Studies on the Amino Acids Fermentation of Pentose Materials

Part III. The Specificity of the Fermentation of Sugars by *Brevibacterium pentoso-aminoacidicum* nov. sp.

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This work was undertaken to determine whether the degradation of sugars by *Brevibacterium pentoso-aminoacidicum* nov. sp., a bacterium capable of producing amino acids from pentoses and hexoses, was due to constitutive or inducible enzymes. It was also intended to clarify the reason for the substrate specificity in the fermentation of sugars by this bacterium. After a series of experiments using washed resting cells grown on various kinds of sugars or their cell-free extracts, it was found that the enzymes involved in the degradation of pentoses were inducible, while those of hexose metabolism were constitutive. The activities of several enzymes related to the pathways of pentose metabolism were demonstrated and the substrate specificity of sugar degradation by this strain was explained satisfactorily by the inducer specificity of these enzymes.

INTRODUCTION

As reported in our previous paper^{1,2)}, *Brevibacterium pentoso-aminoacidicum* No. 115-1 nov. sp. produces remarkable amounts of L-glutamic acid and DL-alanine from both pentoses and hexoses. The ability of this bacterium to convert pentoses into amino acids seems to be a unique property and we took a deep interest in the specificity of fermentation of sugars by this strain.

Studies on the fermentation or oxidation of pentoses by bacteria belonging to the genera of *Lactobacillus*³⁻⁵⁾, *Aerobacter*⁶⁻⁸⁾,

*Escherichia*⁹⁻¹²⁾, *Acetobacter*¹³⁾, *Clostridium*^{14,15)} and *Pseudomonas*^{16,17)} have been conducted by many investigators and a great amount of detailed knowledge has accumulated in the case of lactic acid bacteria: the formation of phosphate esters of ketopentoses by isomerases, kinases and epimerases is essential in the early steps of pentose degradation. In most of these bacteria, it is now known that pentoses are degraded inducibly while the enzymes involved in the fermentation of hexoses are constitutive¹⁸⁾.

The utilization of pentoses concerning the

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amino acids fermentation, however, has received less attention and the knowledge about this subject is completely lacking. Thus it was felt that the metabolism of pentoses by *Brev. pentoso-aminoacidicum* was worthy of study in the aspects of both academic and practical interests.

In the present paper, we are reporting on the fermentative specificity of cells of *Brev. pentoso-aminoacidicum* grown on various kinds of sugars. This strain was found to metabolize pentoses inducibly and hexoses constitutively in the experiments using either resting cells or cell-free extracts.

MATERIALS AND METHODS

Materials.

D-Xylose, D-ribose, L-arabinose, D-fructose and D-glucose used were high grade commercial preparations. L-Ribulose and D-xylulose were prepared according to the method of Glatthaar and Reichstein¹⁸⁾ by the epimerization of L-arabinose or D-xylose in a pyridine solution under reflux. Its purity was assayed chromatographically using phenol saturated with water as the solvent and spraying with aniline hydrogen phthalate reagent²⁰⁾.

Adenosine triphosphate was obtained from the Wakamoto Pharmaceutical Company.

Preparation of Resting Cells.

Cultures of *Brev. pentoso-aminoacidicum* No. 115-1 were maintained on agar slants with the composition of 1% of meat extract, 1% of polypeptone and 0.5% of NaCl. Cells from a 24 hours slant culture were inoculated into a 500 ml flask containing 100 ml of the medium with 1% of each sugar. The composition of this medium is shown in Table I. The cultures were incubated aerobically for 20 hours at 30°C on a reciprocal shaker at a rate of 144 r.p.m. As was reported in the previous paper²⁾, this condition for cultivation was favorable for amino acids accumulation and the cells had high activities.

The cells were harvested in a centrifuge, washed three times with chilled 0.9% NaCl solution and suspended in 0.1 M phosphate buffer (pH 7.0).

