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Enzymatic Activity of *Lactobacillus* Grown in a Sweet Potato Base Medium

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Authors' contributions

Authors SAH and SAI managed the literature research, designed the study, performed the experiments and statistical analysis, wrote the protocol, and wrote the manuscript. Authors AS and MW managed the analyses of the study and edited the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To study the impact of sweet potatoes on *α*-glucosidase, *β*-glucosidase, acid phosphatase, and phytase activity of *Lactobacillus*.
Study Design: Enzymatic activity of seven strains of *Lactobacillus* grown in a sweet potato medium (SPM) was determined and compared to the standard lactobacilli MRS. Strains having the highest enzymatic activity were further enhanced by metal ions.
Place and Duration of Study: Food Microbiology and Biotechnology Laboratory, North Carolina A&T State University, Greensboro, NC, USA, between September 2012 and May 2013.
Methodology: *Lactobacillus* strains were grown in SPM and MRS at 37ºC for 16 h. At the end of incubation, bacterial population (log CFU/mL) was determined by plating and enzymatic activity was determined spectrophotometrically using the corresponding substrate.
Results: *Lactobacillus* strains continue to grow in SPM and MRS and reached averages of 10.98±0.49 and 10.92±0.55 log CFU/mL respectively. Growth of *Lactobacillus* strains in SPM led to higher *β*-glucosidase, acid phosphatase, and phytase activity than MRS. Strains of *L. reuteri* (CF2-7F and SD2112) grown in SPM showed the highest acid phosphatase (15.84±1.05 and 20.56±1.49 Ph U/mL), and phytase (0.66±0.14 and 0.65±0.11 Ph U/mL) respectively. The highest *β*-glucosidase (36.04±3.16 Glu U/mL) activity was obtained from

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L. delbrueckii subsp. *bulgaricus* SR35 grown in SPM. In addition, acid phosphatase and phytase produced by *L. reuteri* CF2-7F growing in SPM were further increased by the addition of Mn^{2+} (70.1 and 41.8%) or Mg^{2+} (94.7 and 20.9%) respectively. *β*-glucosidase activity of *L. reuteri* was increased in a range of 4.1 to 130.6% due to the addition of metal ions.

Conclusion: Components in sweet potatoes could increase enzymatic activity of *Lactobacillus* and the addition of metal ions could further produce an enhanced level of these enzymes.

Keywords: Sweet potato medium; Lactobacillus; enzymatic activity; metal ions and L. reuteri.

1. INTRODUCTION

Lactobacillus is probiotic bacteria with many applications in food and feed [1]. Among the important probiotic characteristics of *Lactobacillus* strains is their ability to produce different functional enzymes that can improve digestibility of complex sugars [1-4]. *Lactobacillus* strains have been recognized to produce functional enzymes such as *α*–glucosidase, *β*-glucosidase, acid phosphatase and phytase. *α*–Glucosidase (*α*-D-glucoside glucohydrolase, EC 3.2.1.20) is responsible for hydrolyzing glycosidic bonds in oligosaccharides (starch, disaccharides, and glycogen) to release *α*-glucose [5,6]. *α*–Glucosidase has been used therapeutically in the treatment of glycogen storage disease type II that caused by a deficiency of this enzyme [5]. *β*-Glucosidase (*β*-D-glucoside glucohydrolase, EC 3.2.1.21) hydrolyzes all four *β*-linked glucose dimmers in cellulose to release glucose monomers [7]. Since cellulose is the primary component in plants, *β*-glucosidase has many applications in agriculture, biotechnology, industry, and human health [8]. Hydrolyzing cellulose can produce valuable products such as petrochemicals, ethanol, stone-washed textiles, and recycled paper [6]. In addition, hydrolyzing cellulose in fermented foods or in the human gut could provide health benefits [9]. Acid phosphatase (orthophosphoric monoester phosphohydrolase, EC. 3.1.3.2) and phytase (*myo*-inositol hexakisphosphate 6-phosphohydrolases; EC 3.1.3.26) hydrolyze phytate, a common fiber in cereals, legumes, and nuts [10-12]. The specificity of acid phosphatase and phytase can partially overlapped since acid phosphatase produced by microorganisms also have phytase activity [13]. Phytate acts as an antinutrient binding with protein, minerals, and vitamins and thereby diminishing their nutritional bioavailability [11,14]. Hydrolyzing phytate reduces its antinutritional properties. These enzymes could be found in low quantities in humans, resulting in health problems [12-14] such as osteoporosis [12]. Thus, the use of microbial sources of these functional enzymes such as *Lactobacillus* appears to be a promising solution for certain human health issues [6,8].

