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Isolation and Identification of *Lactobacillus* Strains from Dairy Products and Evaluation of Carbon Sources Effects on Bacterial Growth and Phytase Activity: Supplement for Fish Feed

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ABSTRACT

***Lactobacillus* sp. are safe organisms for using as probiotic due to their different properties such as useful enzyme products. For example, phytase is an enzyme that has an important role in fish feed digestion. The aim of this study was to isolate and identify *Lactobacillus* species with phytase activity from dairy products such as milk, yogurt, and cheese and to evaluate the effects of different carbon sources on bacterial growth and phytase activity. For this purpose, *Lactobacillus* species were screened from dairy products using biochemical tests, and *16S rRNA* gene analysis was used to confirm the lactobacilli class. Three of the isolates that showed the best bacterial growth and phytase activity were selected and their *16S rRNA* gene sequences were deposited in GeneBank. Then, to determine the optimum growth conditions, three carbon sources including glucose and sucrose at concentrations of 10, 20, 30, 40 g L^{-1} and wheat bran at concentrations of 10, 20, and 30 g L^{-1} were used in three replicates. Phytase activity of isolated bacteria including *Lactobacillus* sp. strains AM11, AM13, and AM14 were measured using zymoplate as well as extracellular enzyme assay. *Lactobacillus* sp. strain AM11 showed higher phytase activity and growth compared to the other isolates ($P< 0.05$). According to the results, *Lactobacillus* sp. strain AM11 isolates can be used as a feed supplement to improve minerals availability in fish nutrition.**

Keywords: 16S rRNA, Fish nutrition, Probiotic.

INTRODUCTION

Among probiotic microorganisms, Lactic Acid Bacteria (LAB) are known as the most important group, of which *Lactobacillus* is one of the most commonly used organisms as a probiotic (Klaenhammer, 2000; Briens *et al*., 2008; Ye *et al*., 2008). They are generally recognized as safe organisms and can be safely used as probiotics for medical and veterinary applications (Bernardeau *et al*., 2006). LAB have also been used as probiotics in foods as they produce bacteriocins that inhibit harmful bacteria (Heredia-Castro *et al*., 2015). Also, they are known to increase digestibility (Gaggia *et al*., 2010). Lactobacilli comprise a large and diverse group of Gram positive, non-spore forming, catalase negative, rod bacteria, able to produce lactic acid as the main end-product of the carbohydrate fermentation (Pelinescu *et al*., 2009). Lactobacilli are present in a variety of sources, including digestive system (Majidzadeh Heravi *et al*., 2016), dairy products (Widodo and Anindita, 2014), fermented rice (Meidong *et al*., 2017) and meat samples (Vaughan *et al*., 1994). Among prokaryotic genes, the *16S rRNA* gene

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is known as a target gene in bacterial diversity studies. This gene is a general marker with a protected sequence and also has high stability and is referred to as a timer of evolution (Durme *et al*., 2001; Bulut, 2003). The *16S rRNA* gene sequence analysis can better identify poorly described, rarely isolated, or phenotypically aberrant strains, and can lead to the recognition of novel pathogens and non-cultured bacteria (Jill and Clarridge, 2004).

Phytase is a special class of phosphatases that catalyzes the sequential hydrolysis of myo-Inositol-(1, 2, 3, 4, 5, 6)-hexakisPhosphate or phytic acid (InsP6) to less phosphorylated myo-inositol derivatives and inorganic phosphate (Haros *et al*., 2007). Phytate degrading activity has been detected in plants, microorganisms, and in some animal tissues and phytases have been purified and characterized from several plants and microbial species (Hill *et al*., 2007). Although phytase is currently used mainly as feed additives in diets of monogastric animals, there is a great potential for the use of this class of enzymes in processing and manufacturing of food for human consumption (Jorquera *et al*., 2008). Nevertheless, there are still limited sources of phytase that are suitable to be used in animal feed (Afinah *et al*., 2010).

Several studies have reported that the media has been found to support the growth of *Lactobacillus* species. These include carbohydrate and/or nitrogen sources such as wheat flour hydrolysate (Hofvendahl and Hahn-Hagerdal, 1997), wheat bran (Naveena *et al*., 2004), wheat bran hydrolysate combined with corn steep liquor (Li *et al*., 2010), lactose and whey permeate (Fu and Mathews, 1999), corn steep liquor, cane molasses with animal and marine by-products (Demirci *et al*., 1998). It is essential to note that all of the previous studies aimed at optimizing the medium for lactic acid production. Sreedevi and Reddy (2012) remarked that pH, temperature, time, and composition of the medium could have an efficient effect on bacterial growth.