TABLE I. THE COMPOSITION OF THE MEDIUM FOR GROWING CELLS

Sugar	1% (sterilized separately)
K ₂ HPO ₄	0.1
MgSO ₄ ·7H ₂ O	0.05
Urea	0.3
Polypeptone, pH=7.0	0.2

Preparation of Cell-free Extracts.

Two general methods were adopted for the preparation of extracts. In one way, the cells were suspended in a buffer solution and subjected to sonic oscillation. In another way, extracts were prepared by grinding the cells with quartz sand.

Manometric Measurements of Oxygen Uptake.

Oxygen uptake was measured by the conventional Warburg manometric techniques²¹⁾. The composition of the reaction mixture is shown in Table II.

TABLE II. THE COMPOSITION OF THE REACTION MIXTURES

Sugar 50 μ M in buffer solution	1.0 ml
Cell suspension in buffer solution	0.5
0.1 M phosphate buffer (pH 7.0)	1.0
20% KOH (in center well)	0.2

Determination of Pentoses.

For the determination of pentose, the orcinol colorimetric method of Mejbaum²²⁾ was adopted.

Determination of Glucose.

Glucose was determined by the modified manometric method of Keilin and Hartree²³⁾ using "Deoxin" (Nagase-sangyo Co.), a glucose oxidase preparation containing catalase. The main compartment of the manometric flask was filled with 1 ml of 0.1 M phosphate buffer (pH 5.8), 1 ml of the unknown solution (ca. pH 5.8), the side arm with 0.5 ml of "Deoxin" solution (2 mg per ml) and the center well with 0.2 ml of 20% KOH. The determination of oxygen uptake was carried out at 30°C.

Demonstration of Enzyme Activities.

Pentose isomerase activity was measured according to Cohen²⁰⁾; the rate of formation of ketopentoses from pentoses was estimated by the cysteine-carbazole reaction.

Phosphoriboisomerase activity was demonstrated

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using the method of Axelrod²⁴; the colorimetric determination of ribulose-5-phosphate formed from ribose-5-phosphate by the action of the isomerase was performed by a modification of carbazole procedure of Dische and Borenfreund²⁵.

The demonstration of ribokinase activity²⁶ was performed by the removal of protein and phosphorylated compounds formed by the action of this kinase from ribose with the $\text{Ba}(\text{OH})_2\text{ZnSO}_4$ reagent of Somogyi²⁷ and measurement of residual ribose in the filtrates by the alkaline copper reagent of Nelson²⁸.

Ribulokinase and xylulokinase were demonstrated by the method of Stumpf and Horecker²⁹. This method depends on the disappearance of free pentose or ketopentose as assayed by the orcinol reaction or the cysteine-carbazole reaction after the removal of phosphate esters as alcohol-insoluble barium salts.

RESULTS AND DISCUSSION

(I) Oxidation of Sugars by Resting Cells Grown on Glucose.

Fig. 1 shows the oxygen uptake with various substances by resting cells grown on glucose. Glucose and fructose were rapidly utilized whereas xylose, L-arabinose and ribose were nearly inert. Of these three pentoses, how-

FIG. 1. Oxygen Absorption by Resting Cells Grown on Glucose.

a: Glucose b: Fructose c: Xylose
d: Ribose e: L-Arabinose
Each vessel contained 9.0 mg of cells.

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ever, xylose was most active. It was thought that the ability of these cells to attack xylose could have been due to the small amounts of pentoses present in the components of the medium. But neither xylose nor any other pentose was detected by paper chromatography in the medium, therefore it was concluded that the ability of these cells to ferment xylose did not depend on the presence of contaminated pentoses.

