BETA-GLYCOSIDASE ENZYME ACTIVITIES OF *LACTOBACILLUS* AND HYDROLYSIS OF ISOFLAVONE

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INTRODUCTION

Phytochemicals, a type of isoflavones, representing a major group of phytoestrogens are the beneficial composites to health[1]. Soy isoflavone complements are used to treat several chronic diseases; cancer cells cardiovascular diseases and osteoporosis[2]. Biological activities of glycosides and aglycones that are in two groups of isoflavones are originating from their aglycones (genistein, daidzein), but not from their glycoside forms (genistin, daidzin). Isoflavone aglycones have been shown to be more quickly and efficiently absorbed into intestines than isoflavone glucosides. β-glucosidases can be used to convert isoflavone glucosides to aglycones[3,4]. Microorganisms are synthesized in cells with β-glucosidase enzymes β-glycoside bond breaking glucosides isoflavones ensure aglycones transformation[5]. Thus, the utility increases with the concentration of isoflavones in free form.

MATERIALS & METHODS

Bacteria

Pure cultures of 39 *Lactobacillus* spp. were obtained from the Gazi University Culture Collection.

Assay of ß-glucosidase activity

β-glucosidase activity was determined at 24 h of incubation in de Man, Rogosa and Sharpe (MRS). The β-glucosidase activity was determined by measuring the rate of hydrolysis of p-nitrophenyl β-D-glucopyranoside (pNPG). The amount of p-nitrophenol released was measured using a spectrophotometer (Hithachi) at 420 nm. One unit of the enzyme activity was defined as the amount of β-glucosidase that released 1 nmol of p-nitrophenol from the substrate pNPG per milliliter per min under assay conditions. The specific activity was expressed as units of enzyme per milligram of the protein[6]. The protein concentration was determined with Bradford Reagent. The enzymatic activity was determined in the supernatant of the cultures and in the cells free extract.

Hydrolysis of isoflavone glucosides

The highest β-glycosidase specific enzyme activity determined *L. rhamnosus* MBA9, *L. casei* SC1 and *L. rhamnosus* EA1 strains grown in MRS medium were inoculated at 2% (v/v) and incubated for 24 h at 37°C. Culture broth, 0.2 mL, was added to 1.8 mL 0.5 M potassium phosphate buffer, pH 7,5 containing 100 µg genistin or daidzin. The mixture was held at 45°C for 30 min and then boiled for 10 min. The composition of isoflavones was analyzed by HPLC[7].

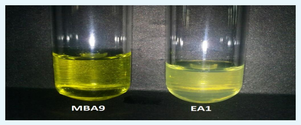
RESULTS

In the present study human-being, nutritional and animal originated 39 *Lactobacillus* species were used. β- glucosidases enzyme activities of the cultures were identified by using p-nitrophenyl-β-D glikopiranozit (p-NPG) as a substrate. In these strains, β-glycosidase specific enzyme activity were determined varies from 0.250-4.500 U/mg. For β-glycosidase enzyme belonging to *L. rhamnosus* MBA9 (4.500 U/mg), *L. rhamnosus* EA1 (2.670 U/mg), and *L. casei* SC1 (3.000 U/mg) strains was showed high β-glycosidase specific enzyme activity (Table 1).

Table 1. β-Glycosidase enzyme, specific activity and protein content in *Lactobacillus* spp.

BACTERIA		Enzyme Activity (U/mL)	Protein Content (mg/mL)	Specific Activity (U/mg)
L. acidophilus	BB1	0.050±0.001	0.050±0.003	1.000±0.001
	BB2	0.020±0.001	0.040±0.006	0.500±0.001
	BB3	0.070±0.005	0.040±0.003	1.750±0.002
	BB4	0.010±0.001	0.030±0.002	0.330±0.001
	BB5	0.020±0.003	0.050±0.001	0.400±0.001
	BB6	0.020±0.000	0.040±0.001	0.500±0.000
	BB7	0.020±0.000	0.020±0.001	1.000±0.000
	BB8	0.010±0.002	0.020±0.004	0.500±0.001
	BB9	0.020±0.001	0.060±0.006	0.330±0.004
	BB10	0.020±0.000	0.040±0.001	0.500±0.000
	BEB2	0.010±0.002	0.020±0.006	0.500±0.007
L. casei	SC1	0.030±0.001	0.010±0.001	3.000±0.001*
	SC2	0.080±0.000	0.060±0.000	1.330±0.000
	SC3	0.070±0.001	0.080±0.001	0.880±0.001
	SC4	0.080±0.000	0.060±0.001	1.330±0.000
	SC5	0.070±0.001	0.080±0.001	0.880±0.001
	SC6	0.080±0.001	0.060±0.002	1.330±0.005
	SC7	0.050±0.001	0.070±0.002	0.710±0.001
	SC8	0.060±0.001	0.070±0.001	0.860±0.001
	SC9	0.090±0.009	0.090±0.001	1.000±0.008
	KC1	0.056±0.001	0.028±0.001	2.000±0.001
	KC2	0.080±0.001	0.060±0.001	1.330±0.002
	KC3	0.090±0.000	0.110±0.001	0.820±0.000
	KC4	0.060±0.001	0.080±0.003	0.750±0.002
L. rhamnosus	MBA9	0.027±0.002	0.006±0.001	4.500±0.002*
	YAC2	0.080±0.005	0.070±0.001	1.140±0.002
	YAC4	0.060±0.007	0.060±0.001	1.000±0.002
	EA1	0.069±0.001	0.026±0.001	2.670±0.001*
L. salivarius	CAC1	0.040±0.000	0.080±0.001	0.500±0.000
	CAC2	0.030±0.000	0.020±0.001	1.500±0.000
	CAC3	0.050±0.002	0.120±0.001	0.420±0.001
L. delbrueckii ssp. *delbrueckii*	ZHC1	0.010±0.003	0.020±0.001	0.500±0.002
	ZHC2	0.020±0.001	0.020±0.002	1.000±0.001
	ZHC3	0.010±0.001	0.040±0.001	0.250±0.001
L. fermentum	KSY4	0.070±0.000	0.090±0.001	0.780±0.000
	YAC3	0.020±0.001	0.030±0.001	0.670±0.001
L. brevis	SC10	0.010±0.002	0.020±0.005	0.500±0.003
L. delbrueckii ssp. *bulgaricus*	YC1	0.050±0.005	0.110±0.001	0.450±0.003
L. paracasei ssp. *paracasei*	BEB1	0.080±0.000	0.050±0.001	1.600±0.000

