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Inducible secretion of phytate-degrading enzymes from bacteria associated with the medical plant *Rosa damascena* cv. Taifi using rice bran

Abd-ElAziem Farouk[1*], Abdulelah Banaja[2], N. Thoufeek Ahamed[1], Othman AlZahrani[1] and Salih Bazaid[2]

[1]Molecular Biotechnology Research Unit, Department of Biotechnology, Faculty of Science, Taif University, Al-Hawiya 888, Kingdom of Saudi Arabia.
[2]Department of Biology, Faculty of Science, Taif University, Al-Hawiya 888, Kingdom of Saudi Arabia.

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More than 320 bacteria were isolated from the soil (Rhizosphere, endophyte, flowers and leaves) of *Rosa damascena* cv. Taifi and screened for phytase activity. Phytase activity was checked for 24 isolates in *Bacillus* broth media supplemented with and without rice bran. Twelve (12) isolates were found with detectable phytase activities. Among them, four selective bacterial strains were active (BAFA.Taifi94, BAFA.Taifi103, BAFA.Taifi111 and BAFA.Taifi117). The phytase activity of strain BAFA.Taifi111, which was grown in the *Bacillus* broth media and supplemented without rice bran showed 19.7 units/ml initial growths whereas, with an induction of rice bran 37.7 units/ml was observed after 10 days in the shaker at 30°C/150 rpm. The optimum temperature for BAFA.Taifi94 and BAFA.Taifi103 was 60°C, whereas it was 70°C for BAFA.Taifi111. The stability at 80°C was exhibited by BAFA.Taifi117. The optimum pH range was pH 5-6.5 at 60°C. The obtained *Bacillus* species for phytase production have been induced using rice bran and their physical properties such as temperature optima, pH optima and thermo stability were found similar to the previously characterized and published or commercially available *Bacillus* phytases.

Key words: *Bacillus sp.,* phytase activities, soil bacteria, *Bacillus* broth, *Bacillus* broth.

INTRODUCTION

Phytate occurs in cereals such as corn, rice bran, wheat bran and in seeds of cotton and rape, as well as in legume soybean (*Glycine max*) with different concentrations from 0.4 to 6.4%. The phytic acid composition of rice bran varies from 0.14 to 0.99% on dry basis weight. The phytase enzyme hydrolyzes phytate to *myo*-inositol and phosphate (Konietzny and Gernier, 2002). Many bacteria, yeast and fungi have phytase activities (Pandey

*Corresponding author. E-mail: aa_farouk@yahoo.com. Tel: +966569999386.

et al., 2001). Phytases were detected in various bacteria, e.g. *Aerobacter aerogenes* (Greaves et al., 1967), *Pseudomonas* sp. (Irving and Cosgrove, 1971), *Lactobacillus* (Angelis et al., 2003), *Aspergillus (*Casey and Walsh, 2003), *Rhizopus* (Sutardi and Buckle, 1988), *Bacillus sp. (*Choi et al., 2001), *Bacillus subtilis* (Powar and Jagannathan,1982), *Klebsiella* sp (Shah and Parekh,1990), *B. subtilis* (natto) (Shimizu, 1992), *Escherichia coli* (Greiner et al., 1993), *Enterobacter* sp. (Yoon et al., 1996) and *Bacillus* sp. DS 11 (later designated as *Bacillus amyloliquefaciens*) (Kim et al., 1998). The genera *Bacillus* and *Enterobacter* produced extracellular phytases. The *E. coli* phytase is a periplasmic enzyme. Muddy soils containing the majority of natural bacterial strains are capable to produce phosphatase and phytase. Nineteen *Bacillus* bacterial strains isolated from maize root had phytase activity and produce favorable enzyme when 1% of rice bran was supplemented in the growth media as reported (Hussin et al., 2007).

