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RESEARCH ARTICLE

MICROBIAL PRODUCTION OF PHYTASE ENZYME FROM TRADITIONAL INDIAN FERMENTED FOOD (IDLI AND JALEBI BATTER)

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ABSTRACT

Cereals and legumes contain high amount of phosphorus in the form of phytic acid or phytate. Due to the absence of phytatse enzyme, monogastric animals cannot digest phytic acid which is the nutritional constituent of animal diet. Phytic acid act as an anti-nutritional chelating agent of different metal ions like Ca, Mg, Fe, Zn. The main objectives of the research were to isolate and characterize phytase producing bacteria from traditional fermented foods like idli and jalebi batter, and optimize phytase production by the isolated strain of bacteria. A total of 10 samples collected from fermented idli and jalebi batter were streaked on phytase screening medium (PSM). Total 50 isolates having apparently different colony morphology were collected and screened based on their morphological properties. The selected 22 isolates were further evaluated among them, 4 isolates PR2, PR6, PR16, PR24 could be grown on PSM plate with 2.5% agar after incubation of 24 hr at 37°C temperature and shown phytase activity in shake flask experiment. Upon screening of 4 potential phytase producing strains, one isolated strain (PR6) was selected and was identified as *Lactobacillus sp.* PR6 through morphological and physiological characterization.PR6 showed 378 U/mL enzymatic activity upon enzymatic assay. This result was optimized the performance of this isolated at different parameters. PR6 showed best results at pH 5.5 and temp 40°C with glucose and sucrose as carbon source and yeast extract as nitrogen source.

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INTRODUCTION

In plants phytate, a major source is present in the form of phosphorous. The storage form of phosphate in nature is Phytic acid and is mostly present in cereals, nuts, legumes, and oil seeds (Bae *et al*., 1999; Chang *et al*., 2004). Phytic acid reduced the presence of different metal ions such as Fe, Zn, Mg, Ca, and etc., so that nutritive quality of food is reduced (Bindu *et al*., 1998). Due to this, phytase enzyme is required to hydrolyze phytic acid. Phytases (myo-inositol hexakis phosphate phosphohydrolase) also known as phytate hydrolyzing enzymes. Which hydrolysesphytate and releases inorganic phosphate (El-Batal *et al*., 2001). Phytase enzyme is widely produced in nature by bacteria, fungi, yeast, plants, and animals. Phytase producing microorganisms are mostly present in rhizospheric soil of crop plants. Monogastric animals like fish, poultry, pig, human etc., do not have sufficient levels of phytate-degrading enzymes in their digestive tracts to digest phytic acid, due to this phosphorus is not available to them.

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So feed is supplemented with inorganic phosphorus to meet phosphorous requirement. As because Phytic acid forms complexes with proteins, amino acids, and different metal ions, it acts as antinutrient constituent in plant-derived food (Pallauf *et al*., 1997). It causes human nutritional deficiencies of different metal ions, where plant food is staple food (Manary *et al*., 2002). Research is going on upon screening and isolation of phytase producing bacteria as phytases play significant role in improving the nutritive quality of food and feed that contain phytic acid (Alconada *et al*., 1996). India is a diverse country in terms of microbes since ancient era. Different types of research of fermented foods are carried out in the recent era because of its good nutritional qualityand other benefits it imparts to our body and also in the face of the growing insecurity of foods, food scarcity etc. Alongside preservation, fermented foods are beneficial as flavor enhancer, increased digestibility, improving nutritional and pharmacological values. It has many applications of protective cultures in different kinds of food systems (Holzapfel *et al*., 1995).Isolation of these beneficial organismsare done from various food sources such as grains, dairy products and fermenting vegetables.

