All experiments were conducted twice. Comparisons between treatments were analyzed by SAS 9.2 (SAS Inst., Cary, NC). *P* values less than 0.05 were considered statistically significant.

Values are means ± standard deviation. Values with different letters within a column are significantly different ($p < 0.05$).

Initial population was 7.16, 7.12, and 7.23 log CFU/g for cilantro, dill, and parsley respectively. (Average initial population=7.17 log CFU/g)

IMPACT OF SWEET POTATO AND METAL IONS ON THE GROWTH AND ENZYMATIC ACTIVITY OF LACTOBACILLUS REUTERI ATCC 55730

Saeed A. Hayek*, Sulaiman O. Aljaloud**, Salam A. Ibrahim*,Mohammad Ali Shariati***

****Food Microbiology Laboratory, North Carolina Agricultural and Technical University, Greensboro, NC, USA***

*****College of Sports Sciences and Physical Activity, King Saud University, Riyadh, Saudi Arabia***

******Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.***

Abstract *The objective of this study was to determine the effect of sweet potatoes and metal ions on the growth and enzymatic activity of Lactobacillus reuteri ATCC 55730. Extract from baked sweet potatoes was supplemented with 4 g/L each of beef extract, yeast extract, and proteose peptone #3 to form the sweet potato medium (SPM). The growth and enzymatic activity of L. reuteri ATCC 55730 in SPM was then compared to standard MRS. The enzymatic activity of L. reuteri ATCC 55730 including α–glucosidase, β-glucosidase, acid phosphatase, and phytase was determined spectrophotometrically using a corresponding substrate. Our results showed similar growth patterns for L. reuteri ATCC 55730 growing in SPM and MRS. Final bacterial population reached 8.59±0.21 and 8.55±0.31 log CFU/mL in SPM and MRS respectively after 16 h of incubation at 37ºC. L. reuteri ATCC 55730 grown in SPM showed a 103.5, 74.5, and 43.6 % increase in β-glucosidase, acid phosphatase, and phytase activity*

respectively compared to MRS. The increase in β-glucosidase due to the addition of Mn^{2+}, Na^{+}, Mg^{2+} and Ca^{2+} ranged between 167 and 208 %. Phytase increased by 69 and 62 % in the presence of Mn^{2+} and Ca^{2+} respectively. These findings indicated that sweet potatoes can improve the enzymatic activity of L. reuteri ATCC 55730 and the addition of selected metal ions could produce an enhanced level of these enzymes.

Key words: *L. reuteri ATCC 55730, sweet potato medium (SPM), MRS, growth rate, bacterial population, and enzymatic activity.*

Introduction:

L. reuteri has been extensively studied for its probiotic characteristics, including: safe administration to healthy individuals, ability to colonize the intestine, usage as a diarrhea therapeutic agent and, inhibitor of pathogenic bacteria, and the immunological modulation of the gastrointestinal mucosa (Morita et al. 2008). The first isolated strain of *L. reuteri* for human use was *L. reuteri* ATCC 55730. This strain was isolated from human breast milk in 1995 and deposited at the American Type Culture Collection (ATCC) (Arvidsson 2002)Lund</pub-location><publisher>Stockholm University</publisher><urls></urls></record></Cite></EndNote>. *L. reuteri* has since reportedly produced different functional enzymes such as α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, acid phosphatase, and phytase (Alazzeh et al. 2009; Hayek et al. 2013b; Otieno et al. 2005; Palacios et al. 2007). Because these enzymes typically occur at low levels in humans, microbial sources of such functional enzymes could be promising agents for the support of human health (Mahajan et al. 2010; Jeng et al. 2011).

Plant components have been shown to enhance the growth and functionality of probiotics including *Lactobacillus* spp. (Gyawali and Ibrahim 2012). Sweet potatoes (*Ipomoea batatas*) are an abundant agricultural product that plays a major role in the food and feed industries. Sweet potatoes are a rich source of carbohydrates, some amino acids, vitamins, minerals, and dietary fiber (Broihier 2006; Padmaja 2009). We have previously reported the successful use of sweet potatoes as a basic component in the formation of an alternative medium for the cultivation of *Lactobacillus* (Hayek et al. 2013a). Thus, the objective of this study was to determine the impact of sweet potato and metal ions on the growth and enzymatic activity of *L. reuteri* ATCC 55730.

Material and method

Media preparation Sweet potato medium (SPM) was prepared according to the method previously described by Hayek et al. (2013a). In this pro-

cedure, 1.0 L of SPM contains 350g of baked sweet potato, beef extract (4.0 g), yeast extract (4.0 g), proteose peptone # 3 (4.0 g), sodium acetate (5g), potassium monophosphate (2g), ammonium citrate (2g), disodium phosphate (Na_2HPO_4) (0.2g), Tween 80 (1.0 mL), magnesium sulfate ($MgSO_4.7H_2O$) (0.1g), manganese sulfate ($MnSO_4.5H_2O$) (0.05g), and L-Cysteine (1.0g). NSPM without nitrogen source was also formed. MRS was prepared by dissolving 55.0 g MRS broth and 1.0 g L-Cysteine in 1.0 L DDW. NSPM, SPM, and MRS were sterilized at 121ºC for 15 min, cooled down, and stored at 4ºC to be used within 24h.