Though many factors might be responsible for the inertness of these cells on pentoses,

FIG. 2. Effect of Energy Source on the Induced Oxidation of Xylose by Glucose-grown Cells.

a: Glucose 5 μM b: Xylose 45 μM + Glucose 5 μM
c: Xylose 45 μM

FIG. 3. Effect of Amino Acids on the Induced Oxidation of Xylose by Glucose-Grown Cells.

a: Casate 1.3 mgM
b: Casate 1.3 mgM + Xylose 45 μM
c: Xylose 45 μM

one of the most probable factors could be that the enzymes involved in the pentose metabolism might be inducible. It may be seen from Fig. 1 that the enzymes were not induced by pentose added even after a considerable time. It was thought that the sources of energy or nitrogen might be required for induction. To examine this possibility, small amounts of glucose or amino acids were added with xylose and the oxygen uptake of these mixtures was determined. But the results shown in Fig. 2 and Fig. 3 indicate that the cells did not acquire the ability to metabolize xylose under these conditions.

In addition dried cells showed exactly the same behaviors towards several kinds of sugars as washed resting cells and impermeability of cells to pentoses did not seem to explain the inability of cells to oxidize pentoses.

(II) Oxidation of Sugars by Resting Cells Grown on Xylose.

As shown in Fig. 4, pentoses as well as hexoses were utilized rapidly by xylose-grown cells, and the rate of oxidation of xylose was especially increased as compared with that

FIG. 4. Oxygen Absorption by Resting Cells Grown on Xylose.

a: Glucose b: Xylose c: Fructose
d: L-Arabinose e: Ribose
Each vessel contained 9.0 mg of cells.

by glucose-grown cells. The rate of oxidation of hexoses did not seem to be affected by the kinds of sugars utilized for growth.

(III) Oxidation of Sugars by Resting Cells Grown on L-Arabinose.

As shown in Fig. 5, pentoses and hexoses were attacked readily by the cells grown on L-arabinose. It is worthy of notice that oxygen uptake was rapid especially with L-arabinose. From the results obtained with these cells, it is probable that the rate of oxidation of hexoses was not decreased by the substitution of pentoses for hexoses as the sugars for growth. On the other hand, the rate of oxidation of pentoses was much influenced by the sugars utilized for growth.

FIG. 5. Oxygen Absorption by Resting Cells Grown on L-Arabinose.

a: Glucose b: L-Arabinose c: Fructose
d: Xylose e: Ribose
Each vessel contained 9.8 mg of cells.

(IV) Oxidation of Sugars by Resting Cells Grown on Ribose.

In the case of ribose-grown cells, too, hexoses were utilized rapidly (Fig. 6). The rate of oxidation of pentoses by these cells was more rapid than that by cells grown on glucose, but the effect was not so marked as that by cells grown on the other pentoses. And it is noticed that in general ribose was

attacked more slowly than the other pentoses.

The extent of growth on ribose was poorer than on the other pentoses and it might be said that ribose was the most unfavorable sugar of these three pentoses.

FIG. 6. Oxygen Absorption by Resting Cells Grown on Ribose.

a: Glucose b: Fructose c: L-Arabinose
d: Xylose e: Ribose
Each vessel contained 6.9 mg of cells.

(V) Oxidation of Sugars by Resting Cells Grown without Sugar.

As shown in Fig. 7, cells grown without sugar utilized hexoses rapidly whereas the

FIG. 7. Oxygen Absorption by Resting Cells Grown without Sugar.

a: Glucose b: Fructose c: Xylose
d: Ribose e: L-Arabinose
Each vessel contained 7.2 mg of cells.

ability of pentose degradation was poor. This phenomenon was almost the same as with cells grown on glucose.

(VI) Specific Oxidation of Various Kinds of Sugars by Resting Cells.

To summarize the results obtained in the experiments described above, comparisons of the rates of the oxidation of various kinds of sugars were performed in terms of Q_{O_2} (μ l O_2 take up per mg dry weight of cells per hour) (Table III).