Another study reported, isolation of LAB (Lactic acid bacteria) were done from vegetables and different forms of traditional fermented foods like dhokla batter, idli batter, dahi, jalebi batter, lassi, yogurt and cabbage (Patel *et al*., 2012; Neha *et al*.,2014). Whereas a different study reported isolation of LAB from Dosa (Appam) batter and vegetable pickle (Vijai Pal *et al*., 2005).Khodaii *et al*. (2013) isolated 43 Lactic Acid Bacteria from commercial probiotic preparation and dairy products. The enzyme activity of the probiotic bacteria ranged between 1.1-5.4mU. Jeevaratnam *et al*. (2015) isolated some Lactic Acid Bacteria from Uttapam batter. Singh *et al*. (2013) had isolated 32 bacteria those were enable to produce the enzyme phytase from various kind of soil samples such as cattle shed, pulse crop field etc. He found *Bacillus subtilis* as the most potent strain with enzyme activity of 378U/mL enzymatic activity. Muthuraman *et al*. (2013) isolated *Pseudomonas fluorescens* from poultry faeces and the enzyme activity found to be 32.94 U/ml at the optimized conditions. Duong *et al*. (2014) used a recombinant expression of *B.subtilis* phytase in *Lactobacillus* and demonstrated that the administration of phytate-degrading probiotic cultures can increase the bioavailability of phytate phosphorus and improve the performance of non-ruminant livestock animals fed a phosphorus-deficient diet. Kaya *et al*. (2012) isolated *Lactobacillus brevis* from Hatay boiled cheese, produced high levels of extracellular and intracellular phytase. The main objective of present study is to isolate a phytase producing probiotic bacteria from Idli and Jalebi batter which is a fermented product very common in Kolkata market, identify the isolated strain and optimize some process parameters for maximum production of phytase enzyme.

MATERIALS AND METHODS

Isolation of a bacteria strain capable of producing phytase: Idli and Jalebi is the traditional fermented foods made by Rice and pulse based batter. All the 10 samples were stored at 4°C in refrigerator and processed within two hours. Samples 10 % (w/v) were homogenized in phosphate buffer (0.1 M, pH 7.2), then serial dilution and pour plating was done on MRS agar and then incubated at 37°C temperature for a time period of 24 to 48 h anaerobically. Selected colonies with different morphological characteristics were transferred on MRS agar slants and kept at refrigerated condition for storage. All cultures were subcultured at regular intervals. They were activated in MRS broth before being used in the experiment. For phytase production the isolates were screened initially as described by Anastasio *et al*. (2010).The spot around zone were cleared after incubation with 2% cobalt chloride solution for 20 min indicates positive for phytase production.

Phytase assay: The isolate was grown in phytate-containing Chalmers broth or 24 h at 35 °C and the CFS (centrifuge sediment) was collected by centrifugation at 10,000 rpm for 15 min. 1 ml of CFS was mixed with 0.6 ml of reaction mixture (3 Mm phytate in 0.2 M sodium acetate buffer pH 4.5) and incubated at 35 °C for 15 min. There action was terminated by addition of 1 ml of 10% TCA (Trichloroacetic acid). The released inorganic phosphate was measured by addition of 0.75 ml of coloring reagent prepared freshly (4 volumes of ammonium molybdate in 5.5 % sulfuric acid and 1 volume of 2.7 % ferrous sulfate solution). The absorbance was measured at 700 nm. Phytase activity was measured in µmol of inorganic phosphate released from phytate. The Chalmers broth was used as negative control.

One unit of phytase is defined as the amount of enzyme required to produce 1 µmol of inorganic phosphate per minute under assay conditions (Anastasio *et al*., 2010).

Identification of the selected strain: The most potent isolates were identified by using morphological and physiological studies according to the Bergey's Manual of Determinative Bacteriology (1993).

Optimization of process parameters

Optimization of Temperature: The selected strain of the organism was grown on MRS broth medium and incubated at different temperatures i.e. 20 to 45ºC (in 5ºC intervals) for 24 h. After 24 h the fermented broth was centrifuged and the clear supernatant was used for assay of the phytase enzyme.

Optimization of pH: The selected strain of the organism was grown on MRS broth medium at different pH 4.0, 5.5, 7.0, 8.5 and incubated at 40 ºC for 24 h. After 24 h the fermented broth was centrifuged and the clear supernatant was used for assay of the phytase enzyme.