Bacterial culture activation and preparation *L. reuteri* ATCC 55730 was provided by BioGaia (Raleigh, NC) and activated in lactobacilli MRS. Prior to each experimental replication, *L. reuteri* ATCC 55730 was streaked on MRS agar and SPM agar then incubated for 48 h at 37°C. An isolated colony was then transferred to 10 mL MRS broth and SPM broth and then incubated at 37°C for next day use.

Culture conditions and growth monitoring SPM and MRS were inoculated with *L. reuteri* ATCC 55730 at low inoculum level (2-3 log CFU/mL) and incubated at 37ºC for 16h. Initial bacterial populations were determined. Bacterial growth was monitored by measuring turbidity (optical density at 610 nm) at 2 h interval, bacterial populations at 8, 12, and 16 h, and final pH.

Enzymatic activity assay *L. reuteri* ATCC 55730 was cultured in batches of 80 mL SPM and MRS and incubated at 37ºC for 16 h. Metal ions, 10 mM of $FeSO_4.4H_2O$, $MgSO_4.7H_2O$, K_2SO_4, or Na_2SO_4, and 5 mM of $MnSO4._4H_2O$ or $CaSO_4.7H_2O$, were added separately to batches of 80 mL SPM broth, inoculated with *L. reuteri* ATCC 55730 and incubated at 37ºC for 16 h. α-Glucosidase and β-glucosidase were determined by monitoring the rate of hydrolysis of $\boldsymbol{\rho}$-nitrophenyl-α-D-glucopyranoside (α-NPGP) and $\boldsymbol{\rho}$-nitrophenyl-**β**-D-glucopyranoside (**β**-NPGP) respectively (Mahajan et al. 2010). Acid phosphatase was determined by monitoring the rate of hydrolysis of $\boldsymbol{\rho}$-nitrophenyl phosphate (ρ-NPP), and phytase was determined by measuring the amount of liberated inorganic phosphate (Pi) from sodium phytate (Haros et al. 2008).

Statistical analysis Each test was conducted three times in randomized block design and the mean values and standard deviations were calculated. R Project for Statistical Computing version R-2.15.2 (www.r-project.org) was used to determine significant differences in the growth and enzymatic activity using one way ANOVA (analysis of variance) with a significance level of $p < 0.05$.

Results and discussion

Figure 1 shows the growth of *L. reuteri* ATCC 55730 in SPM and MRS during 16 h of incubation at 37ºC. After 16 h of incubation, the turbidity reached 0.929±0.034, and 0.932±0.023 OD 610 nm in SPM and MRS respectively. The final bacterial populations of *L. reuteri* ATCC 55730 growing in SPM and MRS (Table 1) showed no significant ($p > 0.05$) differences throughout the incubation time. *L. reuteri* ATCC 55730 observed similar growth patterns in both MRS and SPM. After 24h of incubation, the pH value in SPM and MRS dropped to 4.53±0.08 and 4.02±0.13 respectively. Thus, SPM could perform better than MRS in maintaining the optimum pH while showing a similar growth rate.

Table 3 shows the enzymatic activity of *L. reuteri* ATCC 55730 growing in SPM and MRS. *L. reuteri* ATCC 55730 grown in SPM exhibited higher β-glucosidase, acid phosphatase, and phytase and lower α-glucosidase compared to MRS. β-Glucosidase, acid phosphatase, and phytase in SPM increased by 103.5, 74.5 and 43.6 % respectively compared to MRS. α-Glucosidase activity in SPM was 24.4% lower than MRS. The higher enzymatic activity in SPM compared to MRS may be due to the fact that sweet potatoes are rich in many nutrients that may enhance activity of the studied enzymes.

Figure 2 shows the relative effect of metal ions on the enzymatic activity of *L. reuteri* ATCC 55730 growing in SPM compared to control (SPM without metal ions). With regard to β-glucosidase, the addition of metal ions caused a significant increase in this enzyme with Mn^{2+} showing the highest increase. Mn^{2+}, Mg^{2+}, Ca^{2+}, and Na^{2+} caused an increase in β-glucosidase activity ranging between 167 and 208 %. α-Glucosidase activity of *L. reuteri* ATCC 55730 increased in the presence of Na^{2+}, Mn^{2+}, and Mg^{2+} and decreased in the presence of Fe^{2+}, Ca^{2+}, and K^{+}. The effect of tested metal ions (except Fe^{2+}) on acid phosphatase ranged between of 12 - 44% increase. Fe^{2+} caused a slight decrease in acid phosphatase activity. The addition of metal ions enhanced phytase by 3 – 67 %. Mn^{2+} and Ca^{2+} caused a 67 and 62 % increase in phytase respectively. These results indicate that the selection of metal ions is required to maximize the activity of the target enzyme. However, including Mn^{2+} and Mg^{2+} in SPM may produce an increased level of *L. reuteri* ATCC 55730 enzymatic activity.