Species of *Lactobacillus* that produce functional enzymes could also have many beneficial applications in the food industry and probiotics. Degradation of complex carbohydrates such as cellulose and phytate that are not digestible by human enzymes has been recognized as an important benefit to human health [1,2]. However, the production capacity of *Lactobacillus* of such hydrolyzing enzymes can be affected by the growth environment and growth media composition [3,6,9,10]. Even though the nutritional requirements of *Lactobacillus* have been established, there are many limitations and challenges associated with controlling, optimizing, and maximizing its enzymatic activity [3]. Nutrients available in growth media such as vitamins, minerals, sugars, and proteins have an important role in enhancing the enzymatic activity of *Lactobacillus* [2,3,6,10,15,16]. Sweet potatoes (*Ipomoea batatas*) are abundant agricultural products that play a major role in the food industry and human

nutrition. Sweet potatoes are rich sources of carbohydrates (mainly starch and sugars), some amino acids, vitamins (vitamin A, vitamin C, thiamin (B1), riboflavin (B2), niacin, and vitamin E), minerals (calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc), and dietary fiber [17,18]. This nutritional tuber also contains other bioactive compounds such as antioxidants, triglycerides, linoleic acid, and palmitic acid [17,18]. Previous studies have shown that nutrients and other plant components could support the growth and functionality of probiotic bacteria [3,19]. In addition, sweet potatoes could be used to form an alternative low cost medium that can support the growth of *Lactobacillus* [20]. Sweet potatoes have the potential to enhance the enzymatic activity of *Lactobacillus* and thus may improve their probiotic characteristics. Therefore, the objective of this study was to determine the enzymatic activity of *Lactobacillus* strains growing in a sweet potato based medium.

2. MATERIALS AND METHODS

2.1 Media Preparation

Sweet potato medium (SPM) was prepared according to the procedure previously developed in our laboratory [20]. In this procedure, fresh sweet potatoes (Burch Farms, Faison, NC, USA) were baked in a conventional oven at 400ºC for 1h. The potatoes were then peeled and blended in a kitchen blender with deionized distilled water (DDW) at a ratio 1:2. The solution was centrifuged at 7800×g for 10 min using Sorvall RC 6 Plus Centrifuge (Thermo Scientific, Asheville, NC, USA) and supernatant was collected. One liter of supernatant was mixed with the following ingredients: sodium acetate (5g), potassium phosphate (2g), disodium phosphate (2g), ammonium citrate (2g), Tween 80 (1mL), magnesium sulfate (0.1g), manganese sulfate (0.05g), L-Cysteine (1g), beef extract (Neogen, Lansing, MI, USA) (4g), yeast extract (Neogen) (4g), and proteose peptone #3 (4g) to form the sweet potato medium (SPM).

MRS was prepared by dissolving 55g lactobacilli MRS broth (Neogen) and 1g L-Cysteine in 1 L DDW. SPM and MRS were sterilized at 121ºC for 15 min, cooled down, and stored at 4ºC to be used within 3 days. The initial pH values of SPM and MRS after sterilization were 6.43±0.07 and 6.45±0.04 respectively. All ingredients were obtained from Thermo Scientific unless otherwise stated.

2.2 Bacterial Culture Activation and Preparation

Lactobacillus strains (Table 1) were available in the stock collection of our laboratory. Strains of *L. reuteri* were provided by BioGaia (Raleigh, NC, USA), *L. delbrueckii* subsp. *bulgaricus* and *L. plantarum* were obtained from Visby culture Co. (Netherlands), *L. rhamnosus* GG was provided by Mead Johnson (Evansville, IN, USA), and *L. acidophilus* was isolated in our laboratory from a commercial product. Strains were maintained in glycerol solution and stored at -80ºC in our stock collection freezer. Prior to the experiment, the strains were activated in lactobacilli MRS broth by transferring 100 µL of stock culture to 10 mL MRS broth, incubated at 37ºC for 24 h, then stored at 4ºC. Before each experimental replication, bacterial strains were streaked onto MRS agar and incubated for 48 h at 37ºC. One isolated colony was then transferred to 10 mL MRS broth and incubated at 37ºC for 24 h. *Lactobacillus* strains were individually subcultured twice in batches of 10 mL SPM and batches of 10 mL MRS, incubated at 37ºC for 24h in order for the bacterial strains to adapt to the medium. Cultures grown in SPM and MRS were used throughout the study to inoculate the same medium.

2.3 Bacterial Enumeration

Bacterial population was determined by plating onto MRS agar. Samples were individually diluted by transferring 1 mL into serials of 9 mL (0.1% peptone water solution), then 100 µL of appropriate dilution was plated onto MRS agar and incubated at 37ºC for 48 h. Plates having 25 – 250 colonies were considered for colony counting at the end of incubation. Bacterial populations were expressed as log CFU/mL.

2.4 Culture Conditions

Batches of 80 mL SPM and batches of 80 mL MRS, in 250 mL bottles, were inoculated with 3% v/v individual precultured strains and incubated at 37ºC for 16 h. Bacterial growth was monitored by measuring the turbidity (optical density at 610 nm) at 2 h intervals using a 96-well microplate reader (BioTek Institute,Winooski, VT), and final bacterial populations were determined at 16 h of incubation. Values obtained from optical density were used to calculate the maximum specific growth rates (μ_{max}) of tested strains.

