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Alpha-Amylase Activity of Lactic Acid Bacteria Isolated from Different Fermented Products: Characterization of *Latilactobacillus Curvatus* Y2-1B Amylase

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Keywords
Latilactobacillus curvatus,
Amylase,
Specific activity,
Thermostable

Abstract: In this study, ninety-one lactic acid bacteria isolates (LAB) were obtained from different fermented products and their ability to produce α-amylase was qualitatively examined using starch added medium. Of the LAB isolates, 75 LABs were qualitatively confirmed to be amylase positive, and 20 LABs in total were found to exhibit significant α-amylase production ability. Quantitative tests of α-amylase enzyme activity were then conducted and enzyme activity values for LABs were found in the range of 1.283 U/mL to 13.670 U/mL. Then, 3 LAB isolates (P4-2B, Y2-1B, S1-2B) were selected and identified by genotypic methods. The enzyme from the *Latilactobacillus curvatus* Y2-1B isolate was partially purified, and activity tests were conducted to ascertain the impact of pH, temperature, and cations. The lowest enzyme activity was found to be 0.554 U/mL at pH 9 and 0.694 U/mL at pH 6 as the highest value ($p<0.05$). On the other hand, the purified enzyme formed a band of approximately 40 kDa in SDS-PAGE while the presence of the genes *amy* was examined and approved. It was concluded that selected LAB isolates may be useful in industrial biotechnology and microbial enzyme industry due to their α-amylase enzyme activity.

Farklı Fermente Ürünlerden İzole Edilen Laktik Asit Bakterilerinin Alfa-Amilaz Aktivitesi: *Latilactobacillus Curvatus* Y2-1B Amilazının Karakterizasyonu

Anahtar Kelimeler
Latilactobacillus curvatus,
Amilaz,
Spesifik aktivite,
Termostabil

Öz: Bu çalışmada, farklı fermente ürünlerden doksan bir laktik asit bakterisi izolatı (LAB) elde edilmiş ve α-amilaz üretme yetenekleri nişasta eklenmiş ortam kullanılarak kalitatif olarak incelenmiştir. LAB izolatlarından 75 LAB'nin kalitatif olarak amilaz pozitif olduğu doğrulanmış ve toplamda 20 LAB'nin önemli α-amilaz üretim yeteneğine sahip olduğu bulunmuştur. Daha sonra α-amilaz enzim aktivitesinin kantitatif testleri yapılmış ve LAB'lar için kantitatif enzim aktivite değerleri 1.283 U/mL ile 13.670 U/mL arasında bulunmuştur. Daha sonra, 3 LAB izolatı (P4-2B, Y2-1B, S1-2B) seçilerek genotipik yöntemlerle tanımlanmıştır. Tanımlamadan sonra, *Latilactobacillus curvatus* Y2-1B izolasyonundan elde edilen enzim kısmen saflaştırılarak pH, sıcaklık ve katyonların etkisini belirlemek için aktivite testleri yapılmıştır. Enzim aktivitesinin en düşük değeri pH 9'da 0.554 U/mL ve en yüksek değeri pH 6'da 0.694 U/mL olarak bulunmuştur ($p<0.05$). Öte yandan, saflaştırılmış enzim SDS-PAGE'de yaklaşık 40 kDa'lık bir bant oluştururken, *amy* genlerinin varlığı incelenmiş ve doğrulanmıştır. Sonuç olarak seçilen LAB izolatlarının α-amilaz enzim aktivitesi nedeniyle endüstriyel biyoteknoloji ve mikrobiyal enzim endüstrisinde faydalı olabileceği değerlendirilmiştir.

1. INTRODUCTION

Enzymes have specialized catalytic functions that are formed by living organisms and catalyze biological and chemical reactions [1]. They are involved in many biochemical reactions necessary for all living organisms to perform their vital activities. In order to maintain homeostasis, energy production, and cellular function, metabolic enzymes are essential for controlling biochemical reactions. These enzymes fall into two general categories: anabolic enzymes, which help create complex molecules from simpler ones (like DNA polymerase and fatty acid synthase), and catabolic enzymes, which break down macromolecules to liberate energy (like amylase, lipase, and proteases) [2-4]. Sources of enzymes can be plant, animal or microbial. Enzymes obtained from microorganisms have many advantages compared to those of other sources. The use of enzymes derived from microorganisms, especially in the detergent industry, which is the most important field of use, in paper production, leather processing, textile industry, production of bakery products, alcohol production, cheese making, production of agrochemicals, etc. dates back to ancient Greece [5-7]. Many enzymes used in industry today are of microbial origin, selected on the basis of their high catalytic activity, non-toxicity and non-pathogenicity compared to plant- and animal-derived enzymes [8]. Amylases are one of the most widely used enzymes in industry. These enzymes hydrolyze starch molecules into polymers composed of glucose units [9]. Starch-degrading amylolytic enzymes are of great importance in biotechnological applications ranging from food, fermentation and textiles to paper industries. Most of the amylases used in industry originate from microbial sources due to various factors, for example the great microbial genetic diversity found in the environment. α-amylase is a key enzyme in the metabolism of a wide variety of living organisms that utilize starch as a carbon and energy source [10].

