YEAST SUGAR METABOLISM

Biochemistry, Genetics, Biotechnology, and Applications

Edited by

F. K. ZIMMERMANN
K.-D. ENTIAN

YEAST SUGAR METABOLISM

Biochemistry, Genetics, Biotechnology, and Applications

Edited by

Prof. Dr. F. K. ZIMMERMANN
Institut für Mikrobiologie und Genetik
Technische Hochschule Darmstadt

Prof. Dr. K.-D. ENTIAN
Institut für Mikrobiologie
Johann Wolfgang Goethe-Universität Frankfurt

TECHNOMIC PUBLISHING CO., INC.
LANCASTER · BASEL

Yeast Sugar Metabolism
a TECHNOMIC® publication

Published in the Western Hemisphere by
Technomic Publishing Company, Inc.
851 New Holland Avenue, Box 3535
Lancaster, Pennsylvania 17604 U.S.A.

Distributed in the Rest of the World by
Technomic Publishing AG
Missionsstrasse 44
CH-4055 Basel, Switzerland

Printed in the United States of America
10 9 8 7 6 5 4 3 2 1

Main entry under title:
Yeast Sugar Metabolism: Biochemistry, Genetics, Biotechnology, and Applications

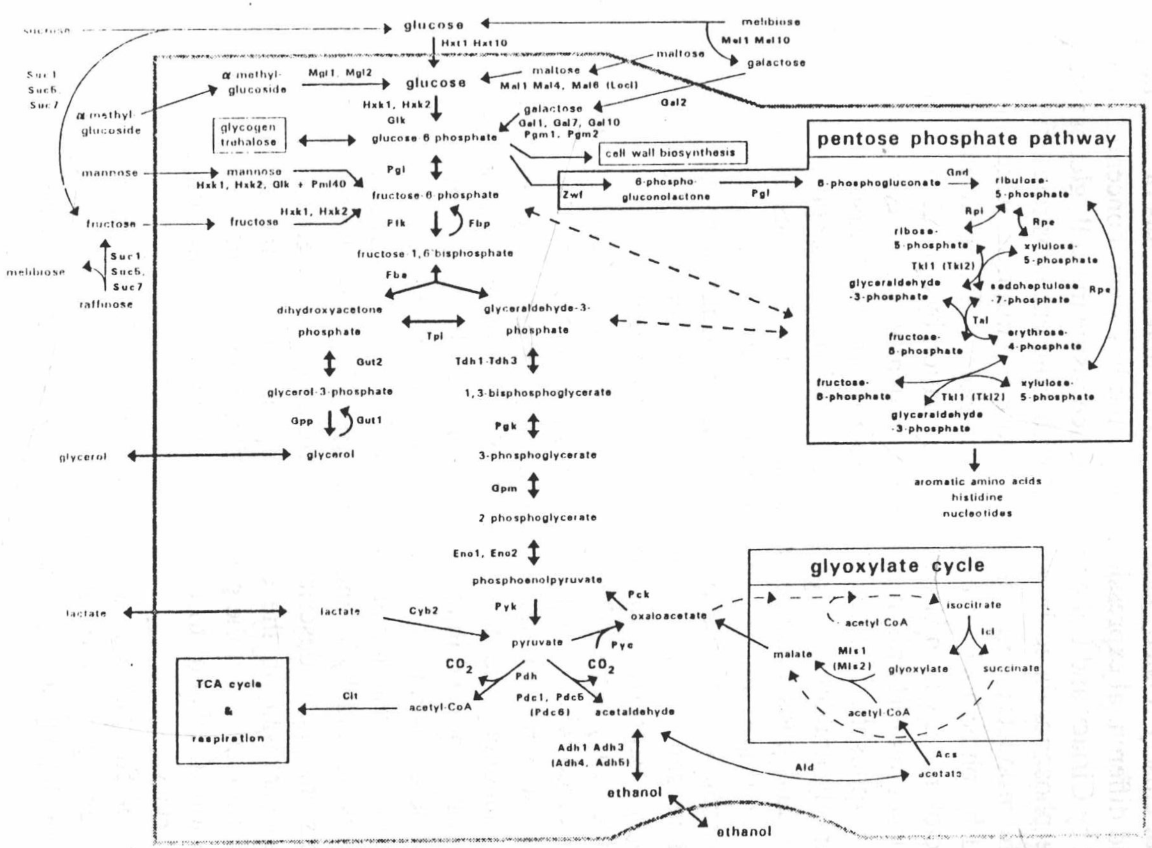
A Technomic Publishing Company book
Bibliography: p.
Includes index p. 561

