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Beta-Glycosidase Activities of *Lactobacillus spp.* and *Bifidobacterium spp.* and The Effect of Different Physiological Conditions on Enzyme Activity

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Abstract

In this research, food (cheese, yoghurt) and animal (chicken) origin 39 *Lactobacillus spp.* and human origin (newborn faeces) three *Bifidobacterium spp.* were used. To designate the β-glycosidase enzyme and specific activities of the cultures, p-nitrophenyl-β-D glikopiranozit (p-NPG) was used as a substrate. The best specific activities between *Lactobacilli* cultures were observed at *Lactobacillus rhamnosus* BAZ78 (4.500 U/mg), *L. rhamnosus* SMP6-5 (2.670 U/mg), *L. casei* LB65 (3.000 U/mg) and *L. casei* LE4 (2.000 U/mg) strains. Bifidobacterium breve A28 (2.670 U/mg) and B. longum BASO15 (2.330 U/mg) strains belonging to the Bifidobacterium cultures had the highest specific activity capabilities. Optimization studies were performed to designate the impact of different pH, temperature, and carbon sources on the β-glucosidase enzyme of *L. rhamnosus* BAZ78 strain (β-Glu-BAZ78), which exhibits high specific activity. As optimum conditions, pH was detected as 7.5, the temperature as 30° C, and the carbon source as 2% glucose for the enzyme. Although the enzyme activity changed as the physiological conditions changed, the β-Glu-BAZ78 showed the highest specificity in the control groups.

Keywords:

Lactobacillus, Bifidobacterium, β-glycosidase enzyme activity, probiotic, optimization

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Introduction

Bifidobacterium spp. is a widespread inhabitant of the human intestinal system during life (Modrackova et al., 2020). Gram-positive lactobacilli are in the Lactic acid bacteria (LAB) group and are outstanding constituents of the intestinal microbiome. Due to their useful health impacts,

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lactobacilli and bifidobacteria are used as probiotics and accepted GRAS (generally regarded as safe), which means they are secure when used properly (Naidu et al., 1999; Garbacz, 2022). Beneficial bacteria that can enhance the microbial environment of the intestinal system, arrange immune-concerned genes and augment resistance to pathogens are called probiotics (Kumar et al., 2917; Meng et al., 2017; Gao et al., 2018). Some inhibitory substances produced by lactic acid bacteria and bifidobacteria have beneficial effects on health such as inhibiting the growth of pathogenic microorganisms in the gastrointestinal tract, playing a role in regulating the immune system, maintaining their vitality in the gastrointestinal tract, and having the ability to grow at low pH values and with bile salts in a wide temperature range. Therefore, they are used as probiotics (Gao et al., 2018; Zhu et al., 2022). In addition, these bacteria are used as human probiotics because of their useful contribution effects such as improving the intestinal microbiota, preventing diarrhoea (Maldonado et al., 2019), strengthening the immune system, lowering the cholesterol level in the blood, improving lactose digestibility, facilitating calcium absorption (O'Callaghan ve van Sinderen, 2016), suppressing cancer cells (Garbacz, 2022), strengthening mineral absorption, and sticking to the intestinal mucosa (Dupont et al., 2014, Kosmerl et al., 2021).

Polysaccharides (especially cellulose) are compounds that are abundant in the biosphere and constitute an important source of recyclable chemicals and fuels. A cellulase enzyme complex secreted by cellulolytic organisms can hydrolyze cellulose to glucose. The enzyme complex includes three enzymes: endoglucanase, cellobiohydrolases, and β-glucosidase (Zang et al., 2018; Ariaeenejad et al., 2020). Endogluconases break off the inner β-1,4-glycosidic bonds of cellulose by hydrolysis to form new chain ends, and exoglucanases form soluble cellobiose units by cutting these new cellulose chains formed at the ends (Teugjas & Väljamäe, 2013; Pang et al., 2017). The enzyme β-glucosidase, which is responsible for the hydrolysis of cellulose, has been the focus of many studies because cellulose is the amplest substrate on earth and an essential renewable energy source in the future (Kara et al., 2011; Tamaki et al., 2016; Seidel & Lee, 2020). β-Glucosidases are a heterogeneous group of hydrolytic enzymes. This enzyme plays a crucial role in various biological activities like cellular signalling, biosynthesis and degradation of structural and storage polysaccharides, and host-pathogen interactions (Singh et al., 2016; Zang et al., 2018).