Fermented items are those made naturally or with the aid of starting cultures from plant and animal sources [11]. They are produced by microorganisms such as bacteria, yeasts and fungi [12]. During the fermentation process, lactic acid bacteria produce various organic acids and metabolites such as flavoring alcohols, aldehydes and esters [13]. LABs are Gram-positive, non-spore-forming, catalase-negative, facultative anaerobic, non-reducingnitrate, acid-resistant and form lactic acid as the main end product of carbohydrate fermentation [14-16]. Lactic acid bacteria used in traditional fermented foods are *Lactobacillus, Lactococcus, Tetragonococcus, Vagococcus, Weissella, Streptococcus, Leuconostoc, Aerococcus, Oenococcus and Pediococcus* [17].

The aim of this study was to isolate LAB from different fermented products and to investigate their ability to produce qualitative and quantitative α-amylase. In this direction, LABs with high activity were identified at molecular level and the effects of different parameters on enzyme activity were characterized.

2. MATERIAL AND METHOD

A total of 94 fermented samples were obtained from Hatay province (Türkiye) and were shown in Table 1 with the codes given. The samples were placed in sterile containers after collection and kept at +4 °C until analyses.

Table 1. Fermented samples from which LAB were isolated within the scope of the study (Ş: turnip, P: cheese, Y: pickled leaf, Z: pickled olive, T: pickle, S: vinegar, Sü: sürk cheese, Ty: salted yogurt, Yo: homemade yogurt)

Samples Provided						
Ş1	Y3	Z7	T6	S1	Sü4	TY8
Ş2	Y4	Z8	T7	S2	Sü5	Yo1
Ş3	Y5	Z9	T8	S3	Sü6	Yo2
Ş4	Y6	Z10	T9	S4	Sü7	Yo3
Ş5	Y7	Z11	T10	S5	Sü8	Yo4
Ş6	Y8	Z12	T11	S6	Sü9	Yo5
Ş7	Y9	Z13	T12	S7	Sü10	Yo6
P1	Y10	Z14	T13	S8	TY1	Yo7
P2	Z1	Z15	T14	S9	TY2	Yo8
P3	Z2	T1	T15	S10	TY3	Yo9
P4	Z3	T2	T16	S11	TY4	
P5	Z4	T3	T17	Sü1	TY5	
Y1	Z5	T4	T18	Sü2	TY6	
Y2	Z6	T5	T19	Sü3	TY7	

2.1 Isolation of Lactic Acid Bacteria

Ten g of sample was taken under sterile conditions and homogenized with 90 mL of Maximum Recovery Diluent (Merck) solution for 5 min. Detailed dilutions up to 10-3-10-5 were then prepared. The prepared dilutions were inoculated on MRS agar for lactic acid bacteria isolation and the inoculated plates were incubated at 30 ºC in anaerobic environment. After the shape and color characteristics of the microorganism colonies were examined, the colonies were purified. The isolates obtained were stored in 20% glycerol added MRS Broth at -80 ºC.

2.2 Qualitative α-Amylase Activity of the Isolates

The methodology described by Sudharhsan et al. [18] was followed to determine the qualitative α-amylase activity of the isoaltes after minor modification. The α-amylase activity of the isolates was first determined qualitatively on starch-MRS medium. The cultures were first inoculated into MRS broth containing 0.25% starch and activated overnight. Then, the cultures were inoculated into solid MRS media containing 0.25% starch. Certain areas of the solid medium were punctured with an agar piercer of approximately 5 mm and 50 µl of samples grown in liquid medium were placed in the cavity and incubated at 30 ºC. After incubation, iodine solution was dripped onto the medium and left for 15-30 minutes and then the formation of a zone around the colonies was considered positive (+) and the absence of a zone was considered as negative (-). The diameter of the zone was measured from different regions and quantitative α-

amylase enzyme activity determination was performed with the selected isolates.

2.3 Quantitative α-Amylase Activity of Selected Isolates

Among the isolates showing qualitative α-amylase activity, quantitative amylase activity of twenty LAB with high zone diameter were detected by DNS method described by Bernfeld [19]. For the test, the isolates were grown overnight in 1% of starch medium. After centrifugation, 100 µl of supernatant was mixed with 200 µl of 0.5% starch (dissolved in pH 7 buffer) solution and incubated at 37 ºC for 30 min. A 400 µl of DNS solution was added to the incubated samples and kept in a boiling water bath for 5 min. Then, 3 mL of distilled water was added to the samples and absorbances were measured at 540 nm using a spectrophotometer. Analyses were performed in three replicates. The different concentrations of maltose were used to prepare a calibration curve used to determine the released sugar concentration and enzyme activity was calculated using the following formula:

$$\text{Enzyme Activity U/mL} = \text{released sugar concentration (mg/mL)} \times \text{RVb (mL)} / (362.32) \times 103 \times (1/t) \times (1/EV) \quad (1)$$

Where RVb is reaction volume, t is duration (min), EV is enzyme volume and 362.32g/mol is the molecular weight of maltose and U/mL is the amount of enzyme releasing 1 µmol of reducing sugar per minute.