Various compounds can be used as a source of carbon. Carbon compounds include simple small molecules like sugars, organic acids, proteins, polysaccharides, and lipids (Barnett, 1981). The presence of sucrose may modify the expression amount and activity of sucrose metabolizing enzymes (Hardy *et al*., 1981; Hudson and Curtis, 1990). Also, wheat bran is helpful as a carbon source in induction of glucose isomerase and phytase production (Viveros *et al*., 2000; Bhasin and Modi, 2012). Glucose is one of the primary molecules that serve as energy sources for almost all organisms, including bacteria. One of the most common growth media used for *Lactobacillus* growth is Man Rogosa Sharp (MRS). The addition of glucose to MRS broth may increase the overall growth rates and biomass of bacteria over time (Song *et al*., 1987; Kuhnt and Anke, 1990; Stadler *et al*., 1994).

The aim of this study was to isolate and identify of *Lactobacillus* strains with phytase activity by morphological and biochemical tests and *16S rRNA* gene analysis and investigate some carbon sources effect on *Lactobacillus* sp. growth and phytase activity. For this purpose, some simple sugars such as glucose, sucrose, and wheat bran were used as the sources of carbon.

MATERIALS AND METHODS

Isolation and Molecular Detection of *Lactobacillus* Strains

Thirty-one *Lactobacillus* species were isolated from sheep and cow's milk, yogurt, and cheese using serial dilutions of samples and screening by conventional culturing on MRS medium for lactobacilli growth. Then, each colony was tested for gram positive (Cappuccio and Sherman, 1998) and catalase activity (Pollock *et al*., 2002). Each isolate was streaked on MRS agar medium and incubated under anaerobic condition using a candle extinction jar with a moistened filter paper to provide a CO_2-enriched, water-vapor saturated atmosphere at 30°C for 48 hours. Single colonies picked off the plates were sub-cultured in MRS broth at 30°C for 24 hours (Hartemink *et al.*, 1997). For *16S rRNA* gene analysis, one pair of specific primers based on the gene sequences of *16S rRNA* in lactobacilli

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presence in dairy products were designed using Generunner version 6 software. *16S rRNA* gene sequences were extracted from NCBI database. The multiple alignment was developed using clustalW in EMBL-EBI (www.ebi.ac.uk/service/tools) and primers designed based on conserved region in sequences. The primers, Forward; 5'-*GGTGAGTAACACGTGGGNAA*-3' and Reverse; 5'-*GCTGATCCGCGATTACTAG*-3' were constructed by Tag Copenhagen company. Bacterial DNA extraction was performed using salt extraction method (http://openwetware.org/wiki/DNA_extraction_-_Salting_Out_protocol) and the quality of extraction was checked in 1% agarose gel electrophoresis.

Amplification of *16S rRNA* Gene by Polymerase Chain Reaction (PCR)

For the amplification of *16S rRNA* gene, the following program was performed. Early denaturing at 95°C for 5 minutes, a run of 36 cycles each cycle consisting of denaturation at 95°C for 1 minutes, annealing at 58°C for 1 minute, and extension 72°C in 90 seconds, and final extension cycle was performed at 72°C for 7 minutes. Amplified products of 1,200 bp were purified using a DNA extraction kit (Favorgen, Taiwan). DNA sequencing on both strands directly was performed by Macrogene (South Korea) according to Sanger sequencing method. The three *16S rRNA* gene sequences were deposited in GeneBank.

Bio-informatic Analysis and Drawing Phylogenetic Tree

At first, the match *16S rRNA* gene sequences were performed in Blast, then, multiple alignment were done with *Lactobacillus 16S rRNA* gene sequences extracted from Blast results as well as those extracted from NCBI. After that, the phylogenetic tree was constructed using phyloDraw software by neighbor joining method.

Experiments on Different Carbon Sources

Different concentrations of each source of carbon including wheat bran with concentrations of 10, 20, and 30 g L^{-1} and glucose and sucrose with concentrations of 10, 20, 30, and 40 g L^{-1} were added to MRS broth media only for isolated and detected bacteria. Then, the isolates were cultured separately in 15 mL flasks and incubated at 15ºC for 48 hours. The absorbance of bacterial growth was measured at 600 nm by spectrophotometer (BEL PHOTONICS-UV-M51 UV/VIS - Italy).

