of fermentation	Hydrolysis of starch	Low carbohydrate
Temperature optimum	Hydrolysis of cellulose	Low alcohol
Osmotic tolerance	Utilization of lactose	Specific flavours
Alcohol tolerance		
Growth rate		
Infection proofing		
Hydrolysis of protein		
Hydrolysis of β-glucan		
Low diacetyl production		

mentation would yield more alcohol from the substrate; infection-proofed yeast would inhibit spoilage organisms and enable more fermentation cycles to be undertaken (less need to have recourse to a propagation cycle) and would enhance the biological stability of the product; yeasts able to secrete protease and β-glucanase would remove haze-forming precursors and reduce the time needed for maturation and stabilization; the formation of diacetyl is an undesirable characteristic of some brewing processes and maturation time would be reduced if yeast unable to produce this compound were available.

Flexible use of raw materials may bring about the replacement of expensive malt carbohydrate with cheaper sources. Engineering the yeast to secrete amylolytic enzymes and cellulases would permit the use of cellulose and starches (provided that they could be extracted in soluble form) from a variety of sources and would remove the dependence of the process on malt enzymes. Similarly, conferring the ability to use lactose on brewing strains would enable whey to be used as a substrate.

Genetic manipulation of brewers' yeast could be used to produce new products: for example, conferring the ability to secrete amyloglucosidase enables much of the residual carbohydrate in beer to be converted to fermentable sugar, which in turn is converted to ethanol; thus the overall carbohydrate content of the beer is lowered (so-called 'low cal' or 'lite' beers). Similar advantages would accrue from the production of strains secreting β-glucanase. Low-alcohol beers could, in theory, be produced by engineering the yeast to prevent it utilizing the major wort-fermentable carbohydrate (maltose), and beers with specifically enhanced flavours may be produced by the appropriate enhancement or restriction of yeast metabolic processes.

To date there are no reports of any genetically engineered brewing strains being used. The areas under most active investigation have been the incorporation of β-glucanase activity from *Bacillus* spp. (Cantwell *et al.*, 1985; Hinchcliffe, 1985), incorporation of dextrinase activity (Freeman, 1981; Tubb *et al.*, 1981; Stewart, Panchal and Russell, 1983) and infection-proofing (Young, 1981, 1983a,b; Hammond and Eckersley, 1984). In addition, preliminary reports have been made on alcohol tolerance (Korhola, 1983), hydrogen sulphide production (Takahashi, Hojito and Sakai, 1980) and diketone production (Holmberg, 1984).

Although genetic engineering offers great potential in these areas of brewing, many of the objectives may be achieved by other technically simpler and more economic means, e.g. by the use of exogenous enzymes or selection of other naturally occurring yeast strains. Furthermore, the products produced by genetically engineered yeasts may need to be subjected to rigorous (and expensive) testing before the strains are acceptable and thus the financial gains to be made from the production of such products must be significant.

The desirable characteristics of the yeasts used in other industries have been reviewed by Johnston and Oberman (1979), Rose (1979) and Spencer and Spencer (1983). For example, distillers' yeast would be capable of using