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Phytase Characterization and Production from Lactobacillus plantarum Strain on Corn Steep Liquor

Fatima Masume USLU Ecem Gizem KIZILKAYA* Esra Sunduz YIGITTEKIN Mustafa GENCOGLU
Sevil TOROGLU Sadik DINCER
Çukurova University, Adana, Turkey

*Corresponding Author:
E-mail:ecemgizemm@gmail.com

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Abstract

In this study, effect of corn steep liquor on phytase enzyme production from Lactobacillus plantarum strain isolated from fermented food products was investigated and produced phytase was characterized. The enzyme produced in MRS broth containing %30 corn steep liquor and %0,2 glucose for 24 hours at 30°C.The intracellular enzyme isolated from Lactobacillus plantarum strain used in this study. The optimum activity of enzyme was emerged at 40°C and pH 5. This enzyme was 92% stable for 30 minutes at 100°C. Enzyme activity decreased with 1mM concetration of $MgCI_2$ (3.6%), $CoCI_2$ (13.5%) $HgCl_2$ (14%), $NiCI_2$ (20%), $MnCl_2$ (15%), $CaCI_2$ (15%), $ZnCl_2$ (9%), $CuCl_2$ (24%), $FeCl_2$ (15%) and EDTA (13%) but activity increased with 5mM concentration of $MgCI_2$ (5%), $CoCI_2$ (13.5%) $HgCl_2$ (22%), $NiCI_2$ (16%), $MnCl_2$ (18%), $CaCI_2$ (20%), $ZnCl_2$ (39%), $CuCl_2$ (10%), $FeCl_2$ (10%) and EDTA (12%). As a result, it is seen that due to the characteristic features of the produced pytase enzyme can be proposed that it is convenient to be used in Industrial area.

Keywords: Lactobacillus plantarum, Phytase, Corn steep liquor, Phytase characterization

INTRODUCTION

Phytases catalyse segregating of phosphate from phytic acid step-by-step or hydrolysis of phytate into curtailed inositol phosphate esters and inorganic phosphat [5,7]. It was reported that phytase activity was found in rice bran and blood of calf for the first time [8,10]. Later existence of phytase activity was found in plant, bacteria, ferment and fungus. In addition to this efficient phytase sources that are produced as endogenic by microflora that is related to mucosa of the small intestine and large intestine are found in human and animals. On the contrary phytase activity of microbial and plant endogenous phytase activity of people and animal is less important in general [12]. Moreover phytase which is intestine digestive enzyme does not exist on people, dogs, pigs, birds and agastric animals.

Corn Steep Liquor is a by-product of the wet milling of maize for obtaining starch it is rich in vitamins and minerals particularly high in nitrogenous compounds so ,widely used as compenent of animal feed and culture enviroment for microorganisms. İt is an inexpensive alternative to much more expensive materials, such as yeast extract and peptone in microbiology. Microorganisms, especially lactobacilli, are detected in the process and contribute to the fermentation of the corn extract throughout the remainder of the steeping. So, it was decided to produce bacterial phytase by using corn steep liquor as a medium for Lactabacillus plantarum.

MATERIAL and METHOD

Bacteria Strain

Lactobacillus plantarum which was isolated and stocked in Bacteriology Laboratory of Biology Department of Faculty of Science and Letters of CukurovaUniversity was used in this research.

Environment and Culture Conditions

Content of production environment which was used in this research (MRS Broth) (1L) contain Peptone10.0 g; Ferment extract 4.0 g; Meatextract 8.0 g; D-Glucose 20.0 g; Dipotassium hydrogen phosphate ($K_2HPO_4.3H_2O$) 2.0 g; Tween 80 1 mL; Di-ammonium hydrogen citrate 2.0; Sodiumacetate $3H_2O$ 5.0 g; Magnesium sulphate ($MgSO_4.7H_2O$) 0,2 g; Manganese sulphate ($MnSO_4.4H_2O$) 0,04 g (1,2,3,4,6,12)

To revitalise strain of Lactobacillus plantarum, we planted it from glycerol stock and incubated in MRS Broth medium at 37°C and 150 rpm for 24 hours.

Phytase Production Medium

MRS Broth+%0,2 glucose content was used as a control group. Phytase production was done in the content that is added Corn Steep Liquor at1%-10%-20%-30% rates into MRS Broth+%0,2 glucose which are inoculated from revitalised Lactobacillus plantarum at %2 rate and 50 mL incubated at 30°C and at 150 rpm for 24 hours by planting into mediums. From these mediums that produced bacteria after incubation, the ones that are equivalent to itself planted at %1 rate and 250 mL incubated for 24 hours at 30°C and at 150 rpm. production medium was centrifuged at 9000 rpm for 10 minutes and pellet part was taken into test tub. To obtain intracellular enzyme , cell lysis was done with sonicator after that 5ml physiological saline solution was added into pellets tubes. finallay test tubes centrifuged (9000 rpm for 10 minute) again and supernatant part (raw enzyme) was used in the experiments that were conducted .