TABLE III. SPECIFIC OXIDATION OF VARIOUS KINDS OF SUGARS BY *Brev. pentoso-aminoacidicum*

Carbohydrates in medium	Rate of oxygen absorption, Q_{O_2} (μ l/mg·h)				
	Xylose	L-Arabinose	Ribose	Glucose	Fructose
Xylose	11.8	1.0	2.7	19.1	6.9
L-arabinose	6.0	23.3	3.9	36.6	10.0
Ribose	5.8	5.8	2.6	19.7	17.1
Glucose	1.1	0	0.6	29.4	14.2
None	1.7	0	1.7	13.9	12.0

Several features were clarified from Table III:

First, hexoses were utilized rapidly irrespective of carbon sources used for growth.

Secondly, xylose was degraded rapidly by cells grown on xylose and moderately well by cells grown on ribose or L-arabinose, while cells grown on glucose or without sugar had a poor ability of metabolizing xylose.

Thirdly, L-arabinose was metabolized by cells grown on various kinds of sugars in the same mode as was seen with xylose and in addition L-arabinose was found to be more fermentable than the other pentoses.

Fourthly, though ribose was not fermented as well as the other pentoses and the effect of sugars used for growth was small, pentose-grown cells were more active than glucose-grown cells in regard to the ability of degrading ribose.

In short, all cells oxidized hexoses and a pentose present in the growth medium rapid-

ly. Moreover it was also seen that a pentose was metabolized considerably well by cells grown on the other pentoses. There may be many explanations for these results, but the following two must be most probable:

(1) Permeability changes occurred during growth of the cells under the various conditions.

(2) Induced enzyme formation or activation took place (In this paper, no clear-cut distinction was made between enzyme formation and activation).

The results obtained in the experiments with resting cells did not make it possible to conclude which factor was responsible. Direct evidence on this point must be obtained through the experiments with cell-free extracts though it was felt that permeability barrier did not exist because dried cells showed the same characters as resting cells did. Therefore, activities of several enzymes concerning pentose degradation were examined in extracts of cells grown on various kinds of sugars.

(VII) Preparation of Cell-free Extracts.

Two general methods of preparing cell-free extracts, grinding with quartz sand and sonic treatment, were examined in the adaptability for this purpose. Extracts were prepared by grinding in the following way: a paste containing 3.6 g of dried cells was ground manually with 7.2 g of quartz sand. The mixture was extracted with 100 ml of a 0.1 M phosphate buffer (pH 7.0). A clear supernatant solution was obtained after centrifugation at $14,500 \times G$ for 20 minutes. Sonic treatment was performed as follows: 25 ml of cell suspension containing 900 mg of dried cells were treated in a sonic oscillator (Tôyôriko Type 50-3, 10 KC, 300 W) under cooling. The cell debris was removed by centrifugation at $14,500 \times G$ for 20 minutes.

The results of this preliminary examination

are shown in Table IV. Phosphoriboisomerase activity was used as the index of the extracted enzymes because this enzyme was constitutive (It will be shown later).

TABLE IV. PREPARATION OF CELL-FREE EXTRACTS

Carbohydrates in medium	Type of extraction	Phosphoriboisomerase activity
		(Ribulose-5-phosphate formed in 10 min)
Xylose	grinding with quartz sand	53.8 μ g
Glucose	"	38.7
Xylose	Sonic oscillation 20 min.	40.2
Glucose	" 20	35.3
Xylose	" 40	69.4
Glucose	" 40	58.5

As shown in Table IV, the method of grinding was fully effective for this purpose and sonic oscillation was also found to be satisfactory after 40 minutes of treatment. From the point of reproducibility and practicability, therefore, the method of sonic treatment for 40 minutes was adopted in the following experiments.

(VIII) L-Arabinose Isomerase Activity.

The presence of L-arabinose isomerase activity was established in extracts obtained from cells grown on various sugars. Fig. 8 shows the activity of this enzyme in extracts of L-arabinose-grown cells. Table V demon-

FIG. 8. L-Arabinose Isomerase Activity of Extracts of Cells Grown on L-arabinose.

