LINDEGREN, C. C. & LINDEGREN, G. (1956). *J. gen. Microbiol.* 15, 19-28

Eight Genes Controlling the Presence or Absence of Carbohydrate Fermentation in *Saccharomyces*

BY C. C. LINDEGREN AND GERTRUDE LINDEGREN

*Biological Research Laboratory, Southern Illinois University,
Carbondale, Illinois, U.S.A.*

SUMMARY: Genes controlling carbohydrate fermentation in *Saccharomyces* often control the fermentation of more than one carbohydrate. The gene MZ has at least five different manifestations involving its ability to respond to five different carbohydrate inducers for the production of a single enzyme; a series of multiple alleles of MZ differ from one another in ability to respond to the different inducers. The gene DX controls the fermentation of dextrin and glycogen. The gene ST controls the fermentation of starch; some starch-positive cultures are Schardinger-dextrin-positive. The gene SU controls the production of a constitutive enzyme which splits both sucrose and raffinose.

The gene GA

The Durham tube and melting-point tube methods (Lindgren, 1956) distinguish eight heritable differences in the fermentation of carbohydrates in the Carbondale breeding stock. The genes occur either in the dominant (capable of producing the enzyme) or the recessive (incapable of producing the enzyme) form. The gene-pair controlling galactose-fermentation (Lindgren & Lindgren, 1946) was the first analysed; the dominant form is now designated by the two-letter symbol GA (previously G), while the recessive is identified by ga (previously g). Because different genes may control the ability to ferment the same sugar a system for distinguishing phenotype and genotype has been introduced. The phenotypes are designated by three-letter abbreviations of the substrate fermented (the designation of the fermenter is by capitals and the non-fermenter by lower-case letters), while two-letter abbreviations describe the genotypes. GA controls the production of an adaptive enzyme-system which has the end result of isomerizing galactose into a utilizable hexose. Whether the enzyme system is the galactowaldenase of Cardini, Caputto, Paladini & Leloir (1950) which converts galactose-1-phosphate to glucose-1-phosphate and which needs uridine diphosphoglucose (UDPG) as coenzyme or 'galactokinase' (galactose + ATP → galactose-1-phosphate) or enzymes needed for the synthesis of UDPG has not been determined. Unadapted GA cultures (and cultures carrying ga) lack the enzyme system for this fermentation. Only one gene-pair controlling the presence or absence of ability to ferment galactose has been discovered in the Carbondale stock. Cultures in the Carbondale stock which ferment galactose on full nutrient medium within 8 days in the Durham tube and within 6 hr. in the melting-point tube are identified as GA; any culture which fails to ferment galactose under these standard conditions is identified as ga.

All the cultures of the Carbondale stock are able to ferment D-glucose and D-fructose; release of one of these hexoses from an oligosaccharide results in a positive fermentation test. Seven of the genes control the fermentation of carbohydrates by releasing fermentable hexose from an oligosaccharide. In Table 1 the genes and the substrates on which they act and some of the characteristics of the hypothetical enzymes are indicated. The vertical arrow indicates the point at which the molecule may be split.

The gene MZ

MZ controls the production of the adaptive enzyme, melezitase (Palleroni & Lindegren, 1958). This gene exists in the form of a series of multiple alleles. Cells carrying the most 'complete' form of the gene are able to adapt to (and grow on) at least five different substrates: maltose, turanose, methyl- α -D-glucopyranoside, sucrose and melezitose. Growth on any one of these substrates produces adapted cells which are able to ferment any one of the other four and which can, in addition, split isomaltose and panose. Because of difficulty in obtaining sufficient amounts of the last two, it is not known whether there are MZ alleles conferring the capacity for growth in isomaltose and panose. One allele of MZ (Mz^d) makes the cells capable of growth on maltose and turanose but incapable of growth on methyl- α -D-glucopyranoside, sucrose or melezitose, and thus a culture carrying it superficially resembles one carrying MA mz. An ma MZ^d culture can be distinguished from an Ma mz culture by transferring to melezitose (or sucrose, or methyl- α -D-glucopyranoside) after growth on maltose: If the culture is ma Mz^d, melezitose will be split; if it is MA mz, melezitose will not be split. The different alleles of MZ (Lindegren & Lindegren, 1953) which have been identified are indicated in the following tabulation:

