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Investigation of oligosaccharides hydrolysis by Botryodiplodia theobromae and its implication

ZYGMUNT MACHOY, NORBERT WYSZYŃSKI

Department of Biochemistry and Department of General Chemistry,
Pomeranian Medical Academy, 70-111 Szczecin,
Powstańców Wielkopolskich 72, Poland

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From the hydrolysis rate of the oligosaccharides used it was found out which enzymes of *Botryodiplodia theobromae* Pat. participated at that process and the order in which they attacked the individual bonds in oligosaccharides.

INTRODUCTION

The literature provides us with reports concerning the utilization of saccharides by *Botryodiplodia theobromae* Pat. Monosaccharides (glucose, fructose, galactose) are used up directly in the course of fungus metabolic processes. The polysaccharides or oligosaccharides are, as a rule, subjected to preliminary enzymatic hydrolysis into monosaccharides.

In this paper the observations accomplished by others (Chaturvedi 1966; Srivastava, Tandon 1969) and authors' own experiments covering the laboratory culture of *B. theobromae* on saccharide media have been taken into account (Machoy et al. 1975). The interpretation of the obtained results has been intended rather to determine the sort of the hydrolysing enzymes that *B. theobromae* has at its disposal during its growth on the sugar medium, and which of the bonds in oligosaccharides are being cleft in the first line, not really to define the usefulness of respective saccharides as available sources of carbon.

MATERIAL AND METHODS

CULTURE

B. theobromae was cultured on a liquid sterilized oligosaccharide – mineral medium according to Capek at temperature of 28°C as described previously (Machoy et al. 1975; Machoy et al. 1980). The oligosaccharide compound of medium i.e. saccharose was substituted in respective series of culture by other oligosaccharides.

OLIGOSACCHARIDES

Trehalose (α-D-glucopyranosyl α-D-glucopyranoside, abbreviation Glc (α1 → 1α)Glc; melibiose (6-O-α-D-galactopyranosyl-D-glucose, Gal (α1 → 6) Glc; lactose (4-O-β-D-galactopyranosyl-D-glucose, Gal (β1-4) Glc; cellobiose (4-O-β-D-glucopyranosyl-D-glucose, Glc (β1 → 4)Glc; melezitose (O-α-D-glucopyranosyl-)1 → 3(-O-β-D-fructofuranosyl-)2 → 1 (– α-D-glucopyranoside, Glc (α1 → 3)Fru(β2 → α1)Glc.

THIN-LAYER CHROMATOGRAPHY

At two-day intervals we took from the fungus culture 0.5 cm^3 of medium, wherein the saccharides were determined by thin-layer chromatography according to Weill and Hanke (1962). After the development of chromatograms using the mixture butanol:piridine:water = 75:15:10 and upon staining in the way reported by Raadsveld and Klomp (1971) the respective saccharides were seen either to appear or disappear. The qualitative analysis of the mono- and oligosaccharides was carried out on the basis of relevant standards. Relying on the analyses performed in the metioned manner, it was concluded which of the enzymes did actively participate in the hydrolysis of oligosaccharides.

RESULTS

The rate at which the oligosaccharides are being utilized during the growth of the fungus was as follows: trehalose > cellobiose > melibiose > melezitose > lactose. (Fig. 1). Whole area of the rectangular figures indicated the used oligosaccharide as the medium compound present in the solution. During the fungus growth glucose was found to appear in all five media. Galactose was revealed in cases in which it