Reaction mixture: 0.066 M L-arabinose 0.1 ml, 0.1 M borate buffer (pH 8.0) 0.8 ml and cell free extract 0.1 ml, at 37°C.

—●— — extracts

TABLE V. L-ARABINOSE ISOMERASE ACTIVITY OF CELLS GROWN ON VARIOUS KINDS OF SUGARS

Carbohydrates in medium	L-ribulose formed in 30 minutes
L-Arabinose	40.0 μ g
Xylose	10.5
Ribose	1.0
Glucose	0.2

strates the specificity of the isomerase to the sugars on which the cells were grown. Practically no activity was found in glucose-grown cells and L-arabinose-grown cells showed the strongest activity of the isomerase. Xylose- or ribose-grown cells were found to possess the activity to a degree less than cells grown on L-arabinose. These facts must be sufficient to conclude that L-arabinose isomerase is an inducible enzyme and L-arabinose, and ribose and xylose in less extent, can act as inducers.

(IX) Xylose Isomerase Activity.

Fig. 9 and Table VI show the presence and specificity of xylose isomerase. For the same reason as in the case of L-arabinose isomerase, xylose isomerase was concluded to be also an inducible enzyme.

FIG. 9. Xylose Isomerase Activity of Extracts of Cells Grown on Xylose.

Reaction mixture: 0.066 M xylose 0.1 ml, 0.1 M borate buffer (pH 8.0) 0.8 ml and cell-free extracts 0.1 ml, at 37°C.

—●— extracts

TABLE VI. XYLOSE ISOMERASE ACTIVITY OF EXTRACTS OF CELLS GROWN ON VARIOUS KINDS OF SUGARS

Carbohydrates in medium	Xylulose formed in 30 minutes
Xylose	8.4 μ g
L-Arabinose	4.2
Ribose	2.0
Glucose	0.8

(X) Phosphoriboisomerase Activity.

Though no activity of isomerizing free ribose was found in extracts of cells grown on any sugars examined, even on ribose, the activity of phosphoriboisomerase which isomerizes ribose-5-phosphate to ribulose-5-phosphate was found in extracts of all cells (Fig. 10 and Table VII). It may be worthy of remark that even hexose-grown cells had the

FIG. 10. Phosphoriboisomerase Activity of Extracts of Cells Grown on Glucose.

—●— extracts

TABLE VII. PHOSPHORIBOISOMERASE ACTIVITY OF EXTRACTS OF CELLS GROWN ON VARIOUS KINDS OF SUGARS

Carbohydrates in medium	Ribulose-5-phosphate formed in 10 minutes
Xylose	69.4 μ g
L-Arabinose	36.7
Ribose	57.3
Glucose	58.5

Reaction mixture: 1 mg/ml ribose-5-phosphate 0.5 ml and cell-free extract 0.2 ml, at 37°C.

activity of this isomerase, and it was concluded that this enzyme was constitutive. This fact could be understood well in respect to its role in the hexose-monophosphate shunt, one of the important pathways of hexose metabolism.

(XI) Ribulokinase Activity.

In the presence of ATP and MgCl_2 , L-ribulose was phosphorylated by extracts of cells grown on pentoses especially on L-arabinose (Fig. 11 and Table VIII). As shown in Fig. 11, the presence of MgCl_2 was essential for the full activity of the kinase. Table VIII indicates that this kinase was also under the control of induction by pentoses.

FIG. 11. Ribulokinase Activity of Extracts of Cells Grown on L-Arabinose.

a: -ATP b: -extracts
c: - MgCl_2 d: complete

TABLE VIII. RIBULOKINASE ACTIVITY OF EXTRACTS OF CELLS GROWN ON VARIOUS KINDS OF SUGARS

Carbohydrates in medium	Pentose disappeared in 20 minutes
L-Arabinose	6.5 μg
Xylose	3.2
Ribose	2.0
Glucose	0.4

Reaction mixture: 0.5% L-arabinose (or L-ribulose) 0.5 ml, 0.4 M tris buffer (pH 7.5) 0.5 ml, 0.1 M MgCl_2 1.0 ml, 0.05 M ATP 0.5 ml, H_2O 3.5 ml and cell-free extract 2.0 ml, at 30°C.

