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ORIGINAL ARTICLE

Optimization of physical parameters for the production of Phytase from isolate BGS3 (*Bacillus* sp.) isolated from North Gujarat region.

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ABSTRACT
Phytase-producing bacteria were screened using soil samples from the rhizosphere region of various agricultural areas, cow dung sheds, agricultural and dairy waste dump sites, etc. in the north Gujarat area. Quantitative screening using phytase screening media and qualitative screening for phytase production under submerged fermentation was carried which confirms BGS3 to be a potential phytase producer. Based on molecular identification isolate was found to be Bacillus sp. Optimization of physiological factors such as pH, temperature, incubation period, and inoculum percentage were further investigated. Which confirms it highest phytase production at 37°C, 7 pH, 5 % inoculum and 72 hrs.
Keywords: *Phytase, Optimization, Screening, OVAT*

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INTRODUCTION

Phytate (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) is a phosphate and inositol-based compound found in cereals, legumes, and other foods that are commonly used as main ingredients in animal feeds for commercial purposes. Regretfully, because the gastrointestinal tracts of monogastric animals have relatively little activity of phytate-degrading enzymes, phytates cannot be hydrolysed effectively in these animals. In this case, phosphorus deficit must be prevented by exogenous phosphate addition [1]. The leaching of phosphates from phytates, which may be broken down by ambient microorganisms, results in significant environmental degradation in addition to raising feed costs. Conversely, phytate is well acknowledged to be a potent chelator of cations, such as $Ca^{2+,}$ Zn^{2+}, $Fe^{2+,}$ $Cu^{2+,}$ and $Mg^{2+,}$ rendering them inaccessible to animals that are monogastric. Additionally, it has the propensity to combine with proteins to create insoluble complexes, which lowers the bioavailability of these vital nutrients [2]. Among the unique class of phosphatases that includes purple acid phosphatase (PAP), b-propeller phytase (BPP), and histidine acid phosphatase (HAP), is phytase (myo-inositol hexakisphosphate phosphohydrolases, EC 3.1.3.26, EC 3.1.3.8). Its function is to catalyse the hydrolysis of phytate to phosphorylated myo-inositol derivatives. Because of its unique enzymatic activity, phytase is regarded as a green feed additive that can both efficiently increase phytate-P availability and remove phytate's anti-nutritional function, which lowers production costs and improves environmental protection. leading to increased environmental protection and decreased production costs. Additionally, adding phytases to the feed for monogastric animals can lower the excretion of phosphorus and increase the availability of energy, minerals, trace elements, and amino acids [3]. Despite the fact that there are many sources of phytases, microbes are typically regarded as the greatest sources due to their convenient manufacturing and advantageous catalytic qualities.

A particular class of phosphatase known as phytase hydrolyses phytate to produce phosphoric acid and myo-inositol. The antinutritional effect of phytate can be mitigated by adding phytase to animal feed [4].

As feed supplements, a number of commercially available fungal phytases have been used, but their effectiveness has been limited. These include Allzyme SSF, Finase PL, and Natuphos from *Aspergillus niger*, Phyzyme and Ronozyme from *Aspergillus oryzae*, and Finase from *Trichoderma reesei* [5] [6]. Because of their higher substrate specificity, increased resistance to proteolysis, high thermal stability, improved pH optimum, and catalytic effectiveness, bacterial phytases have thus gained more attention than their fungal counterparts [7].

Malnutrition and silent hunger can be solved in developing nations by adding phytase as a dietary ingredient. In addition, the addition of phytase to animal feed lowers cattle sector costs and partially addresses the issue of phosphorus contamination, or eutrophication [8].

MATERIAL AND METHODS

Chemicals: Sodium phytate (Sodium salt of phytic acid) was acquired from HiMedia Chemical Laboratories Pvt. Ltd. Company, Mumbai and remaining chemicals used were all of analytical grade from SRL and Sigma.

Isolation, identification and molecular characterization

The phytase producing bacterial strain used in the present investigation was isolated from Agriculture soil Gozaria village Mehsana. The isolates were tested for their ability to produce phytase on the Wheat Bran Extract Agar media and phytase screening medium, the streaked plates were incubated overnight at 37 ◦C and the zone of hydrolysis of the plate gave a visual indication of extracellular phytase production. Phytase activity in the liquid media was determined as per methodology adopted in earlier work. The isolate was further proceeded for the molecular identification.

