Identification of Enzymes Degrading Resistant Starch From Human Colonic Bacterium *Ruminococcus bromii*

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ABSTRACT

Ruminococcus bromii is considered as an important species in the human gut that degrades efficiently resistant starch (RS) that escape the digestion by host enzymes. When RS Type 3 has been used as a source of energy, *R. bromii* have been shown to express extracellular glycoside hydrolase (GH) enzymes. Among the extracellular GH enzymes, Amy 9 has high activity at 55℃ at pH 5 in sodium acetate and high specificity towards α-1.4-glucosidic linkages, whereas Amy 10 and Amy 12 have high activity at 40℃ at pH 5 in sodium acetate and strong activity to α-1.6-glucosidic linkage about short branched chain. Although each Amy10, Amy12 were not able to degrade RS type 3, the mixture of various extracellular enzymes with Amy 9 (Amy 9 + Amy 10 or Amy 9 + Amy 12) was higher than activity of single extracellular enzyme indicating the synergistic properties of these enzymes. Our study was conducted to understand the characterization of GH enzymes which are responsible for RS degradation and the synergistic relationship between extracellular GH proteins from *R. bromii*.

RESEARCH OBJECTIVES

- Characterization of RS3 degrading enzymes in *R. bromii*
- Investigation of the role of each enzyme in RS3 degradation

INTRODUCTION

- Resistant starch (RS) is the part of starch indigestible by human enzymes and generally, it is classified into four fractions, RS1, RS2, RS3 and RS4. (Table 1)
- *Ruminococcus bromii* is known as a dominant member of the human gut microbiota and secretes various enzymes to degrade the RS3 that is the most resistant starch fraction and entirely resistant to digestion by pancreatic amylases.
- Among the various extracellular glycoside hydrolases in *R. bromii*, Amy9 could hydrolyze RS3 whereas each Amy10 and Amy12 were not active in RS3 digestion.
- When enzymes (Amy9, Amy10, and Amy12) simultaneously reacted, hydrolysis of resistant starch type 3 is more effective than single enzyme reaction.

Table 1. Type of resistant starch

Resistant starch type	Classification
Type 1	Physically inaccessible or indigestible resistant starch
Type 2	Inaccessible to enzymes due to starch conformation
Type 3	Retrograded starch
Type 4	Chemically modified to resist digestion

RESULTS

Expression of RS3 degrading enzymes

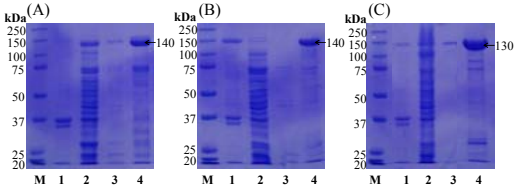

Figure 1.SDS-PAGE analysis of purified enzymes.
(A): Amy9; 140 kDa, (B): Amy10; 140 kDa,
(C) : Amy12; 130 kDa
Lane M: the molecular standard marker, Lane 1: Cell lysate supernatant of the *E. coli* cell, Lane 2: Inclusion body, Lane 3: Cell lysate passing through Ni-NTA affinity column, Lane 4: Purified enzyme

Enzymes properties

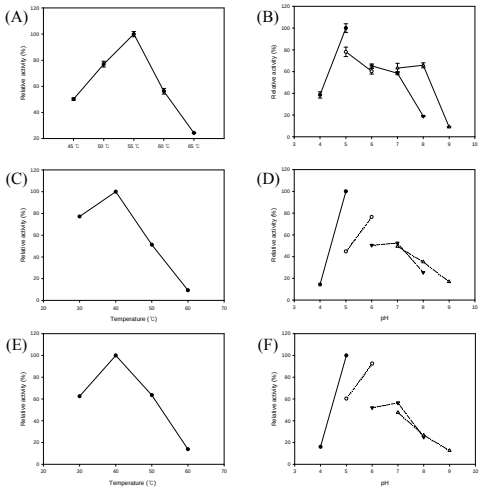

Figure 2. Effect of temperature and pH on the activity. Amy9 - (A) (B); Amy10 - (C), (D); Amy12 - (E) (F).
●, sodium acetate pH 4.0 to 5.0; ○, sodium citrate pH 5.0 to 6.0; ▼, sodium phosphate pH 6.0 to 8.0; △, Tris-HCl pH 7.0 to 9.0.

Ability of hydrolysis on RS3

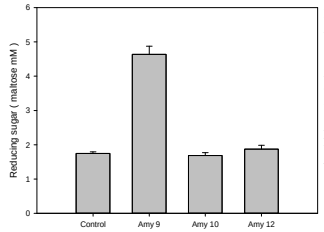

Figure 2. Degradation ability of Amy9, Amy10 and Amy12 on resistant starch type 3

A typical reaction mixture contained 0.5% Novelose (RS typ3) and 0.05 mg each of enzymes in 120 ㎕ of 50 mM sodium phosphate buffer (pH 7.0). reaction were incubated at 37 ℃ for up to 48 h.

