DETERMINATION OF BETA-GLUCOSIDASE ACTIVITIES OF 3 LACTOBACILLI STRAINS IN SIMULATING GASTRIC AND INTESTINAL DIGESTION

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INTRODUCTION

β-glucosidase enzyme responsible for hydrolysis of cellulose is an enzyme that are produced by microorganisms and changes isoflavone glucosidases into the form of bioactive isoflavone aglycone which are beneficial in terms of health[1]. β-glucosidase enzyme has a commercially important role with its usage in various fields such as; biotechnology, food industry and pharmacology. It is quite efficient in prevention of various chronic diseases such as cancer because it blocks the enzymes that support tumor growth[2].

METHODS

Bacteria

In this research, 3 strains (originated from human, food and animal) that belong to *Lactobacillus* species were used. Molecular definitions of them have been performed according to 16S rDNA zone.

Simulating gastric and intestinal digestion

In vitro conditions for simulating gastric and intestinal digestion were designed. The reaction mixture was incubated with 3 g/L pepsin (Sigma. 1:10000 ICN) (pH 2, pH 3, pH 4, and pH 7) for 30 min at 30°C defined as the gastric phase. The reaction mixture was incubated with 1 g/L pancreatin (Sigma, P-1500 USP). 0.30% bile salt (pH 5.5, pH 6.5, pH 7.5 and pH 8.0) for 30 min at 30°C described as the intestinal phase[3]. β- glucosidase enzyme activities of 3 strains were evaluated by using p-nitrophenyl-β-D glikopiranozit (p-NPG) as a substrate[4]. The specific activity (U/mg) is expressed as units of activity per milligram of protein[5]. The protein concentration was designated with Bradford Reagent (Amresco). The enzymatic activity was determined in the supernatant of the cultures for extracellular β-Glu activity and in the cells free extract for intracellular β-Glu activity.

RESULTS

β-glucosidase activity

In the present study, 3 *Lactobacillus* cultures were screened for *β*-glucosidase (*β*-Glu) activity. These cultures possessing enzymatic activity had a yellow color during the reaction due to the release of p-nitrophenol from the substrate pNPG. The activity was determined in the cell-free extract and was not detected in culture supernatant. The lowest and highest values of *β*-Glu activity of these cultures are portrays in Table 1. The cultures used in this study shown different levels of *β*-Glu specific activities which ranged 2.670, 3.000, and 4.500 U/mg.
L. rhamnosus MBA9 (4.500 U/mg) explained the highest specific activity, while *L. rhamnosus* EA1 (2.670 U/mg) the lowest specific activity after 24 h of incubation under the specified growth conditions

Table 1. β-glucosidase enzyme, spesific activity and protein content in MRS medium

Strains	Code	Isolation Source	Enzyme Activity (U/mL)	Protein Content (mg/mL)	Spesific Activity (U/mg)
L. casei	SC1	Human	0.030±0.001	0.010±0.001	3.000±0.001
L. rhamnosus	EA1	Food	0.069±0.001	0.026±0.001	2.670±0.001
L. rhamnosus	MBA9	Animal	0.027±0.002	0.006±0.001	4.500±0.002

Simulating gastric and intestinal digestion

β-glucosidase activities studies of enzyme activities in artificial gastric fluid and artificial intestinal fluid have been performed for 3 strains (*L. rhamnosus* MBA9, *L. rhamnosus* EA1, *L. casei* SC1). It has been determined that 3 strains can show high β-glucosidase activities by holding their liveliness in negative conditions of gastro-intestinal system (acid, bile salts).

In artificial gastric fluid, it has been determined that *L. rhamnosus* MBA9 (1.280 U/mg), *L. casei* SC1 (2.200 U/mg) and *L. rhamnosus* EA1 (1.250 U/mg) have the highest specific enzyme activity ability in pH 4.0, while *L. rhamnosus* MBA9 (0.630 U/mg), *L. casei* SC1 (1.060 U/mg) and *L. rhamnosus* EA1 (0.880 U/mg) have the lowest specific enzyme activity ability in pH 2.0 (Table 2, 3, 4).