2.4 Identification of Isolates by Genotypic Methods and PCR Amplification

Phenol chloroform method was used for genomic DNA isolation of lactic acid bacteria [20]. 16S PEU7 (GCA AAC AGG ATT AGA TAC CC) and 16S DG74 (AGG AGG TGA TCC AAC CGC A) were used as primers for PCR amplification of 16S rRNA region of LAB isolates [21]. The final volume of the PCR mixture was 25 µL consisting of 2 µL buffer, 2 µL dNTP, 0.5 µL Taq polymerase, 17.5 µL nuclease-free water, 1 µL each primer and 1 µL template DNA. PCR reaction conditions were 95 °C for 10 min, 94 °C for 40 s, Tm °C for 1 min, 72 °C for 35 s, 35 cycles and final extension at 72 °C for 10 min. To test for amplification, a 2% agarose gel was run in 1xTBE buffer. The bands formed after the running process were analyzed by taking an image of the gel with an imaging device.

For sequence analysis, the PCR samples were sent to Sentebiolab Co. and the results were compared with the sequences obtained in FASTA format using the BLAST program with the sequences registered in the NCBI (National Center for Biotechnology Information) database and the genetically closest species were determined.

2.5 Determination of α-Amylase Gene Region

PCR amplification was performed with primer α-amy F' (AGATCAGGCGCAAGTTCAGT), R' (TTTTTATGGGCACACCACTCA) [22]. belonging to the α-amylase gene region in the identified isolates. The final volume of the PCR mixture was 25 µL using 2 µL buffer, 2 µL dNTP, 0.5 µL Taq polymerase, 17.5 µL nuclease-free water, 1 µL each primer and 2 µL template DNA. PCR reaction conditions were 94 °C for 30 s, 94 °C for 15 s, Tm °C for 30 s, 72 °C for 90 s, 35 cycles and final extension at 72 °C for 10 min. To test for amplification, a 2% agarose gel was run in 1xTBE buffer. The bands formed after running were analyzed by taking an image of the gel with an imaging device.

2.6 Partial Purification of α -Amylase from İsolates

Among the identified samples, *L. curvatus* Y2-1B isolate showing high α-amylase enzyme activity was partially purified. For purification, 1000 µl of activated *L. curvatus* Y2-1B isolate was added to 9 mL of liquid medium containing 1% starch in MRS broth and incubated at 30 ºC and 80 rpm for 24 hours for enzyme production. It was then centrifuged at 10000 rpm for 10 min at 4 ºC. The supernatant was filtered through a 0.22 µm filter and cold acetone (80%) was added to the filtrate and the sample-acetone mixture was incubated at -20 ºC for one night. Then, the supernatant was centrifuged at 10000 rpm for 10 min and 10 mL of sodium phosphate buffer (pH 5.5) was added to the precipitated enzyme. The acetone-purified α-amylase was stored at +4 ºC for analyses.

2.7 Molecular Weight of *L. Curvatus* Y2-1B α -Amylase

The molecular weight of α-amylase enzyme produced by partially purified *L. curvatus* Y2-1B was calculated by SDS-PAGE. SDS-PAGE analysis was performed according to the method described by LaemmLi [23] using 7.5% and 20% separating gel. The samples in powder form (10 mg/mL) were dissolved in distilled water and mixed (1:3 v/v) with the sample buffer (0.5 M Tris–HCl pH 6.8, 40% glycerol, 2% SDS, 0.2% bromophenol blue, 5% (w/v), β-mercaptoethanol). Then, the samples, which were kept in boiling water for 5 minutes, cooled and made ready for analysis. 5 µL of marker and 20 µL of prepared sample solutions were loaded in loading wells and a vertical electrophoresis system (Mini-PROTEAN®System, Bio-Rad) was run with a constant current of 20 mA/gel. After the running process was completed, the bands stained with the staining solution (25% Coomassie brilliant blue, 50% methanol, 50% acetic acid) were then kept in the destaining solution (50% methanol, 10%acetic acid) to remove excess dye. The bands were stained with staining solution (25% Coomassie brilliant blue, 50% methanol, 50% acetic acid) and then washed with destaining solution (50%methanol, 10%acetic acid) to remove excess dye. Biorad Gel Doc EZ imaging system was used to image the bands.

2.8 Effect of pH, Temperature and Cations on α-Amylase Enzyme Activity

The α-amylase activity of the purified *L. curvatus* Y2-1B isolate was examined at different buffer pHs, different temperatures and different ratios of cation ions ($CaCl_2$ and $CoCl_2$). For pH test; 0.5% starch solution was prepared using KH_2PO_4-Na_2HPO_4 as buffer at pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. Then, the α-amylase activity of the isolates was measured according to the Bernfeld method [19]. For different temperature experiments, the α-amylase enzyme activity of *L. curvatus* isolate at 30 °C, 40 °C, 50 °C, 60 °C, 70 °C was measured according to the Bernfeld method. For different cations; 10 mM and 1 mM $CoCl_2$, 10 mM and 1 mM $CaCl_2$ stock solutions were prepared. The final concentration of the enzyme and solution mixture was 1 mM (1 µL solution and 99 µL enzyme) and pre-incubated at 37 °C for 1 h. After incubation, α-amylase enzyme activity was measured according to the Bernfeld method [19].