RESULTS AND DISCUSSION

Isolation and identification of a bacteria strain capable of producing phytase: A total of 10 samples of Rice and pulse based batter of traditional fermented foods like Idli and Jalebi batter were collected from households and local markets Idli and Jalebi batter were plated on MRS agar and after 48 h of incubation, typical colonies showing different morphological characteristics were picked up (Table 1). Total 50 isolates having apparently different colony morphology were collected and screened based on their morphological properties. The result was shown in Table 1. The selected 22 isolates were further evaluated.

Phytase assay: The selected 22 isolates were further evaluated among them, 4 isolates PR2,R6, PR16, PR24 could be grown on PSM plate with 2.5% agar after incubation of 24 h at 40°C temperature and shown phytase activity in shake flask experiment. Upon screening of these four strains the one strain PR6 which was producing maximum amount of the enzyme was selected.

Identification of the selected strain: Morphological characteristics of the isolated strain PR6 was done according to Bergey's Manual of Determinative Bacteriology (1993)For the identification purpose. Ninth Edition and the strain was found as *Lactobacillus sp.*PR6. The result of the Morphological study was shown in Table 2. The picture of the isolated strain was shown in Fig.1.

Growth at different temperature: To optimize the temperature for maximum production of Phytase enzyme by the isolated strain the organism was grown at different temperatures i.e. 20 to 45 ºC (in 5 intervals). The results of these experiments are shown in Fig. 2. It was found that with increase in temperature amount of enzyme produced increased up to 40 ºC then it reduced with further increase in temperature. So 40ºC temperature was selected as the optimum temperature for production of the Phytase enzyme by the isolated *Lactobacillussp.*PR6 and used in further studies.

Table 1. Selection of Phytase producing strain from different source

Sample No	Type of sample	Source	No. of Sample	No. of Isolates collected
1	IdliBatter	Homemade	1	6
		Local Market	3	13
2	Jalebi Batter	Homemade	2	11
		Local Market	3	15
		Laboratory made	1	5
	Total		10	50

Table 2. Morphological study of the isolated strain PR6

Strain Number	Size	Shape	Pigmentation	Form	Margin	Elevation	Gram Staining Characteristics
PR6	Moderate	Rod	No pigmentation	Regular	Entire	Flat	Gram positive

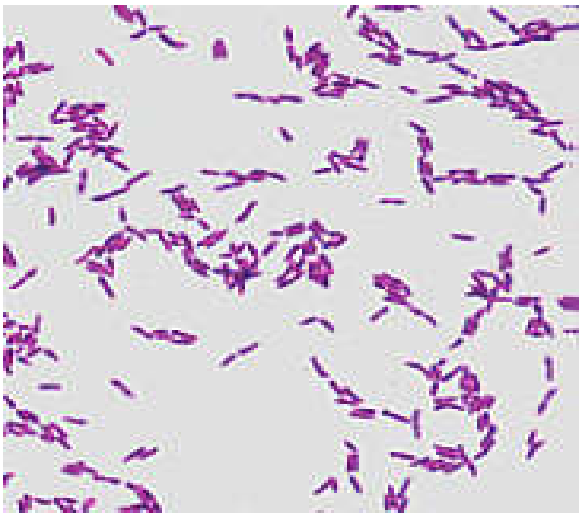

Figure 1. Image of the isolated strain after Gram staining

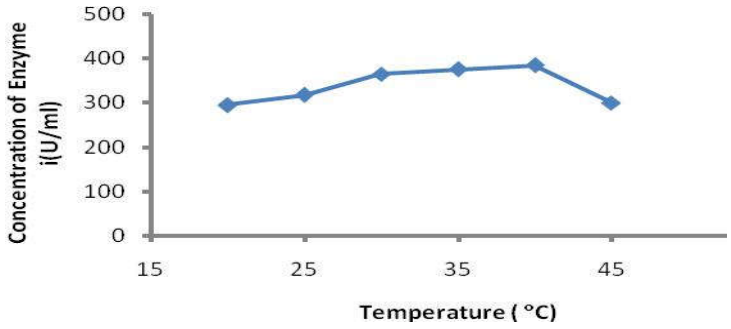

Figure 2. Optimization of temperature for the maximum production of phytase enzyme by the isolated strain PR6