Substrate	Genotype				
	MZ	MZ ^a	MZ ^b	MZ ^c	MZ ^d
Maltose	+	+	+	+	+
Turanose	+	+	+	+	+
Sucrose	+	+	+	+	-
Methyl- α -D-glucopyranoside	+	-	+	-	-
Melezitose	+	+	-	-	-
Raffinose	-	-	-	-	-

Melezitase splits any terminal non-reducing α -D-glucopyranosyl radical from a di- or trisaccharide, irrespective of whether it is attached to another hexose or simply to a methyl group and irrespective of the type of attachments to the hexose, except that it is not active against large molecules nor against a trisaccharide such as raffinose in which the non-reducing radical is blocked.

The gene MA

MA (Lindegren & Lindegren, 1949) controls the production of an adaptive enzyme system which splits maltose and turanose. A culture carrying MA mz is distinguished from one carrying MA MZ or ma MZ by the fact that the

Table 1. Genes, enzymes and substrates

Substrates	Enzyme	Gene
Maltose	Melezitase, splits single hexoses from small molecules at the α -D-Gp linkage irrespective of the point of attachment of the second radical; not active against O-dextrin, nor against raffinose in which the α -D-Gp radical is blocked	MZ
Turanose		
Methyl α -D-glucopyranoside		
Sucrose		
Melezitose		
Isomaltose		
Panose		
Maltose		
Turanose		
Dextrin		
Glycogen	Maltase	MA
Amylopectin	Dextrinase splits single hexoses from relatively short chain complexes. Only slowly active against maltose	DX
Starch		
Sucrose	Amylase splits single hexoses from large complexes. Not active against maltose	ST
Raffinose		
Melibiose	Invertase, β -D-fructofuranosidase	SU
D-Galactose	Melibiase, not active against raffinose, O- β -fructofuranosyl-melibiose; apparently blocked by the β -D-fructofuranosyl radical	ME
Methyl α -D-glucopyranoside		
Ethyl α -D-glucopyranoside		
Phenyl α -D-glucopyranoside		
	?	GA
	Methyl α -D-glucopyranosidase	MG

MA mz cultures do not achieve adaptation to melezitose by growth on maltose. Any culture which ferments maltose and turanose, and which (after growth on maltose) does not ferment melezitose, is MA mz.

Hestrin & Lindegren (1950, 1952) found that an MA MG su mz culture when grown on maltose achieved the ability to ferment methyl- α -D-glucopyranoside, but that an MA mg su mz culture was incapable of heterologous adaptation. This is the only case in the fermentation genotypes of detected interaction between two genes and is interpreted to indicate that the gene MG probably functions to produce a precursor of the maltase produced by MA. This inference is based on the fact that methyl- α -D-glucopyranoside is very rare in nature and it seems improbable that a gene would be selected and perpetuated which produced an adaptive enzyme specific for it; it seems more probable that the gene MG functions primarily to enhance the activity of the MA gene.

The gene DX

DX (Lindegren, Lindegren, Drysdale, Hughes & Brenes-Pomales, 1956) controls the synthesis of a dextrinase capable of splitting dextrin and glycogen but neither starch nor amylopectin. The failure of dextrinase to act against the substrates split by melezitose suggests that it splits off exposed reducing radicals; its slow action against maltose suggests that it is inhibited by the proximity of a non-reducing radical.

The gene ST

The gene ST produces an amylase which splits fermentable molecules from starch and amylopectin but not from either dextrin or glycogen.