Phytase Enzyme Assay

Phytase activity of *Lactobacillus* species were detected by using a specific method described by Bae *et al*. (1999). To extract the enzyme, a single colony of *Lactobacillus* strains was cultivated in MRS broth containing 0.1% (w/v) sodium phytate at 30°C for 48 or 24 hours. After incubation, the bacteria were separated from the supernatant by centrifugation in 5,000 rpm for 10 minutes, at 4°C. The supernatant was isolated and filtered through a filter (pore size: 0.45 µm) under vacuum. Then, the same volume of ethanol 96% was added and kept at -20°C for one day. Subsequently, the suspension was centrifuged at 10,000 rpm for 15 minutes at 4°C and the extracellular enzyme was isolated. The concentrated extracellular enzyme was suspended in 0.1M sodium acetate buffer for pH 6.0, and was kept for activity assays of enzyme at -20°C (Bae *et al*. 1999).

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Phytase activity was assayed by measuring the amount of phosphate released from sodium phytate (Raghavendra and Halami, 2009). A reaction mixture containing 400 µL of extracellular enzyme, 200 µL of 100 mM sodium acetate buffer, and 200 µL sodium acetate buffer containing 2 mM sodium phytate as substrate was incubated at 15°C for 15 minutes. The reaction was stopped by adding 800 µL of 10% (w/v) trichloroacetic acid solution (Raghavendra and Halami, 2009). The released inorganic phosphate was measured by adding 800 µL of color reagent, prepared daily by mixing 4 volumes of solution A and one volume of solution B. The fresh solution A was prepared daily by dissolving 2 g ammonium molybdate in 80 mL distilled water and adding 5.5 mL sulfuric acid (98%) and diluting to 20 mL with water. Solution B contained 2% ferrous sulfate (2 g in 100 mL distilled water). The mixture was centrifuged at 10,000 rpm for 5 minutes at 4°C. After 15 minutes, the absorbance of tubes containing the reactions were measured in the 660 nm range, using a spectrophotometer (BEL PHOTONICS-UV-M51 UV/VIS spectrophotometer- Italy) (Choi *et al*., 2001). One unit of phytase activity was defined as the amount of enzyme producing 1 µmol of inorganic phosphorous per 15 min. The unit of enzyme was estimated in one mL of bacteria culture. The results were compared to a standard curve prepared with inorganic phosphate (K2HPO4).

Statistical Analysis

This experiment was conducted with a completely randomized design and all experiments were done in triplicates. Data were first normalized using Kolmogorov-Smirnov method, then, Two-way ANOVA was used to specify the effect of different carbon sources and their concentrations with the interaction between them on growth and phytase enzyme activity. To compare differences between the mean values of treatments, Duncan's test was applied. All data were reported mean±*SD* using SPSS version 17.

RESULTS

Isolation and Molecular Detection of *Lactobacillus* Strains

In the 31 LAB isolated from sheep and cow's milk, the minimum and maximum ranges of phytase activities and growth rates after 24 hours were 0.15±0.007–0.912±0.14 U mL^{-1} and 0.08±0.01–2.42±0.3, respectively. The phytase activities of the three strains that showed the best bacterial growth were 0.901±0.10, 0.912±0.14 and 0.771±0.05 U mL^{-1}, with growth rates of 2.42±0.030, 2.30±0.002 and 1.42±0.23, respectively. Also, the isolated strains were Gram-positive, catalase-negative, facultative anaerobe, and rod-shape bacteria.

The amplification of *16S rRNA* gene was performed by PCR and the major bands of 1,200 bp were detected in 1% agarose gel (Figure 1).

The result from *16S rRNA* gene analysis showed that our isolates had very close (99%) identity to *Lactobacillus* species reported on NCBI database (Figure 2).