β-Glucosidases are extensively used in different processes like the hydrolysis of isoflavone glycosides, the generation of fuel ethanol from agricultural residues, and the dismissal of aromatic compounds from unwanted precursors (Singhania et al., 2013; Chen et al., 2021). β-Glucosidases could be generated by several organisms, such as fungi (*Aspergillus niger, Penicillium decumbens*), yeasts (*Candida spp.)* archea, and a few bacteria (Singh et al., 2016; Zang et al., 2018; Chen et al., 2021). Also, plants can accumulate β-glucosides and β-glucosinolates in their bodies and release them into the environment when needed. β-glucosidic substrates and β-glucosidases are stockpiled in different substructures or tissue sections of the cell. As a result of damage to the cell when pathogens or herbivores come to plant tissues, the substrate and the enzyme combine. At this time, the hydrolysis of the substrates begins and the aglycons or other degradation products released as

a result of hydrolysis create a toxic effect and prevent the entry and dispersal of harmful organisms to the plants. Thus, β-glucosidase enzymes play a significant role in the defence system against plant pests. β-glucosidases are highly effective in improving the quality of wine, tea, and fruit juice. Aglycones are structures that are highly effective in food quality and production. However, aglycones must be hydrolyzed by β-glucosidase enzymes to form. When the β-glucosidase enzyme is added during or after production, an increase is observed in the taste, flavour, aroma, and other quality factors of the products (Grohmann et al., 1999; Sener, 2015).

In this research, it was aimed to detect β-glucosidase enzyme activities with p-nitrophenyl-β-D glycopyranoside (p-NPG) substrate of *Lactobacillus* and *Bifidobacterium* strains isolated from various sources. Optimization studies of enzyme activities of *L. rhamnosus* BAZ78 strain showing high specific activity at different pH, temperature, and carbon sources were also aimed at.

Materials and Methods

In this study, 39 *Lactobacillus spp*. (*L. casei* (13), *L. acidophilus* (11), *L. rhamnosus* (4), *L. delbrueckii* subsp. *delbrueckii* (3), *L. brevis* (1), *L. paracasei* subsp. *paracasei* (1), *L. delbrueckii* subsp. *bulgaricus* (1), *Ligilactobacillus salivarius* (3), *Limosilactobacillus fermentum* (2)), human (17), food (4) and animal (18) origin, and 3 *Bifidobacterium spp.* (*B. breve* (2) and *B. longum* (1)) strains, isolated from newborn faeces, were used. The strains were obtained from the Gazi University Faculty of Science Biotechnology Laboratory Culture Collection.

Man & Rogosa and Sharp (MRS) medium was used to encourage the growth of lactobacilli and the determination of β-glucosidase enzyme activities. To determine the effect of media used in enzyme optimization studies on enzyme activity, 2% sucrose, lactose, fructose, and cellobiose added media were used instead of glucose in the MRS medium. Trypticase Phytone Yeast Extract medium (TPY) was used to encourage the growth of bifidobacteria cultures and to determine β-glucosidase activities, and Man & Rogosa and Sharp medium with Modified Cysteine (MMRSC) and Man & Rogosa and Sharp medium with Cysteine (MRSC) were used for enzyme optimization studies. In addition, all experimental studies with bifidobacteria were carried out using an anaerobic kit (Oxoid, Anaerobic generating kit), which provides 10% CO2 release to the environment in the anaerobic jar (Oxoid, Anaerojar).

Determination of B-Glucosidase Activities of Strains

To designate the enzyme activities of the bacterial cultures, after the cultures were developed in the appropriate medium, they were centrifuged at 5000 rpm for 20 min at 4ºC (Sigma 2-16 KC). Cell pellet and supernatant were separately washed twice with 0.5 M potassium phosphate buffer (PBS) (0.02% KCl, 0.144% Na_2HPO_4, 0.8% NaCl, 0.024% KH_2PO_4, pH 7.0) and their optical densities were adjusted to Mc Farland 5 (~ 15 log cfu/mL). To break down the cell wall of bacteria, the samples were kept for 5 min in an ultrasonication device (Vibra-Cell, Sonics & Materials Inc.

Danbury, CT USA brand) tuned to 50 MHz frequency and ice was added. Centrifugation was applied at 1000 rpm for 10 min at 4ºC to precipitate cell wastes. For the measurement of β-glucosidase activity, p-NPG was used as a substrate (Strahsburger et al., 2017). Then 0.5 mL of the cell suspension was added to the mixture containing 2 mL 2.5 mM p-NPG in 0.5 M PBS (pH 7.5), and the mixture was incubated for 30 min at 30°C. The reaction was stopped by keeping it at 95ºC for 5 min. The β-glucosidase activities of the cultures were detected by measuring at a wavelength of 420 nm in a spectrophotometer (Hitachi UV-1800, Japan) (Choi, 2002). It was prepared by using 1 mL of 0.5 M PBS (pH 7.5) instead of the crude extract as a blind.