The genes DX and ST originated from a culture of *Saccharomyces diastaticus* (Andrews & Gilliland, 1952). Cultures which break down large carbohydrate polymers are exceedingly rare in *Saccharomyces*. The first *Saccharomyces* producing an enzyme of this type carried two such genes, and it is difficult to avoid the conclusion that one originated from the other. One parent of the pedigree (Tables 2 and 3) was a dextrin-fermenter originating from *S. diastaticus*. The other was descended from our inbred breeding stock. The hybrid was homozygous for the genes GA, mg and me; it was heterozygous for the genes indicated below:

Genotypes								
16119	a	DX	ST	ma	SU	AN	HI	ur
16060	α	dx	st	MA	su	an	hi	UR

The fermentative phenotypes are indicated below:

Phenotypes									
16119	DXN	STA	MAL ^L	tur	SUC	RAF	GAL	mgs	meb
16060	dxn	sta	MAL	TUR	SUC ^L	raf	GAL	mgs	meb

Table 2. *Pedigree of a hybrid heterozygous for DX/dx, ST/st*

16201	dx	ST	TUR	SU	a	hi	an	
16202	dx	st	tur	su	α	HI	AN	
16203	DX	st	TUR	su	α	hi	AN	
16204	DX	ST	tur	SU	a	HI	an	
16206	DX	ST	TUR	su	α	hi	an	UR
16207	Died							
16208	dx	ST	tur	su	α	hi	an	ur
16209	dx	st	TUR	SU	a	HI	AN	UR
16210	dx	ST	tur	su	α	hi	AN	ur
16211	dx	st	tur	su	α	hi	AN	UR
16212	DX	st	TUR	SU	a	HI	an	UR
16213	DX	ST	TUR	SU	a	HI	an	ur
16222	DX	st	TUR	su	α	HI	AN	ur
16223	dx	ST	tur	su	a	HI	AN	UR
16224	dx	ST?	TUR	SU	a	hi	an	UR
16225	DX	ST	tur	SU	α	hi	an	ur
16226	dx	ST?	TUR	SU	a	hi	AN	UR
16227	DX	st	TUR	su	α	hi	an	UR ^L
16228	dx	st	tur	SU	α	hi	an	ur
16229	DX	ST?	tur	su	?	HI	AN	ur
16242	dx	ST	TUR	SU	a	HI	AN	ur
16243	DX	st	tur	su	a	hi	an	UR
16244	dx	ST	tur	SU	α	hi	an	UR
16245	DX	ST	TUR	su	α	HI	AN	UR
16246	DX	st	tur	SU	α	HI	AN	ur
16247	dx	st	TUR	SU	a?	HI	AN	UR
16248	Died							
16249	dx	st	TUR	su	α	hi	an	ur
16251	DX	st	TUR	su	a	HI	AN	ur
16252	dx	st	TUR	SU	α	hi	an	UR
16253	dx	st	tur	su	a?	HI	AN	UR
16254	DX	st	tur	SU	α	hi	an	.
16255	DX	st	tur	su	α	HI	AN	ur
16256	dx	st	tur	SU	α	hi	an	ur
16257	DX	st	TUR	su	a?	hi	an	UR
16258	dx	st	TUR	SU	a	HI	AN	UR
16259	dx	st	TUR	SU	a?	HI	an	ur
16260	DX	st	TUR	su	a	hi	AN	UR
16261	DX	ST	tur	SU	α	HI	AN	ur
16262	dx	st	tur	su	α	hi	an	UR
16267	dx	ST	tur	su	a?	hi	AN	ur
16268	DX	ST	TUR	SU	α	HI	AN	UR
16269	dx	st	TUR	su	α	hi	an	UR
16270	DX	st	tur	SU	a	HI	an	ur
16271	dx	st	.	su	α	HI	AN	UR
16272	DX	ST	tur	SU	a	HI	AN	ur
16273	dx	ST	TUR	SU	a	hi	an	ur
16274	.	.	.	.	.	.	.	UR
16275	Died							
16276	dx	st	.	.	.	.	.	ur
16277	dx	st	.	.	.	.	.	UR
16278	DX	st	.	.	.	.	.	ur
16299	dx	ST?	TUR	su	a	hi	an	ur
16300	DX	st	tur	SU	α	hi	an	UR
16301	dx	st	TUR	SU	a	hi	an	ur
16302	DX	st	tur	su	α	HI	AN	ur

The abbreviations listing genotype first are DX, DXN-dextrin; ST, STA-starch; MA, MAL-maltose; TUR-turanose; SUC-sucrose; RAF-raffinose; AN-anthranilic acid; HI-histidine; UR-uracil; GA, GAL-galactose; MG, MGS-methyl- α -D-glucopyranoside; ME, MEB-melibiose.

