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STUDIES ON BIOMASS UTILIZATION BY MICROBIOLOGICAL ACTIVITIES

Part III. Raw Starch Digestion by Microbial and Plant β-Amylases

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Abstract

With an aim of utilization of bacterial and plant β-amylases, digestion of various raw starches by β-amylase from *Bacillus cereus* BQ10-S1 Spo II was compared with those by soybean and barley β-amylases, α-amylase from *Bacillus subtilis* and glucoamylase I from *Rhizopus delemar*. The bacterial β-amylase showed much higher digestibility than plant β-amylases but less digestibility than glucoamylase I.

The bacterial β-amylase was found to digest most easily rice starch samples followed by wheat, corn, potato and sweet potato starches. Among five rice starch samples obtained in 75% polishing stages of Yamadanishiki, Nihonbare and LA 110, the rice brans of 90-75% layer, especially that of Nihonbare were most easily digested by the bacterial β-amylase.

Introduction

Since the oil crisis in 1973 much attention has been paid to researches on energy saving or replacing conventional fossil fuels to other fuels. For instance, alcohol from biomass that is one of renewable resources has become of interest as an alternative energy.[15,16] New production systems of alcohol with high efficiency using raw starch materials have been developed and found to save a large amount of energy.[5,13,21,25,26] Raw starch digestion by commercial enzyme preparations was also applied to Sake brewing and the resulting Sake was reported to contain more than 18% of alcohol with an excellent quality.[1,4,12] However, how varieties of rice grains, their degrees of polishing and so forth will effect on digestion by commercial enzyme preparations still remains to be investigated. Especially, little is known on raw starch digestion by β-amylases. Though no plant β-amylase was reported to digest raw starches,[20] our recent results have shown that microbial β-amylases can digest raw starches much more than plant β-amylases.[17]

In the present paper we deal with raw starch digestion by β-amylases and comparison of their digestibility by α-amylase and glucoamylase with that by β-amylases. We also deal with comparison of digestibility of raw rice starch samples obtained in 75% polishing stages of two rice varieties (Yamadanishiki and Nihonbare) suitable for Sake brewing and one long rice variety (LA 110).

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Meterials and Methods

Rice varieties and raw starch samples

Brown rice samples of Yamadanishiki, Nihonbare and LA 110 (a long rice variety from the

U. S. A.), which were harvested in 1981 in the Experiment Farm for Sake-rice, Hyogo Prefectural Agricultural Center, were used in the experiments. Each sample was milled with a Satake Grain Test Mill and the degree of polishing (%) was calculated by weight differences between polished rice and rice bran obtained. The rice bran samples at each polishing stage were also used as raw starch samples. Wheat, corn, sweet potato and potato starches used in the experiments were of practical grade from Wako Pure Chemical Industries, Ltd.

Enzymes

Bacterial β-amylase was obtained in our laboratory from the fermentation broth of *Bacillus cereus* BQ10-Sl Spo II in an EYELA jar fermenter (Type M-160) (Tokyo Rika Co. Ltd.).[7,8] Other enzymes were commercial preparations ; soybean β-amylase from Wako Pure Chemical Industries, Ltd., barley β-amylase (Type II-B) and α-amylase (Type XI-A) of *Bacillus subtilis* from Sigma Chemical Company and glucoamylase I of *Rhizopus delemar* from Toyobo Co., Ltd., respectively. Each β-amylase preparation was examined on formation of only maltose as the enzyme reaction product by paper chromatography.

Raw starch digestion

Raw starch digestion was carried out as follows. The reaction mixture (total volume : 2 ml) in the test tube containing 10 mg of each raw starch sample and 60 units of each amylase (estimated by Bernfeld method[2]) in 25 mM acetate buffer (pH 5.0) or phosphate buffer (pH7.0) was incubated at 35 ℃ with stirring. The test tube was set at an angle of 35° on the shaker (Taiyo Incubator Personel) at 164 reciprocation/min and 3.1 cm amplitude. After a given time of incubation the reaction mixture was centrifuged at 4000 rpm for 25 min and the supernatant was subjected to the determination of reducing sugar content.

Measurement of reducing sugar

Reducing sugar in 1 ml of the supernatant obtained above was determined by Somogyi-Nelson method.[19] To the test tube 1 ml of the supernatant and 1ml of the copper reagent was added. After standing for about 30 min, it was diluted up to 24 ml with deionized water and subjected to the colorimetric determination at 660 nm.