In conclusion, *L. reuteri* ATCC 55730 exhibited a similar growth pattern and similar bacterial populations in both SPM and MRS. Sweet potato enhanced the enzymatic activity of *L. reuteri* ATCC 55730 and the addition of metal ions to SPM resulted in additional enhancement. Selection of a specific metal ion is required in order to produce a maximized enzymatic activity.

The results also suggest that consumption of sweet potatoes may serve the probiotic functionality of *L. reuteri* ATCC 55730, and this functionally can be further enhanced in the presence of metal ions. More work is needed to determine possible combinations and optimum concentrations of metal ions to maximize the enzymatic activity of *L. reuteri* ATCC 55730.

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Table 1 - Bacterial populations of L. reuteri ATCC 55730 in MRS and SPM at 8, 12, and 16 h of incubation at 37ºC.

	Incubation time (h)			
	0	**8**	**12**	**16**
Media*	**Bacterial population (log CFU/mL)**			
MRS	2.25±0.23 [a]	4.38±0.23 [a]	5.98±0.33 [a]	8.55±0.31 [a]
SPM	2.48±0.29 [a]	4.45±0.09 [a]	5.95±0.37 [a]	8.59±0.21 [a]

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

Table 2 - Enzymatic activity of L. reuteri ATCC 55730 in SPM and MRS after 16 h of incubation at 37ºC

Enzyme	MRS	SPM	Relative activity %
α-Glucosidase (Glu U/mL)	47.30±3.66 [a]	31.05±2.15 [b]	-24.4
β-Glucosidase (Glu U/mL)	2.82±0.38 [b]	5.74±0.76 [a]	103.5
Acid phosphatase (Ph U/mL)	13.50±0.84 [b]	23.56±1.49 [a]	74.5
Phytase (Ph U/mL)	0.39±0.05 [b]	0.56±0.11 [a]	43.6

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

The relative activity was calculated as the enzymatic activity in SPM divided by the enzymatic activity in MRS multiplied by 100 then minus 100.

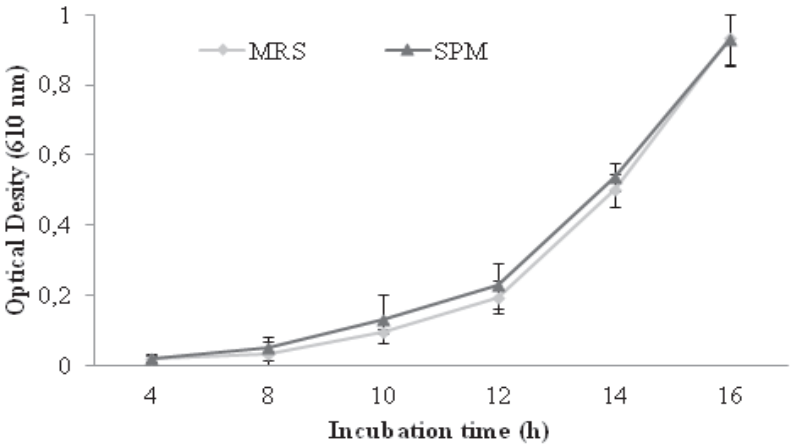

Figure 1 - Growth of L. reuteri ATCC 55730 during 16 h of incubation at 37ºC. Data points are the average of three replicates with a standard error bar.

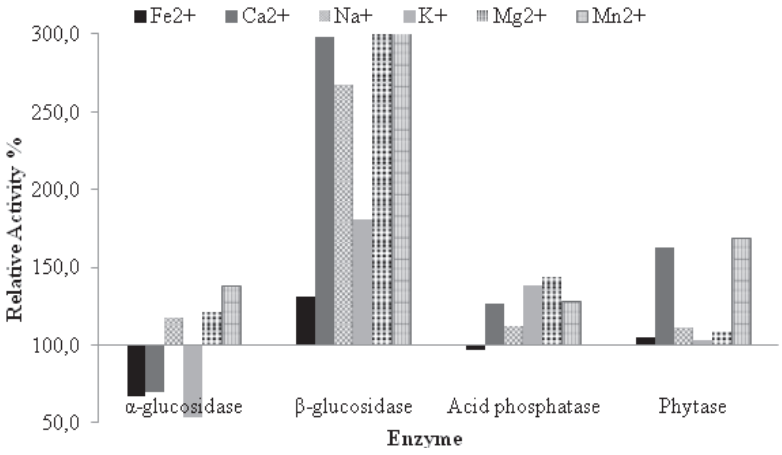

Figure 2 - Relative enzymatic activity (%) of L. reuteri ATCC 55730 with added different metal ions compared to the control group without metal ions.

The relative activity was calculated as the enzymatic activity in SPM with metal ions divided by the enzymatic activity in SPM multiplied by 100.