2.9 Protein Determination

The protein content of the partially purified enzyme was determined according to the Lowry method using bovine serum albumin (BSA) as a standard [24]. The analyses were carried out in three replicates.

3. RESULTS

A total of 91 LAB isolates were obtained from the fermented products. The morphologically different isolates were selected and purified (Figure 1). Qualitative enzyme activity of isolates was tested, and positive ones included to next steps. (Figure 2). Among the isolates showing α-amylase activity, twenty LAB with high zone diameter were selected and their extracellular α-amylase activities were determined spectrophotometrically (Figure 3). The lowest enzyme activity was determined as to be 1.28 U/mL and the highest activity was 13.67 U/mL.

tgr

Figure 1. LAB colonies

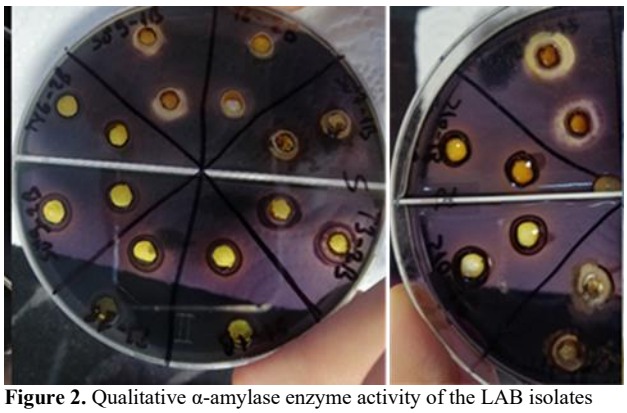

Figure 2. Qualitative α-amylase enzyme activity of the LAB isolates

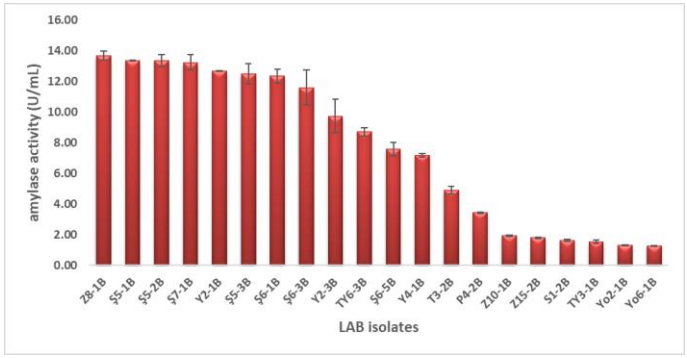

Figure 3. Quantitative α-amylase activity of the selected LAB isolates

Three selected LAB isolates of gel images of PCR products are shown in Figure 4 and three the isolates (P4-2B, Y2-1B, S1-2B) were identified (Table 2). The agarose gel image of the PCR amplification performed with the α-amy primer belonging to the α-amylase gene region in the identified isolates is shown in Figure 5. In the positive strains, 500 bp for *Lactiplantibacillus plantarum* S2-1B and approximaetley 350 bp for *Leuconostoc mesenteroides* P4-2B *Latilactobacillus curvatus* Y2-1B amplicon sizes were detected for amylase.

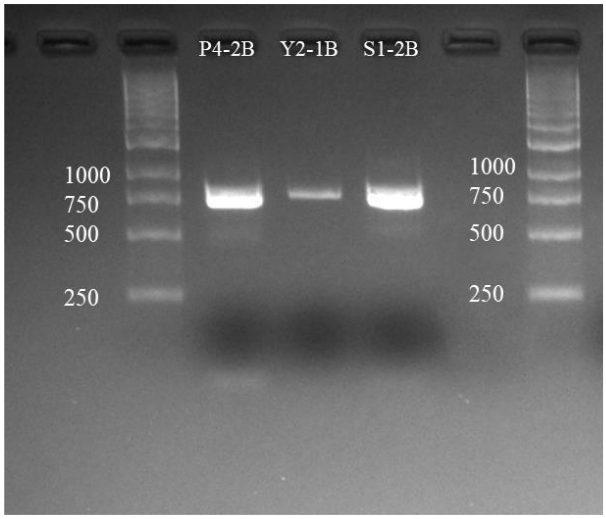

Figure 4. PCR image of the LAB isolates (*Leuconostoc mesenteroides* P4-2B *Latilactobacillus curvatus* Y2-1B, *Lactiplantibacillus plantarum* S2-1B)

Table 2. Information about the identified isolates

Isolates	Samples	Description	Percent homology with GenBank sequence	GenBank Accession No.
P4-2B	Cheese	*Leuconostoc mesenteroides*	99.31%	AB362705.1
Y2-1B	Pickled Leaf	*Latilactobacillus curvatus*	95.96%	MG031203.1
S1-2B	Vinegar	*Lactiplantibacillus plantarum*	99.86%	HG798398.1