16119 was turanose-negative and therefore ma. It is characteristic of cultures carrying the gene DX to produce gas slowly from maltose. This may be due to the presence of small amounts of dextrin in the maltose.

Genetical separation of dextrinase and amylase. Regular segregation of genes DX/dx occurred in every ascus. Although this was not true of ST/st, it is clear that DX and ST segregate independently. Since most of the cultures fermented maltose, the diagnosis for MA was made by action on turanose. Regular segregation of the other genes indicated in the pedigree show that a true hybrid was produced and that the failure of the ST gene to segregate regularly was not due to polyploidy. In Table 3 detailed analysis of the diagnosis of the genes DX and ST is given. The same fourteen asci were analysed, using the melting-point tube technique. The tubes were discarded after 7 days, and if fermentation had not occurred up to that time were registered as negatives and indicated by the minus sign.

Table 3. *Pedigree showing fermentation of carbohydrate polymers*

Each minus sign indicates a separate test. Two series of tests were made and the results are listed under A and B, respectively. The day on which fermentation occurred is indicated by Arabic numerals and if more than one positive fermentation occurred more than one numeral is listed. For instance, culture 16208 is listed as a positive fermenter of dextrin as indicated by the numerals 333. This means that three tests were made and all proved positive on the third day.

	Dextrin		Glycogen		Starch		Amylopectin		Schardinger dextrin	
	A	B	A	B	A	B	A	B	A	B
16201	≡	=	≡	=	5	—	=	≡	=	=
16202	≡	=	≡	=	=	=	4	=	=	=
16203	333	11	≡	=	=	=	=	=	=	=
16204	333	11	333	55	5	—	=	=	5	—
16206	333	11	555	23	5	—	33	=	=	=
16207	Died									
16208	=	=	=	=	5	—	—	44	=	=
16209	=	=	=	=	=	45	=	=	=	=
16210	—	=	≡	=	5	—	=	5	—	55
16211	≡	=	≡	=	=	=	=	=	=	=
16212	333	22	77	—	=	=	=	=	=	=
16213	333	22	555	=	5	—	=	4	—	=
16222	.	.	.	.	.	.	.	.	.	.
16223	≡	=	≡	=	5	—	4	—	=	=
16224	=	=	=	=	=	4	—	=	=	=
16225	333	11	333	55	5	—	4	=	=	=
16226	=	=	≡	=	≡	=	=	5	—	=
16227	333	11	66	—	66	=	=	=	=	=
16228	=	=	=	=	=	=	=	=	=	=
16229	333	11	444	55	=	4	—	=	=	=

Table 3 (cont.)

	Dextrin		Glycogen		Starch		Amylopectin		Schardinger dextrin	
	A	B	A	B	A	B	A	B	A	B
16242	≡	=	≡	=	77	=	=	3?	=	=
16243	333	33	666	=	=	=	=	=	=	=
16244	≡	=	≡	=	=	47	=	=	=	=
16245	222	11	33	22	5—	=	=	=	=	=
16246	22	11	66	44	=	=	=	=	=	=
16247	=	=	=	=	=	=	=	=	=	=
16248	Died									
16249	=	=	=	=	=	=	=	=	=	=
16251	333	11	333	44	=	=	=	=	=	=
16252	=	=	=	=	=	=	=	=	=	=
16253	=	=	=	=	=	=	=	=	=	=
16254	333	11	333	77	=	=	=	=	=	=
16255	.	11	.	33	.	.	.	=	.	=
16256	.	.	.	.	.	.	.	.	.	.
16257	11=	11	55=	22	.	=	.	=	=	=
16258	=	=	=	=	=	=	=	=	=	=
16259	6—	=	=	=	=	=	=	=	=	=
16260	33	11	33	55	=	=	=	=	=	=
16261	11	44	444	=	=	45	=	44	=	=
16262	=	=	.	=	.	=	.	.	.	.
16267	≡	=	≡	=	55	5	=	5	=	=
16268	333	11	333	44	55	5—	44	4—	=	=
16269	5	=	=	3	=	=	=	=	=	=
16270	333	11	≡	22	=	=	=	=	=	=
16271	≡	=	≡	=	.	=	.	=	.	=
16272	44	23	=	=	=	66	=	2	=	=
16273	=	=	=	=	=	77	55	=	=	=
16274	.	=	.	=	.	=	.	=	.	=
16275	Died									
16276	≡	=	≡	=	=	=	=	=	=	=
16277	=	=	=	=	.	=	.	=	.	=
16278	44	44	777	55	=	=	=	=	=	=
16299	=	=	=	=	=	=	=	66	=	=
16300	11	11	22	3	=	=	=	=	=	=
16301	=	=	=	=	=	=	=	=	=	=
16302	11	11	22	22	=	=	=	=	=	=