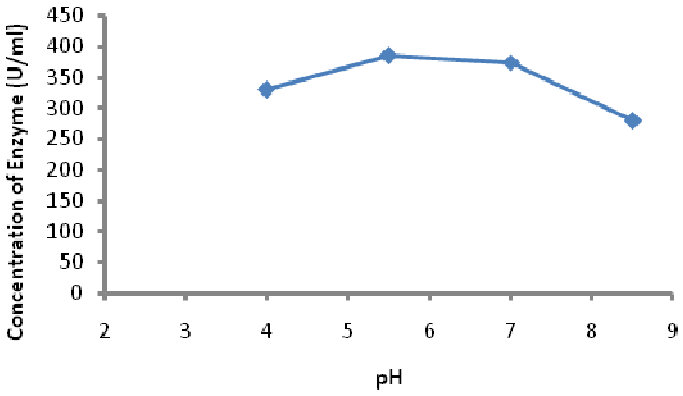

Figure 3. Optimization of pH for the maximum production of phytase enzyme by the isolated strain PR6

Growth at different pH: To optimize the pH for maximum production of Phytase enzyme by the isolated strain experiment was carried at different pH i.e. at 4.0, 5.5, 7.0 and 8.5. The results of these experiments are shown in Fig.3. It was found that with increase in medium of enzyme produced increased up to pH 5.5 then it reduced with further increase in pH. So pH 5.5 was selected as the optimum pH for production of the Phytase enzyme by the isolated *Lactobacillussp.*PR6 and used in further studies.

DISCUSSION

Phytase, which plays an important role in biochemistry of inositol phosphates, has been both extensively and intensively studied. A lot of sources of this enzyme were subsequently discovered. The physico-chemical and biochemical properties of some of the described phytases seem to be well known, especially the phytase produced by *Aspergillus sp.*Bacteria Investigation in the field of molecular genetics yielded the possibility to overproduce this enzyme. This program aiming at improving the industrial production of phytase on a cost-effective level, will continue because the efforts to use phytase in feed and food industries have been successful. Also the preparation of inositol phosphates using phytase to hydrolyze phytic acid seems to be interesting. As lower inositol phosphates and phospholipids play important role in transmembrane cell signalling and calcium mobilization from intracellular stock, the investigation of the potential role of phytase in this mechanism would be interesting.

Phytases were originally proposed as an animal feed additive to enhance the value of plant material in animal feed by liberating phosphate (Mitchell *et al*. 1997). Phytase is present in about 75% of all the diets for simple-stomach animals and its market volume exceeds US$350 million annually (Shivange *et al*. 2012). The current global phytase market has been estimated to account for more than 60% of the total enzyme market. The increase in economic pressure and increased concern over the environmental impact of life-stock production, have paved the way for the economic success of phytases as an animal feed additive. Phytases used for animal feed application differ in their enzymatic properties such as pH profile, stability under stomach conditions,temperature stability, kinetic constants, and substrate specificity. 'Ideal' phytases for animalfeed applications should fulfil a series of quality criteria.Thus locally available source especially from *Lactobacillus sp strain PR6* that isrecognized can have tremendous benefit in terms of production in large quantities.

Conclusion

This present study focuses on the screening and isolation of the phytase producing organisms from fermented Idli and Jalebi batter, locally available in Kolkata and identification of those isolates by morphological study. The quantitative enzymatic assay ensures the most potent strain and it was identified as *Lactobacillus sp.* PR6. Further two parameters i.e. temperature and pH was optimized for maximum production of the enzyme by the isolated strain and those optimum values were 40^{o}C and 5.5 respectively.

Disclosure statement: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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