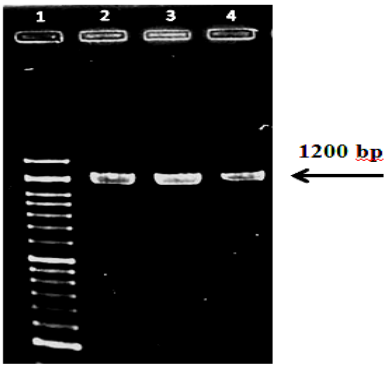

Figure 1. *16S rRNA* gene amplification using PCR. Lane 1: DNA size marker (Sinaclone, 50-1,500 bp, PR901633), Lane 2: PCR product of *Lactobacillus* sp. Strain AM11, Lane 3: PCR product of *Lactobacillus* sp. Strain AM13, and Lane 4: PCR product of *Lactobacillus* sp. Strain AM14.

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The *16S rRNA* gene sequences from our *Lactobacillus* sp. strains AM11, AM13 and AM14 isolates were deposited in GeneBank under accession numbers of MF449508, MF455195 and MF445153, respectively.

Effect of Carbon Sources on Bacterial Growth

Different effect of carbon sources on isolated bacterial growth are shown in Table 1. No significant changes in bacterial growth were detected in different concentrations of glucose in *Lactobacillus* sp. AM11 and AM14 (Table 1; P> 0.05). In strain AM13, the maximum growth rate of bacteria was observed at 10 and 20 g L^{-1} of glucose concentrations (P< 0.05). Glucose trials of *Lactobacillus* sp. AM11 showed that the isolate AM11 had greater overall growth and maximum biomass compared to *Lactobacillus* sp. AM13 and AM14. Moreover, AM13 had higher growth and biomass than AM14 (P< 0.05). Two-way ANOVA revealed significant interactions between glucose concentration and the bacterial strains (P= 0.00). Bacterial growth was significantly different among groups exposed to different glucose concentrations and strains (P= 0.00 and 0.00, respectively).

Adding different concentrations of sucrose in the medium showed that growth of *Lactobacillus* sp. AM11 was higher than the other two isolates (Table 1; P< 0.05). Different concentrations of sucrose showed that *Lactobacillus* sp. AM11 biomass were higher and those of AM13 and AM14 were lower at concentration of 10 g L^{-1} than the others. No significant differences were found at different concentrations of sucrose 20, 30 g L^{-1} in AM13 and 20, 30, 40 g L^{-1} in AM11 and AM14 (P< 0.05). Two-way ANOVA revealed significant interactions between sucrose concentrations and bacterial strains (P= 0.61). Bacterial growth was significantly different among groups exposed to different sucrose concentrations and strains (P= 0.00 and 0.00, respectively).

Lactobacillus sp. AM11 at the wheat bran solution concentration of 20 and 30 g L^{-1} and AM13 at the concentration of 30 g L^{-1} attained the highest growth rate (Table 1). The growth of *Lactobacillus* sp. AM14 showed no significant (P< 0.05) difference in growth of bacteria among different concentrations of wheat bran. The lowest growth was observed in AM14 in all concentrations (P< 0.05). Two-way ANOVA revealed significant interactions between wheat bran concentrations and bacterial strains (P= 0.61). Bacterial growth was significantly different among groups exposed to different wheat bran concentrations and strains (P= 0.00 and 0.00, respectively).

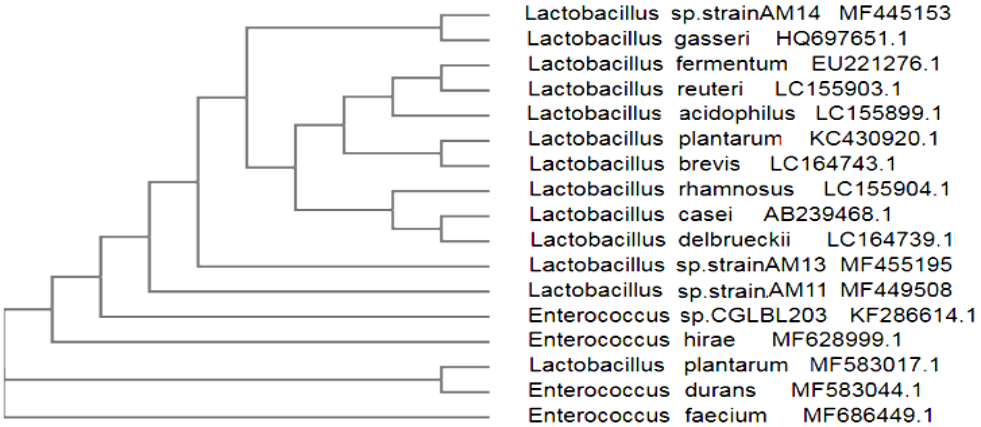

Figure 2. Phylogenetic tree of *Lactobacillus* sp. strains AM11, AM13, and AM14 with related lactobacilli species inferred from sequence of *16S rRNA* gene created by neighbor joining method.

