TRANSGLYCOSIDATION RELATING TO RIBOFLAVIN BY *ESCHERICHIA COLI*

IX. OCCURRENCE OF TRANSGLYCOSIDASE IN MICROORGANISMS

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In the previous papers (1—3), the authors have reported the investigations concerning the transglycosidases of *Escherichia coli*, which require B₂¹ as a specific acceptor of glycosyl groups, especially on their isolations and on their characteristics. The wide occurrence of such transglycosidases in various microorganisms suggests that the transglycosidation relating to B₂ may be one of the synthetic pathways of polysaccharides in microorganisms.

In an attempt to gauge the actual significance of the transglycosidase in cellular metabolisms of microorganisms, a study is instituted concerning the occurrence of the transglycosidases in various microorganisms including the typical strains of fermentation microorganisms, along with the natures of their enzymes in comparison with that of *E. coli* which was previously reported (1—3).

EXPERIMENTAL

Materials and Methods

Organisms — The 26 strains of the microorganisms which had been kept in this laboratory were used, including 12 strains of molds, 5 strains of yeasts and 9 strains of bacteria.

Each strain of the molds was grown statically at 30° for 7 days 100 ml of the cultural medium containing 3 g glucose, 0.1 g NH₄NO₃, 0.1 g KH₂PO₄, 0.1 g NaNO₃, 0.05 g MgSO₄, 0.05 g KCl and 1 g CaCO₃. Each strain of the yeasts was grown at 30° for 24 hr in 1 l of malt extract. Each strain of *Lactobacillus* sp. was grown at 37° for 48 hr in 1 l of the cultural medium composing of malt extract containing 20 g glucose, 2.5 g yeast extract and 20 g CaCO₃.

Clostridium acetobutylicum was grown at 37° for 48 hr in 1 l of the cultural medium containing 20 g glucose, 5 g polypeptone, 0.5 g K₂HPO₄, 0.5 g KH₂PO₄, 0.2 g MgSO₄, 0.01 g NaCl, 0.01 g FeSO₄ and 0.01 g MnSO₄. *E. coli*,

¹ The following abbreviations are used: riboflavin, B₂; riboflavinyl, B₂-

Aerobacter aerogenes and *Bacillus subtilis* were grown statically at 37° for 48 hr in a nutrient bouillon.

The cells of the microorganisms were harvested by a centrifuge and washed by distilled water. The cells were dried with an electric fan at room temperature.

Assay of Transglycosidase — The assay of the transglycosidase was carried out in the similar way to that described in the previous paper (1). Reaction mixture (total volume, 5 ml) consisting of B₂, donor saccharide, buffer and the cell suspension containing 25 mg of the dried cells, was incubated for 5 hr under the optimal conditions. B₂-glycoside formed was determined by paper-chromatographic procedure.

RESULTS

1. Occurrence of Transglucosidase in Microorganisms

The transglucosidase catalyzing glucosyl transfer from maltose to B₂ was

TABLE I
Occurrence of Transglucosidase in Microorganisms

Reaction mixture containing 200 μ M B₂, 25 mM maltose, 0.1 M glycine or acetate buffer and 1 ml of cell suspension (25 mg of the dried cells), was incubated for 5 hr.

Microorganism	Optimum		B ₂ -glucoside synthesized	Specific activity
	pH	Temperature		
		°C	μ M	
<i>Escherichia coli</i>	7.0	30	29	100
<i>Aerobacter aerogenes</i>	"	"	41	137
<i>Bacillus subtilis</i>	"	"	32	110
<i>Clostridium acetobutylicum</i>	"	37	15	51
<i>Lactobacillus casei</i>	6.5	"	13	44
<i>L. fermenti</i>	"	"	14	47
<i>L. brevis</i>	"	"	12	42
<i>Leuconostoc mesenteroides</i>	"	"	14	47
<i>Streptococcus lactis</i>	"	"	18	54
<i>Zygosaccharomyces pombe</i>	4.5	30	27	92
<i>Saccharomyces cerevisiae</i> (baker's yeast)	"	"	25	85
<i>S. cerevisiae</i>	"	"	26	87
<i>S. sake</i>	"	"	23	79
<i>Torula colliculosa</i>	"	"	24	83
<i>Aspergillus oryzae</i>	4.5	45	28	97
<i>A. niger</i>	"	"	49	167
<i>A. awamori</i>	"	"	32	110
<i>A. candida</i>	"	"	27	93
<i>Rhizopus oryzae</i>	"	"	29	99
<i>R. javanicus</i>	"	"	28	97
<i>R. batatas</i>	"	"	29	99
<i>R. nigricans</i>	"	"	31	106
<i>R. chinensis</i>	"	"	31	107
<i>Penicillium chrysogenum</i>	"	"	33	114
<i>P. purpurogenes</i>	"	"	36	124
<i>Mucor javanicus</i>	"	"	72	247