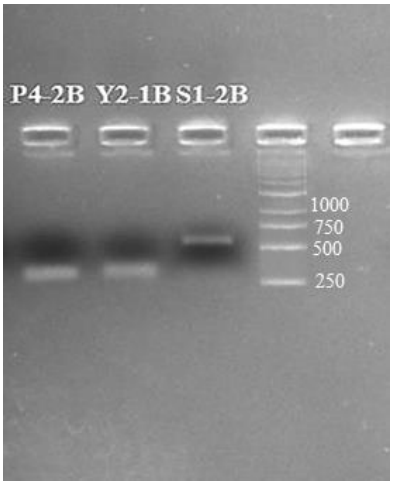

Figure 5. PCR image of *α-amy* gene region of the LAB isolates (*Leuconostoc mesenteroides* P4-2B, *Latilactobacillus curvatus* Y2-1B, *Lactiplantibacillus plantarum* S2-1B)

L. curvatus, which showed α-amylase enzyme activity and had a high zone diameter among the identified isolates, was selected and partial purification was performed and the effect of pH, temperature and cation on extracellular α-amylase activity was determined spectrophotometrically (Figure 6). The lowest enzyme activity in *L. curvatus* was found to be 0.554 U/mL at the pH 9 and 0.694 U/mL at pH 6 as a highest value. Regarding the temperature data of the enzyme activity, the lowest activity was 0.591 U/mL at 70 °C and the highest activity was 0.735 U/mL at 30 °C for *L. curvatus.* In cation effect, the lowest enzyme activity in *L. curvatus* was 0.662 U/mL at 10 mM $CaCl_2$ and the highest was 0.779 U/mL at 1 mM $CoCl_2$. Specific enzyme activity calculations were made upon determination of protein amount, and these values were determined as 1.354 U/mg at pH 6 and 1.082 U/mg at pH 9. On the effect of temperature, *L. curvatus* was determined as 1.154 U/mg at 70 °C and 1.434 U/mg at 30 °C. These values were determined as 1.291 U/mg at 10 mM $CaCl_2$ and 1.520 U/mg at 1 mM $CoCl_2$ (Figure 7). The relative activity value of the extracellular α-amylase enzyme of *L. curvatus* isolate was determined by incubation in KH_2PO_4-Na_2HPO_4 buffer at 37 °C for 30 min. It was found that *L. curvatus* had the highest enzyme activity at pH 6 (100%) (Figure 8). *L. curvatus* had the highest enzyme activity at 30 °C (100%), while *L. curvatus* had the highest enzyme activity at 1 mM $CaCl_2$ and 1 mM $CoCl_2$ (100%). The relative enzyme activity at pH 9 and 8 were 80 % and 86 %, respectively.

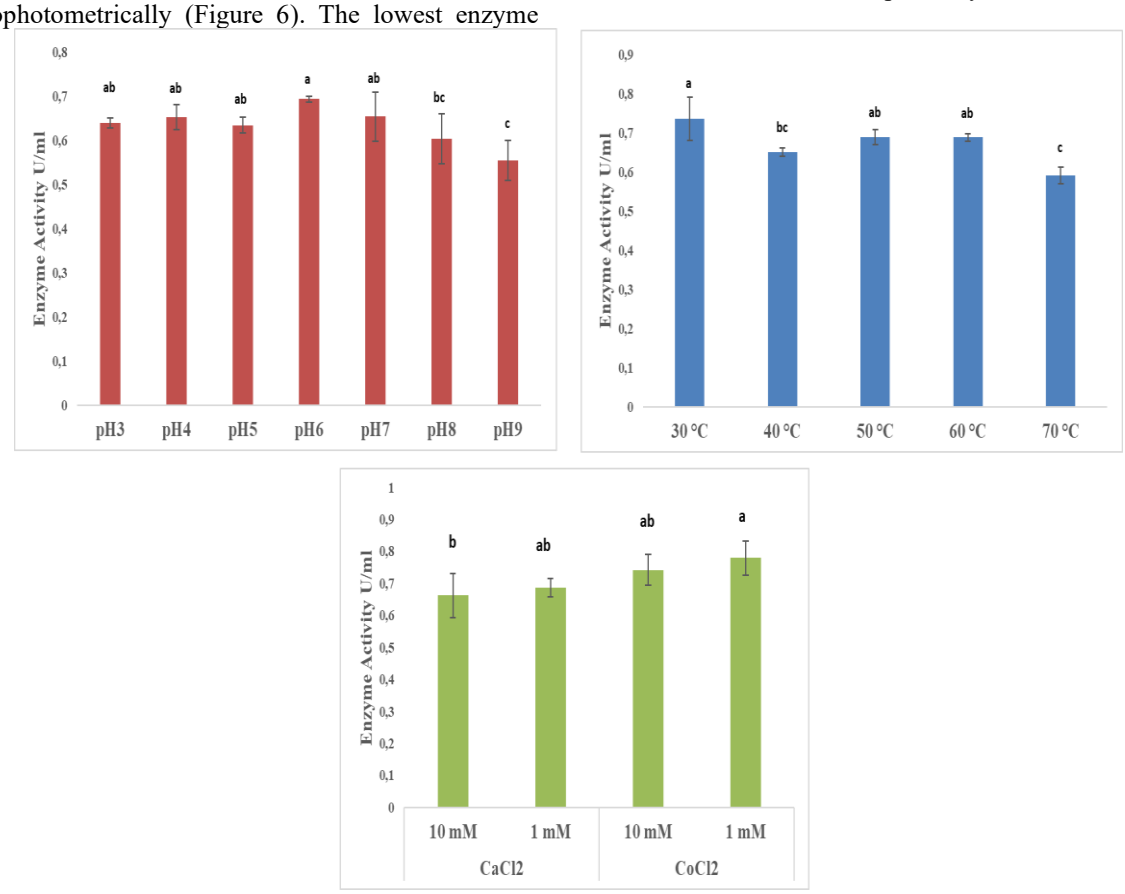

Figure 6. Effect of pH, temperature and different cations on α-amylase activity of *L. curvatus* Y2-1B isolate