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Table 1. Lactobacilli strains' growth at different concentrations of glucose, sucrose and wheat bran after 24 hours. [a]

Concentration (g L^{-1})		$OD_{600\ nm}$ *Lactobacillus* sp. strain			*P* value		
		AM11	AM13	AM14	Concentrations	Strains	Concentrations × Strains
glucose	10	2.055 ± 0.017^{Aa}	0.982 ± 0.080^{Ba}	0.287 ± 0.032^{Ca}	0.00	0.00	0.00
	20	2.055 ± 0.014^{Aa}	0.915 ± 0.021^{Ba}	0.234 ± 0.117^{Ca}			
	30	2.063 ± 0.028^{Aa}	0.751 ± 0.010^{Bb}	0.298 ± 0.100^{Ca}			
	40	2.061 ± 0.017^{Aa}	0.643 ± 0.013^{Bc}	0.236 ± 0.085^{Ca}			
sucrose	10	2.10 ± 0.011^{Aa}	0.764 ± 0.007^{Bc}	0.143 ± 0.037^{Cb}	0.00	0.00	0.00
	20	2.05 ± 0.009^{Ab}	0.857 ± 0.007^{Ba}	0.221 ± 0.033^{Ca}			
	30	2.05 ± 0.010^{Ab}	0.860 ± 0.008^{Ba}	0.228 ± 0.043^{Ca}			
	40	2.03 ± 0.018^{Ab}	0.802 ± 0.008^{Bb}	0.270 ± 0.045^{Ca}			
wheat bran	10	2.28 ± 0.035^{Ab}	2.16 ± 0.010^{Ac}	$1.06\pm0.\ 23^{Ba}$	0.00	0.00	0.00
	20	2.38 ± 0.162^{Aa}	2.24 ± 0.090^{Bb}	1.17 ± 0.04^{Ca}			
	30	2.42 ± 0.030^{Aa}	2.30 ± 0.002^{Aa}	1.42 ± 0.23^{Ba}			

[a] Values are mean±*SD*. Different lowercase and uppercase letters show significant differences in each column and raw, respectively (P< 0.05).

Effect of Carbon Sources on Bacterial Phytase Activity

The effect of different carbon sources (glucose, sucrose, and wheat bran) on phytase activity are shown in Table 2. Except 30 g L^{-1} glucose concentration (Table 2), the phytase activity was not significantly (P< 0.05) different among all bacterial strains, and at this concentration, the lowest phytase activity was observed in the strain AM 13 and AM14 and the highest activity was observed for AM 11 (P< 0.05). Also, no significant (P< 0.05) differences were found between AM11 and AM14 in all concentrations of carbon sources. The highest (P< 0.05) phytase activity was observed at 10 and 20 g L^{-1} glucose concentrations for strain AM13. Two-way ANOVA revealed significant interactions between glucose concentration and the bacterial strains (P= 0.00). The phytase activity was significantly different among groups exposed to different glucose concentrations and strains (P= 0.00 and 0.00, respectively).

The effect of sucrose on phytase activity (Table 2) showed that 10 g L^{-1} sucrose concentration had the highest enzyme activity in AM13 and no significant difference was found in phytase activity for AM11 and AM14 strains at10, 20, and 30 g L^{-1} concentrations and among 20, 30 and 40 g L^{-1} sucrose concentrations (P< 0.05). The isolate *Lactobacillus* sp. AM11 showed the highest phytase activity in all concentrations (P< 0.05). Two-way ANOVA revealed no significant interactions between sucrose concentrations and bacterial strains (P= 0.61). The phytase activity was significantly different among groups exposed to different sucrose concentrations and strains (P= 0.00 and 0.00, respectively).