Results and Discussion

Raw starch digestion by bacterial and plant β-amylases

Wheat, sweet potato, corn and potato starches were subjected to digestion by β-amylases from different sources. As shown in Fig. 1, wheat starch was most easily digested by bacterial β-amylase and the amount of maltose produced was about two times greater than those by soybean and barley β-amylases (I in Fig. 1). As seen in II, III and IV in Fig. 1, there was no marked difference in digestibility among the three starches and β-amylases. All the enzyme reactions were found to level off during the first six hours of incubation. Though bacterial β-amylase was reported to digest more strongly

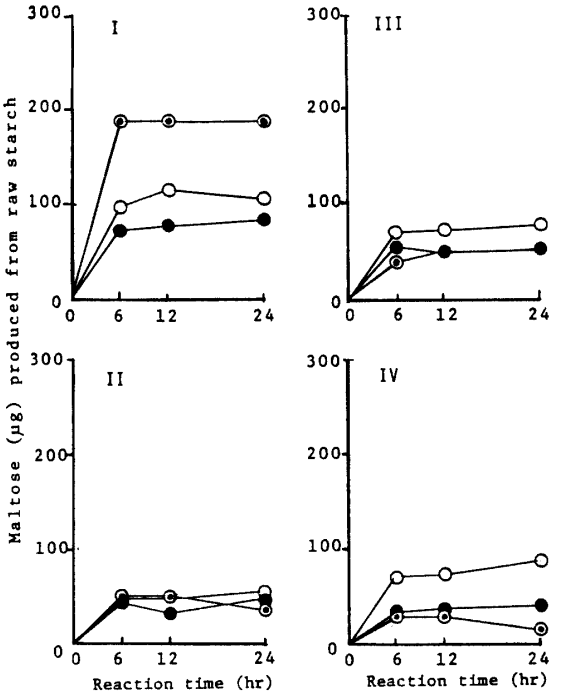

Fig. 1. Hydrolysis of raw starches with bacterial and plant β-amylases.

I : wheat starch II : sweet potato starch
III : corn starch IV : potato starch

⊙—⊙ bacterial β-amylase
●—● soybean β-amylase
○—○ barley β-amylase

sweet potato starch than plant β-amylases,[26] our results suggest that wheat starch is more suitable for examination of β-amylase digestibility because sweet potato starch is less digestive than other starches (II in Fig. 1).

Raw starch digestion of rice samples at various polishing stages

In 75% polishing stages of rice grains, four starch samples in addition to unpolished grains (A) were obtained ; rice brans of 100-90% layer (B) and 90-75% layer (D), 90% polished (C) and 75% polished rice grains (E) which were both milled and passed through 150 meshes for the enzyme reaction.

i) *Yamadanishiki* The five starch samples from Yamadanishiki were subjected to β-amylase digestion and the results obtained are shown in A to E in Fig. 2. The digestion of the rice bran of 90-75 % layer by bacterial β-amylase after 24 hr reaction was the highest (D in Fig.