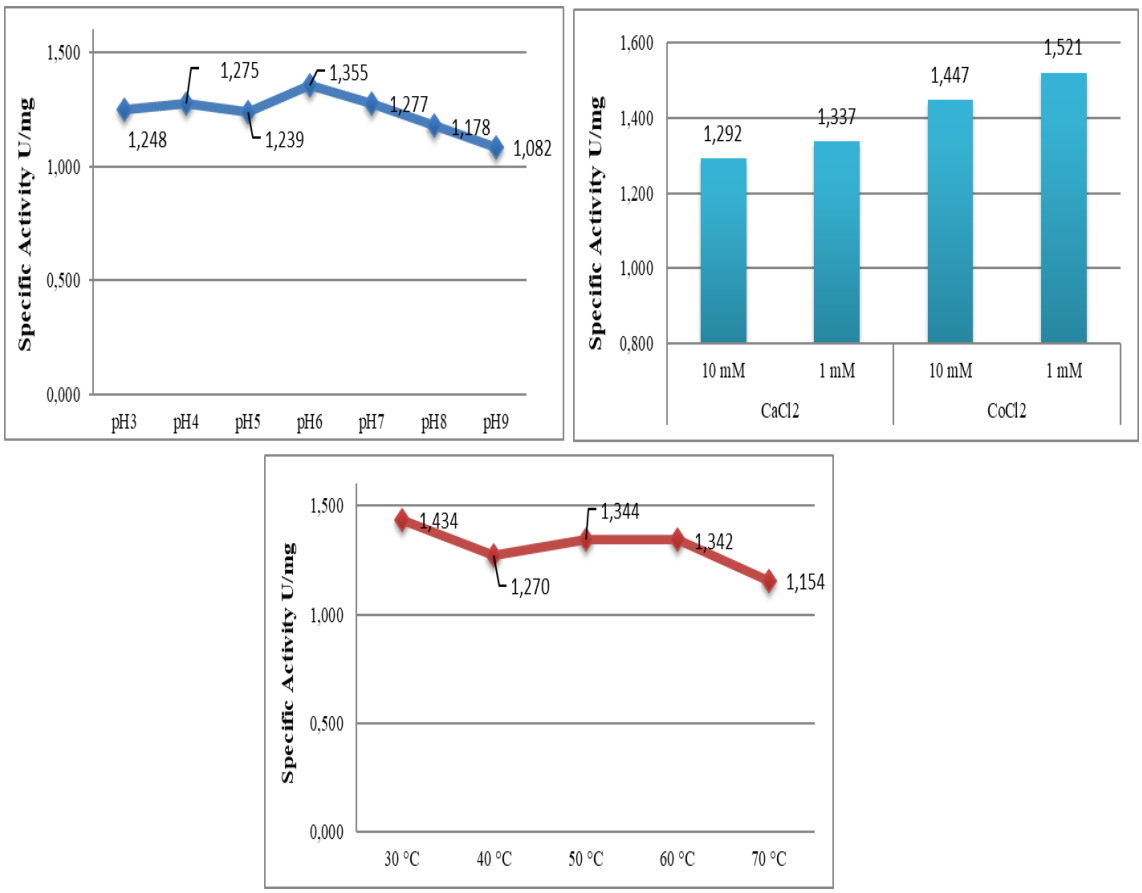

Figure 7. Specific α-amylase activity values of *L. curvatus* Y2-1B at different conditions