2.5 Culture with Metal Ions

The impact of different metal ions on *α*-glucosidase, *β*-glucosidase, acid phosphatase, and phytase produced by *L. reuteri* (CF2-7F and SD2112) were studied in SPM. In this procedure 10 mM of either $FeSO_4.4H_2O$, $MgSO_4.7H_2O$, K_2SO_4, or Na_2SO_4, or 5 mM of either $MnSO4._4H_2O$ or $CaSO_4.7H_2O$ were prepared separately into batches of 80 mL nonsterile preprepared SPM broth in 250 mL bottles. The use of 10 mM of metal ions or less was established to avoid hypertonic pressure on the bacterial cells [15]. We used 5 mM of $MnSO_4.4H_2O$ and $CaSO_4.7H_2O$ since higher concentrations did not dissolve in SPM. Batches of 80 mL SPM without metal ions served as a control. Samples were sterilized at 121ºC for 15 min using LV 250 Laboratory System Sterilizer (Steris, Mentor, OH, USA), cooled down to room temperature, then inoculated with 3% v/v individual precultured *L. reuteri* strains and incubated at 37ºC for 16h.

2.6 Enzyme Samples Preparation

At the end of incubation, cultures were divided into two portions of 40 mL each. One portion was used for *α*-glucosidase and *β*-glucosidase determination and the other portion was used for acid phosphatase and phytase determination.

Samples used for *α*-glucosidase and *β*-glucosidase determination were centrifuged at 7800×g for 10 min at 4°C using Sorvall RC 6 Plus Centrifuge to harvest the bacterial cells. The cells were washed twice with 0.5 M sodium phosphate buffer (pH 6.0) and suspended in 1 mL of the same buffer. Suspended cells were maintained in Eppendorf tubes containing 0.1 mm glass beads and treated with a mini-Beadbeater-8 (Biospec Products, Bartlesville, OK, USA) for a total of 3 min in order to disrupt the cells. During cell disruption process, samples were allowed to rest after each minute for 15 s in an ice bath to avoid overheating. Samples were then centrifuged at 12,000×g for 20 min using Microcentrifuge 5415 R (Eppendorf, Hamburg, Germany) and supernatant was used for enzyme assay analysis of *α*-glucosidase. Disrupted cells were then suspended in a minimum amount of sodium phosphate buffer and used for the enzyme assay analysis of *β*-glucosidase.

Samples used for acid phosphate and phytase determination were centrifuged at 7800×g for 10 min at 4ºC to harvest the bacterial cells. The cells were washed with 50 mM Tris-HCl (pH 6.5) and suspended in 1 mL 50 mM sodium acetate-acetic acid (pH 5.5). Suspended cells were disrupted and centrifuged using same procedure as that used for *α*-glucosidase and *β*-glucosidase samples. Supernatants were used for enzyme assay analysis of acid phosphatase and phytase.

2.7 Determination of *α*-glucosidase and *β*-glucosidase

α-Glucosidase and *β*-glucosidase were determined according to the procedure described by Mahajan and others (2010) with some modifications [6].

In this procedure *α*-glucosidase activity was determined by monitoring the hydrolysis rate of *ρ*-nitrophenyl-*α*-D-glucopyranoside (*α*-*ρ*NPG) as a substrate. The enzyme reaction contains 1 mL of 10 mM *α*-*ρ*NPG and 0.5 mL of enzyme sample was incubated in a water bath at 37ºC for 20 min. The reaction was stopped by the addition of cold 2.5 mL of 0.5 M Na_2CO_3. The amount of *ρ*-nitrophenol released from the reaction was determined by measuring the optical density at 420 nm using GENESYS 10S UV-Vis spectrophotometer (Thermo Scientific).

β-Glucosidase activity was determined by monitoring the rate of hydrolysis of *β*-*ρ*NPG as a substrate. Enzyme reaction was assayed following the same procedure as that used for *α*-glucosidase. After stopping the reaction, samples were centrifuged at 10,000×g for 20 min at 4ºC. The amount of *ρ*-nitrophenol in the supernatant was determined by measuring the optical density at 420 nm.

One unit of *α*-glucosidase or *β*-glucosidase (Glu U/mL) was defined as 1.0 µM of *ρ*-nitrophenol liberated per minute under assay conditions.

2.8 Determination of Acid Phosphatase and Phytase

Acid phosphatase and phytase were determined according to the procedure described by Haros and others (2008) with some modifications [21].

Acid phosphatase was determined by monitoring the hydrolysis rate of *ρ*-nitrophenyl phosphate (*ρ*-NPP) as a substrate. In this procedure, 250 µL of 0.1 M sodium acetate buffer (pH 5.5) containing 5 mM *ρ*-NPP was mixed with 250 µL of enzyme sample. Samples were incubated in a water bath at 50ºC for 30 min and reactions were stopped by adding 500 µL of 1.0 M NaOH. The released *ρ*-nitrophenol was measured at 420 nm. One unit of acid phosphatase (Ph U/mL) was defined as 1.0 µM of *ρ*-nitrophenol liberated per minute under assay conditions.