Strains belonging to the genus *Lactobacillus*, p-nitrophenyl-β-D-glucopyranoside (p-NPG) as a substrate using the β-glucosidase enzyme activities and their product (p-nitrophenol) formed. p-nitrophenyl-β-D-glucopyranoside (p-NPG) containing the mixture turned into a yellow color formation is observed in the product (Picture 1).



Picture 1. *L. rhamnosus* MBA9 ve *L. rhamnosus* EA1 strains yellow color formation with β-glycosidase enzyme activity at pH 7.5

The strains that showed high β-glycosidase specific enzyme activity was determined ability hydrolyzed the isoflavone glucosides, genistin and daidzin, using high pressure liquid chromatography (HPLC). These strains hydrolyzed 42.6-56.0% of genistin and 59.8-74.0 % of daidzin (Table 2). These results support that β-glucosidase is an important enzyme which produced by *Lactobacillus* strains and can be used to transform isoflavone glucosides to beneficial for health aglycones.

Table 2. Hydrolysis of genistin and daidzin at 100 µg/mL by *Lactobacillus* strains cultured in MRS media for 24 h at 37°C.

Bacteria	Genistin			Daidzin		
	t_0 min amount (µg/mL)	t_{30} min amount (µg/mL)	Hydrolysis (%)	t_0 min amount (µg/mL)	t_{30} min amount (µg/mL)	Hydrolysis (%)
SC1	50.0	22.7	56.0	50.0	13.2	74.0
MBA9	50.0	21.3	42.6	50.0	20.1	59.8
EA1	50.0	22.4	56.0	50.0	20.8	60.0

L. casei SC1 and *L. rhamnosus* EA1 strains chromatography on hydrolyzation isoflavones genistin (Figure 1, 3) and daidzin (Figure 2,4).

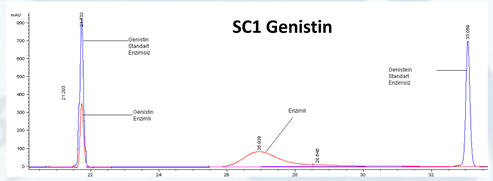


Figure 1. Chromatogram showing the hydrolysis of isoflavones genistin strain of *L. casei* SC1

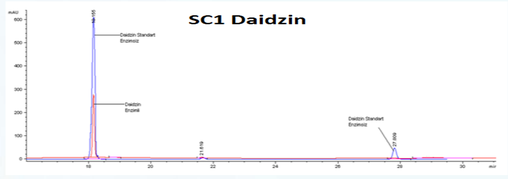


Figure 2. Chromatogram showing the hydrolysis of isoflavones daidzin strain of *L. casei* SC1

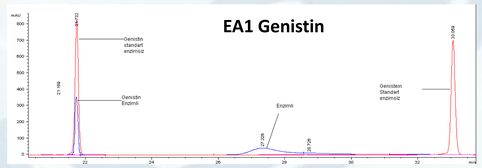


Figure 3. Chromatogram showing the hydrolysis of isoflavones genistin strain *of* *L. rhamnosus* EA1

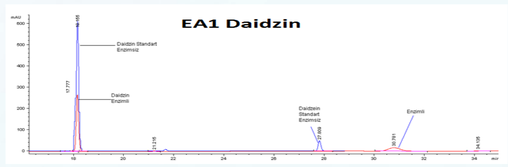


Figure 4. Chromatogram showing the hydrolysis of isoflavones daidzin strain of *L. rhamnosus* EA1

CONCLUSIONS

Marazza et al.,[8] the highest specific activity value of β-glucosidase in the *L. rhamnosus* strain CRL981 studies (22.93 U/mg); Tsangalis et al.,[9] strains of *Bifidobacterium longum*-b 4.625 ± 0.034 U/mg; Choi et al.,[7] (2002) the highest β-glucosidase enzyme activity, *L. delbrueckii* ssp. *delbrueckii* KCTC 1047 strain (0.3 unit) has determined that indicate.

Choi et al.,[7] *L. delbrueckii* ssp. *delbrueckii* KCTC 1047 hydrolyzed genistin and daidzin completely while *L. bulgaricus* KCTC 3188, *L. casei* KCTC 3109, *L. delbrueckii* KCTC 1058, *L. lactis* KCTC 2181 hydrolyzed 70–80% of genistin into genistein and 25–40% of daidzin into daidzein.

According to our results, high β-glucosidase specific activity showed *L. casei* SC1, *L. rhamnosus* MBA9, and *L. rhamnosus* EA1 strains found to be high ability to hydrolyze, these strains as biological food products with the use of starter cultures will become easier the formation of the active isoflavones.

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