THE MELEZITOSE LOCUS IN SACCHAROMYCES: ONE GENE PRODUCING MORE THAN ONE ENZYME[1]

TOMMY M. YAU and CARL C. LINDEGREN
Biological Research Laboratory, Southern Illinois University, Carbondale.

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INTRODUCTION

The Melezitose Locus (MZ) of Saccharomyces controls the utilization of a number of alpha-glucosides (Palleroni and Lindegren, 1953; Lindegren and Lindegren, 1953; Hwang and Lindegren, 1964). By the most refined recombinatorial tests (including more than a thousand tetrad analyses), this locus has been established as the locus of a single gene (Hwang, Lindegren, Bhattacharjee and Roshanmanesh, 1964). Previous interpretations of the data had been based on the assumption that this gene produced a single enzyme capable of hydrolizing a variety of alpha-glucosidic substrates. In this communication, evidence is presented which indicates that at least three different alpha-glucosidases are produced by induced cells. The alpha-glucosidases induced in the wild-type and mutant strains in the presence of the various competent inductors were studied by DEAE-cellulose chromatography and starch-gel electrophoresis.

MATERIALS AND METHODS

All yeast cultures used in this study were haploid strains from the Lindegren Breeding Stock. The wild-type MZ culture D1008Y-2 (TMSGZ) responds to turanose, maltose, sucrose, alpha-methyl glucoside or melezitose with the production of alpha-glucosidic activities. Culture 14 UV (TMsgz) cannot adapt to sucrose, alpha-methyl glucoside or melezitose while D1008Z-1 (TMSgz) cannot

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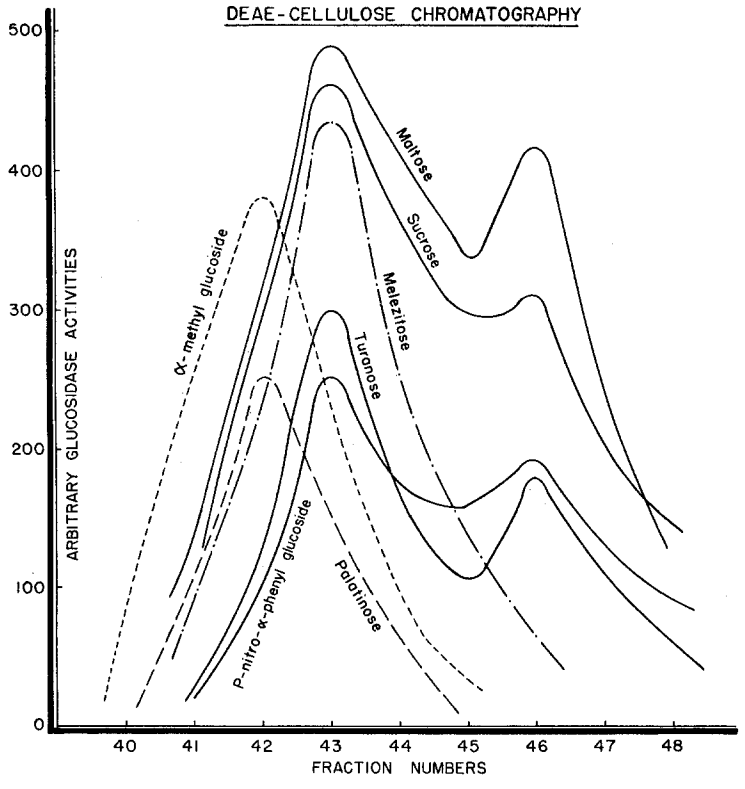
adapt to alpha-methyl glucoside or melezitose. D1008Y-2 was grown in the presence of 4% turanose, maltose, sucrose, alpha-methyl glucoside or melezitose in separate experiments. D1008Z-1 and 14UV were grown in the presence of 4% maltose. The induced cells were disrupted by sonicating with a Branson Sonifier for 4 minutes. The cell-free supernatant solution after centrifugation was lyophilized and stored at -10° C.