Phytase activity was determined by measuring the amount of liberated inorganic phosphate (Pi) from sodium phytate. In this procedure, 400 µL of 0.1 M sodium acetate (pH 5.5) containing 1.2 mM sodium phytate was mixed with 200 µL of enzyme sample. Samples were incubated in a water bath at 50ºC for 30 min and reactions were stopped by adding 100 µL of 20% trichloroacetic acid solution. An aliquot was analyzed to determine the liberated Pi by the ammonium molybdate method, at 420 nm [22]. One unit phytase (Ph U/mL) was defined as 1.0 µM of Pi liberated per minute under assay conditions.

2.9 Statistical Analysis

Each experimental test was conducted three times in randomized block design to study the enzymatic activity of *Lactobacillus* strains growing in SPM and MRS. Mean values and standard deviations were calculated from the triplicate tested samples. R-Project for Statistical Computing version R-2.15.2 (www.r-project.org) was used to determine the significance differences in the growth and enzymatic activity among the tested lactobacilli strains using one way ANOVA (analysis of variance) with a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Growth of *Lactobacillus* in a Sweet Potato Medium

Table 1 shows final bacterial populations and μ_{max} values of *Lactobacillus* grown in SPM and MRS. *Lactobacillus* strains continued to grow in SPM and MRS to reach averages of 10.98±0.49 and 10.92±0.55 log CFU/mL respectively after 16 h of incubation at 37ºC. The averages of μ_{max} values for tested strains in SPM and MRS were 0.367±0.021 and 0.358±0.021 respectively. Bacterial populations and μ_{max} were slightly higher in SPM than in MRS. However, differences in the bacterial populations and μ_{max} values in SPM and MRS were not significant ($p > 0.05$).

Sweet potatoes are rich source of sugars, vitamins, and minerals but contain small amount of protein (1.54%). We have previously showed that supplementing of sweet potatoes with external nitrogen sources could ensure similar lactobacilli growth as that in MRS [19]. Thus, SPM, supplemented with 4 g/L of each peptone, yeast extract, and beef extract, could support lactobacilli growth.

3.2 Impact of SPM on *α*-Glucosidase and *β*-Glucosidase

Table 2 shows *α*-glucosidase and *β*-glucosidase activity of *Lactobacillus* strains growing in SPM and MRS. The *α*-glucosidase activity of *Lactobacillus* strains ranged between 5.88±0.58 (*L. plantarum* 299v in SPM) and 56.93±3.16 (*L. reuteri* CF2-7F in MRS) Glu U/mL. Strains of *L. reuteri* (CF2-7F and SD2112) showed the highest *α*-glucosidase compared to other tested strains. *L. reuteri* (CF2-7F and SD2112) showed significantly ($p < 0.05$) higher *α*-glucosidase (56.93±3.16 and 47.30±3.66 Glu U/mL respectively) in MRS than in SPM (31.87±2.52 and 29.05±2.14 Glu U/mL respectively). The growth of *L. reuteri* (CF2-7F and SD2112) in SPM led to a relative decrease in *α*-glucosidase by 44.0 and 38.6 % respectively (Fig. 1). *L. acidophilus* SD16 and *L. rhamnosus* GG B103 grown in SPM showed a slight relative increase in *α*-glucosidase activity by 10.6 and 14.6% respectively. On average, the growth of *Lactobacillus* strains in SPM led to a relative decrease in *α*-glucosidase by an average of 8.7% compared to that in MRS.

Table 1. List of *Lactobacillus* strains, original sources, final bacterial populations, and maximum specific growth rate (μ_{max})

***Lactobacillus* Strain**	**Original source**	**Bacterial population log CFU/mL***		**Maximum specific growth rate (μ_{max})/h**	
		MRS	**SPM**	**MRS**	**SPM**
L. plantarum 299v	Human patient	10.73±0.42[a]	11.08±0.43[a]	0.347±0.021[a]	0.363±0.027[a]
L. acidophilus SD16	Commercial source	10.66±0.41[a]	10.98±0.44[a]	0.378±0.027[a]	0.387±0.025[a]
L. acidophilus EF7	Commercial source	10.96±0.48[a]	11.03±0.56[a]	0.373±0.017[a]	0.363±0.023[a]
L. rhamnosus GG B103	Child fecal isolate	11.00±0.61[a]	10.81±0.61[a]	0.383±0.021[a]	0.395±0.019[a]
L. delbrueckii subsp. *bulgaricus* SR35	Yogurt culture	10.88±0.44[a]	10.99±0.14[a]	0.314±0.041[a]	0.334±0.045[a]
L. reuteri CF2-7F	Child fecal isolate	11.14±0.29[a]	11.03±0.61[a]	0.354±0.017[a]	0.351±0.025[a]
L. reuteri SD2112	Mother's milk	11.06±0.57[a]	10.94±0.41[a]	0.359±0.011[a]	0.377±0.031[a]
Average		10.92±0.55[a]	10.98±0.49[a]	0.358±0.024[a]	0.367±0.021[a]