One unit of β-glucosidase enzyme is the amount of enzyme that catalyzes the formation of 1 μmol of product per minute or the conversion of 1 μmol of the substrate (U=μmol/min). The specific activity of an enzyme is the number of enzyme units per 1 mg of protein. The β-glucosidase enzyme activity of the cultures was calculated using the Beer-Lambert law as stated below (Temizkan et al., 2008).

Enzyme unit in the sample = (Vt x dA/dt x 1000 x dilution factor)/(ελ x vs x d)= (U/L)

dA/dt: Change in absorbance per unit time (minute) (min^{-1})

ελ: Absorption coefficient (cm^2 mol^{-1})

d: The light path of the cuvette (usually 1 cm)

Vt: Total volume of the reaction mixture (mL)

vs: Volume of the sample (enzyme) involved in the reaction (mL)

Specific Activity (U/mg) = (Enzyme activity (U/mL)/(Protein amount (mg/mL)

Moreover, the relative activity of the strain with the highest specific activity was calculated according to the below formula (Yüksekdağ & Yüksekdağ, 2021).

Relative activity (%) = (Investigated parameter's enzyme activity/Enzyme activity at optimum conditions) x 100.

The Effect of Different Physiological Conditions on the B-Glucosidase Enzyme

L. rhamnosus BAZ78 strain, which showed the highest specific activity, was selected from the cultures whose β-glucosidase activities were determined, and enzyme optimization studies were carried out at different pH, temperature, and carbon sources. To determine the activities at different pHs, the pH of 0.5 M PBS was adjusted to 4.0, 5.0, 6.0, 7.0, 7.5 (control), and 8.0 using 3 M HCl and 3 M NaOH. After adding 0.5 mL of the cell suspension to the mixture including 2 mL 2.5 mM p-NPG in 0.5 M PBS (pH 7.5), the mixture was mixed at different temperatures (30ºC-control,

37ºC, 40ºC, 50ºC, and 60ºC) and the impact of temperature on the activity was designated by measuring the enzyme activity of the mixture, which was left to incubate at different temperature values. To specify the β-glucosidase activities of the samples in different carbon sources, instead of 2% glucose (control), fructose, sucrose, lactose, and cellobiose were added into MRS broth, which is the most suitable carbon source for the development of BAZ78.

Statistical Analysis

All studies were carried out in three parallels and three replications and the average results of the studies were given. The data obtained were analyzed using the SPSSe statistical software (IBM SPSS Statistics Data Editor, version 22). With the Pearson correlation, it was investigated whether there was a correlation between the enzyme activity-protein amount and protein amount-specific activities of the strains.

Results

Due to their probiotic properties, lactobacilli and bifidobacteria could be an alternative to plants as a source of β-Glucosidase enzyme, which has a very different commercial use potential. For this reason, this study, it was aimed to investigate safe and new lactobacilli and bifidobacteria with high enzyme activity.

B-Glucosidase Enzyme Activity

Strains belonging to *Lactobacillus* and *Bifidobacterium* genera formed a product (p-nitrophenol) with their β-glucosidase enzyme activities by using p-NPG to be a substrate. Product formation was seen when the mixture containing p-NPG turned yellow. β-glucosidase enzyme activities, protein amounts, and specific activities observed in the pellets of the cultures are given in Table 1.

Among the cultures belonging to the genus *Lactobacillus*, the highest specific activity was in L. rhamnosus BAZ78 (4.500 U/mg), and the lowest specific activity was in *L. delbrueckii* subsp. *delbrueckii* ZYN31 (0.250 U/mg). It was determined that *B. breve* A28 (2.670 U/mg) and *B. longum* BASO15 (2.330 U/mg) strains had the highest specific activity ability among the cultures belonging to the *Bifidobacterium* genus (Table 1). When the specific activities of the bacteria were evaluated, significant differences in activity were observed between strains in both species ($p<0.01$).