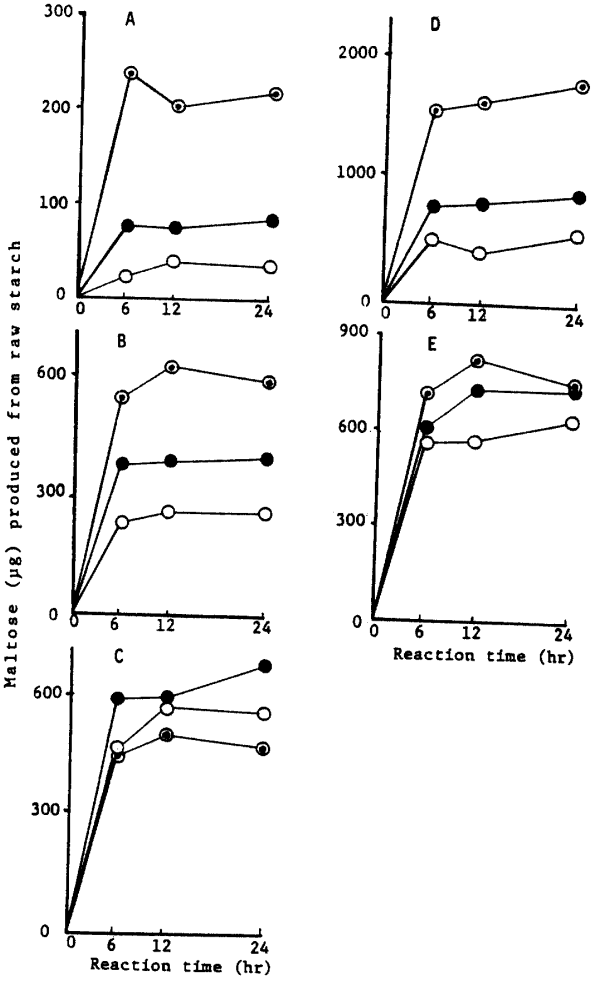

Fig. 2. β-Amylase digestion of raw starches in polished rice and rice bran samples (Nihonbare).
A : unpolished rice grain
B : rice bran of 100-90% layer
C : 90% polished rice grain
D : rice bran of 90-75% layer
E : 75% polished rice grain
⊙—⊙ bacterial β-amylase
●—● soybean β-amylase
○—○ barley β-amylase

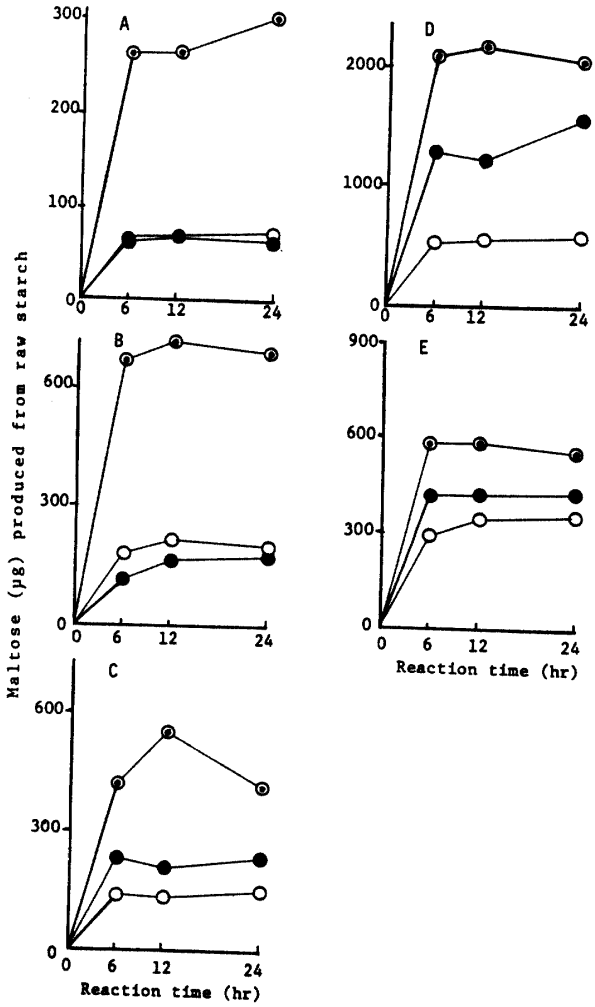

Fig. 3. β-Amylase digestion of raw starches in polished rice and rice bran samples (Yamadanishiki).
A : unpolished rice grain
B : rice bran of 100-90% layer
C : 90% polished rice grain
D : rice bran of 90-75% layer
E : 75% polished rice grain
⊙—⊙ bacterial β-amylase
●—● soybean β-amylase
○—○ barley β-amylase

2), the value of which was about seven times greater than that of unpolished rice grains (A in Fig. 2). The digestibility of starch samples by bacterial β-amylase was D, E, B, C and A in decreasing order except the case of C where soybean β-amylase showed the highest activity inversely.

ii) *Nihonbare* The highest digestion, as seen in Fig. 3, was also seen in D by bacterial β-amylase that was about forty times greater than the lowest digestion in A. Bacterial β-amylase also showed higher activities against all the samples than the other enzymes. The amount of maltose produced by bacterial β-amylase in A was about six times greater than those by the other enzymes, but these differences, as seen in E, became much less in the samples from inner layers of the grains.

iii) *LA 110* The results on LA 110 are shown in Fig. 4, where bacterial β-amylase showed the highest digestibility in D and the difference between bacterial β-amylase and barley β-amylase came to about ten times in A. But the amount of maltose produced by the former was less than those in the cases of Yamadanishiki and Nihonbare. Except that there were little differences among three enzyme activities against C, other results on LA 110 were similar to those of two other rice varieties.