In artificial intestinal fluid, *L. rhamnosus* MBA9 (0.800 U/mg), *L. casei* SC1 (1.170 U/mg) and *L. rhamnosus* EA1 (0.730 U/mg) have the highest specific enzyme activity in pH 8.0, while *L. rhamnosus* MBA9 (0.610 U/mg), *L. casei* SC1 (0.660 U/mg) and *L. rhamnosus* EA1 (0.560 U/mg) have the lowest specific enzyme activity ability in pH 5.5 (Table 2, 3, 4).

Table 2. *L. casei* SC1 strain protein content, β-glycosidase enzyme and specific activity in the artificial gastric fluid and artificial intestinal fluid

L. casei SC1	Artificial Gastric Fluid				Artificial Intestinal Fluid			
	pH 2.0	pH 3.0	pH 4.0	pH 7.0	pH 5.5	pH 6.5	pH 7.5	pH 8.0
Enzyme Activity (U/mL)	0.035±0.000	0.037±0.000	0.055±0.005	0.050±0.001	0.063±0.001	0.046±0.000	0.043±0.001	0.035±0.001
Protein Content (mg/mL)	0.030±0.003	0.031±0.000	0.025±0.001	0.027±0.001	0.095±0.001	0.062±0.000	0.055±0.001	0.030±0.001
Specific Activity (U/mg)	1.060±0.000	1.190±0.000	2.200±0.005	1.850±0.001	0.660±0.001	0.740±0.000	0.780±0.001	1.170±0.001

Table 3. *L. rhamnosus* EA1 strain protein content, β-glycosidase enzyme and specific activity in the artificial gastric fluid and artificial intestinal fluid

L. rhamnosus EA1	Artificial Gastric Fluid				Artificial Intestinal Fluid			
	pH 2.0	pH 3.0	pH 4.0	pH 7.0	pH 5.5	pH 6.5	pH 7.5	pH 8.0
Enzyme Activity (U/mL)	0.070±0.004	0.070±0.002	0.075±0.000	0.072±0.002	0.054±0.000	0.056±0.002	0.060±0.002	0.051±0.000
Protein Content (mg/mL)	0.080±0.002	0.068±0.002	0.060±0.001	0.065±0.001	0.097±0.002	0.080±0.002	0.085±0.001	0.070±0.001
Specific Activity (U/mg)	0.880±0.002	1.030±0.001	1.250±0.000	1.110±0.002	0.560±0.000	0.700±0.001	0.710±0.002	0.730±0.000

Table 4. *L. rhamnosus* MBA9 strain protein content, β-glycosidase enzyme and specific activity in the artificial gastric fluid and artificial intestinal fluid

L. rhamnosus MBA9	Artificial Gastric Fluid				Artificial Intestinal Fluid			
	pH 2.0	pH 3.0	pH 4.0	pH 7.0	pH 5.5	pH 6.5	pH 7.5	pH 8.0
Enzyme Activity (U/mL)	0.049±0.003	0.027±0.001	0.055±0.000	0.031±0.000	0.039±0.003	0.044±0.001	0.051±0.000	0.048±0.003
Protein Content (mg/mL)	0.078±0.001	0.035±0.001	0.043±0.001	0.040±0.000	0.064±0.002	0.067±0.001	0.068±0.000	0.060±0.001
Specific Activity (U/mg)	0.630±0.003	0.770±0.001	1.280±0.001	0.780±0.000	0.610±0.002	0.660±0.001	0.750±0.000	0.800±0.003

CONCLUSIONS

In the study, it has been determined that, the bacteria show high β-glucosidase enzyme activity by protecting their aliveness in gastro-intestinal environment. This is the first study on β-Glu activity in artificial gastric and intestinal fluids from lactobacilli. The bacteria had the ability to survive in simulating stomach and intestine conditions by exhibiting a high β-Glu activity. An alternative perspective has been gained for their probiotic characteristics in terms of tolerating the gastric acid environment and the gall in the intestine.

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