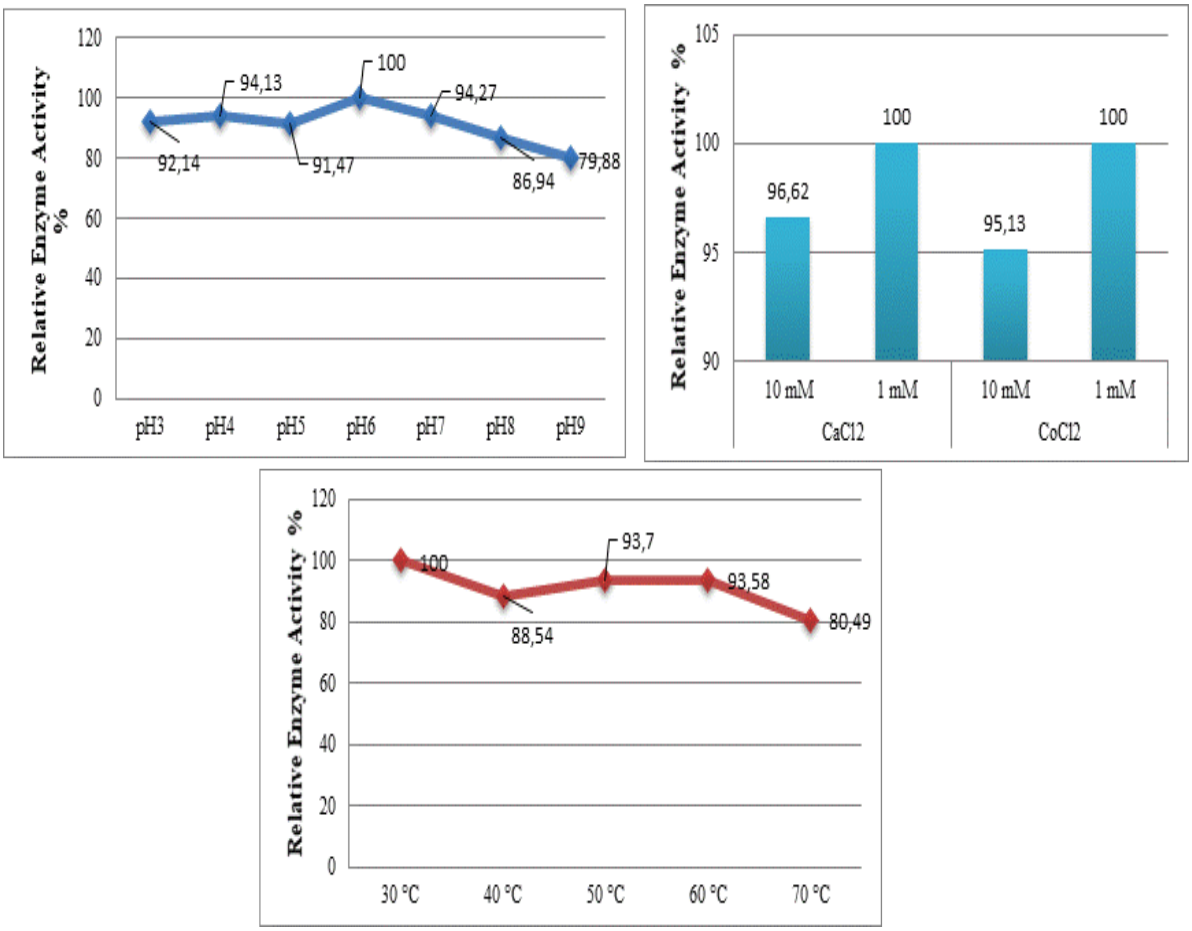

Figure 8. Relative enzyme activity for extracellular α-amylase of *L. curvatus* Y2-1B strain

It was determined that the purified enzyme formed a band of approximately 40 kDa in SDS-PAGE (Figure 9).

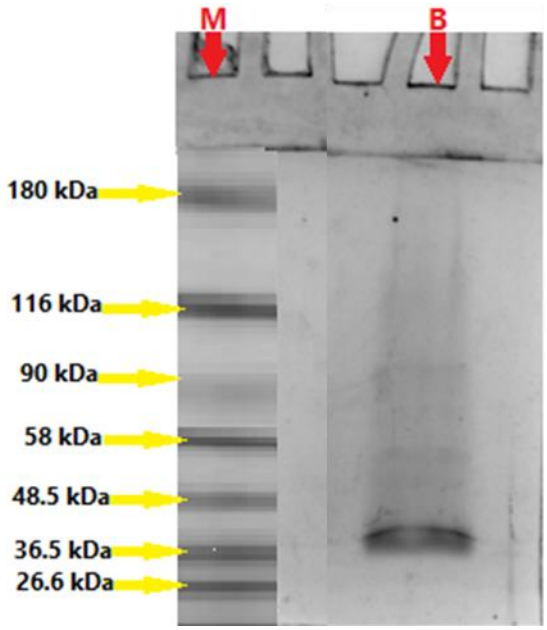

Figure 9. SDS-PAGE electrophoresis image of α-amylase isolated from *L. curvatus* Y2-1B. M: Marker, B: *L. curvatus*, Arrows are ordered by the molecular weight of the standard protein. (Sigma Marker TM wide range 26,6-180,000 Da)

4. DISCUSSION AND CONCLUSION

In this study, LAB were isolated from different fermented products, and their ability to produce α-amylase was investigated. LABs with high α-amylase activity were identified, characterized, and their effects on enzyme activities under different conditions were characterized. Vishnu et al. [25] determined the total amylase activity of *Lactobacillus amylophilus* strain as to be 0.59 U/mL. Orhan [26] found that *Lb. plantarum* ssp. *plantarum*, *Lb. casei, Lb. coryniformis* ssp. *tourquens, Pediococcus pentosaceus* ve *L. mesenteroides* ssp. *dextranicum* species showed amylase activity on starchy medium. In another study by Songré-Ouattara et al. [27], 30 LAB were selected for production of amylase, phytase and α-galactosidase were characterized. Two *L. plantarum* and three *L. fermentum* isolates that could produce one or more of these enzymes were selected and they found that the amylase activities of these isolates were lower than 0.05 Ceralpha Units/mL. These values were found to be low compared to our data.

Velikova et al. [28] stated that the strong expression of amy1 conformed to the presence of extracellular amylase activity (7–8 U/mL) as similar to our study.