Dextrin ferments more readily than the other large polymers, occasionally showing a positive fermentation on the first day and generally showing a positive fermentation on the third day. Glycogen ferments less rapidly, no positives occurring on the first day. Whole cell preparations were used and the possibility that the same enzyme was involved in both fermentations is not excluded. A series of tests on starch and amylopectin revealed a general correspondence between the fermentability of these two substrates and indicated that dextrin-positive, glycogen-positive cultures were occasionally starch- and amylopectin-negative and, conversely, that some starch- and amylopectin-positive cultures were occasionally dextrin- and glycogen-negative. Two positive tests were obtained with Schardinger dextrin; one

of the Schardinger positives was dextrin-positive and the other dextrin-negative. These data indicate that dextrin is a reliable and precise indicator of the presence of the gene DX. The fermentation of starch and amylopectin is either so difficult or the expressiveness of the gene is so restricted that unless some special purpose can be served by analysing for this genotype, it will not be particularly helpful in mapping experiments. There are some indications that the enzymes produced by DX and ST are constitutive.

The tetratype ascus 16201-16204 produced a culture capable of fermenting both dextrin and starch (DX ST), one capable of fermenting dextrin but not starch (DX st), one incapable of fermenting dextrin but capable of fermenting starch (dx ST) and one incapable of fermenting either starch or dextrin (dx st). The pedigree was completely regular with regard to the segregation of ability to ferment dextrin; two fermenters and two non-fermenters were found in every ascus. The ability to ferment starch was irregularly segregated, some tetrads producing more than two fermenters per ascus and others not producing any fermenters. The ability to ferment turanose is the diagnostic criterion for the gene MA. It is clear that a starch fermenter in a regular tetrad such as 16201-16204 may be turanose-negative and, therefore, *ma*. Similarly, a dextrin-fermenter in a regular ascus such as 16201-16204 may be turanose-negative and, therefore, *ma*. Although these data clearly indicate the independence of the genes controlling the fermentation of starch and dextrin, they also suggest that there are diagnostic difficulties involved in analysing hybrids heterozygous for the ability to ferment starch. Amylase probably splits single hexoses from large D-glucan molecules at the 1, 4 linkage. If primary action is on 1, 4 linkages, action on 1, 6 linkages seems unlikely since the 1, 6 linkages are buried inside the polysaccharide molecule. The gene DX does not confer the ability to ferment turanose. Any dextrin-positive culture which is turanose-negative is DX *ma*, although it may act slowly on maltose.

Five *dxn STA tur* cultures (serial numbers 16208, 16210, 16223, 16244 and 16267) were studied critically for their capacity to ferment maltose and found to be maltose-negative. This observation reveals that starch-positive cultures may be completely incapable of acting on maltose, and that possession of the gene ST does not confer the ability to split maltose. It appears that starch is not disintegrated by breaking off single maltose molecules but presumably by splitting off single hexose molecules. It has been suggested that DX produces an enzyme which acts slowly on maltose because it may be inhibited by the proximity of a non-reducing radical. The complete absence of any action by the gene ST on maltose suggests that the same restriction applies to a much greater degree to the enzyme produced by ST and that it is able to split off terminal reducing radicals of starch only because they are so far removed from the non-reducing radicals. The experiment was controlled by testing the ability of the *dxn STA TUR* cultures 16299, 16278 and 16242 to act on maltose. They fermented maltose rapidly. Cultures 16249 and 16258, which are *dxn sta TUR*, were also rapid fermenters of maltose.