Table 1. The lowest and highest values of the β-glycosidase enzyme, specific enzyme activity, and protein content of *Lactobacilli* and *Bifidobacteria*

Bacteria	***Protein content*** **(mg/mL)**	***Enzyme activity*** **(U/mL)**	***Specific activity*** **(U/mg)**
L. acidophilus BAZ54	0.050±0.003	0.050±0.001	1.000±0.001
L. acidophilus BAZ51	0.020±0.006	0.010±0.002	0.500±0.007
L. acidophilus BAZ59	0.030±0.002	0.010±0.001	0.330±0.001
L. acidophilus BAZ63	0.020±0.001	0.020±0.000	1.000±0.000
L. acidophilus BAZ29	0.040±0.006	0.020±0.001	0.500±0.001
L. acidophilus BAZ43	0.020±0.005	0.010±0.002	0.500±0.003
L. acidophilus BAZ22	0.020±0.004	0.010±0.002	0.500±0.001
L. acidophilus BAZ61	0.050±0.001	0.020±0.003	0.400±0.001
L. acidophilus BAZ36	0.040±0.001	0.020±0.000	0.500±0.000
L. acidophilus ZYN13	0.060±0.006	0.020±0.001	0.330±0.004
L. acidophilus ACS6	0.040±0.003	0.070±0.005	1.750±0.002
L. casei LB65	0.010±0.001	0.030±0.001	3.000±0.001
L. casei LB68	0.060±0.001	0.080±0.000	1.330±0.000
L. casei LE4	0.028±0.001	0.056±0.001	2.000±0.001
L. casei LE7	0.060±0.001	0.080±0.001	1.330±0.002
L. casei LB17	0.110±0.001	0.090±0.000	0.820±0.000
L. casei LB19	0.060±0.002	0.080±0.001	1.330±0.005
L. casei LB6	0.080±0.001	0.070±0.001	0.880±0.001
L. casei LB23	0.070±0.001	0.060±0.001	0.860±0.001
L. casei LB49	0.080±0.001	0.070±0.001	0.880±0.001
L. casei LB61	0.080±0.003	0.060±0.001	0.750±0.002
L. casei LB83	0.060±0.000	0.080±0.000	1.330±0.000
L. casei LB74	0.090±0.001	0.090±0.009	1.000±0.008
L. casei LB64	0.070±0.002	0.050±0.001	0.710±0.001
L. rhamnosus GD11	0.070±0.001	0.080±0.005	1.140±0.002
L. rhamnosus LP2	0.060±0.001	0.060±0.007	1.000±0.002
L. rhamnosus BAZ78	0.006±0.001	0.027±0.002	4.500±0.002
L. rhamnosus SMP6-5	0.026±0.001	0.069±0.001	2.670±0.001
L. salivarius ZYN9	0.020±0.001	0.030±0.000	1.500±0.000
L. salivarius ZYN15	0.120±0.001	0.050±0.002	0.420±0.001
L. salivarius ZYN23	0.080±0.01	0.040±0.000	0.500±0.000
L. delbrueckii subsp. delbrueckii ZYN33	0.020±0.002	0.020±0.001	1.000±0.001
L. delbrueckii subsp. delbrueckii ZYN31	0.040±0.001	0.010±0.001	0.250±0.001
L. delbrueckii subsp. delbrueckii BAZ32	0.020±0.001	0.010±0.003	0.500±0.002
L. fermentum ZYN17	0.030±0.001	0.020±0.001	0.670±0.001
L. fermentum LB16	0.090±0.001	0.070±0.000	0.780±0.000
L. brevis LB63	0.080±0.002	0.050±0.001	1.600±0.001
L. delbrueckii subsp. bulgaricus B3	0.110±0.001	0.050±0.005	0.450±0.003
L. paracasei subsp. paracasei BKS20	0.050±0.001	0.080±0.000	1.600±0.000
B. breve A26	0.050±0.000	0.060±0.000	1.200±0.000
B. breve A28	0.027±0.000	0.072±0.000	2.670±0.000
B. longum BASO15	0.030±0.005	0.070±0.000	2.330±0.000

The Effect of Different Physiological Conditions on Enzyme Activity

L. rhamnosus BAZ78 strain with the highest specific activity was selected for use in enzyme optimization (pH, temperature, and carbon source) studies. Enzyme activities, specific activities, and protein amounts were determined by using 0.5 M PBS adjusted to various pH values (4.0, 5.0, 6.0, 7.0, 7.5, and 8.0) of the L. rhamnosus BAZ78 strain (Table 2).