Identification of the isolate was carried out as per the methodology developed and employed in earlier research work, based on the qualitative and quantitative assay for the enzyme production BGS3 was found to be the potential phytase producer [9].

2.3 Optimization of phytase production:

Isolate was processed for optimization of cultural conditions using One Variable At Time (OVAT) approach, to study the effect of important physiological and nutritional parameter with an aim to identify maximum phytase producing condition.

Optimization of physiological condition for phytase production Parameters studied under the objective were pH (3,4,5,6,7,8 and 9), temperature (30°C, 35°C, 37°C, 40°C, 45 °C, 50°C, 55°C and 60°C) inoculum size (1%,5%,10%,15% and 20%) and incubation time (24 hrs,48 hrs,72 hrs, 96 hrs, and 120hrs).

RESULTS AND DISCUSSION

In the present study, phytase producing bacterial isolate collected from various region of north Gujarat were taken under study. isolated from Agriculture soil Gozaria Village Mehsana from Rhizosphere soil, based on the plate studies it showed the highest percent of zone of hydrolysis in plate assay (Fig 1), followed by quantitative assay where it showed the 0.479 U/mL enzyme production in liquid media. Based on molecular identification of isolate using MEGA 7 [10] software analysis it was found to be *Bacillus* sp. (Fig 2) hence sequence identified was submitted in NCBI with accession no. PQ223664.

Fig 1. Zone of Hydrolysis on PSM plate

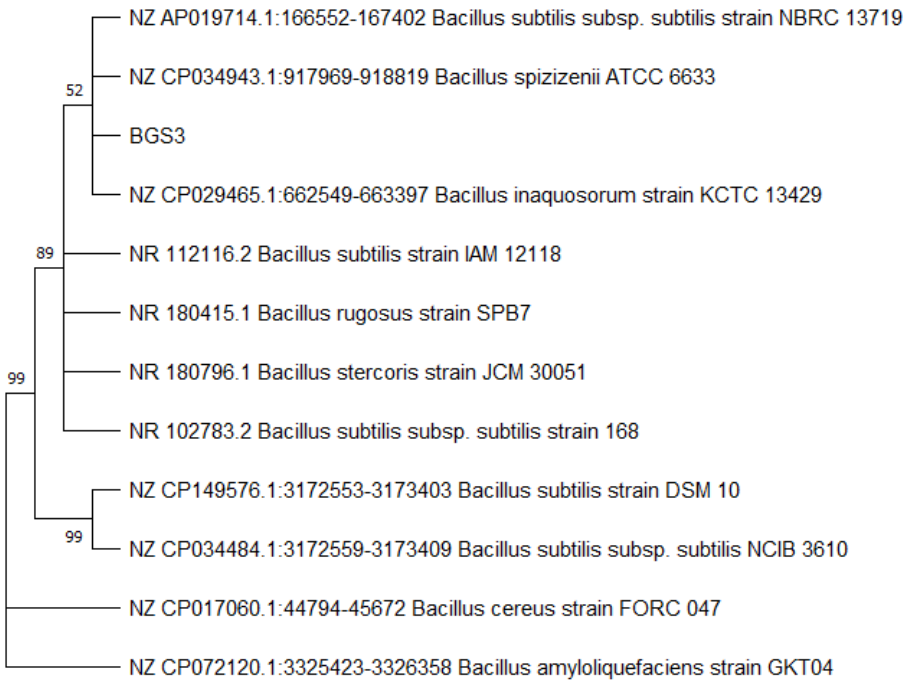

Fig 2. Evolutionary relationship of BGS3

For the higher yield of the enzyme, it is very much required that the microorganism should be provided with the optimum cultural conditions, including physical environment and nutritional requirement. Any variation in the growth conditions of the microorganisms such as change of temperature, pH, fermentation time, inoculum concentration or in nutritional requirements, and nitrogen sources, Carbon source or inducers etc. may influence the metabolic activity of the microorganism and ultimately enzyme production. The effect also varies widely from species to species and each of the organism and for different isolates of the same species. Hence it is necessary to optimize the fermentation parameters for the maximum production of phytase with a view to develop economically feasible technologies.