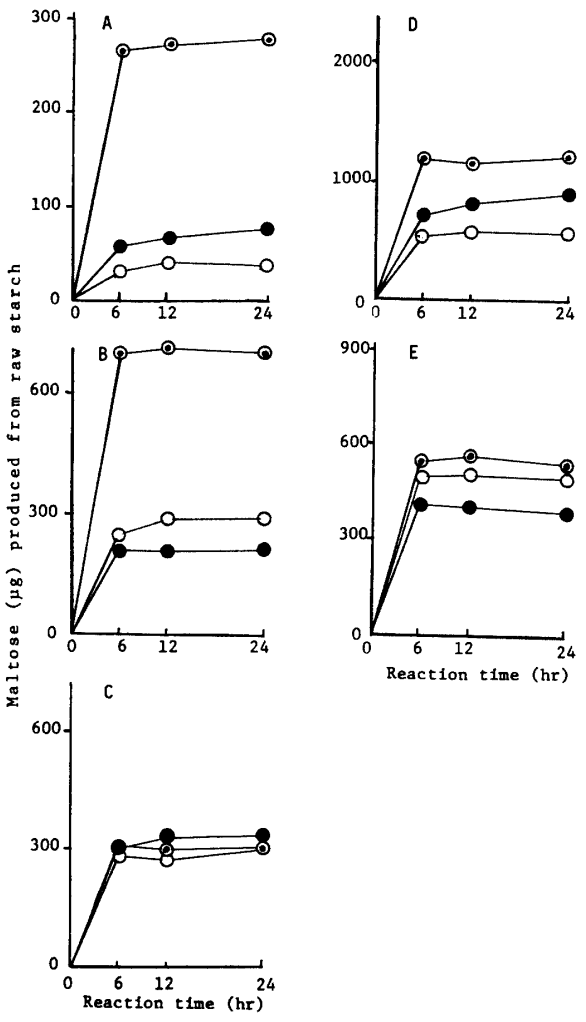

Fig. 4. β-Amylase digestion of raw starches in polished rice and rice bran samples (LA 110).
A : unpolished rice grain
B : rice bran of 100-90% layer
C : 90% polished rice grain
D : rice bran of 90-75% layer
E : 75% polished rice grain
⊙——⊙ bacterial β-amylase
●——● soybean β-amylase
○——○ barley β-amylase

Comparison of raw starch digestion by β-amylases

In order to compare digestibility of the rice samples by β-amylase, the results concerned are summarized in Fig. 5. Although no plant β-amylase has been reported to digest raw starches,[3,20,24] soybean and barley β-amylases were found to digest raw starch samples in our experiments. However, bacterial β-amylase expectedly showed the highest digestibility among the three β-amylases used and the rice brans of 90-75% layer, especially that of Nihonbare were most easily digested. Of interest is that unpolished rice starch samples from the three rice varieties were least digestive. Proteins in rice grains were reported to adsorb amylases and lower the enzyme reactions.[14,30,31] If we accept this view, the rice bran of 100-90% layer or 90-75% layer containing more proteins should be less digestive. Our results, on the contrary, showed that 75% polished rice grains containing less proteins were more digestive. Though these discrepancies must be elucidated in future, white rice brans of 90-75% layer, which are obtained as one of by-products of Sake brewing or rice polishing companies, would be expected to be a good substrate for the bacterial β-amylase in industrial maltose production.