Library of Congress Catalog Card No. 96-61823
ISBN No. 1-56676-466-1

Preface

THE yeast *Saccharomyces cerevisiae* has played a central role in the evolution of microbiology, biochemistry, and genetics, in addition to its use as a technical microbe for the production of alcoholic beverages and leavening of dough. Sugar metabolism is certainly the most prominent metabolic activity of *Saccharomyces cerevisiae*, and it was in this area that scientific exploration was most prominent. The highlights of this field of yeast research are covered in the contribution of Barnett. During the past 25 years, *Saccharomyces cerevisiae* has become one of the model organisms for molecular genetics and cell biology, which culminated on 24th April, 1996, with the release of the first complete nuclear genome sequence of a eukaryote (Dujon, 1996; Johnston, 1996; Hieter et al., 1996). *Saccharomyces cerevisiae*, "life with 6000 genes" (Goffeau et al., 1996), has now arrived at the front stage of life sciences, and the complete sequence of its nuclear genome can be accessed in data banks.

This book deals with *Saccharomyces cerevisiae*'s most prominent activity: sugar metabolism. It thus addresses workers in the field of basic yeast industries producing baking, beer, and wine yeasts, who want to exploit the enormous advances of yeast molecular biology and genetics to improve the performance of their products. Consequently, emphasis in this book is strongly on the genetical side, and all the authors have contributed decisively to the exploration of the intricacies of yeast sugar metabolism in particular and carbon metabolism in general. Therefore, this book is also intended for workers in the fields of basic research in physiology, metabolic regulation, and molecular genetics. *Saccharomyces cerevisiae* is already widely used as a host for "foreign gene expression" to produce proteins of technical and medical interest and even as a model organism in pharmaceutical development.



The central glycolytic pathway with connected metabolic routes and key reactions of gluconeogenesis are depicted. Enzyme designations were adapted from gene designations, with numbering only where isoenzymes are involved. Enzymes shown in parentheses may not normally function in main stream metabolism and serve other purposes. Cofactors and energy balances have been omitted but are described in the respective book chapters. Figure provided by J. Heinisch.

Heinisch has provided a graphical overview of the yeast carbohydrate metabolism. *Saccharomyces cerevisiae* uses glucose and fructose most efficiently, and uptake of these hexoses has been a topic of dispute until the really complex basis was resolved. There are multiple genes coding for different, but closely related, facilitators with different levels of substrate affinities and differential expression at high and low sugar concentrations, as reviewed by Ciriacy and Reifenberger. Once inside the cell, glucose and fructose are phosphorylated by kinases with different substrate affinities (Entian). The products are glucose- or fructose-6-phosphate, which are interconverted by phosphoglucose isomerase (Boles). The next enzyme is phosphofructokinase, which is subject to regulation by several metabolites (Kopperschläger and Heinisch). The subsequently acting enzymes are fructose-1,6-bisphosphate aldolase, triosephosphate isomerase, the three very similar glyceraldehyde-3-phosphate dehydrogenases, phosphoglycerate kinase, and phosphoglycerate mutase. These enzymes are largely constitutive and are covered by Heinisch and Rodicio, except for phosphoglycerate kinase, which is dealt with by Chambers because the promoter of its gene *PGK1* has become an important part of *Saccharomyces cerevisiae* expression casettes for foreign genes.

The last four enzymes of the glycolytic reaction chain to ethanol are only fully induced when fermentable sugars are present in the medium. There are two isozymes for enolase, one acting as a glycolytic enzyme, the other in the gluconeogenic direction, as discussed by Müller and Entian. Pyruvate kinase is induced in the presence of glycolytic substrates and requires allosteric activation by fructose-1,6-bisphosphate, the product of the phosphofructokinase reaction and dealt with by Boles. Pyruvate decarboxylase produces acetaldehyde and carbon dioxide. The complex genetic system of this enzyme is described by Hohmann. There are two alcohol dehydrogenases present in the cytoplasm; one—like in the case of the enolases—is more active in the glycolytic direction, the other more active in the oxidation of ethanol to acetaldehyde. This complex system is reviewed by Ciriacy.

All strains of *Saccharomyces cerevisiae* utilize glucose, fructose, and mannose. Other sugars can be used by some strains, and Barnett provides an overview of the physiology and biochemistry of the utilization of such sugars.

Entian and Schüller report on the genetic background of the regulation of the enzymatic machinery for the utilization of di- and trisaccharides. The genetic system for the assimilation of galactose and its intricate regulation is covered by Melcher.