Except for the concentration of 10 g L^{-1} wheat bran, the activity of phytase in the remaining concentrations in different strains showed a significant difference (P< 0.05) (Table 2). The isolate of Lactobacillus sp. AM11 showed that phytase activity was higher (P< 0.05) than other strains at concentrations of 20 and 30 g L^{-1}. No significant difference was found in phytase activities of the strains AM13 and AM14 in concentrations of 20 and 30 g L^{-1} of wheat

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Table 2. Lactobacilli strains' phytase activity (U mL^{-1}) at different concentrations of glucose , sucrose and wheat bran after 24 hours. [a]

Concentration ($g L^{-1}$)		Phytase activity (U mL^{-1}) *Lactobacillus* sp. strain			*P* value		
		AM11	AM13	AM14	Concentrations	Strains	Concentrations × Strains
glucose	10	0.267 ± 0.09^{Aa}	0.213 ± 0.01^{Aa}	0.258 ± 0.05^{Aa}	0.00	0.00	0.00
	20	0.222 ± 0.03^{Aa}	0.206 ± 0.00^{Aab}	0.227 ± 0.02^{Aa}			
	30	0.322 ± 0.02^{Aa}	0.178 ± 0.01^{Bc}	0.228 ± 0.04^{Ba}			
	40	0.261 ± 0.017^{Aa}	0.186 ± 0.013^{Bbc}	0.236 ± 0.085^{Ca}			
sucrose	10	0.71 ± 0.10^{Aa}	0.38 ± 0.01^{Ba}	0.41 ± 0.05^{Ba}	0.00	0.00	0.61
	20	0.63 ± 0.04^{Aab}	0.16 ± 0.01^{Cb}	0.27 ± 0.05^{Bab}			
	30	0.56 ± 0.06^{Aab}	0.16 ± 0.01^{Bb}	0.24 ± 0.15^{Bab}			
	40	0.53 ± 0.10^{Ab}	0.15 ± 0.007^{Bb}	0.13 ± 0.10^{Bb}			
wheat bran	10	0.167 ± 0.03^{Ab}	0.117 ± 0.01^{Ab}	0.131 ± 0.04^{Aa}	0.00	0.00	0.00
	20	0.402 ± 0.08^{Aa}	0.131 ± 0.01^{Bab}	0.143 ± 0.02^{Ba}			
	30	0.387 ± 0.08^{Aa}	0.150 ± 0.01^{Ba}	0.123 ± 0.03^{Ba}			

[a] Values are mean±*SD*. Different lowercase and uppercase letters show significant differences in each column and raw, respectively (P< 0.05).

bran (P< 0.05). Also, no significant difference was found in phytase activities of the strains AM14 and AM11 in all concentrations, however, in the case of AM 13, 30 g L^{-1} of wheat bran showed significantly (P< 0.05) higher activity and 10 and 20 g L^{-1} of wheat bran didn't have significant difference with others. Two-way ANOVA revealed significant interactions between wheat bran concentration and the bacterial strains (P= 0.00). The phytase activity was significantly different among groups exposed to different wheat bran concentrations and strains (P= 0.00 and 0.00, respectively).

DISCUSSION

In the present study, thirty-one LAB were isolated from sheep and cow's milk and yogurt. Among these bacteria, three isolates had higher levels of phytase activity and bacterial growth and were *Lactobacillus* sp., which was confirmed using *16S rRNA* gene molecular analysis. The gene sequences were submitted to the GeneBank with the names of *Lactobacillus* sp. AM11, AM13, and AM14. Phytase activity is influenced by several physicochemical factors including the composition of the growth medium, the type of strain, cell growth, methods of cultivation, inoculum concentration, time of incubation, pH, temperature, salinity, carbon, nitrogen and mineral sources (Lambrechts *et al*., 1993; Gargova and Sariyska, 2003).

Carbon source as a component of culture medium plays an important role in the growth and activity of phytase enzyme. In this study, the addition of carbon source to MRS broth increased the rate of growth of bacteria and they were able to use all sugars for biomass and phytase activity. Sreedevi and Reddy (2012) demonstrated that supplementing the *Bacillus* sp. c43 medium with glucose and sucrose caused significant increase in phytase production. Addition of glucose was also found to be useful for enzyme activity. In this study, supplementing the medium with glucose caused no significant difference in phytase activity and bacterial growth at different concentrations in *Lactobacillus* sp. AM11 and AM14. The results showed that sucrose

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was the best carbon source with maximum phytase activity 0.71±0.10 U mL^{-1}.