(XII) Xylulokinase Activity.

Xylulokinase activity was demonstrated in extracts of pentose-grown cells. This kinase was shown to behave like ribulokinase and found to be an inducible enzyme (Fig. 12 and Table IX).

FIG. 12. Xylulokinase Activity of Extracts of Cells Grown on Xylose.

a: -ATP b: -extracts
c: - MgCl_2 d: complete

TABLE IX. XYLULOKINASE ACTIVITY OF EXTRACTS OF CELLS GROWN ON VARIOUS KINDS OF SUGARS

Carbohydrates in medium	Pentose disappeared in 20 minutes
Xylose	4.0 μg
L-Arabinose	2.2
Ribose	1.8
Glucose	0.2

Reaction mixture: 0.5% xylose (or xylulose) 0.5 ml, 0.4 M tris buffer (pH 7.5) 0.5 ml, 0.1 M MgCl_2 1.0 ml, 0.05 M ATP 0.5 ml, H_2O 3.5 ml and cell-free extract 2.0, at 30°C.

(XIII) Ribokinase Activity.

Extracts of cells grown on pentoses phosphorylated ribose in the presence of ATP and MgCl_2 (Fig. 13 and Table X). This kinase was also identified to be inducible.

(XIV) Specific Activities of Various Enzymes in Cell-free Extracts.

Summarizing the results of the experiments using cell-free extracts, Table XI shows the specificity of enzyme activity of *Brev. pentosaminoacidicum*: the basis of this comparison

FIG. 13. Ribokinase Activity of Extracts of Cells Grown on Ribose.

a: -ATP b: -extracts
c: -MgCl₂ d: complete

TABLE X. RIBOKINASE ACTIVITY OF EXTRACTS OF CELLS GROWN ON VARIOUS KINDS OF SUGARS

Carbohydrate in medium	Ribose disappeared in 20 minutes
Ribose	3.7 μg
Xylose	3.6
L-Arabinose	2.3
Glucose	0.6

Reaction mixture: 0.5% ribose 0.5 ml, 0.4 M tris buffer (pH 7.5) 0.2 ml, 0.1 M MgCl₂ 0.25 ml, 0.05 M ATP 0.5 ml, 0.1 M NaF 0.25 ml and cell-free extract 2.0 ml, at 30°C.

TABLE XI. SPECIFICITY OF ENZYME ACTIVITY OF *Brev. pentoso-aminoacidicum*

Enzyme activity	Carbohydrates in medium.			
	Xylose	L-Arabinose	Ribose	Glucose
Isomerases:				
Xylose isomerase	10.5	5.2	2.5	1.0
Arabinose isomerase	52.5	200.0	5.0	1.0
Phosphoriboisomerase	1.2	0.6	1.0	1.0
Kinases:				
Xylulokinase	20.0	11.0	9.0	1.0
Ribulokinase	8.0	16.2	5.0	1.0
Ribokinase	6.0	3.8	6.2	1.0

was the activity of extracts of glucose-grown

cells. Several facts were revealed by this comparison:

(1) Enzymes involved in the degradation of pentoses were classified into two groups: constitutive enzymes and inducible ones.

Phosphoriboisomerase was found to be a constitutive enzyme and L-arabinose isomerase, xylose isomerase, ribokinase, xylulokinase and ribulokinase were demonstrated to be inducible.

(2) Extracts of xylose-grown cells had strong activities of xylose isomerase and xylulokinase, and moderate activities of L-arabinose isomerase, ribulokinase and ribokinase.