Determination of enzyme activity

To determining phytase activity, after mixing 250 substrate (2mM Na-phytate) µL and 250µL raw enzyme were reacted for 15 minute. To stop the reaction 500 µL of 10% TCA (trichloroacetic acid) After stopped reaction 1 ml of coloring solution (a mixture of 4 volumes of 2.5% ammonium molybdate and 1 volume 2.5% iron sulfate solution) was added and after waiting 10 minutes the measurements of activity which was calculated according to the formula given below done at 700 nm wave length.

Phytase activity = $\frac{W \times V \times 1000}{VE \times t \times M}$

W: The amount of phosphate released (OD700/ The slope of the standard phosphate chart)

VE: volume of enzyme

V: volume of reaction mixture

T: reaction time

M: atomic weight of phosphorus

Characterization Of Produced Phytase

İn ordor to charecterization of phytase, optimum pH and temprature was investigated .To determine the effect of temperature and pH on enzyme activity, experiments were carried out at various temperatures (20°C-100°C) and pH (3,3.4,3.8, 4.2 ,4.6, 5.0, 5.4,5.8, 6.2, 6,4, 7.0, 7.4, 7.6, 8.0, 8.4, 8.8, 9.2, 9.6, 10.0, 10.2 and 10.4).

Produced phytase pre-incubated at 80,90 and 100 ° C for various times (5,10,15,20 and 30 minute) and thermal stability of phytase was determined.Moreover effect of various inhibitors and divalent cations ($MgCI_2$,$CoCI_2$ HgCl2 NiCI2 ,MnCl2 CaCI2 ZnCl2 ,CuCl2 FeCl2 and EDTA) on phytase activity were investigated.

RESULTS and DISCUSSION

In this research the optimum activity of enzyme which was isolated from bacteria was emerged at 40°C and pH 5. Maxcimum phytase activity was determined as 415 U/mL Temperature and pH dependent test results was given below (figure 2 and Figure 3) İn adition to optimum corn step liquor concetration was determined as % 30. (Figure 1)

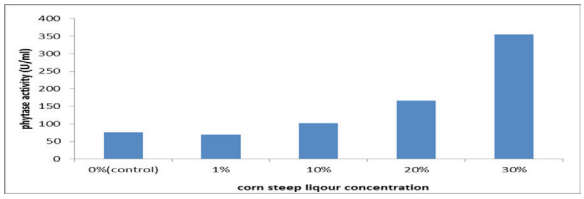

Figure 1. Efect of corn step liqour concentration on phytase activity

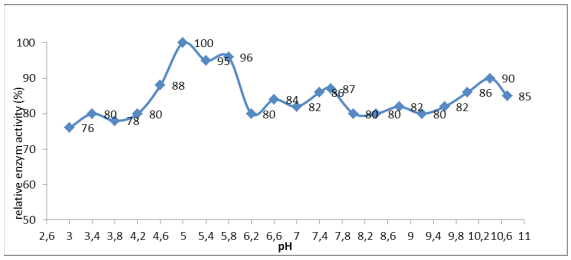

Figure 2. Effect of pH on phytase activity

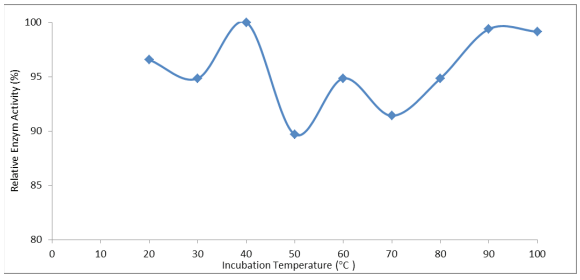

Figure 3. Effect of temperature of on phytase activity

The experiments showed that the thermostability the enzyme activity was 92% stable for 30 minutes at 100°C. (figüre 4)

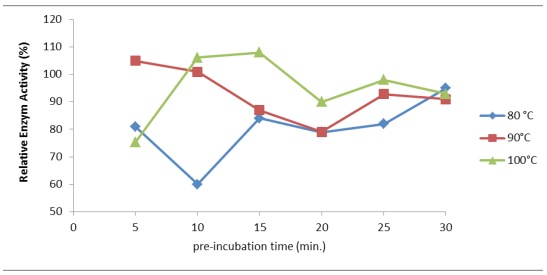

Figure 4. Thermostability of phytase

To determine the effect of inhibitors and divalent cations on the enzyme activity, the produced phytase enzyme was preincubated in both 1mm and 5mm concentrations at 37 °C for 40 minutes. Experiment results were given figure 5.

	Relative Activity (%)	Relative Activity (%)
Chemical	**1 mMconcentration**	**5 mMconcentration**
Control	100	100
EDTA	87	112
CaCl2	85	120
HgCl2	86	122
MnCl2	85	118
ZnCl2	91	139
MgCl2	96	105
NiCl2	80	116
CuCl2	76	110
CoCl2	87	114
FeCl2	85	110

Figure 5. Effects of inhibitor and divalent cations on phytase activity

Many plants, microorganisms and animal species are found as phytase supply. Production and usage of phytase for industrial purposes are the leading usage areas of phytase. In addition to this it is important for bait industry at the most.

In this study conducted phytase production from Lactobacillus plantarum was performed. In this production phytase activity was increased by Corn Steep Liquour. Thus by providing phosphate supply from Corn Steep Liquour which was a waste positive effect ensured in phytase activity as well as waste utilisation provided.

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