All the available data suggests that the maximum enzyme production is attained at 72hrs for bacterial isolates [11][12][13], while some of the isolates are cultured for 48hrs as well for the enzyme production, while the fungal cultures shows the max, enzyme production upto 96 -120 hrs. Present results also imply with the phytase activity reached a maximum at 72hrs (3 days) of fermentation (Fig 3) however the lactobacillus isolates are well known for enzyme production in 48hrs [14]. Extending the fermentation resulted in a slight decrease in phytase activity, which might be due to proteolytic degradation of the enzyme or accumulation of secondary metabolites. All the physiological parameters have played a phenomenon role for uplifting the enzyme production. Optimization of incubation time is important as it reduces production time for maximum phytase production. Similarly, understanding of optimum condition of temperature, pH and percent inoculum helps to minimize efforts to have maximum phytase production. The literature study supports the various combination of physiological condition for maximum enzyme production. Where *Bacillus* HCYL03 favors 72 hrs of incubation time at 45 °C and Ph 5 for maximum enzyme production[15], *B. licheniformis* produces well under the condition of 96 hrs, with a temperature range of 45 ° C and pH 5.5 [16], for *Bacillus* SDN-014 best suitable condition for phytase production was found to be 84 hrs , 37°C, pH 7[17], *B. tequliensis* at 60 °C pH 5 in 48 hrs of incubation[18], for *Klebsiella* 72hrs, 55°C, pH 3.5-5.5 [19]. In the current study, under the various physiological conditions studied for higher enzyme production for the isolate BGS3 was found to be temp 37°C (Fig. 4), 7 pH (Fig.5) and 5 % inoculum (Fig. 6) and 72 hrs.