**Data points with different lower case letters in the same row are significantly ($p<0.05$) different.*

The *β*-glucosidase activity of *Lactobacillus* strains ranged between 2.61±0.40 (*L. plantarum* 299v in MRS) and 36.04±3.16 (*L. delbrueckii* subsp*. bulgaricus* SR35 in SPM) Glu U/mL. Strains of *L. reuteri* (CF2-7F and SD2112) showed a significantly ($p < 0.05$) higher *β*-glucosidase (7.90±0.76 and 5.47±0.96 Glu U/mL respectively) in SPM than in MRS (4.72±0.95 and 2.82±0.38 Glu U/mL respectively). By contrast, strains of *L. acidophilus* (SD16 and EF7) showed a significantly ($p < 0.05$) higher *β*-glucosidase (4.60±0.77 and 9.30±0.57 Glu U/mL respectively) in MRS than in SPM (2.66±0.44 and 6.71±0.79 Glu U/mL respectively). The growth of *L. reuteri* (CF2-7F and SD2112) in SPM led to a relative increase in *β*-glucosidase by 67.4 and 94.0% respectively (Fig. 1). The growth of *L. acidophilus* SD16 *and* EF7 in SPM led to a relative decrease in *β*-glucosidase activity by 42.4 and 27.8% respectively. On average, *Lactobacillus* strains showed an 11.7% increase in *β*-glucosidase in SPM compared to MRS.

Differences in *α*- and *β*-glucosidases were also shown among strains of the same species. For example, *L. acidophilus* EF7 and *L. reuteri* CF2-7F showed higher *α*- and *β*-glucosidases compared to *L. acidophilus* SD16 and *L. reuteri* SD2112 respectively. These results are in agreement with other studies indicating that the ability of *Lactobacillus* strains to produce *α*- or *β*-glucosidases is a strain dependent [6,9,23,24]. On the other hand, *L. reuteri* and *L. delbrueckii* subsp. *bulgaricus* SR35 should be given more attention with regard to having the highest *α*-glucosidase and *β*-glucosidase activity respectively. *L. delbrueckii* was also reported in another study to have higher *β*-glucosidase activity compared to other lactobacilli species [25]. The high production of *α*-glucosidase by *L. reuteri* could be of special interest in probiotic applications since these strains were also reported to produce high quantities of *α*-galactosidase and *β*-galactosidase [2]. Thus, SPM could support an increase in the production of *β*-glucosidase by *Lactobacillus* strains but may cause a slight decrease in *α*-glucosidase. Strain selection might be considered to maximize *α*- and *β*-glucosidases.

Table 2. *α*-Glucosidase and *β*-glucosidase activity of *Lactobacillus* strains in SPM and MRS after 16h on incubation at 37ºC

Lactobacilli Strain	*α*-Glucosidase (Glu U/mL)*		*β*-Glucosidase (Glu U/mL)*	
	MRS	SPM	MRS	SPM
L. plantarum 299v	6.30 ± 0.80^{aD}	5.88 ± 0.58^{aD}	2.61 ± 0.40^{aD}	2.63 ± 0.51^{aD}
L. acidophilus SD16	7.72 ± 0.55^{aD}	8.54 ± 0.89^{aBC}	4.60 ± 0.77^{aCD}	2.66 ± 0.44^{bD}
L. acidophilus EF7	9.45 ± 0.73^{aCD}	9.63 ± 0.65^{aBC}	9.30 ± 0.57^{aC}	6.71 ± 0.79^{bC}
L. rhamnosus GG B103	10.50 ± 0.61^{aCD}	12.03 ± 0.56^{aB}	17.76 ± 1.04^{aB}	15.71 ± 1.76^{aB}
L. delbrueckii subsp. *bulgaricus* SR35	12.45 ± 0.79^{aC}	12.63 ± 0.86^{aB}	35.45 ± 2.70^{aA}	36.04 ± 3.16^{aA}
L. reuteri CF2-7F	56.93 ± 3.16^{aA}	31.87 ± 2.52^{bA}	4.72 ± 0.95^{bCD}	7.90 ± 0.76^{aC}
L. reuteri SD2112	47.30 ± 3.66^{aAB}	29.05 ± 2.14^{bA}	2.82 ± 0.38^{bD}	5.47 ± 0.96^{aCD}

**Data points with different lower case letters in the same row for the same enzyme are significantly ($p < 0.05$) different. Data points with different upper case letters in the same column are significantly ($p < 0.05$) different.*