Table 2. Effect of pH. temperature and media on the β-glycosidase enzyme, specific enzyme activity, and protein content in *Lactobacillus rhamnosus* BAZ78 strain

	pH					
	4.0	**5.0**	**6.0**	**7.0**	**7.5 (Control)**	**8.0**
Protein content (mg/mL)	0.010±0.001	0.008±0.001	0.005±0.003	0.006±0.001	0.006±0.002	0.007±0.001
Enzyme activity (U/mL)	0.014±0.007	0.014±0.001	0.017±0.010	0.024±0.005	0.027±0.004	0.014±0.010
Specific activity (U/mg)	1.400±0.007	1.800±0.001	3.400±0.005	4.000±0.001	4.500±0.002	2.000±0.009

	Temperature (° C)				
	30 (Control)	**37**	**40**	**50**	**60**
Protein content (mg/mL)	0.006±0.002	0.006±0.000	0.007±0.002	0.009±0.002	0.010±0.001
Enzyme activity (U/mL)	0.027±0.004	0.021±0.000	0.018±0.003	0.020±0.005	0.018±0.001
Specific activity (U/mg)	4.500±0.002	3.500±0.000	2.570±0.002	2.220±0.003	1.800±0.001

	Carbon Sources				
	Glucose (Control)	**Fructose**	**Lactose**	**Sucrose**	**Cellobiose**
Protein content (mg/mL)	0.006±0.002	0.025±0.001	0.032±0.001	0.028±0.001	0.030±0.001
Enzyme activity (U/mL)	0.027±0.004	0.040±0.001	0.041±0.003	0.032±0.005	0.036±0.001
Specific activity (U/mg)	4.500±0.002	1.600±0.001	1.280±0.003	1.143±0.005	1.200±0.001

The protein amount of the β-Glu-BAZ78 at different pH values was determined as 0.005-0.010 mg/mL, β-glucosidase enzyme activity as 0.014-0.027 U/mL, and specific activity as 1.400-4.500 U/mg. It was observed that the β-Glu-BAZ78 did not lose its activity at different levels of pH, and the specific activity was the best at pH 7.5 and the lowest at pH 4.0 (Figure 1).

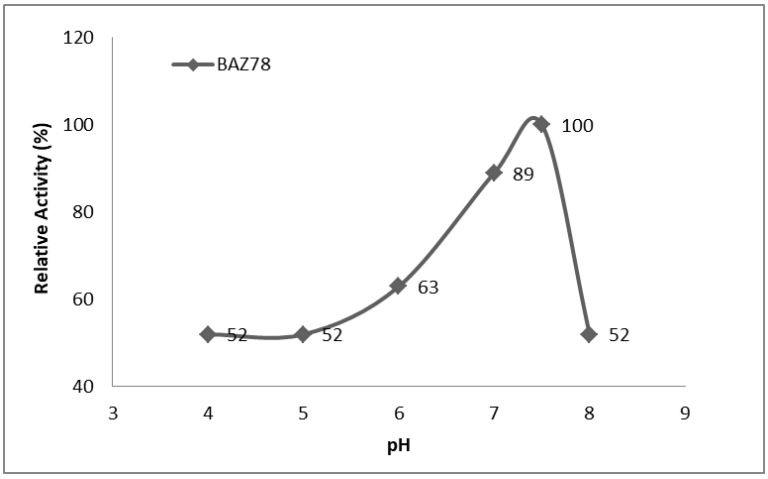

Figure 1. Effect of pH on β-glucosidase enzyme

The mixture containing 2 mL 2.5 mM of p-NPG, in 0.5 M potassium phosphate buffer (pH 7.5) was added to 0.5 mL of the BAZ 78 cell suspension. The mixture was then incubated at different temperatures (30º C, 37º C, 40º C, 50º C, and 60º C). The impacts of different temperatures on the enzyme activity are given in Table 2. The protein amount of the β-Glu-BAZ78 was designated as 0.006-0.010 mg/mL, enzyme activity was detected as 0.018-0.027 U/mL, and specific activity was 1.800-4.500 U/mg at varying temperature values. The optimum temperature of the β-Glu-BAZ78 was found to be 30º C (Figure 2).