(3) Extracts of L-arabinose-grown cells had strong activities of L-arabinose isomerase and ribulokinase, and moderate activities of xylose isomerase, xylulokinase and ribokinase.

(4) Extracts of ribose-grown cells had these enzyme activities in a moderate degree.

(5) Practically no activities of these enzymes were demonstrated in extracts of glucose-grown cells.

(6) It must be worthy of being mentioned that not only the pentose related to an enzyme directly but the other pentoses could act as inducers of the enzyme. No attempt was made to clarify the reason of these facts but the similarity of the chemical structure of pentoses or the action of intermediates of pentose metabolism might be a key to solve this problem.

(XV) Pathways of Pentose Metabolism in *Brev. pentoso-aminoacidicum*.

The activities of isomerases and kinases shown in Fig. 14 were demonstrated in cell-free extracts of *Brev. pentoso-aminoacidicum*. The metabolic pathways in this figure is just the same as in the case of lactic acid bacteria and it was postulated that the same pathways were present in *Brev. pentoso-aminoacidicum*.

The specific fermentation of sugars by rest-

FIG. 14. Pathways of Pentose Metabolism in *Brev. pentoso-aminoacidicum*.

* Inducible enzyme

ing cells was thought to be caused by the specific induction of these inducible enzymes in this figure and the fermentation of pentoses by this strain may be called induced fermentation. Furthermore, the specific activity of degradation of sugars was explained by this scheme quite satisfactorily: for instance, the reason why glucose-grown cells could not oxidize pentoses was that all the inducible enzymes in this scheme were absent. Xylose grown-cells were induced to have strong xylose

FIG. 15. Oxygen Absorption by Resting Cells grown on Glucose.

a: Glucose 1 μ M b: Ribose 1 μ M
c: Ribose-5-phosphate 1 μ M
Each vessel contained 10 mg of cells.

FIG. 16. Oxygen Absorption by Extracts of Cells Grown on Glucose.

a: Glucose 1 μ M b: Ribose-5-phosphate
c: Ribose 1 μ M

Each vessel contained cell-free extracts equivalent to 1.8 mg of cell.

isomerase and xylulokinase, so they could degrade xylose rapidly. L-Arabinose-grown cells were thought to behave like xylose-grown cells. The moderate activity of degradation of some pentose was the result of having the moderate activity of the enzymes related to the metabolism of the pentose. As for the degradation of ribose, phosphoriboisomerase was constitutive, so the induced formation of only ribokinase was necessary. Namely, glucose-grown cells could not degrade ribose by

only one reason that ribokinase was not induced in them, and from this presumption, ribose-5-phosphate, not ribose, must be degraded by not only pentose-grown cells but also glucose-grown cells. Fig. 15 and Fig. 16 show the activity of oxidizing ribose and ribose-5-phosphate by glucose-grown cells. Though resting cells did not show any activity to these substances, cell-free extracts could degrade ribose-5-phosphate rapidly confirming the above hypothesis. The reason of no activity of resting cells to ribose-5-phosphate might be that these cells were impermeable to this substance.

SUMMARY

Brevibacterium pentose-aminoacidicum was grown on various kinds of sugars as its energy sources. Oxidation of xylose, L-arabinose, ribose, glucose and fructose was studied with the resting cells.

Pentose present in the growth medium and hexoses were oxidized rapidly by all the cells.

Pentoses not present in the growth medium were also oxidized by cells grown on the other pentoses though not so rapidly.

Hexose-grown cells were not able to oxidize any of the pentoses.

Activities of xylose isomerase, L-arabinose isomerase, phosphoriboisomerase, ribokinase, xylulokinase and ribulokinase were demonstrated in cell-free extracts and all these enzymes except phosphoriboisomerase were found to be inducible enzymes.

The specific induction of these enzymes was closely related to the specific oxidation of sugars by resting cells and the degradation of pentoses might be called the induced fermentation: namely, for the degradation of pentoses, the induction of these enzymes were found necessary.