Gradient chromatography was performed in a 35 x 2.5 cm column packed with DEAE-cellulose (Bio-Rad Cellex D) equilibrated with 0.01M KH_2PO_4 (pH 6.0). One-half ml of dialyzed enzyme preparation obtained from maltose-induced cells of D1008Y-2 (containing 4.0 mg of protein) was applied to the column. The column was eluted with an ionic gradient produced by constantly mixing a reservoir of 0.01M KH_2PO_4 buffer with increments of 0.5M KH_2PO_4 buffer, both at pH 6.0. A peristaltic pump was used to keep the rate of mixing constant and equal to the flow rate which was 0.7 ml/minute. Seven ml fractions were collected. Alpha-glucosidase activities were assayed (1) by the Glucostat reagent (Worthington Biochemical Corporation) and, (2) by the p-nitro-phenyl-alpha-D-glucopyranoside (PNPG) method as described by Halvorson and Ellias (1958).

Starch-gel electrophoresis was performed according to the methods of Smithies (1955). A 13% gel in 0.03M sodium borate (pH 8.48) was prepared from hydrolyzed starch (Connaught Medical Research Laboratory). The bridge buffer was 0.3M sodium borate (pH 8.12). The best results were obtained by applying 250 volts and allowing the current to pass through the buffer for 15 hours. After electrophoresis, the gels were cut horizontally and enzyme activities towards various substrates were located by overlaying the horizontally cut gels with 1% agar dissolved in pH 5.8 phosphate buffer containing the Glucostat reagent plus 3% substrate. Zones of alpha-glucosidase activities were detected by the appearance of brown bands. To demonstrate activities towards PNPG, the gels were overlayed with 0.05% PNPG in 1% agar alone. Enzyme activities were located by the appearance of yellow bands.

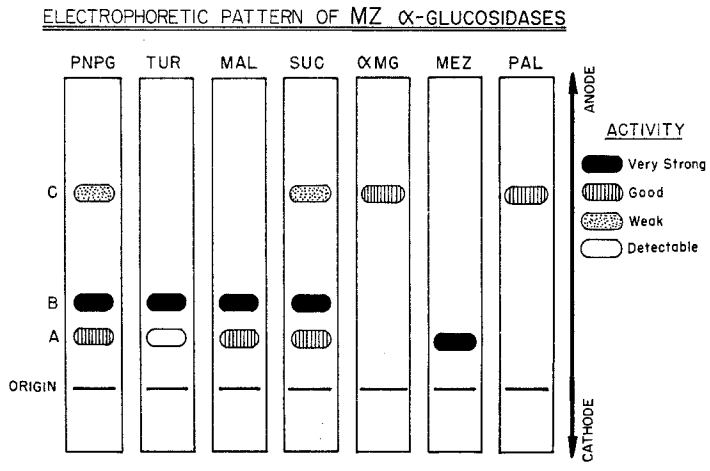
RESULTS

Enzyme activities of the chromatographic fractions collected were tested against the various substrates. The results are shown in Figure 1. The enzymic activities against turanose, sucrose, maltose and PNPG are dis-



tributed in two peaks (fraction #43 and #46). Activity against melezitose was found to peak in one fraction (#43) while activities against palatinose and alpha-methyl glucoside peaked in another fraction (#42).

The results of the starch-gel electrophoretic analysis were in agreement with the chromatographic experiments and are shown diagrammatically in Figure 2. Identical electrophoretic patterns were obtained no matter which inductor was used. Moreover, the two mutant alleles D1008Z-1 and 14UV also gave the same electrophoretic patterns as the wild-type D1008Y-2. The results demonstrate that a single gene produced alpha-glucosidases with not only different electrophoretic mobilities but also different substrate specificities. Three



different alpha-glucosidases detected by electrophoresis were designated A, B and C. A is most active towards melezitose, C towards alpha-methyl glucoside and palatinose, and B towards PNPG, turanose, maltose and sucrose.

SUMMARY AND CONCLUSION

Activation of the MZ gene by any competent inductor to which it responds results in the simultaneous induction of at least three different enzymes with overlapping and yet distinct patterns of substrate specificities. This gene is the only gene defined by tetrad analysis which controls the production of more than one enzyme.

Acknowledgments

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References

Halvorson, H. and Ellias, L., Biochim. Biophys. Acta, 30, 28 (1958).

Hwang, D. S., Lindegren, C. C., Nature, 203, 791 (1964).

Hwang, D. S., Lindegren, C. C., Bhattacharjee, J. K., and Roshanmanesh, A., Can. J. Genet. Cytol., 6, 414 (1964).

Lindegren, C. C., and Lindegren, G., Genetica, 26, 430 (1953).

Palleroni, N. J., and Lindegren, C. C., J. Bact., 65, 122 (1953).

Smithies, O., Biochem. J., 61, 629 (1955).