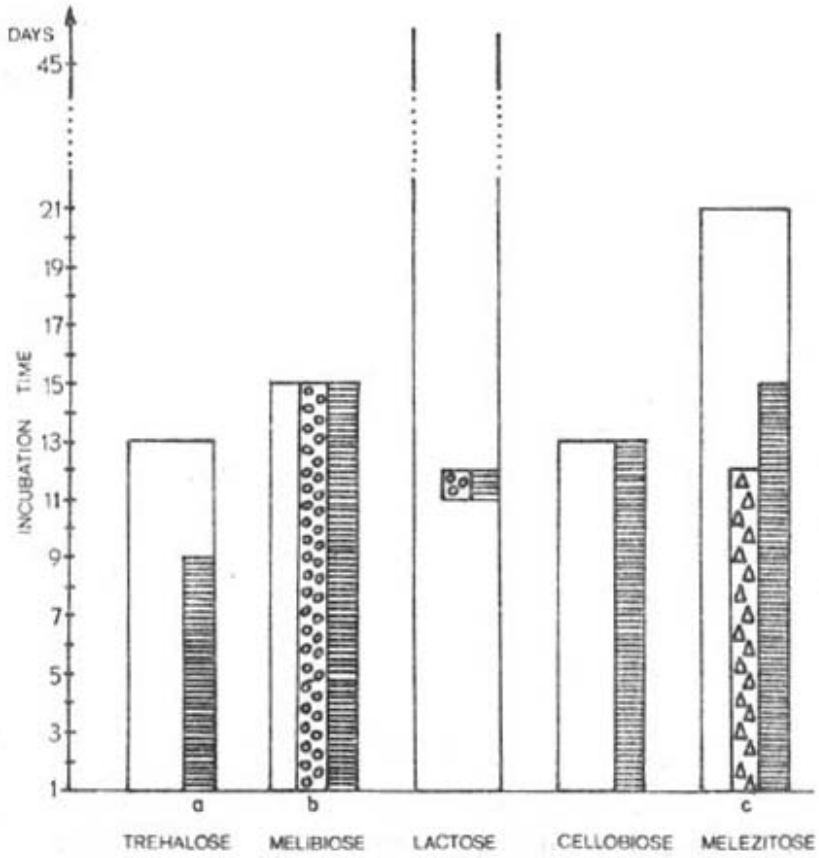

Fig. 1. Rate of oligosaccharides hydrolysis by hydrolases of *Botryodiplodia theobromae* in culture *in vitro*

The whole area of the rectangular figures indicates the presence of the used oligosaccharide as the basic medium sugar in the solution. During fungus growth monosaccharides were found to appear in all media as the result of the oligosaccharides hydrolysis

a – Glucose (Gl); *b* – Galactose (Gal); *c* – Turanose

constituted an added (used up) oligosaccharide component. Lactose practically failed to undergo hydrolysis within the 45-days experiment. In the course of melezitose hydrolysis turanose as a new disaccharide was disclosed in the medium solution. The growth of the fungus on the medium consisting of melibiose, cellobiose, melezitose and lactose resulted in the presence of 1-2 newly synthesized compounds (Table 1). It was only in hydrolysis of trehalose that no additional formation of new compounds was recorded such as oligosaccharides or conjugates of saccharides whose R_f coefficients differed in comparison with oligosaccharides being used in the media.

Table 1

Synthesis of new oligosaccharide compounds or formation of conjugates with higher R_f values during the culture of *Botryodiplodia theobromae* in vitro

Oligosaccharides	Days	Number of newly formed compounds	Time of disclosed presence of new compounds in days
Trehalose	13	0	–
Melibiose	15	2	1-15 11-15
Lactose	interrupted after 45 days	2	1-45 11-45
Cellobiose	13	2	1-13 9-12
Mellezitose	21	1	9-12

DISCUSSION

It was ascertained by Umezurike (1970, 1975) that *B. theobromae* contained cellulase and β-glucosidase. Our earlier investigations showed that the fungus possessed the following enzymes hydrolyzing oligosaccharides glucoamylase (EC 3.2.1.3) and invertase (EC 3.2.1.26) but maltase (EC 3.2.1.20) and β-amylase (EC 3.2.1.2) were only likely to appear. (Enz. Nomencl. 1964). Instead the presence of α-amylase (EC 3.2.1.1), laminaranase (EC 3.2.1.6), inulase (EC 3.2.1.7) and initialy lactase (EC 3.2.1.23) could be excluded (Machoy et al. 1975). The further search for, and the establishing of the presence of other enzymes made it necessary for us to use oligosaccharides of differentiated molecular structure with regard to the bonds between the respective monosaccharides in these oligosaccharides (Staňek et al. 1965).