Phytase, a well-known industrial enzyme is an object of extensive research. Bacterial and fungal (*Aspergillus* and *Penicillium*) enzymes conquered many applications in manufacturing sectors (Alva et al., 2007; Krootdilaganandh, 2000) and used in biofertilizers (Farouk et al., 2013). Many phytases were revealed, characterized in the last 10 years and are commercially produced. Phytase market volume exceeds US$250 million and is growing at around 10% every year. Commercial Phytases from four different sources, *Peniophora lycii* (6-phytase of fungal origin); *Aspergillus niger* (3-phytase of fungal origin, HAP); *E. coli* (6-phytase of bacterial origin, HAP); *Penicillium funiculosum* (3-phytase of fungal origin, HAP) are available. Storage stability of commercial pure phytases (OptiPhos, Phyzyme and Ronozyme) at 23°C was more than 91% for 30 days and up to 180 days approximately, more than 85% were in retaining activity. Pure phytases were more stable than in pre-mixes. Coated phytases are more stable than the non-coated (Sulabo et al., 2011). Extracellular phytase production was optimized at 39.7°C, at an initial pH of 7.1, supplementation with 13.6% rice bran, 320 rpm of agitation and 0 vvm of aeration by *Enterobacter sakazakii* ASUIA279 and it was purified (Farouk et al., 2012). A recombinant *E. coli* DH5α that had shown higher phytase activity after 17 h incubation than *E. sakazakii* ASUIA279 after 5 days of incubation was reported (Ariff et al., 2013). A novel phytase gene (phyMS) from *Mycobacterium smegmatis* showed specific activity of 233.51 U/mg, optimal pH of 3 and 7 at 60°C was reported by Tamrin et al. (2014).

B. subtilis, a widely used food microorganism, generally recognized safe (GRAS) by the American Food and Drug Administration. Researches were carried out on *B. subtilis* from soil and other sources. *B. subtilis* CF92, an isolate from cattle feces, produces phytase with an exhibited optimal activity at 60°C at pH 7.0 and stable over a pH range of 4.0 to 8.0 (Hong et al., 2011). Phytase production by *B. subtilis* US417 (112 U/g of wheat bran) with a higher productivity in (SF) Submerged [2.3 U/(g×h)] than in (SSF) Solid State Fermentation [1.2 U/(g×h)] was reported (Kammoun et al., 2012). Bacterial phytases have considerable potential in commercial applications (Ursula and Griner, 2004). Among different substrates used (wheat bran, rice bran, bengal gram bran, red gram bran, groundnut oil cake, sesame oil cake, coconut oil cake, cotton oil cake, soya bean meal, oatmeal, corn meal and barley meal) for *B. subtilis*, wheat bran was observed as the best substrate for phytase yield. Combination of wheat bran with rice bran and groundnut oil cake in 1:1 ratio resulted in better phytase production (0.50 and 0.79 U/ml respectively) than using wheat bran only (Sreedevi and Reddy, 2012). A *B. subtilis* strain (BPTK4) isolated from boggy water sample produced phytase during the 48th h of incubation at 32°C with the pH of 6.5 was reported (Shamna et al., 2012). A high temperature withstanding neutral phytase isolated from *Bacillus nealsonii* ZJ0702 presented in soil was sequentially purified to homogeneity by ammonium sulfate precipitation (Yu and Chen, 2013). From poultry waste, an extracellular phytase producing bacteria was isolated and phytase was purified by ammonium sulphate fractionation of protein and dialysis followed by SDS-PAGE. The DNA from *Bacillus megaterium* was isolated in the 60th h at 37°C, pH 6.5 and 200 rpm shaking (Dhiraj et al., 2013).

Phytase producing bacteria (21 isolates) were isolated from Soil samples of Bt Rhizosphere, which were collected from NBt cotton growing area of Andhra Pradesh, India. An isolate NBtRS6 yielded phytase more than the other isolates (Ushasri et al., 2013). A strain (DR6) amid the 32 phytase producing bacteria confirmed a 39 mm clear zone on phytase specific medium (PSM-pH 5.5, Temperature 50°C with Glucose and Sucrose as Carbon source and Yeast extract as Nitrogen source) and was identified as *B. subtilis* with an enzymatic activity 378 U/mL as reported (Singh et al., 2013). Phytase activity of 22.165 U/ml was reported for *P. aeruginosa* isolated from rhizospere soil samples (Sasirekha et al., 2012). The initial pH of cultivation media and incubation temperature play a vital role in the phytase productions of the bacterial strains. According to the source of the bacteria isolated, their optimum temperature also varies; high phytase production was at pH 6.5 and then lowered to pH 4.0 after incubation for two days at 37°C. The optimal temperature for many phytases produced by microorganisms is between 25 to 37°C (Choi et al., 1999). Most of the phytases have their pH optima in the range of pH 4.0 to 5.6. Phytase from *Bacillus sp*. normally have optimum pH at 6.5 to 7.5 (Satyanarayana and Vohra, 2001) with a temperature range of 36 to 48°C. Better production was achieved at 37°C with the pH 7; *B. subtilis* (pH 6.0 to 6.5 and 60°C) (Shimizu, 1992), *B. amyloliquefaciens* (pH 7.0 to 8.0 and 70°C) (Kim et al.,

Table 1. The colonies (77 to 171) from leaf, root and flower extracts at different temperature.