Raw starch digestion by α-amylase and glucoamylase I

Table 1 shows the results of raw starch diges-

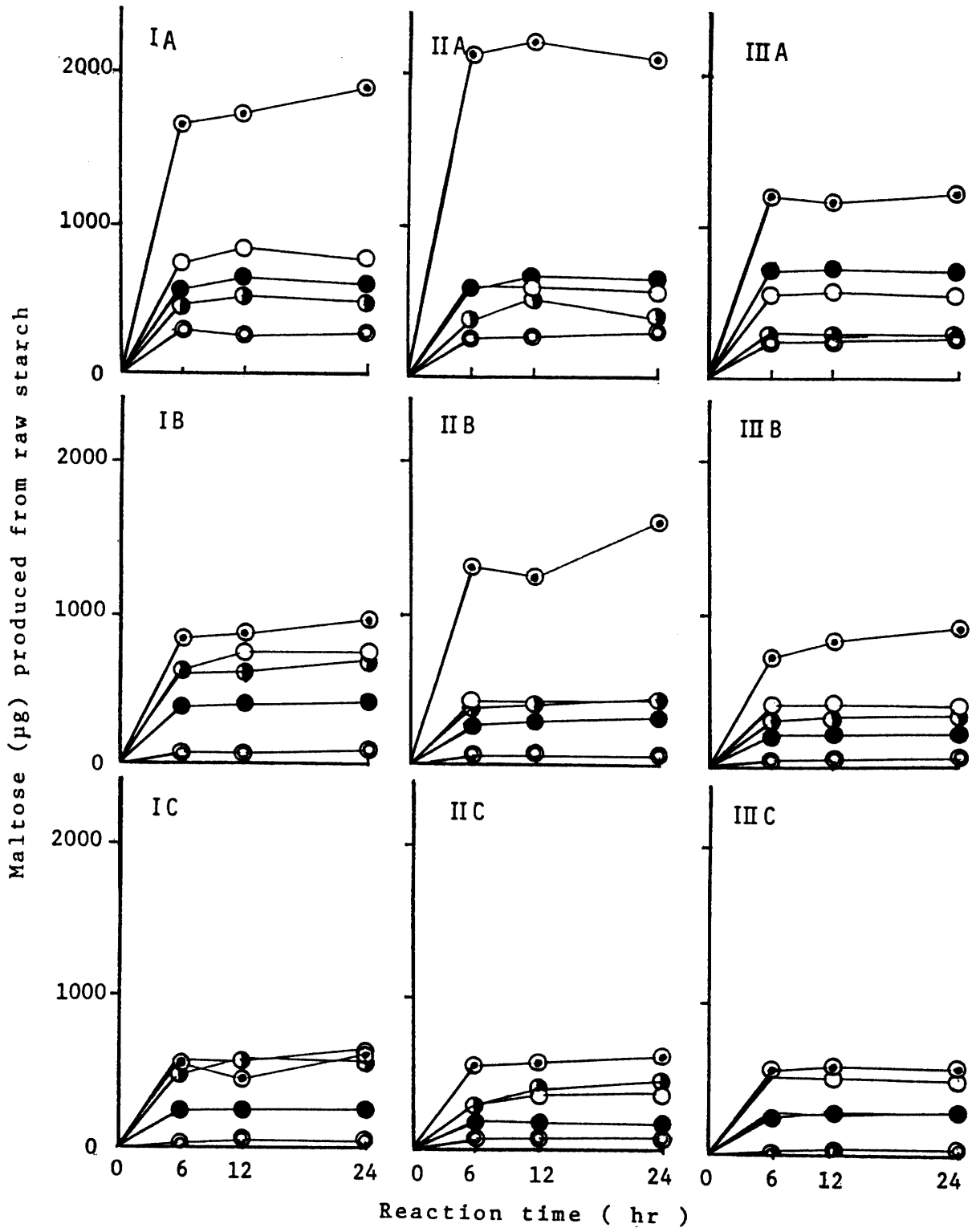

Fig. 5. Comparison of raw starch digestion with bacterial and plant β-amylases.
I : Yamadanishiki II : Nihonbare III : LA 110
A : bacterial β-amylase B : soybean β-amylase C : barley β-amylase
◎—◎ unpolished rice grain ●—● rice bran of 100-90% layer
◑—◑ 90% polished rice grain ⊙—⊙ rice bran of 90-75% layer
○—○ 75% polished rice grain

Table 1. Raw starch digestion with α-amylase and glucoamylase I.