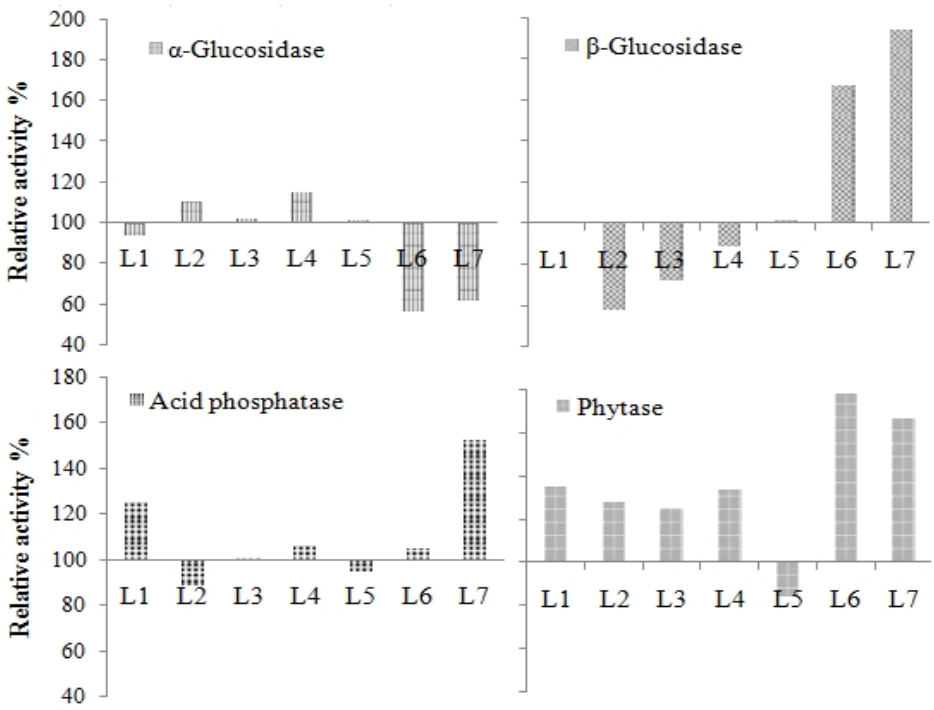

Fig. 1. Relative activity (%) of *α*-glucosidase, *β*-glucosidase, acid phosphatase, and phytase produced by *Lactobacillus* strains grown in SPM compared to that in MRS after 16 h of incubation at 37ºC.

*The relative activity was calculated as enzyme activity in SPM divided by enzyme activity in MRS then multiplied by 100. *L1: L. plantarum 299v, L2: L. acidophilus SD16, L3: L. acidophilus EF7, L4: L. rhamnosus GG B103, L5: L. delbrueckii subsp. bulgaricus SR35, L6: L. reuteri CF2-7F, and L7: L. reuteri SD2112.*

3.3 Impact of SPM on Acid Phosphatase and Phytase

Table 3 shows acid phosphatase and phytase activity of *Lactobacillus* strains in SPM and MRS. Acid phosphatase ranged between 8.67±0.87 (*L. acidophilus* EF7 in MRS) and 20.56±1.49 (*L. reuteri* SD2112 in SPM) Ph U/mL. *L. reuteri* CF2-7F and SD2112 showed the highest acid phosphatase activity compared to other lactobacilli strains. The growth of *L. reuteri* SD2112 in SPM led to a 52.3% increase in acid phosphatase (Fig. 1). In addition, *L. plantarum* 299v, *L. rhamnosus* GG B103, and *L. reuteri* CF2-7F showed relatively higher acid phosphatase activity in SPM compared to MRS. On average *Lactobacillus* strains showed a 35.9% increase in acid phosphatase activity in SPM than MRS.

Phytase activity of *Lactobacillus* strains ranged between 0.16±0.03 (*L. delbrueckii* subsp. *bulgaricus* SR35 in SPM) and 0.66±0.14 (*L. reuteri* CF2-7F in SPM) Ph U/mL. All tested strains produced significantly ($p < 0.05$) higher phytase in SPM compared to MRS except *L. delbrueckii* subsp. *bulgaricus* SR35. The growth of *L. reuteri* (CF2-7F and SD2112) in SPM led to 78% and 67% respectively increases in phytase (Fig. 1). The phytase activity of *Lactobacillus* strains growing in SPM increased by an average of 10.3% compared to MRS.

Table 3. Acid phosphatase and phytase activity of *Lactobacillus* strains in SPM and MRS after 16h on incubation at 37ºC.

Lactobacilli Strain	Acid phosphatase (Ph U/mL)*		Phytase (Ph U/mL)*	
	MRS	SPM	MRS	SPM
L. plantarum 299v	9.44 ± 1.18^{aB}	11.83 ± 1.09^{aC}	0.34 ± 0.03^{bA}	0.46 ± 0.04^{aB}
L. acidophilus SD16	11.22 ± 0.57^{aB}	9.92 ± 0.78^{aC}	0.32 ± 0.02^{bA}	0.41 ± 0.04^{aB}
L. acidophilus EF7	8.67 ± 0.87^{aB}	8.68 ± 0.85^{aC}	0.32 ± 0.05^{bA}	0.40 ± 0.03^{aB}
L. rhamnosus GG B103	9.96 ± 1.07^{aB}	10.60 ± 0.55^{aC}	0.30 ± 0.03^{bA}	0.40 ± 0.02^{aB}
L. delbrueckii subsp. *bulgaricus* SR35	11.19 ± 0.95^{aB}	10.61 ± 0.84^{aC}	0.19 ± 0.02^{aB}	0.16 ± 0.03^{bC}
L. reuteri CF2-7F	15.09 ± 0.95^{aA}	15.84 ± 1.05^{aB}	0.37 ± 0.03^{bA}	0.66 ± 0.14^{aA}
L. reuteri SD2112	13.50 ± 0.84^{bA}	20.56 ± 1.49^{aA}	0.39 ± 0.05^{bA}	0.65 ± 0.11^{aA}