A small fraction of the glucose-6-phosphate is metabolized via the direct oxidation pathway and the nonoxidative pentose phosphate cycle, as presented by Schaaff-Gerstenschläger and Miosga. This reaction sequence is

Designation	Enzyme
Acs	Acetyl-CoA-Synthetase
Adh1-Adh3, Adh4, Adh5	Alcohol-Dehydrogenases
Ald	Aldehyde-Dehydrogenase
Cit	Citrate-Synthase
Cyb2	Lactate-Dehydrogenase
Eno1, Eno2	Enolases
Fba	Fructose-1,6-bisphosphate-Aldolase
Fbp	Fructose-1,6-bisphosphatase
Gal1	Galactokinase
Gal10	Uridinediphosphoglucose-4-Epimerase
Gal2	Galactose permease
Gal7	Galactosephosphate-Uridylyltransferase
Glk	Glucokinase
Gnd	Phosphogluconate-Dehydrogenase
Gpm	Phosphoglycerate-Mutase
Gpp	Glycerolphosphate-Phosphatase
Gut1	Glycerolkinase
Gut2	Glycerol-3-phosphate-Dehydrogenase
Hxk1, Hxk2	Hexokinase PI and PII, respectively
Hxt1-Hxt10	Hexosetransporters
Icl	Isocitrate-Lyase
Mal1-Mal4, Mal6	*MAL*-loci comprising a maltose permease, a maltase structural gene, and a regulatory gene each
Mel1-Mel10	Melibiase
Mgl1, Mgl2	α-Methylglucosidases
Mls1, Mls2	Malate-Synthases
Pck	Phosphoenolpyruvate-Carboxykinase
Pdc1, Pdc5, Pdc6	Pyruvate-Decarboxylases
Pdh	Pyruvate-Dehydrogenase
Pfk	Phosphofructokinase
Pgi	Phosphoglucose-Isomerase
Pgk	Phosphoglycerate-Kinase
Pgl	Phosphogluconolactonase
Pgm1, Pgm2	Phosphoglucomutases
Pmi40	Phosphomannose-Isomerase
Pyc	Pyruvate-Carboxylase
Pyk	Pyruvate-Kinase
Rpe	Ribulose-5-phosphate-Epimerase
Rpi	Ribose-5-phosphate-Isomerase

Designation	Enzyme
Sucl-Suc5, Suc7	Invertase
Tal	Transaldolase
Tdhl-Tdh3	Glyceraldehyde-3-phosphate-Dehydrogenases
Tkll, Tkl2	Transketolases
Tpi	Triosephosphate-Isomerase
Zwf	Glcuose-6-phosphate-Dehydrogenase

important for the generation of NADPH$^+$ and substrates for the formation of histidine, aromatic amino acids, and nucleotides.

There are two storage carbohydrates in *Saccharomyces cerevisiae:* trehalose and glycogen, which play important physiological roles, as discussed by François, Blázquez, Ariño, and C. Gancedo.

An efficient system of osmoregulation is an absolute necessity for *Saccharomyces cerevisiae* since it can grow on and ferment even concentrated sugar solutions. Glycerol is the major osmotic stress protectant, and regulation of its production is under an elaborate control, as reviewed by Prior and Hohmann.

Pyruvate is the metabolite that can be further metabolized, not only as a substrate for ethanol production by pyruvate decarboxylase. It is also a substrate for the pyruvate dehydrogenase complex, covered by Steensma, which produces acetyl-CoA, an important substrate in many biosynthetic reactions.

Saccharomyces cerevisiae can also grow on ethanol as the sole carbon source. This requires the operation of gluconeogenesis where glucose-6-phosphate is formed largely through a reversion of glycolysis. This intricate and very sophisticated regulatory system is dealt with by J. M. Gancedo and C. Gancedo.

In constrast to the commonly held view, glycolysis is not a constitutive pathway. It operates only fully when fermentable sugars are available. Recent developments in the understanding of this regulatory circuit are discussed by Boles, Zimmermann, and Thevelein.

Drastic changes in carbon metabolism are induced when cells growing on a nonfermentable carbon source are exposed to fermentable sugars, especially fructose or glucose. Gluconeogenesis, respiration, and the systems for the utilization of galactose, maltose, and sucrose are repressed. This regulatory circuit has been extensively studied and is dealt with by Entian and Schüller.

Prior and Kötter report on the attempts to construct *Saccharomyces cerevisiae* strains that can utilize pentoses and form ethanol, a topic of great biotechnological importance and promise. Another goal of genetic engineering of *Saccharomyces cerevisiae* is the utilization of polysaccharides. Great success is in sight, as documented in the contribution by Pretorius.

Hansen and Kielland-Brandt report on activities to improve the quality of brewing yeast by genetic engineering, and Henschke gives an overview of the properties of wine yeast and also on the efforts to construct better wine yeasts by genetic engineering.

REFERENCES

Dujon, B. The yeast genome project what did we learn? *Trends Genet.* 12:263–270 (1996).

Goffeau, A., Barrell, B. G., Bussey, H., et al. Life with 6000 genes. *Science* 274:562–657 (1996).

Hieter, P., D. E. Basset, Jr. and D. Valle. The yeast genome—a common currency. *Nature Genet.* 13:253–255 (1996).

Johnston, M. Genome Sequencing: The complete code for a eukaryotic cell. *Curr. Biol.* 6:500–503 (1996).

Table of Contents