The current paper disclosed in *B. theobromae* the existence of the following enzymes taking part in degradation and synthesis of oligosaccharides; trehalase (EC 3.2.1.28), melibiase (EC 3.2.1.22), cellobiase (EC 3.2.1.21), α-1,3 glucosidase (EC 3.2.1.27) and invertase (EC 3.2.1.26). Trehalose hydrolysis proceeded due to the presence of trehalase. Melibiase was responsible for the hydrolysis of melibiose. Cellobiose was hydrolyzed by cellobiase. Trisaccharide melezitose was hydrolyzed by two enzymes. The first of them, more active, appeared to be invertase. As a result glucose and disaccharide turanose were formed. Disaccharide turanose was hydrolyzed by α-1,3 glucosidase. The presence of lactose was observed throughout the experiment.

Only after 10-days culture, trace quantities of glucose and galactose in the medium were found in one test. That could be caused not only by the low activity of lactase but by the participation of another enzyme, namely cellobiase. This enzyme is known to be of low specificity and to act also on β-D-galactoside, apart of being active also in transfer reactions of transferation, i.e., in the synthesis of higher compounds likely higher oligosaccharides. In the presence of four oligosaccharides (trehalose being the exception) transferases synthesized from 1 to 2 higher compounds (Table 1). We suspect that for melibiose and lactose those were the reactions of galactosyltransferases, for cellobiose reactions of glucosyltransferases, but whenever melezitose was used, the reactions of fructosyltransferases.

B. theobromae enzymes most rapidly hydrolyzed this bond Fru(β2 → 1α)Glc (that being a similar system of binding as in saccharose). Next in order the bonds Glc(α1 → 1)Glc as well as Glc(β1 → 4)Glc, then Gal(α1 → 6)Glc and finally Glc(α1 → 3)Fru. The fungus did not hydrolyze the bond Gal(β1 → 4)Glc (Fig. 1).

It may be concluded that the hydrolases of *B. theobromae* hydrolyze the following saccharides: 1 – trehalase hydrolyzes trehalose; 2 – melibiase hydrolyzes melibiose (as well as the following saccharides in which the same bonds occur: raffinose, lychnose, fructosylraffinose); 3 – lactase hydrolyzes lactose (as well as lactosucrose, fucosyllactose, lactaminyl-lactose, lactodifucotetraose); 4 – cellobiase hydrolyzes cellobiose (and lactose); 5 – invertase hydrolyzes melezitose (as well as gentianose, lactosucrose, planteose, raffinose, umbelliferose, lychnose, stachyose and sucrose); 6 – α-1,3 glucosidase hydrolyzes melezitose (and turanose).

An attentive observations on the hydrolysis of melibiose and melezitose disclos that the coefficients R_f of the two oligosaccharides are subjected to a gradual minimal decrease during the last days of fungus culture as compared to their standards. What has been the cause of changes in the decreasing rate of the oligosaccharides migration is at present hard to elucidate because of the lack of experimental proofs. One cannot exclude minor modifications of molecules or functional groups of sugars or the exchange of various monosaccharides in oligosaccharides molecule on a route of transferations, which in that instance would have a lower R_f coefficient.

In summing-up it has been accepted that:

1 – the established sequence for the rate of bonds hydrolysis in oligosaccharides corresponds to the activity of hydrolytic enzymes recorded in *B. theobromae*;

2 – the knowledge of the kinds of enzymes existing in *B. theobromae* facilitates the prediction: which chemical compounds may provide nutrient

medium for the fungus growth. In technical aspect it may be implemented for biological degradation of food industry sewage and for gaining additional protein of fungus origin.

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Badanie hydrolizy oligosacharydów przez *Botryodiplodia theobromae* i jej znaczenie

Streszczenie

W wyniku hydrolizy użytych oligosacharydów ustalono, które enzymy *Botryodiplodia theobromae* Pat. biorą udział w tym procesie i w jakiej kolejności atakują poszczególne wiązania w cukrach.