Kanpiengjai et al. [29] reported that the amount of extracellular amylolytic enzyme was between 0.04-2.5 U/mL and *L. plantarum* isolate showed the highest activity. In their study about *L. plantarum* isolate, they found that the optimum temperature was between 40-60 oC and the optimum pH value was between 3.5-8, showing a wide pH stability, similar to our results. In another study, it was reported that the optimum pH range of amylase activity of *Enterococcus faecium* isolate was pH 6-7. The authors stated that the enzyme was significantly sensitive to pH and amylase activity was unstable at high temperatures and high pH values. In the same study, they found that Ca^{+2}, Na^{+} and Sr^{+2} metal ions increased amylase activity [30]. Abiodun Onilude et al. [31] reported that the highest amylase enzyme activity of *L. plantarum* isolate was at pH 7, gradually increased from pH 3 to pH 7 and the optimum temperature value was 40 ºC. In the study on the amylase ability of *Geobacillus thermoleovorans*, it was determined that the optimum temperature was 80 ºC and the optimum pH was 6 [32]. In a study conducted to obtain the optimum culture conditions of thermostable α-amylase produced by *Bacillus licheniformis* SO-B3 isolate, it was reported that the optimum temperature was at 70 °C and the optimum pH was in the range of 5.0-6.0 [33]. Pan [34] determined that the optimum enzyme activity value was 3.7 U/mL at pH 7.5 and the total protein amount was 7.0 mg and the specific activity value was 31.62 U/mg for *Bacillus subtilis* amylase enzyme isolated from soil. In the study conducted by Zhao et al. [35], it was determined that $CaCl_2$ played an important role in the production of amylase enzyme of *Bacillus amyloliquefaciens* isolate in addition to carbon and nitrogen sources. In another study, the amylase enzyme ability of *Pleurotus tuberregium* isolate was examined and it was found that the optimum activity was at pH 5, the optimum temperature was 70 ºC and $CaCl_2$ increased the activity [36].

It was informed that *L. curvatus* strain is a candidate probiotic and has several genes associated with carbohydrate utilization and bacteriocin production, and these genes can provide strong carbohydrate fermentative ability and antibacterial capacity [37]. In addition, carbohydrate metabolism enzymes such α-amylase and α-glucosidase enzymes are the subject of research on probiotic isolates. In the study conducted by Ragavan and Das [38], α-amylase and α-glucosidase enzyme of probiotic yeasts *Yarrowia lipolytica, Kluyveromyces lactis, Lipomyces starkeyi, Saccharomycopsis fibuligera* and *Brettanomyces custersianus* isolates were examined and it was determined that *L. starkeyi* showed the highest α-glucosidase enzyme activity of 66% and *K. lactis* showed the highest α-amylase enzyme activity of 75%. Jo et al. [39] examined the properties of *Limosilactobacillus fermentum* strain as an amylase and phytase producing starter for the preparation of rice-based probiotic beverages and stated that it is suitable for fermentation in terms of rapid growth rate, pH and metabolite changes in rice solution, amylase and phytase activities and optimal viscosity changes for beverage as starting properties for rice fermentation. In the same study, the cultivation status of *Limosylactobacillus fermentum* strain in different media was examined and it was reported that α-amylase enzyme showed an enzyme activity of 0.11 U in 0.5% rice added medium, α-amylase enzyme activity in the range of 0.08-0.02 U in MRS medium and phytase enzyme production was 17.65 U, indicating that this isolate can produce α-amylase and phytase enzymes.

Molecular weight of α-amylase expressed in *B. subtilis* WB800 was deterimined as 58.4 kDa [40], in *Bacillus amyloliquefaciens* FW2 was 55 kDa [37], in *Lactobacillus*

sp G3_4_1TO2 was 95 kDa, in *Anoxybacillus ayderensis* FMB1 isolate was found to be 58.5 kDa [32] and in *Bacillus subtilis* was indicated as 50 kDa [41]. It was informed that most microorganisms produce thermostable α-amylase with a molecular weight in the range of 21–160 kDa and most work well on acidic pH [30]. Tallapragada et al. [42] stated that the majority of α-amylases produced by lactic acid bacteria are near to 100 kDa. Supprtingly, Giraud and Cuny [43] informed that the amino acid sequences deduced show that both *amyA* genes encode a long polypeptide with a molecular weight of around 100 kDa. However, in different studies related to lactobacillus amylases, a well-defined band was obtained as 50 kDa [35]. The authors also informed that discrepancies in the estimation of the size can been explained either by migration artefacts of the proteins in SDS-PAGE or by glycosylation of the protein.

Enzyme studies have a crucial role in many industries such as food, textiles, cosmetics, detergents and amylases are one of the industrially important hydrolytic enzymes. Lactic acid bacteria are industrial microorganisms in terms of extracellular enzyme production. In this study, LABs with the ability to produce α-amylase enzyme using different fermented products were identified and it is thought to be important in terms of creating starter cultures for future studies and using them in different microbiology studies. *Latilactobacillus curvatus* Y2-1B isolate can be employed in thermophilic amylase enzyme production investigations since it demonstrated α-amylase enzyme activity at high temperatures.

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