found to be present in the cells of all the microorganisms tested. The activity of the molds were comparatively greater than that of the others and *Aspergillus niger* and *Mucor javanicus* were found to glucosidate as much as 30–50 per cent of B₂ under the optimal conditions.

Depending on the microorganisms employed, the optimal conditions for the transglucosidase were different. For instance, the optimal pH and temperature were pH 7.0 and 30° for *E. coli*, pH 4.5 and 45° for *Aspergillus oryzae* and pH 4.5 and 30° for *Saccharomyces cerevisiae*.

These results were summarized in Table I.

2. Occurrence of Transgalactosidase in Microorganisms

The transgalactosidase catalyzing galactosyl transfer from lactose to B₂ was found in various strains of the bacteria as well as of the molds. Above all, *E. coli*, *Aspergillus awamori* and *Penicillium chrysogenum* had a very high activity.

However, it was noted that the activity of the transgalactosidase was hardly detectable in the cells of the yeasts tested. Since the metabolic ability of lactose by these microorganisms has been considerably low, it is

TABLE II
Occurrence of Transgalactosidase in Microorganisms

Reaction mixture containing 200 μ M B₂, 25 mM lactose, 0.1 M glycine or acetate buffer and 1 ml of cell suspension was incubated for 5 hr.

Microorganism	Optimum		B ₂ -galactoside synthesized	Specific activity
	pH	Temperature		
		°C	μ M	
<i>Escherichia coli</i>	7.2	30	18	100
<i>Aerobacter aerogenes</i>	"	"	17	95
<i>Bacillus subtilis</i>	"	"	15	83
<i>Clostridium acetobutylicum</i>	"	"	7	39
<i>Lactobacillus casei</i>	6.2	"	12	67
<i>L. fermenti</i>	"	"	12	66
<i>L. brevis</i>	"	"	12	67
<i>Leuconostoc mesenteroides</i>	"	"	10	55
<i>Streptococcus lactis</i>	"	"	10	58
<i>Zygosaccharomyces pombe</i>			±	
<i>Saccharomyces cerevisiae</i> (baker's yeast)			±	
<i>S. cerevisiae</i>			±	
<i>S. sake</i>			±	
<i>Torula colliculosa</i>			±	
<i>Aspergillus oryzae</i>	4.2	45	21	117
<i>A. niger</i>	"	"	6	33
<i>A. awamori</i>	"	"	24	133
<i>A. candida</i>	"	"	16	89
<i>Rhizopus oryzae</i>	"	"	8	44
<i>R. javanicus</i>	"	"	8	45
<i>R. batatas</i>	"	"	9	50
<i>R. nigricans</i>	"	"	9	52
<i>R. chinensis</i>	"	"	5	28
<i>Penicillium chrysogenum</i>	"	"	29	162
<i>P. purpurogenes</i>	"	"	5	27
<i>Mucor javanicus</i>	"	"	8	44

possibly accepted that there is a close relation between the activity of the transgalactosidase and the metabolic ability of lactose.

The optimal conditions for the transgalactosidase were found to be pH 7.2 and 30° for *E. coli*, pH 6.2 and 30° for *Lactobacillus casei* and pH 4.2 and 45° for *A. awamori*.

These results were summarized in Table II.

3. Occurrence of Transfructosidase in Microorganisms

The transfructosidase catalyzing fructosyl transfer from sucrose to B₂ was found in various yeasts and molds, as well as in bacteria, such as *E. coli* and *Bacillus subtilis*.

As reported in the previous paper (4), there existed two types of transglycosidase, namely transglucosidase and transfructosidase, in the cells of the microorganisms: transglucosidase transferred the glucosyl part of sucrose to B₂ and transfructosidase transferred the fructosyl part to B₂. The relative amounts of these transglycosidases occurring in the cells of microorganisms varied with different strains. For instance, the yeast cells contained much transfructosidase but less transglucosidase, while the cells of the bacterial

TABLE III
Occurrence of Transfructosidase in Microorganisms

Reaction mixture containing 200 μ M B₂, 25 mM sucrose, 0.1 M glycine or acetate buffer and 1 ml of cell suspension was incubated for 5 hr.