The gene SU

SU controls the production of a constitutive enzyme, β -D-fructofuranosidase (invertase) which splits the exposed β -D-fructofuranosyl radical from either sucrose or raffinose. Any culture fermenting raffinose under standard conditions is SU. Raffinose is the unique substrate which identifies SU; the enzyme produced by MZ also splits sucrose but is incapable of splitting raffinose.

The gene ME

ME controls the production of an adaptive enzyme splitting melibiose but not raffinose. Any melibiose-positive culture is identified as ME (Lindegren, 1949). Melibiase splits the terminal α -D-glucopyranosyl radical from melibiose. It is blocked from acting on raffinose by the β -D-fructofuranosyl radical.

The gene MG

MG (Lindegren & Lindgren, 1949) controls the production of an adaptive enzyme methyl- α -D-glucopyranosidase; some data indicate that both phenyl- and ethyl- α -D-glucopyranoside are also split; the enzyme is capable of splitting non-hexose radicals from substituted α -D-glucopyranosides but not from disaccharides. Any maltose-negative culture which is methyl- α -D-glucopyranoside-positive is identified as MG.

We are indebted to Dr Maurice Ogur for many suggestions concerning the modes of action of the gene-controlled enzymes. We have corresponded with many carbohydrate chemists and enzymologists and wish to acknowledge their helpful suggestions but hesitate to name them directly to avoid the implication that they are in agreement with the inferences made. We are indebted to Dr Allene Jeanes, Dr N. K. Richtmyer, Dr E. J. Hehre, Dr M. L. Killey, Dr R. J. Dimler, Dr W. W. Pigman and to E. R. Squibb and Sons for gifts of rare sugars.

This work has been supported by research grants from the National Cancer Institute of the National Institutes of Health, Public Health Service C-1179 and the U.S. Atomic Energy Commission AT (11-1) 176.

REFERENCES

- ANDREWS, J. & GILLILAND, R. B. (1952). Super-attenuation of beer: a study of three organisms capable of causing abnormal attenuations. *J. Inst. Brew.* **58**, 189.
- CARDINI, C. E., CAPUTTO, R., PALADINI, A. C. & LELOIR, L. F. (1950). Uridine diphosphate glucose: the coenzyme of the galactose-glucose phosphate isomerization. *Nature, Lond.* **165**, 191.
- HESTRIN, S. & LINDEGREN, C. C. (1950). Carbohydrases in *Saccharomyces* haploid stocks of defined genotype. I. Fermentation and hydrolysis of α -glucosides by yeast 6233. *Arch. Biochem.* **29**, 315.
- HESTRIN, S. & LINDEGREN, C. C. (1952). Carbohydrases in *Saccharomyces*. II. Gene-controlled induction of glucosidases by α -glucosides. *Arch. Biochem. Biophys.* **38**, 317.
- LINDEGREN, C. C. (1949). *The Yeast Cell, Its Genetics and Cytology*. St. Louis, Mo.: Educational Publishers, Inc.

- LINDEGREN, C. C. (1956). Methods for the diagnosis of gene-controlled fermentative ability in *Saccharomyces*. *Wallerstein Labs. Commun.* **19**, 49.
- LINDEGREN, C. C. & LINDEGREN, G. (1946). The cytogene theory. *Cold Spr. Harb. Symp. quant. Biol.* **11**, 115.
- LINDEGREN, C. C. & LINDEGREN, G. (1949). Unusual gene-controlled combinations or carbohydrate fermentations in yeast hybrids. *Proc. nat. Acad. Sci., Wash.* **35**, 23.
- LINDEGREN, C. C. & LINDEGREN, G. (1953). The genetics of melezitose fermentation in *Saccharomyces*. *Genetica*, **26**, 430.
- LINDEGREN, C. C., LINDEGREN, G., DRYSDALE, R. B., HUGHES, J. P. & BRENES-POMALES, A. (1956). Genetical analysis of the clones from a single tetrad of *Saccharomyces* showing non-Mendelian segregation. *Genetica* (in the Press).
- PALLERONI, N. J. & LINDEGREN, C. C. (1953). A single adaptive enzyme in *Saccharomyces* elicited by several related substrates. *J. Bact.* **65**, 122.

(Received 15 September 1955)