Bacillus agar plates with incubation temperature (°C)	Colonies from leaves	Colonies from roots	Colonies from roots
BAS 30	77-98	133-148	149-171
BAS 40	99-120	-	-
BAS 50	121-132	-	-

1998) and *E. coli* (pH 5.0 and 70°C) as reported (Choi et al., 1999). The six bacterial strains among 30 strains which were isolated from Malaysian maize plantation were grown in Luria Bertani (LB) and Luria Bertani+Rice Bran (LBRB) media for five days produced highest phytase activity was also reported (Hussin et al., 2009). The aim of the current work is to isolate bacteria from soil of *Rosa damascena* plant using rice bran and study the phytase activity along with the temperature and pH optimization.

MATERIALS AND METHODS

Materials, chemicals and rose extract

Chemicals used were of analytical grade and commercially available. Glycerol (from Scharlab, S.L. Barcelona, Spain); phytic acid, citric acid (from Sigma-Aldrich, U.S.A); sodium acetate 100 mM (from Loba Chemie Pvt. Ltd, Mumbai), and liquid nitrogen (from Air liquid, Jeddah).

Preparation of the rose extract

Initially, water was sterilized by autoclaving at 121°C at 15 psig for 20 min for the extraction. The Taif roses, *R. damascena* cv. Taifi with a delicate and passionate fragrance from the rose pot in early morning hours were plucked and collected. The plant itself, the green leaves, flowers and roots were washed with sterilized distilled water and then with ethyl alcohol. All the parts which were taken in mortar and pestle were homogenized to fine powder separately, with liquid nitrogen and extracted using sterilized distilled water. The rose extract collected was kept in a shaking incubator at 30°C for 2 h and then stored at -80°C until further use. This rose extract was used for further study of phytase activity.

Media preparations and screening

Initially, the mud of *Rosa damascena cv. Taifi* was collected from the cleaned root and it was washed with 3.5 L of sterilized distilled water (Farouk et al., 2014b). The supernatant was collected after filtration using Whatman filter paper No.1. The different nutrients media such as *Bacillus* agar, Nutrient agar, Tryptic soya agar and Potato dextrose agar were made by autoclaving at 121°C at 15 psig for 20 min.

The sand water filtrate was poured into the Bacillus agar, potato dextrose agar and nutrient agar plates and kept in incubation temperatures at 30, 40 and 50°C. The growth in *Bacillus* agar at 50°C was predominant. An aliquot of extracts from flowers, leaves, and roots were smeared in *Bacillus* agar, potato dextrose agar and nutrient agar and kept at 30°C for 24 h. All the plates containing a total of 321 colonies were marked. Colonies were picked out from the plates and transferred to sterilized potato dextrose agar, nutrient agar and peptone broths which were prepared by autoclaving at 121°C at 15 psig for 20 min. All the 321 tubes containing the colonies were organized and kept at -80°C. From the 321 tubes, colonies were inoculated in *Bacillus* agar plates. The colonies (77-171) from leaf, root and flower extracts at different temperature are shown in Table 1.

The *Bacillus* agar medium was prepared using the premix from Fluka's Hichrome *Bacillus* agar (49.2 g of premix was added to 1l of distilled deionized water, the pH was adjusted to 7.1 and the solution was autoclaved at 12°C for 15 min) and freshly prepared extracts were used during this project .

The *Bacillus* broth medium was prepared using the premix from Fluka's Hichrome *Bacillus* agar (49.2g of premix was added to 1l of distilled deionized water, the pH was adjusted to 7.1 and the solution was autoclaved at 121°C for 15 min. (The agar was removed by filtering using Whatman filter paper to get clear broth). The *Bacillus* broth was poured into sterilized 20 ml bottles.

The Hichrome *Bacillus* agar and broth media contained Peptic digest animal tissue (10 g/l); meat extract (1 g/l); D-Mannitol (10 g/l); Nacl (10 g/L); phenol red (0.025 g/l) and agar (15 g/l) as composition. Final pH was (at 25°C) 7.4±0.2. The solutes were shaken until they dissolved and the pH was adjusted to 7.4 using 1 N NaOH (8 ml). The volume was then adjusted to 1 L by adding deionized water. The sterilization was done by autoclaving at 121°C for 20 min at 15 psi (1.05 kg/cm) on liquid cycle. The composition of Hichrome *Bacillus* broth media was the same as Hichrome agar media except the agar.