Microorganism	Optimum		B ₂ -fructoside synthesized	Specific activity
	pH	Temperature		
		°C	μ M	
<i>Escherichia coli</i>	7.4	37	11	100
<i>Aerobacter aerogenes</i>	"	"	7	64
<i>Bacillus subtilis</i>	"	"	9	82
<i>Clostridium acetobutylicum</i>			±	
<i>Lactobacillus casei</i>			±	
<i>L. fermenti</i>			±	
<i>L. brevis</i>			±	
<i>Leuconostoc mesenteroides</i>			±	
<i>Streptococcus lactis</i>			±	
<i>Zygosaccharomyces pombe</i>	4.5	30	14	127
<i>Saccharomyces cerevisiae</i> (baker's yeast)	"	"	15	136
<i>S. cerevisiae</i>	"	"	15	132
<i>S. sake</i>	"	"	14	131
<i>Torula colliculosa</i>	"	"	13	121
<i>Aspergillus oryzae</i>	5.2	45	11	102
<i>A. niger</i>	"	"	12	107
<i>A. awamori</i>	"	"	11	102
<i>A. candida</i>	"	"	13	117
<i>Rhizopus oryzae</i>	"	"	14	124
<i>R. javanicus</i>	"	"	6	52
<i>R. batatas</i>	"	"	8	68
<i>R. chinensis</i>	"	"	7	67
<i>Fenicillium chrysogenum</i>	"	"	5	41
<i>P. purpurogenes</i>	"	"	14	131
<i>Mucor javanicus</i>	"	"	6	51
			7	60

strains such as *E. coli*, revealed lesser activity of transfructosidase.

The optimal conditions for the transfructosidase were found to be pH 7.4 and 37° for *E. coli*, pH 4.5 and 30° for *S. cerevisiae* and pH 5.2 and 45° for *A. oryzae*.

The experimental results were summarized in Table III.

DISCUSSION

The wide distribution of transglycosidase in microorganisms, strongly suggests some important relation of this enzymatic activity to the microbial cells.

The optimal conditions for the transglycosidases, however, vary with different strains employed and their enzymatic activities also vary considerably. On the whole, it is inviting to assume that the uneven occurrence of the transglycosidases would relate to the differences in the metabolic abilities of the donor saccharides, *i.e.*, maltose, lactose and sucrose, by the microorganisms, especially to their efficiencies in synthesizing polysaccharides.

Moreover, there existed some differences in the natures of the products of the transglycosidases among different strains. For instance, the transgalactosidase of *E. coli* can produce not only B₂-galactoside but also a series of B₂-compounds of oligo-galactosides in a short period of transgalactosidation, while by *A. oryzae*, B₂-galactoside is the major product of the reaction; but this is not the essential difference of the transglycosidation itself, as the molds reveals some peculiarity of the presence of strong glycosidase which attacks B₂-oligoglycoside to liberate B₂ and the oligoglycoside (5).

A comparison of the results with the synthetic abilities of oligo- or polysaccharides by the microorganisms might, therefore, contribute to evaluate the significance of the transglycosidase for polysaccharide biosynthesis.

On the other hand, there is no direct informations about the presence of B₂-glycoside in the cells of microorganisms. Peel (6) estimated the amount of different flavins in various microorganisms and found that flavin mononucleotide and flavin-adenine dinucleotide comprised the major part of total flavins. However, as previously described (5), B₂-glycoside is only the initial product in the successive transglycosidation and is easily consumed to synthesize B₂-compounds of oligoglycosides. The B₂-oligoglycosides thus formed may be enzymatically hydrolyzed to B₂ and the oligoglycosides in the growing cells. Therefore, B₂-glycoside forms a part of the unphosphorylated flavins in the cells of microorganisms, but in very small amounts.

SUMMARY

1. The transglycosidase requiring B₂ as the specific acceptor of glycosyl groups was found in various kinds of microorganisms.
2. The optimal conditions and the activities of the transglycosidases were found to vary with the different strains of the microorganisms.
3. A close relationship was assumed to exist between the activity of the

transglycosidases and the metabolic ability of the donor saccharides in microorganisms.

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