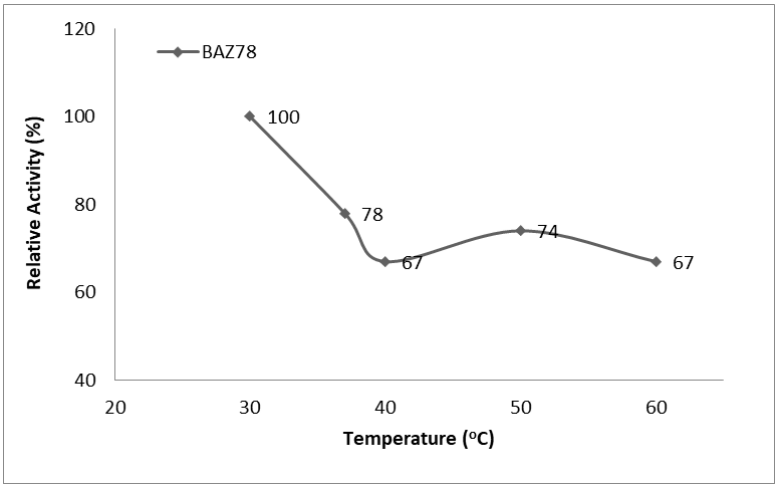

Figure 2. Effect of temperature on β-glucosidase enzyme

The specific activities, enzyme activities, and protein amounts of the strains were determined by using different carbon sources (lactose, sucrose, fructose, cellobiose) at the same rate instead of 2% glucose in the MRS medium in which the *L. rhamnosus* BAZ78 strain was developed (Table 2). The protein amount of the enzyme in different carbon source MRS medium of BAZ78 strain was determined as 0.006-0.032 mg/mL, enzyme activity as 0.027-0.041 U/mL, and specific activity as 1.143-4.500 U/mg. It was detected that the most suitable carbon source for the β-Glu-BAZ78 was MRS medium containing 2% glucose. The lowest specific activity was observed in the medium containing 2% sucrose (Figure 3).

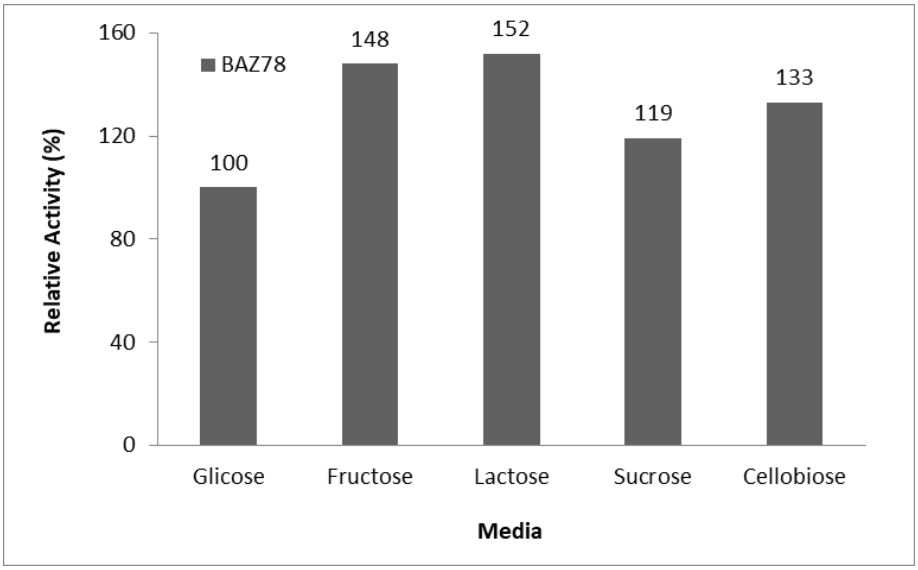

Figure 3. Effect of different media on β-glucosidase enzyme

Discussion

Isoflavones are present as the forms of glycosides and aglycones. The high content of isoflavones exists in glycoside forms but the biological activity of isoflavones is primarily from aglycones. Thus, it is important and necessary to transform isoflavone glycosides into the aglycones form (Lu et al., 2022). The β-glucosidase enzyme is a substantial enzyme that could convert the glucoside form of isoflavone to the aglycone form, which is beneficial for health and generated by microorganisms. Although the β-glucosidase enzyme is more common in plants, moulds, and yeasts, it can be generated by lactic acid bacteria and bifidobacteria (Esteve-Zarzoso et al., 1998; Marazza et al., 2009; Yüksekdağ et al., 2018).