The Potato dextrose agar media with a composition of 24 g/l potato dextrose broth from Fluka, (Sigma Aldrich, U.S.A.) and 28 g nutrient agar from Himedia, India was used. The pH was adjusted to 7.4 and the solution was autoclaved for 20 min at 15 psi (1.05 kg/cm) at 121°C on liquid cycle.

The Tryptic soya agar media with a composition of 40 g/L Tryptic soya agar from Fluka, (Sigma Aldrich, U.S.A.) in distilled water was adjusted to pH 7.4 and the solution was autoclaved for 20 min at 15 psi (1.05 kg/cm) at 121°C on liquid cycle.

The agar plates were made by weighing 28 g of nutrient agar obtained from Himedia, India, and dissolving it in 1l of sterilized distilled water. The pH was adjusted to 7.4 and the solution was autoclaved for 20 min at 15 psi (1.05 kg/cm) at 121°C on liquid cycle. After autoclaving, the medium was swirled gently to distribute the melted agar evenly throughout the solution. It was allowed to cool down to 50 to 60°C. Then, 20 ml of this medium was poured on to 90 mm Petri dishes under sterile conditions and allowed to cool. After the medium strengthens completely, they were inverted and stored at 4°C. They were removed from storage 1 to 2 h prior to use (Sambrook and Russell, 2001**)**. Suspension of the bacterial cultures were covered completely on the agar plates and allowed to dry. The agar plates were then inverted and incubated for 24 h at 37°C. After incubation, growth appeared. The colonies were marked.

Sample preparation for enzyme assays

The production of enzymes was carried out in the production medium

without addition of agar using the shaken flask fermentation method. The inoculum of the selected strain was produced using sterile *Bacillus* broth in 20 ml sterilized sample bottles. Five percent of inoculums were inoculated on 20 ml of production medium. The bottles were incubated at 30°C for 48 h with shaking at 150 rpm for better aeration and growth of organism.

The samples of enzyme activity assays were prepared by centrifuging 1.5 ml bacterial culture for 1 min at 13,000 rpm (Idriss et al., 2002). The supernatant was used for assay. The amount of phytase produced was assayed using chemical assay using ammonium molybdenum method (Heinonen and Lahti, 1981).

Induction of phytate degrading enzymes using rice bran

Production of phytate-degrading enzyme from Malaysian soil bacteria using rice bran containing media was reported (Hussin et al., 2010). In this study, the autoclaved 5% rice bran was supplemented to the production medium and incubated at 30°C for 48 h at shaker conditions at 150 rpm for better aeration and growth of organism. The amount of phytase produced was assayed again using ammonium molybdenum method (Heinonen and Lahti, 1981). The 5% rice bran was prepared by mixing in distilled water and the pH was adjusted to 6.42. The solution was autoclaved for 15 min at 15psi (1.05 kg/cm^2) on liquid cycle.

Phytase activity assay

Phytase measurements were carried out at 28°C. The reaction was initiated with the addition of phytase enzyme day after a day. After 30 min incubation, the liberated inorganic phosphate was measured using a modification of the ammonium molybdate method (Heinonen and Lahti, 1981). A freshly prepared solution of acetone: 5 N sulfuric acid: 10 mM ammonium molybdate (2:1:1 v/v) and thereafter 100 µl of 1.0 M citric acid were added to 400 µl of the phytase assay mixture. The cloudiness was removed by centrifugation at 10,000 rpm for 10 min prior to the measurement of absorbance at 355 nm in a UV double beam spectrophotometer. In order to quantify the phosphate released; a calibration curve was constructed within the range of 5 to 1200 mM phosphate.

The control was prepared by adding the stop solution prior to adding the enzyme into the assay mixture. The phytase activity was calculated from the average of at least three phytase assay measurements. One unit (U) of phytase activity was defined as the concentration of inorganic phosphate, in µmol, released per min per mL of enzyme preparation (U/ml). The activity was calculated using the slope of the straight line resulting from the assay with the sample (absorbance/minute) and the slope of the straight line arising from the standard curve (absorbance/µmol of P).

The phytase activity of BAFA.Taifi strains in *Bacillus* media supplemented with rice bran and in the absence of rice bran was carried out. The culture filtrates were measured for phytase activity in microplate reader MR96A (Shenzhen Mindray Bio-Medical electronics Co., Ltd.) at a primary wavelength of 405 nm and a secondary wavelength of 450 nm.