Specific activity of enzymes in polymers

Substrates		Amy 9	Amy 10	Amy 12
Soluble starch		6.65 ±0.17	3.22 ±0.27	0.41 ±0.01
Pullulan		ND	13.18 ±0.65	11.82 ±0.05
Amylose	In Water	ND	ND	ND
	In DMSO	3.92 ±0.37	0.39 ±0.01	0.1 ±0.01
Amylopectin	In Water	ND	ND	ND
	In DMSO	0.911 ±0.01	ND	ND

ND : Not detected
Unit / mg

Table 2. Comparison of specific activity on polymer
A reaction mixture contained 0.5% carbon source (polymer) and 0.05 mg each of enzymes in 120 ㎕ of 50 mM Sodium phosphate buffer (pH 7.0). Reaction were performed at 37 ℃. Soluble starch is gelatinized at 121 ℃ for 15 min by autoclave and Pullulan was dissolved in water. Untreated Amylose and Amylopectin were dissolved in water and 90% dimethyl sulfoxide.

A-1.4-linked-malto-oligosaccharide hydrolysis pattern

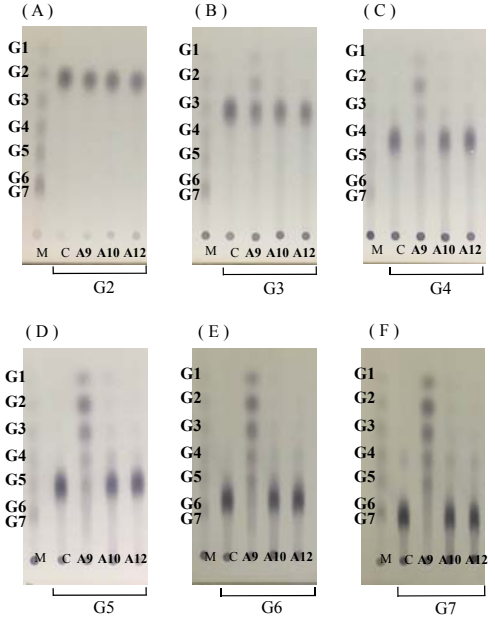

Figure 3. TLC analysis of the hydrolysis products from maltooligosaccharide.
Lane M : G1~G7 standard; Lane c: control; Lane A9: Amy9; Lane A10: Amy 10; Lane A12: Amy12

Cyclodextrin base substrate hydrolysis pattern

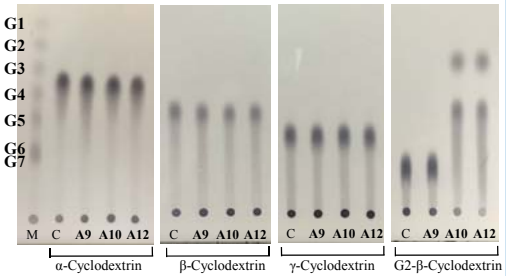

Figure 4. TLC analysis of the hydrolysis products from cyclodextrin base substrates. Lane M : G1~G7 standard; Lane c: control; Lane A9: Amy9; Lane A10: Amy 10; Lane A12: Amy12

Synergistic effect of enzymes on RS3

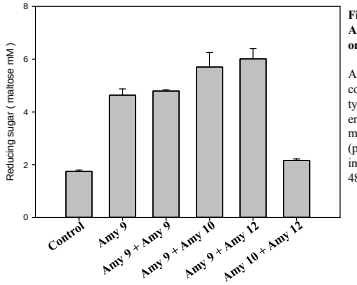

Figure 5. Synergistic effect of Amy9, Amy10 and Amy12 on resistant starch type 3

A typical reaction mixture contained 0.5% Novelose (RS typ3) and 0.05 mg each of enzymes in 1,000 ㎕ of 50 mM sodium phosphate buffer (pH 7.0). reaction were incubated at 37 ℃ for up to 48h.

CONCLUSIONS

- Amy 9 has high activity at 55℃ at pH 5 in sodium acetate and Amy 10 and Amy 12 have high activity at 40℃ at pH 5 in sodium acetate.
- Although each Amy10, Amy12 were not able to degrade RS type 3, Amy was able to degrade RS type 3.
- Amy9 had high specificity towards α-1.4-glucosidic linkages, whereas Amy10 and Amy12 showed main activity to hydrolyze the α-1.6-glucosidic linkage of short branched chain.
- The mixture of various enzymes with Amy 9 (Amy 9 + Amy 10 or Amy 9 + Amy 12) was higher than activity of single enzyme indicating the synergistic properties of these enzymes.

REFERENCES

- Ze X, et al (2012) *International Society for Microbial Ecology*. 6, 1535-1543.
- Ze X, et al (2015) *Microbiology*. 6(5), 1-11.
- Moraïs S, et al (2016) *Microbiology*. 18, 542-556.
- Moraïs S, et al (2010) Microbiology. 1, e00285-e00210.
- Murashima K, et al (2002) Journal of Bacteriology. 184, 5088-5095.