Sreeramulu *et al*. (1996) used LAB and found *L. amylovorus* B4552 to be the best bacteria strain, which produced 125-126 units mL^{-1} phytase in glucose medium supplemented with inorganic phosphorus. In this study, phytase activity was lower than *L. amylovorus* B4552. Ebune *et al*. (1995) reported that glucose was very often used as a nutrient for growing *Aspergillus-ficuum* and activity of phytase enzyme and found that 5.2% (w/v) of glucose or lower percentages had positive effects on the rate of biomass growth, enzyme activity, and reduction of phytic acid. Also, the presence of glucose caused high level of phytate-degrading activity in *E. coli* (Touati *et al*., 1987) and *Lactobacillus amylovoras* (Sreeramulu *et al*., 1996). Greiner (2007) suggested that bran was excellent substrate for production of extracellular phytate degrading enzyme in microorganisms. The phytate in bran was less soluble than the synthesized phytate like sodium-phytate, therefore, phosphates were released more slowly than from bran phytate. In the study of Nampoothiri *et al*. (2004) about thermostable phytase activity by *Thermoascus auranticus* in submerged fermentation, wheat bran was used as a carbon source supplemented with different mono, di, and polysaccharides such as glucose and sucrose.

In this study, addition of 10 g L^{-1} sucrose and glucose to media culture of the three isolated bacteria, and 20 g L^{-1} of wheat bran for AM11 and AM13, and 10 g L^{-1} of wheat bran for AM14 revealed the best concentrations of carbon sources for increasing phytase activity. Also, for increasing bacterial growth, addition of 10 g L^{-1} of glucose to media culture of the three isolated bacteria, 10 g L^{-1} of sucrose for AM11, 20 g L^{-1} for AM13 and AM14, and 20 and 30 g L^{-1} of wheat bran for AM11 and AM13, respectively, and 10 g/L of wheat bran for AM14 were the best concentrations of carbon sources.

Moreover, all lactobacilli isolates could grow at 15ºC, which agrees with those found by Bulut (2003). Therefore, these isolates can be used as supplementary food for *Oncorhynchus mykiss*, which is named as cold-water fish.

CONCLUSIONS

The results obtained demonstrate that optimization of medium components and cultivation conditions is a feasible way to enhance enzyme activity as well as yield. In addition, the results can be useful for the use of these isolates as probiotics to improve the use of phosphorus in the feeding of cold-water fish.

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جداسازی و شناسایی سویه‌های لاکتوباسیلوس به عنوان مکمل غذایی ماهی و ارزیابی اثر منابع کربن بر رشد باکتری و فعالیت فیتازی

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چکیده

گونه لاکتوباسیلوس به علت خواص متفاوت آنها مانند محصولات آنزیمی مفید، ارگانیسم‌های امن برای استفاده به عنوان پروبیوتیک می‌باشند. برای مثال، فیتاز آنزیمی است که نقش مهمی در هضم غذایی ماهی دارد. هدف از این مطالعه، جداسازی و شناسایی گونه‌های لاکتوباسیلوس با فعالیت فیتازی از محصولات لبنی مانند شیر، ماست و پنیر و نیز بررسی تأثیر منابع مختلف کربن بر رشد باکتری و فعالیت فیتازی بود. برای این منظور، گونه‌های *Lactobacillus* از محصولات لبنی با استفاده از تست‌های بیوشیمیایی غربالگری شدند و آنالیز ژن 16S rRNA برای تأیید کلاس لاکتوباسیل‌ها استفاده شد. سه جدایه که بیشترین رشد باکتریایی و فعالیت فیتازی را نشان دادند انتخاب شدند و توالی ژن16S rRNA در بانک جهانی ژن ذخیره شدند. سپس برای تعیین شرایط بهینه رشد، از سه منبع کربن شامل غلظت گلوکز و سوکروز در غلظت‌های ۱۰، ۲۰، ۳۰ و ۴۰ گرم در لیتر و سبوس گندم در غلظت‌های ۱۰، ۲۰ و ۳۰ گرم در لیتر در سه تکرار استفاده شد. فعالیت فیتازی باکتری‌های جدا شده شامل: سویه‌های لاکتوباسیلوس AM11، AM13 و AM14 با استفاده از زیموپلیت و هم‌چنین آنزیم خارج سلولی اندازه-گیری شد. *Lactobacillus* sp. strain AM11 بالاترین فعالیت فیتازی و رشد را در مقایسه با سایر سویه‌ها نشان داد. طبق نتایج سویه *Lactobacillus* sp. strain AM11 می‌تواند به عنوان مکمل غذایی برای بهبود دسترسی مواد معدنی در تغذیه ماهی استفاده شود.

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