Optimization of phytase production

In order to determine the effect of temperature on phytase production, the selected bacterial isolate was grown in production media and incubated at different temperatures: 4°C, ROOM Temperature 25, 30, 40, 50, 60, 70, 80 and 90°C for 1 h at pH 6.5. Culture filtrates were later measured for phytase activity in microplate reader MR96A (Shenzhen Mindray Bio-Medical electronics Co., Ltd.) at a primary wavelength of 405 nm and a secondary wavelength of 450 nm. Bacterial strains used were BAFA.Taifi94, BAFA.Taifi103, BAFA.Taifi111 and BAFA.Taifi117.

The effect of initial media pH on phytase production was conducted by adjusting the production media to pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0 and 8.5 before bacterial inoculation. After 1 h of incubation at 50°C, culture filtrates were measured for phytase activity in spectro UV–Vis double beam PC 8 scanning auto cell spectrometer (Labomed, Inc; U.S.A) at wavelength 630 nm. Bacterial strains used were BAFA.Taifi94, BAFA.Taifi103, BAFA.Taifi111 and BAFA.Taifi117.

The induction of phytase degrading enzymes using rice bran was determined by adding the production media with 5% rice bran and inoculated with test organisms. The media was incubated for 24 h at 37°C. Culture filtrates were later measured for phytase activity in microplate reader MR96A at a primary wavelength of 450 nm and a secondary wavelength of 630 nm.

RESULTS

Phytase activity of bacterial strains

A total of 321 bacterial strains were isolated from the mud sample of *Rosa damascena cv.Taifi*, among them, four strains were found to be positive for phytase production by their zone forming ability in CMC containing media (Farouk et al., 2014a). Among 48 bacterial isolates from *Bacillus* agar plates, an isolate BAFA.Taifi117 exhibited the highest enzyme activity.

Since the growth study was essential for the production of extracellular enzymes, it was studied by using the shaken flask fermentation method. The stationary phase of growth was reached after about 48 h. The production of phytase was detected after 36 h of cultivation.

The production of phytase was considerably low before 36th hours and after 48th hours of production. It was considered as the log phase and its variation also depends on the nutrient present in the medium and the cultural condition of the organism. The environmental parameter also influences the maintenance time of the bacteria. The plate assay and chemical assay confirmed the production of phytase. Although, the production of phytase was detected after 36 h of cultivation, it increased during growth and reached maximum level (37.7 U/ml) in rice bran medium after ten days as shown in Figure 1.

The isolated *Bacillus* strains in *Bacillus* broth medium supplemented with rice bran in shaken flask at 150 rpm produced significant amount of phytase during the 48th hour of incubation at 30°C with pH of 6.5. In *Bacillus* broth medium supplemented with rice bran in shaken flask at 150 rpm, water was used as the source for the isolation of phytase producing bacteria.

Figure 2 shows a comparative study of phytase activity of different BAFA strains with the supplementation of rice bran in media. Increasing moderate phytase activity was observed by the induction of rice bran after ten days for BAFA.Taifi111.