**Data points with different lower case letters in the same row for the same enzyme are significantly ($p < 0.05$) different. Data points with different upper case letters in the same column are significantly ($p < 0.05$) different.*

Strains of *L. reuteri* showed the highest acid phosphates and phytase activity. Previous study also showed that *L. reuteri* could produce higher phytase compared to other lactobacilli species [10]. However, phytase activity in the tested strains was found low. The phytate degrading activity of *Lactobacillus* seems to be due to a non-specific acid phosphatase, which shows high hydrolysis rates with monophosphorylated compounds [10, 26]. In addition, phytase does not seem to be common in *Lactobacillus* strains and phytase activity of *Lactobacillus* is generally low compared to other bacterial genera [10,26]. Phytase and acid phosphatase are particular subgroups of phosphatases, whereas phytase exhibits a preference for phytate. The specificity of both phytase and acid phosphatase can partially overlapped since acid phosphatase also shows phytase activity [13]. Thus, both acid phosphatase and phytase can be useful in the degradation of phytate. An increased level of these enzymes can be produced by *Lactobacillus* while growing in a SPM.

3.4 Effect of Metal Ions on the Enzymatic Activity of *L. reuteri*

Enzymatic activity of the tested *Lactobacillus* strains was relatively higher in SPM than in MRS for most enzymes. In addition, our results showed that strains of *L. reuteri* expressed higher enzymatic activity than other tested *Lactobacillus* species. Thus, further steps were taken to investigate the effect of different metal ions on the enzymatic activity of *L. reuteri* growing in a SPM. Fig. 2 showed the relative effect of metal ions on the enzymatic activity of *L. reuteri* compared to control (SPM without metal ions). The *α*-glucosidase activity of *L. reuteri* increased in the presence of Na^{2+} and Mg^{2+} and decreased in the presence of Fe^{2+}, Ca^{2+}, and K^{+}. Mn^{2+} increased *α*-glucosidase activity of SD2112 and showed a slight decrease in CF2-7F. In regard to *β*-glucosidase activity of *L. reuteri*, all metal ions showed an increase in this enzyme that ranged between 4.1 and 130.6 for CF2-7F in presence of K^{+} and SD2112 in presence of Mn^{2+} respectively. Ca^{2+} and Fe^{2+} caused higher enhancements on *β*-glucosidase than other metal ions. The addition of Ca^{2+} to SPM increased *β*-glucosidase activity of *L. reuteri* CF2-7F and SD2112 by 116.9 and 129.8% respectively.