In this research, the β-Glucosidase enzyme activity of *Lactobacillus* and *Bifidobacterium* bacteria was investigated. Since the β-Glucosidase enzyme is an intracellular enzyme (Marazza et al., 2009; Yüksekdağ et al., 2018; Huang et al., 2021; Lenz et al., 2022), ultrasonication, which is widely used as a mechanical method, was used to break down the cell wall of bacteria in enzyme extraction in our study (Carević vd., 2015). To support the intracellular β-glucosidase enzyme

activity of 42 strains belonging to 39 lactobacilli and 3 bifidobacteria cultures, the enzyme activity was also examined in the culture supernatants. No enzyme activity was found in the culture supernatants. Forty-two strains isolated from different sources (human, food, animal) were used, and differences in enzyme activities (Table 1) were observed according to the isolation source. In general, when the specific activities of the strains are evaluated, it is seen that the enzyme activities of animal origin strains (except *L. rhamnosus* BAZ78) are low, while the enzyme activities of human origin strains are higher than other strains.

L. rhamnosus BAZ78 strain with low protein content and high enzyme activity has high specific activity ability, while the strains with high protein content and low enzyme activity (*B. breve* A28 and *B. longum* BASO15) show low specific activity (Table 1). However, although the enzyme activities were high in some strains, a decrease in specific enzyme activities was observed due to the high protein content. To support this situation, Pearson's correlation test was applied and it was seen that there was a moderate negative correlation between protein amount and specific enzyme activity since r= - 0.582, and this relationship is significant since $p<0.01$. Since r= 0.787 between enzyme activity and protein amount, a high level of positive correlation ($p<0.01$) was determined. Marazza et al. (2009) reported that they observed the highest specific activity in *L. rhamnosus* CRL981 (22.93 UE/mg) strain in their study and they did not find enzyme activity in the cell supernatant. Tsangalis et al. (2002) reported that Bifidobacterium longum-b strain had β-glucosidase enzyme activity at the amount of 4.625±0.034 U/mg. Choi et al. (2002) determined the β-glucosidase activities of *L. bulgaricus* KCTC 3188, *L. casei* KCTC 3109, *L. delbrueckii* KCTC 1047, *L. delbrueckii* KCTC 1058, and *L. lactis* KCTC 2181 in MRS medium. At the end of the study, the highest β-glucosidase enzyme activity was observed by *L. delbrueckii* subsp. delbrueckii KCTC 1047 strain (0.3 units). In addition, it was reported that other strains did not have significant β-glucosidase activity. Yüksekdağ et al. (2018) determined β-glucosidase specific activities of potential probiotic bacteria. The authors reported that β-Glu-specific activity varied from 0.250 to 3.000 U/mg. *L. casei* SC1 (2.750 U/mg), and *L. rhamnosus* EA1 (2.100 U/mg) showed the highest specific activity. In this study, among lactobacilli cultures, the *L. rhamnosus* BAZ78 strain exhibited the best specific activity at 4.500 U/mg and B. longum BASO15 (2.330 U/mg) demonstrated the highest specific activity among the bifidobacteria cultures. It was seen that the results obtained from our research were close to other results.

For the β-glucosidase enzyme to be used in industrial applications, it is necessary to determine the most suitable physical conditions in which the enzyme is active, as well as its high enzyme activity. Enzyme reactions are impressed by pH. Amendments in the ionization of prototropic groups in the active centre of the enzyme at low acidic and high alkaline pHs affect the appropriate conformation of the active centre, the binding of the substrate, and the catalysis of the reaction (Koolman & Roehm, 2005). Because pH affects the rate of an enzyme-catalyzed reaction, it is necessary to know its effect on the activity of an enzyme. In this study, the pH of 0.5 M PBS was adjusted to 4.0-8.0 to observe the impact of pH on β-Glu-BAZ78. An increase in enzyme

activity was observed from pH 4.0 to pH 7.5, the highest activity was determined at pH 7.5 (4.500 U/mg) (Table 2), and relative activity was observed to be 52-100% (Figure 1). A decrease in activity was observed after the optimum pH value. In line with all these results, it can be said that the optimum pH value to determine β-glucosidase enzyme activity is 7.5. L. delbrueckii subsp. delbrueckii KCTC 1047 strain, which showed high β-glucosidase enzyme activity, the optimum pH value was reported as 6.0 (Choi et al. 2002). Marazza et al. (2009) adjusted the pH of 100 mM McIlvaine buffer to 3.0-8.0 to designate the impact of pH on enzyme activity. They determined that the optimum pH value for *L. rhamnosus* CRL981 strain showing high specific activity was 6.4. Sestelo et al. (2004) developed the *Lactobacillus plantarum* strain isolated from wine at pH 4.5-7.5 and determined that the optimum pH value was 5.0. According to the results of the analysis carried out to determine the effect of pH on the specific enzyme activity, it was seen that the optimum pH value differs in all studies. Several amino acids in an enzyme molecule carry a charge. Within the enzyme molecule, positively and negatively charged amino acids will attract (Datta et al., 2017). Changing the pH will impress the ionization state of acidic or basic amino acids. When the pH value of the reaction medium changes, the shape and structure of the enzyme will change. The structure of the enzyme has a strong effect on the enzyme activity and alterations in the structure of the enzyme influence the rate of chemical reactions. It is thought that the differences may be due to these reasons. In addition, differences in the isolation source, buffer, extraction method, and enzyme and substrate concentration of the strains may also be triggers.