Effect of temperature and pH

In the study of the *Rosa damascena*, the optimum

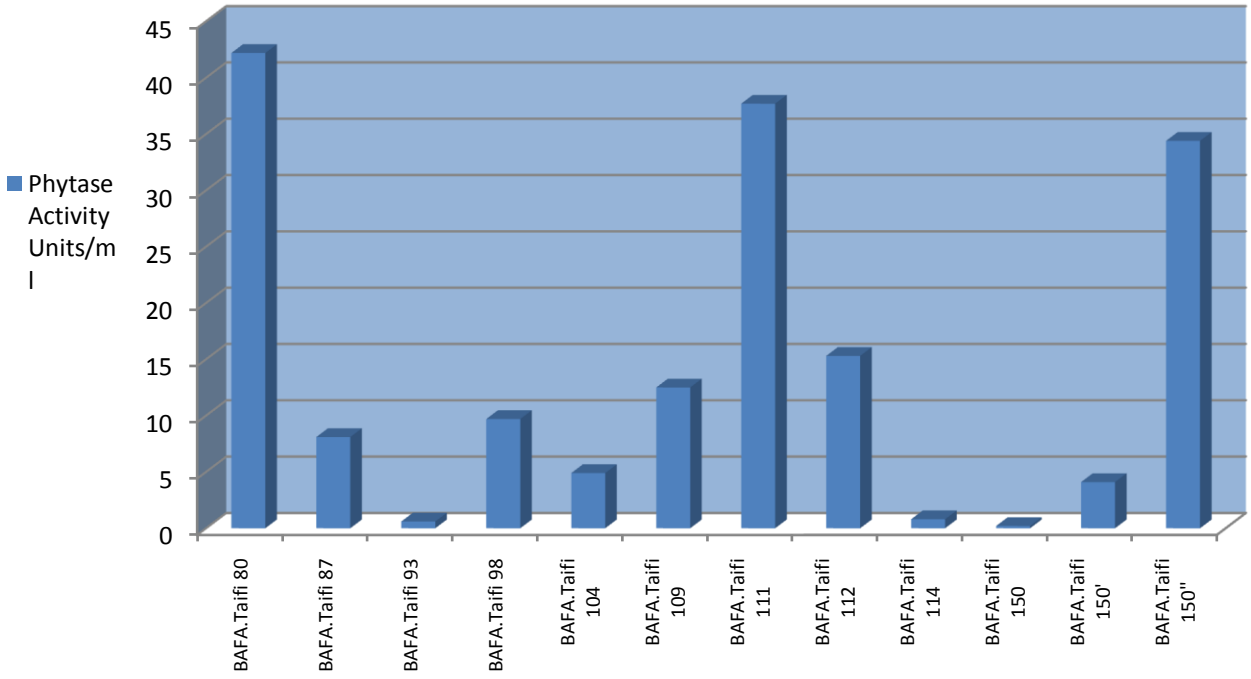

Figure 1. The phytase activity of selected bacterial strains in *Bacillus* broth and rice bran.

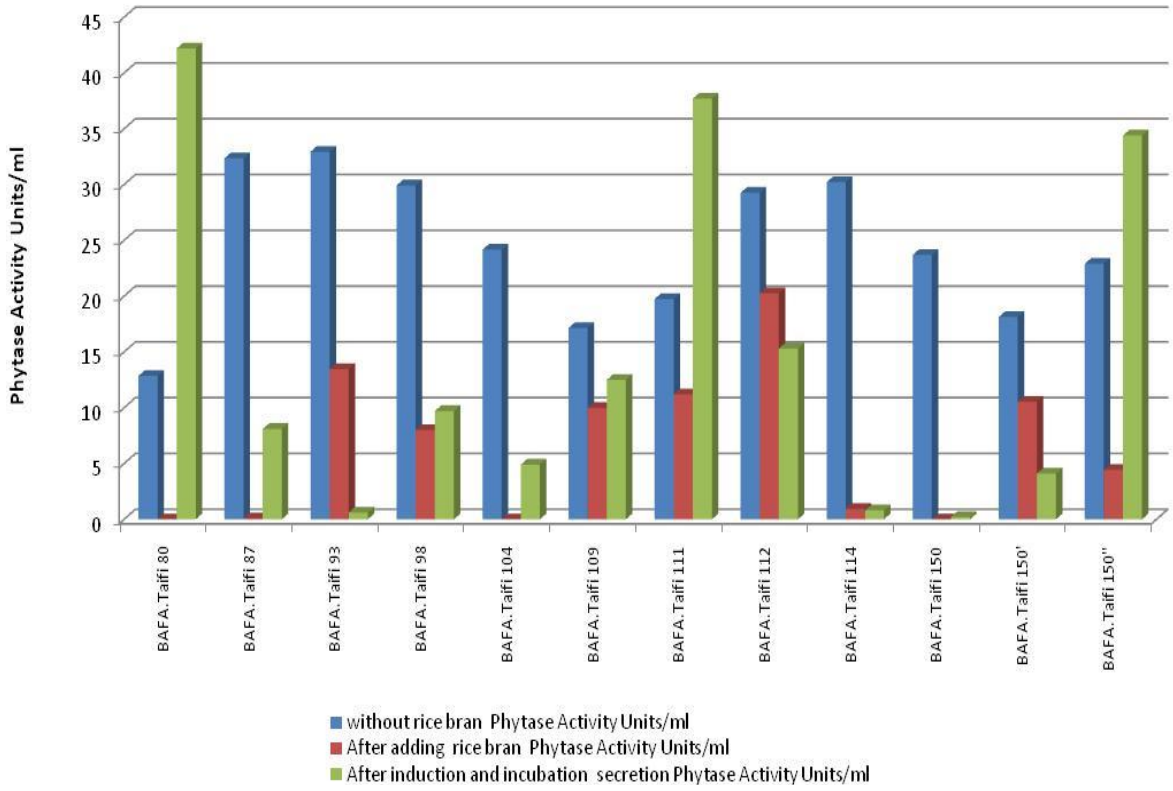

Figure 2. Comparative phytase activity study of BAFA.Taifi strains with and without rice bran.