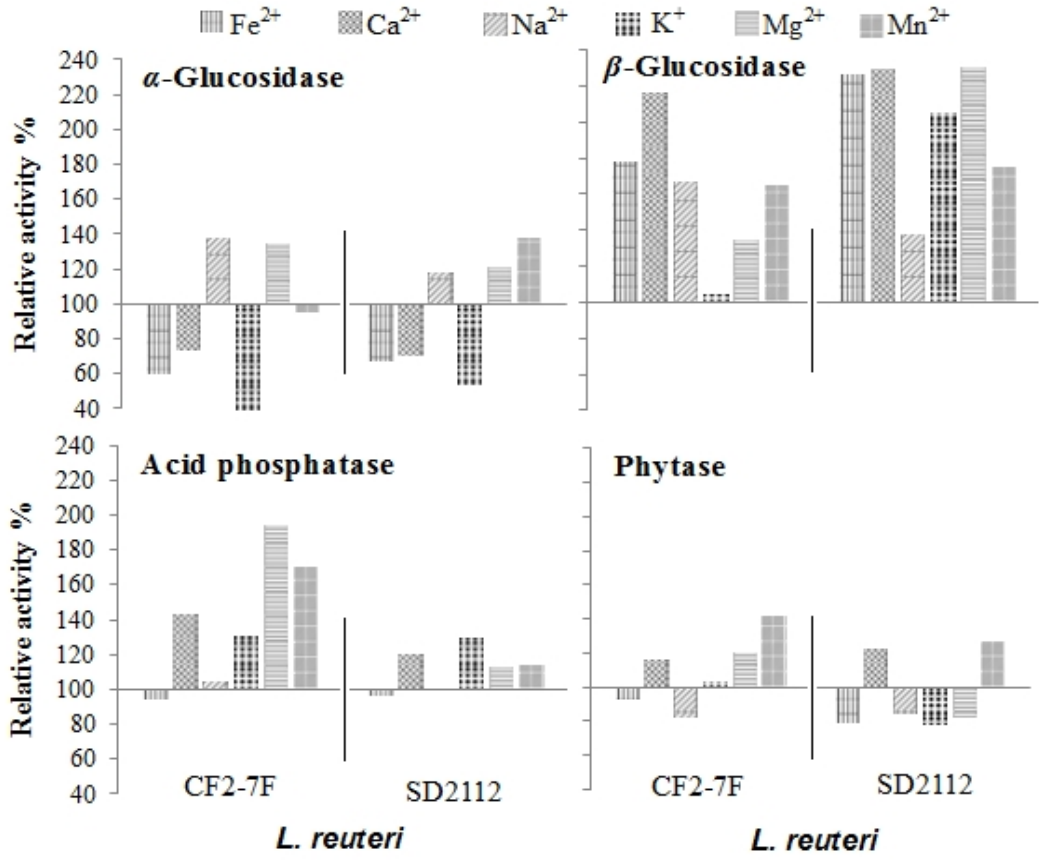

Fig. 2. Relative enhancement effect (%) of metal ions on *α*-glucosidase, *β*-glucosidase, acid phosphatase, and phytase produced by *L. reuteri* after 16h of incubation at 37ºC.
Relative activity was calculated as the enzyme activity in SPM with the addition of metal ions divided by enzyme activity in control samples then multiplied by 100

Previous studies showed that the effect of metal ions on *β*-glucosidase activity varied with the bacterial strain and concentration of the metal ions [16,27]. *α*-Glucosidase activity from *Lactobacillus rhamnosus* R was inhibited by Mn^{2+}, Cu^{2+}, and Fe^{2+} and Zn^{2+} and slightly activated by Li^{+}, Na^{+}, K^{+}, Ca^{2+}, Co^{2+}, and Mg^{2+} [27]. The addition of 10 mM of Mn^{2+} caused a significant enhancement (150% to 230%) of *β*-glucosidase activity while 10 mM of Zn^{2+} or Cu^{2+} caused up to a 90% reduction of *β*-glucosidase [8]. Thus, our results agreed with previous works suggesting that the addition of metal ions could produce an enhanced level of both *α*-glucosidase and *β*-glucosidase. In addition, metal ions showed higher enhancement on *β*-glucosidase activity of *L. reuteri* SD2112 than *α*-glucosidase of the same strain.

The relative effect of metal ions on acid phosphatase and phytase produced by *L. reuteri* growing in SPM was also studied (Fig. 2). All metal ions enhanced acid phosphatase produced by *L. reuteri* except Fe^{2+}. The phytase activity of *L. reuteri* increased in the presence of Ca^{2+} and Mn^{2+} and decreased in the presence of Fe^{2+} and Na^{+}. The presence of Mg^{2+} and K^{+} caused an increase in phytase activity of *L. reuteri* CF2-7F and a decrease in phytase activity of *L. reuteri* SD2112. These results indicated that the effect of metal ions on

phytase activity is a strain specific. However, the presence of metal ions exhibited a significant effect on both acid phosphatase and phytase activity of *L. reuteri*. Previous study showed that phytase activity of *Lactobacillus* strains can be enhanced in the presence of Ca^{2+} [28]. This is in agreement with our results which also showed that phytase activity increased in the presence of Ca^{2+} for both *L. reuteri* strains. It is well established that Mg^{2+} is essential for a vast number of enzymatic reactions [29]. It has also been suggested that a high concentration of Mn^{2+} helps the cell to deal with reactive oxygen species and serves as an alternative for the absence of a gene encoding a superoxide dismutase. In addition, Mn^{2+} was reported to stimulate good phosphatase activity which may be due to the fact that many protein phosphatases have Mn^{2+} [16,30]. Therefore, including Mn^{2+}, Mg^{2+}, and Ca^{2+}in the growth culture of *L. reuteri* could enhance the enzymatic activity. These results suggested that the selection of a combination of specific strain and specific metal ion is required to achieve a maximized level of the target enzyme. Combination of metal ions and different concentrations may also be used depending on the enzymatic response of the strain to different metal ions.

4. CONCLUSION

In this work we studied the growth and enzymatic activity of seven *Lactobacillus* strains grown in SPM and MRS. SPM was supplemented with 4 g/L of each peptone, yeast extract, and beef extract. Our resulted revealed that SPM could support similar growth rate of *Lactobacillus* strains compare to MRS. SPM could also enhance the activity of *β*-glucosidase but may cause a decrease in *α*-glucosidase activity. *Lactobacillus* could also express better acid phosphatase and phytase activity growing in SPM compared to MRS. Strains of *L. reuteri* (CF2-7F and SD2112) showed the highest *α*-glucosidase, acid phosphates, and phytase whereas *L. delbrueckii* subsp. *bulgaricus* SR35 showed the highest *β*-glucosidase. The enzymatic activity of *L. reuteri* growing in SPM could be further enhanced by the addition of metal ions. The effect of metal ions may vary according to different strain and thus was suggested to be a strain specific. However, the addition of Mn^{2+}, Mg^{2+}, and Ca^{2+}in the growth culture of *L. reuteri* could enhance the enzymatic activity. Therefore, SPM could be a suitable medium for the growth and enzymatic activity of *Lactobacillus* and may also support other bioactivity. Further work is required to investigate the impact of SPM on other functionality of *Lactobacillus* and to determine the optimum concentration and combination of metal ions to maximize the enzymatic activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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