Raw starch sample		α-Amylase	Glucoamylase I
Wheat starch		709.2	1606.7
Corn starch		292.5	1231.7
Sweet potato starch		108.3	850.0
Potato starch		27.5	136.7
Yamadanishiki rice grain	Unpolished	304.0	3180.0
	90% polished	375.0	2030.0
	75% polihsed	493.3	1560.0
Nihonbare rice grain	Unpolished	251.7	2633.3
	90% polished	255.0	1400.0
	75% polished	483.3	936.7
LA 110 rice grain	Unpolished	226.7	2496.7
	90% polihsed	253.3	1260.0
	75% polished	315.0	936.7
Yamadanishiki rice bran	100-90% layer	141.7	1623.3
	90-75% layer	523.3	4100.0
Nihonbare rice bran	100-90% layer	143.3	1243.3
	90-75% layer	560.0	3520.0
LA 110 rice bran	100-90% layer	132.3	1153.3
	90-75% layer	568.3	3013.3

Each reaction was carried out at 35°C for 24 hrs and each centrifugal supernatant was subjected to glucose measurement by Somogyi-Nelson's method. Each figure in Table 1 shows μg of glucose produced from raw starches.

tion by α-amylase and glucoamylase I. α-Amylase showed relatively lower activities against sweet potato and potato starches than other starch samples. Among the rice starch samples α-amylase also showed a tendency to digest more easily samples from inner layers of the grains. Among the rice varieties there was no marked difference in digestibility by α-amylase. Pancreatic α-amylase was reported to digest raw corn starch most strongly and followed by malt α-amylase, *Bacillus subtilis* α-amylase and *Aspergillus oryzae* α-amylase.[24] The digestibility of raw corn starch by *B. subtilis* α-amylase was reported to be about 60 % that of pancreatic α-amylase. Since the enzyme used in the experiments was α-amylase from *B. subtilis*, digestibility by bacterial β-amylase is estimated to be stronger than that by *Asp. oryzae* α-amylase and weaker than those by pancreatic and malt α-amylases.

Glucoamylase I showed stronger activities against all the starch samples than α-amylase, and, therefore, was thought to be stronger than bacterial β-amylase, too. As seen in Table 1, glucoamylase I activity was about five to ten times greater than that of α-amylase. Glucoamylase I was also reported to digest raw corn starch more strongly than glucoamylase II or α-amylase, and glucoamylase I in the presence of α-amylase, to digest most effectively raw corn starch.[23] In this respect, an attempt should be made to realize more efficient production of maltose from such raw starch materials as white rice brans by bacterial β-amylase, although maltose production was reported from soluble starch by enzymatic hydrolysis.[28,29] The relationship between protein contents including enzyme proteins like amylases of rice grains[6,18,22] and rice varieties[27] should be investigated for an effective application of raw starch digestion to Sake

brewing in future. Furthermore, from the standpoint of utilization of natural resources, enzymatic utilization of alfalfa root starch is one of the interesting items to be investigated.[9,10,11]

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微生物活性によるバイオマス利用に関する研究

第Ⅲ報　微生物及び植物 β-アミラーゼによる生澱粉の分解

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西川欣一・山根国男・西田清数

要　　約

微生物及び植物 β-アミラーゼの利用，開発を目的とした研究の一つとして，各種生澱粉の分解性について検討した。微生物 β-アミラーゼとして本学醗酵生産学研究室において分離した *Bacillus cereus* BQ10-S1 Spo II の培養濾液から分離・精製した細菌β-アミラーゼを，又植物β-アミラーゼとして市販の大豆及び大麦β-アミラーゼをそれぞれ用いた。さらに，これら酵素活性との比較のために市販の枯草菌α-アミラーゼ及びリゾープス・グルコアミラーゼⅠも実験に供した。

まず，各種生澱粉に対するβ-アミラーゼの分解活性を Somogyi-Nelson 法で測定した結果，細菌 β-アミラーゼが植物 β-アミラーゼの約2倍の分解活性を示し，供試生澱粉中では米と小麦澱粉が馬鈴薯と甘藷澱粉よりも比較的よく分解されることが分かった。また，他酵素との比較では，グルコアミラーゼⅠの生澱粉分解活性が最も強く，細菌β-アミラーゼ，α-アミラーゼ及び植物β-アミラーゼの順であった。

次に，兵庫県酒米試験場で栽培された山田錦，日本晴及びアメリカ原産の多収穫米ＬＡ110 の玄米を用いてそれぞれ75%精米し各5種類の生澱粉試料を得た。これら各試料の分解性を調べた結果，細菌 β-アミラーゼの場合，山田錦と日本晴からの試料の分解性がよく，とくに日本晴の90～75%精米で得られた白糠の分解性が最も高いことが分かった。また，ＬＡ110 の場合はいずれの酵素によっても比較的低い分解性を示すことなどが分かった。