temperature for phytase production was found to be different for the selected strains. The BAFA.Taifi94 and BAFA.Taifi103 have an optimum phytase activity at 60°C; The BAFA.Taifi111 has an optimum phytase activity at

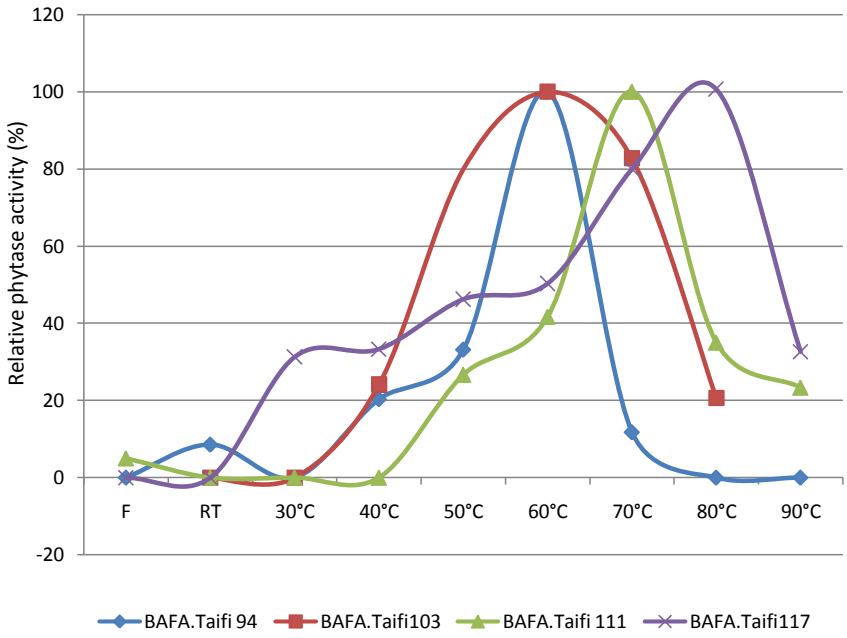

Figure 3. Temperature optimization curve for bacterial strains BAFA.Taifi94, BAFA.Taifi103, BAFA.Taifi111 and BAFA.Taifi117. F represents fridge temperatrue (4°C) and RT is room temperature (25°C).

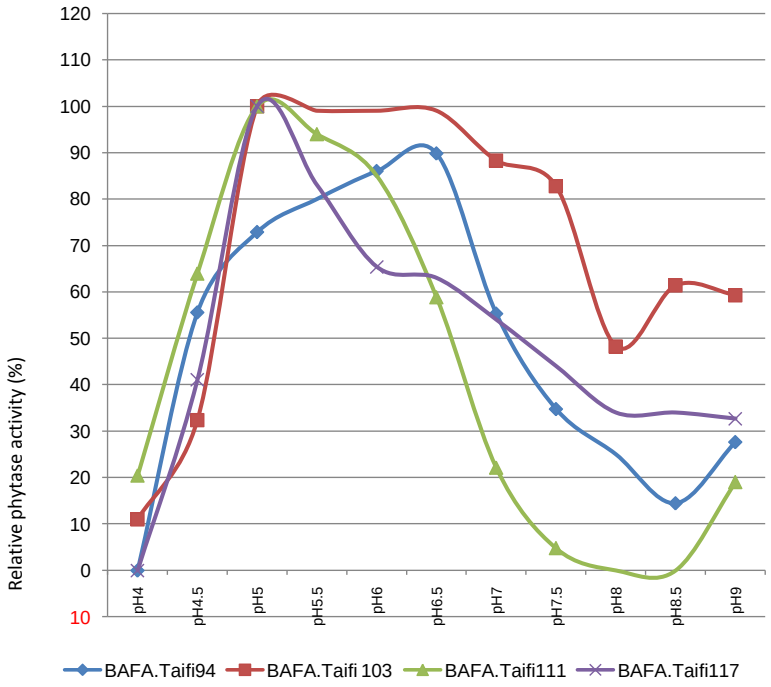

Figure 4. The pH Optimization curve for bacterial strains BAFA.Taifi94, BAFA.Taifi103, BAFA.Taifi111 and BAFA.Taifi117.

70°C and the BAFA.Taifi117 has an optimum phytase activity at 80°C as shown in Figure 3.