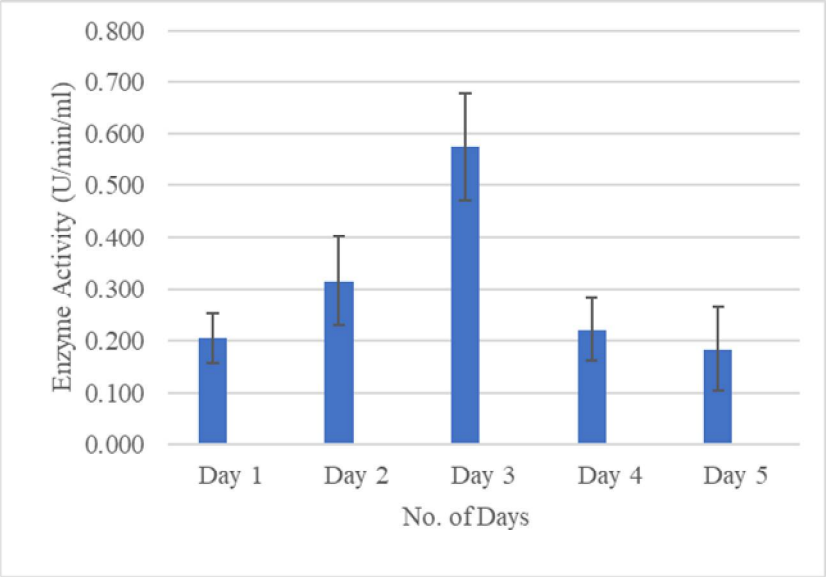

Fig 3. Effect of time on Phytase production

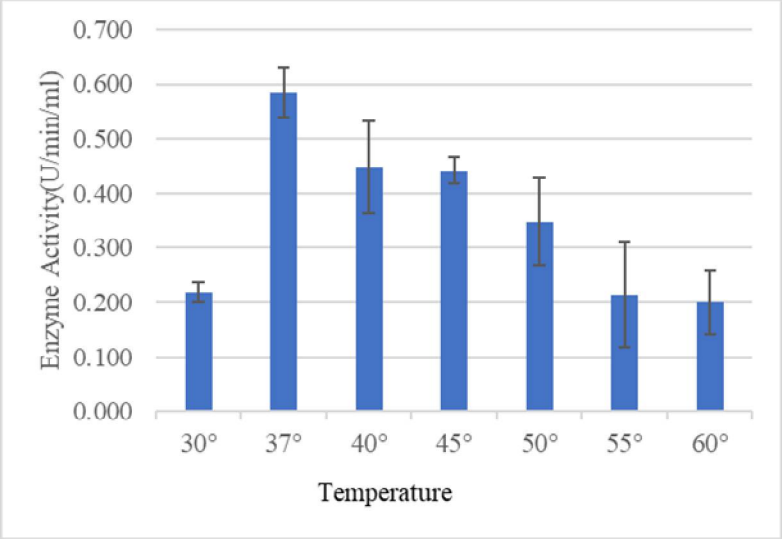

Fig 4. Effect of temperature on Phytase production

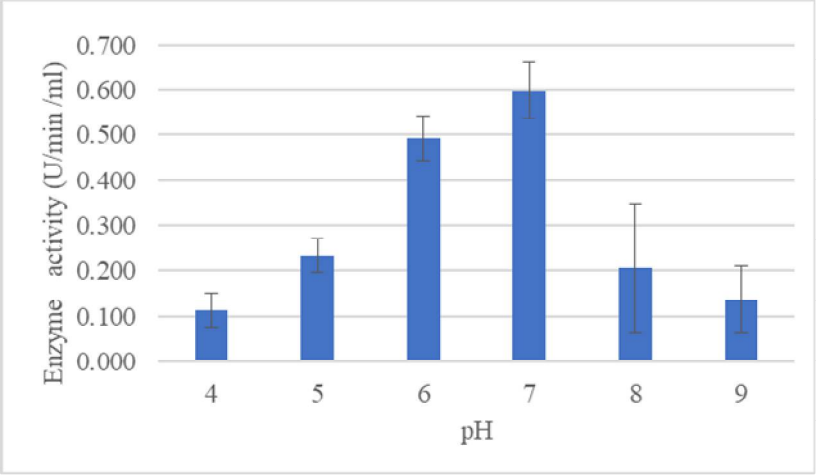

Fig 5. Effect of pH on Phytase production

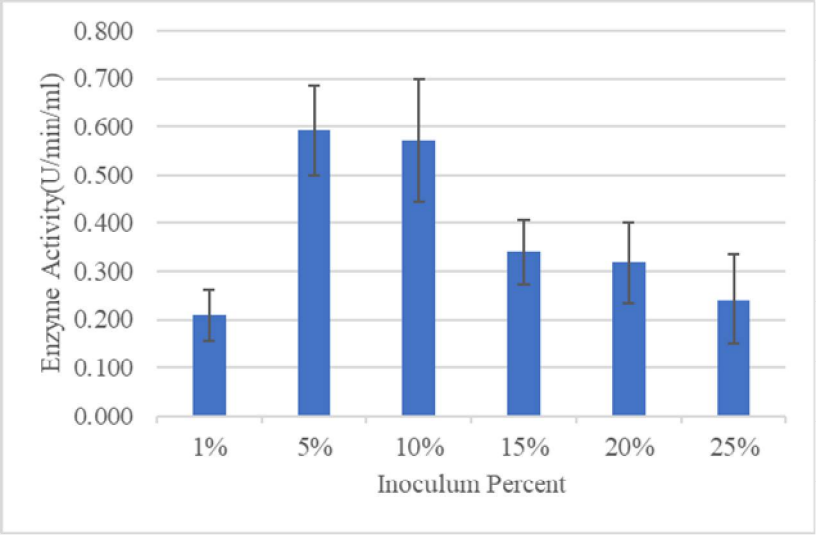

Fig 6. Effect of inoculum percent on Phytase production

CONCLUSION

To conclude, in the current study we have tried to identify the novel bacterial isolates which has the potential to produce the phytase enzyme to degrade the phytic acid a major antinutritional component of many staple crops. Furthermore, to enhance the phytase production physio-chemical parameters were optimized to enhance the phytase production which led to identify the best suitable condition for growth of isolate. Keeping the potential applications of phytase in mind, this work merits special interest towards producing novel phytase enzyme that finds use in biotechnological applications in animal feed additives, human health, pulp and paper industries.

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Conflict of interest - The authors declares that there is no conflict of interest.

Supplementary materials - None

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