Enzyme activity increases with temperature in the temperature range at which enzymes are stable. When the temperature rises above a certain value, a decrease in activity is observed because the enzyme protein will begin to denature (Pamuk, 2011; Kılıç et al., 2014). Therefore, it is significant to determine the temperature value at which the enzyme has high activity and the impact of temperature on enzyme activity.

In the study, the enzyme and specific activities of the β-Glu-BAZ78 were determined at 30º C, 37º C, 40º C, 50º C, and 60º C temperature values. The highest β-glucosidase enzyme and specific activity were observed at 30ºC, while the lowest specific activity was detected at 60º C. Relative activity was calculated to be 67-100% (Figure 2). In addition, although specific activity and enzyme activity decreased in the BAZ78 strain at 60º C, an increase in protein amount was observed. This suggests that protein structures can be resistant to high temperatures and this strain could be used commercially. It has been reported that enzyme activity shows effective activity up to 45º C, and enzyme activation decreases above this temperature value (Choi et al., 2002). Marazza et al. (2009) declared that the optimum temperature value for *L. rhamnosus* CRL981 strain was 42º C, while Coulon et al. (1998) reported that *L. casei* ATCC 393 strain had the highest β-glucosidase activity at 35º C. Sestelo et al. (2004) determined the enzyme activity of *L. plantarum* strain at the temperature values of 30-55º C and determined that the enzyme activity was at the highest value at 45º C. Marazza et al. (2009) enzyme at 50º C for 5 min. They found that the β-glucosidase activity of *L. rhamnosus* CRL981 strain decreased by 20% when they were kept waiting, and Sestelo et al.

(2004) found a 50% decrease in the enzyme activity of *L. plantarum* when they applied the same procedure. It is seen that the suitable temperatures for the enzyme activity are different in the studies, and in this study, the enzyme activity changed depending on the temperature.

Determination of the impact of various carbon sources on the growth of microorganisms can provide information about their biotope, particularly the nutrients necessary for their improvement (Alvarez-Zúñiga et al., 2020). In the study, the impact of carbon sources on enzyme activity was determined by growing *L. rhamnosus* BAZ78 strain in different carbon sources (glucose, fructose, sucrose, lactose, cellobiose). It was determined that the BAZ78 strain showed the highest (4.500 U/mg) β-glucosidase-specific enzyme activity in MRS medium containing 2% glucose, and the lowest (1.140 U/mg) in medium containing 2% sucrose. According to the results, relative activity was designated to be 100-152% (Figure 3). Tsangalis et al. (2002) analyzed five strains of Bifidobacteria (*B. pseudolongum, B. longum-a, B. longum-b, B. animalis, B. infantis*) in MRS liquid to designate the impact of carbon sources used on enzyme activity. The authors used different carbon resources (MRS-glucose, MRS-lactose, and MRS-raffinose). They declared the specific activity ranges of the strains in their media as follows: 0-0.779 U/mg in MRS, 0-4.625 U/mg in MRS-glucose, 0-3.651 U/mg in MRS-lactose, and 0-0.780 U/mg in MRS-raffinose. Researchers reported that the *B. infantis* strain did not show any enzyme activity in the carbon source used, while other strains had high β-glucosidase activity in MRS-glucose liquid media.

In conclusion, the β-glucosidase enzyme has attracted the attention of both scientists and entrepreneurs due to its industrial importance. Therefore, in this study, it is important to identify probiotic strains with high enzyme activity. For this purpose, *Lactobacillus spp.* (39) and *Bifidobacterium spp*. (3) β-glucosidase enzyme activities, protein amounts, and specific activities of strains were determined. *L. rhamnosus* BAZ78 strain with the highest specific activity among these cultures was used for enzyme optimization studies. To the results obtained, β-Glu-BAZ78 could be used for industrial purposes by being purified.

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Author Contributions

B.C.A. performed all the experiments and drafted the main manuscript text. B.C.A. and Z.N.Y. designed the experimental work and final versions of the statistics table. Z.N.Y. reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest/competing interests.

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