Besides temperature, pH is found to be one of the most important factors for phytase detection. The significant production of phytase was observed at the pH of 6.5 (100 U/ml) (Figure 4). It was found that the strain BAFA.Taifi117 requires a slightly acidic pH for phytase production.

Table 2. The selected bacterial strains with phytase activity at pH 6.5.

Bacterial strains	Optimum temperature (°C)	Phytase activity (U/ml)
BAFA.Taifi94	60	100
BAFA .Taifi103	60	100
BAFA.Taifi111	70	100
BAFA.Taifi117	80	101

Table 3. The selected bacterial strains with phytase activity at 37ºC.

Bacterial strains	Optimum pH	Phytase activity at 37ºC (U/ml)
BAFA.Taifi94	6.5	90
BAFA .Taifi103	5	100
BAFA.Taifi111	5	100
BAFA.Taifi117	5	101

The optimum pH on phytase production was found to be at pH 5 and pH 6.5 at 37°C for the selected strains. The BAFA.Taifi103, BAFA.Taifi111 and BAFA.Taifi117 have an optimum phytase activity at pH 5 at 37°C and the BAFA.Taifi94 has an optimum phytase activity at pH 6.5 at 37°C as shown in Figure 4.

Tables 2 and 3 represent the selected Bacterial strains with phytase activity at constant pH 6.5 and temperature at 37°C respectively.

By testing ten BAFA. Taifi strains, it was found that the phytase activity of the BAFA.Taifi strain (BAFA.Taifi80) without rice bran was 12.5 units/ml after one day, but with the induction of rice bran, it was observed to be 42.2 units/ml after 10 days in a shaker at 30°C/150 rpm.

The phytase activity of the BAFA.Taifi strain (BAFA.Taifi111) without rice bran was 19.7 units/ml after one day, but with the induction of rice bran it was observed to be 37.7 units/ml after 10 days in a shaker at 30°C/150 rpm.

The phytase activity of the BAFA strain (BAFA.Taifi150) without rice bran was 22.9 units/ml after one day, but with the induction of rice bran, it was observed to be 34.4 units/ml after 10 days in a shaker at 30°C/150 rpm.

The phytase activity of both strains BAFA.Taifi109 and BAFA.Taifi112 without rice bran after one day was 17.15 and 29.63units/ml. But after 10 days, the phytase activity with the induction of rice bran showed a moderate reduction of 12.5 and 15.3 units/ml, respectively.

The phytase activity of the BAFA.Taifi strains without rice bran was 32.9 units/ml for BAFA.Taifi93; 30.2 units/ml for BAFA.Taifi114; and 32.2 units/ml for BAFA.Taifi87. A drastic reduction in the phytase activity was observed with the induction of rice bran and it was 0.8, 0.6 and 8.1 units/ml respectively after 10 days in a shaker at 30°C/150 rpm.

This study revealed that the addition of 5% of rice bran stimulates the expression of phytase by the bacterial strains of the *Rosa damascena* cv.Taifi*.* The considerable amount of phytase activity reveals that the bacterial sp. can be an effective source of phytase.

DISCUSSION

Phytase in rice bran is present as a less soluble K-Mg salt; normally it is enclosed by starch or combined with protein, which leads to a lower rate of hydrolysis (Wang et al., 1999). So, by using rice bran as a known carbon source, phytase production was favoured continuously in the shaker conditions in the presence of phytate (Konietzny and Greiner, 2002). Rice bran has been used for the stimulation of phytase secretion in *Bacillus subtilis* (Agalya et al*.,* 2013).

However, inducible secretion of phytase-degrading enzymes from bacteria associated with *Rosa damascena* cv.Taifi using rice bran had shown an excellent result against 5% rice bran induction. Rice bran plays a major role in phytase production as observed in certain bacterial strains (Hussin et. al., 2010). The characterization of secreted phytase from BAFA.Taifi strains has confirmed features of previously studied *Bacillus* phytases in our laboratory (Igbasan et al*.,* 2000). The real and beneficial production of phytase using cheap natural media such as rice bran is to be studied further as one of the best known media for phytase production (Hussin et al., 2007). Most of BAFA.Taifi strains taken for this study behaved differently with time and their stimulation for the secretion of phytase in liquid media contains rice bran in their ingredients with various concentrations.

The role of various cereal substrates like sugar cane baggage and wheat bran on phytase production can be studied in the future for the bacilli strains obtained from *Rosa damascena* and other Taif rose varieties. Further

study is to be done for cloning to confirm the purification of phytase degrading enzymes and their specific activity.

Conflict of Interests

The authors have not declared any conflict of interests.

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