

Full Length Research Paper

Potential phytate-degrading enzyme producing bacteria isolated from Malaysian maize plantation

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Phytases catalyze the hydrolysis of phosphomonoester bonds in phytate, thereby releasing lower forms of myo-inositol phosphates and inorganic phosphate. Phytase enzyme preparations have a wide range of applications in animal and human nutrition. The addition of phytate-degrading enzyme can improve the nutritional value of plant-based foods by enhancing protein digestibility and mineral availability through phytate hydrolysis during digestion in stomach or during food processing. 30 strains of potential phytate-degrading enzymes bacteria isolated from Malaysian maize plantation were cultivated in Luria Bertani (LB) and Luria Bertani + Rice Bran (LBRB) media for 5 days and were analyzed for phytase activity. The 6 strains with highest activity were chosen for species identification. Two set of broad-range 16S rRNA PCR primers were used for genotypic identification. ASUIA279 was the strain that had the highest phytase activity in LBRB followed by ASUIA271, ASUIA138, ASUIA260, ASUIA243 and ASUIA30. The genotypic technique revealed *Pantoea stewartii* ASUIA271, *Enterobacter sakazakii* ASUIA279, *Bacillus cereus* ASUIA260, *Bacillus subtilis* ASUIA243, *P. stewartii* ASUIA138 and *B. cereus* ASUIA30.

Key words: Bacterial phytase, rice bran, production, genotypic.

INTRODUCTION

Phytases (myo-inositol hexakisphosphate phosphohydrolase), also known as phytate-degrading enzymes, hydrolyse phytate to myo-inositol and phosphoric acid in a stepwise manner forming myo-inositol phosphate intermediates (Koneitzny and Greiner, 2002). Phytases are widespread in nature because they can be found in animals, plants and microorganisms. Like filamentous fungi (Pasamontes et al., 1997), phytate-degrading enzymes of yeasts (Nakamura et al., 2000; Vohra and Satyanarayana, 2002) gram-negative (Tambe et al., 1994; Sajidan et al., 2004) and gram-positive bacteria (Kerovuo et al., 1998) were also identified and characterized. Phytate is an abundant plant constituent comprising 1 to 5% (w/w) of edible legumes, cereals, oil

seeds, pollens and nuts (Cheryan, 1980). Most foods of plant origin contain 50 to 80% of their total phosphorus as phytate (Harland and Morris, 1995). Phytate-bound phosphorus is poorly utilized by monogastric animals, due to insufficient phytate-degrading activity in the gut (Ravindran et al., 1995; Fandrejewski et al. 1997).

The first commercial phytase product, which was available 10 years ago, offered animal nutritionists the tool to drastically reduce phosphorus excretion of monogastric animals (Haefner, 2005). According to Kornegay (1999), phosphorus excretion can be reduced between 25 and 50% depending on diet, species and level of phytase supplementation. Normally, phytase is incorporated into commercial poultry, swine, and fish diets to improve the availability of phosphorus, minerals, amino acids, and energy.

At present, there is no single phytase that is able to meet the diverse needs for all commercial and environmental applications. Therefore, there is an ongoing

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interest in screening microorganisms, including bacteria for novel and efficient phytases. Different sources of phytases would have differences in some properties, such as substrate specificity, resistance to proteolysis and catalytic efficiency. The aims of the present study were to investigate and identify the potential bacteria strains as phytase producers isolated from Malaysian maize plantation.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Phytic acid, dodecasodium salt, was purchased from Sigma chemical Co. (St. Louis, Mo.). All other chemicals and media were of analytical grade commercially available. LB broth was prepared from 10 g tryptone, 5 g yeast extract, and 10 g NaCl and LBRB was prepared by adding of 50 g of rice bran in 1 l of LB media. Both media were adjusted to pH 7.0 prior to utilization. 30 bacterial strains showing phytase activities were previously isolated from Malaysian maize plantation (Anis Shobirin et al., 2007) were cultivated in 10 ml LB and LB + 5% rice bran media, and incubated at 37°C and agitated at 200 rpm. Samplings for extra-cellular enzyme activity were performed after 2 and 5 days of growth. Samples for enzyme activity assays were prepared by centrifugation of 1.5 ml bacterial culture at 13,000 rpm for 1 min (Idriss et al., 2002). The cell-free supernatant was separated and tested for phytase activity. Potential phytase producing strains (ASUIA260, ASUIA271 and ASUIA279) were grown in LB + 0.1% sodium phytate to determine the Na-phytate efficacy on the ability of those bacteria in producing phytase.

Standard phytase assay

Phytate-degrading activity was determined at 50°C in 350 µl of 100 mM sodium acetate buffer, pH 5.0 containing 1.03 mM sodium phytate. The enzymatic activity was done by adding 10 µl of enzyme solution to the assay mixture. After incubating for 30 min at 50°C, the liberated phosphate was measured according to ammonium molybdate method (Heinonen and Lahti, 1981) with some modifications. Added to the assay mixture was 1.5 ml of a freshly prepared solution of acetone: 5 N H₂SO₄: 10 mM ammonium molybdate (2:1:1 v/v) and 100 µl acetic acid. Any cloudiness was removed by centrifugation prior to the measurement of absorbance at 355 nm. To calculate the enzyme activity, a calibration curve was produced over the range of 5-600 mmol phosphate ($\epsilon = 8.7 \text{ cm}^2/\text{nmol}$). Activity (units) was expressed as 1 µmol phosphate liberated per min. Blanks were run by addition the ammonium molybdate solution prior to adding the enzyme to the assay mixture (Greiner, 2004).

Species identification

6 strains (ASUIA279, ASUIA271, ASUIA138, ASUIA260, ASUIA243 and ASUIA30) were choosing for species identification based on the high phytase activity. The strains were grown on LB broth and harvested by centrifugation at 10000 rpm. DNA was extracted using DNA isolation kit (Qiagen, Hilden, Germany). The DNA was quantified spectrophotometrically (A260) and purity checked by electrophoresis on a 0.8% agarose gel. 2 sets of 16S rRNA PCR primers, PF3: (5' GAGAGTTTGATCCTGGCTCAG 3'), PR4: (5' CTGTTGCTCCCCACGCTTTC 3') and PF5: (5' GTAGCGGTGA AATGCGTAGA 3') PR6: (5' CTACGGCTACCTTGTTACGA 3') were used. PCR amplification was performed using an Eppendoff

mastercycler (Hamburg, Germany). The PCR reaction mixtures contained 2 µl of dNTPs mixture (1.25 mM each), 1 µl of each primer (20 mM), 2.5 µl of DNA, 1 µl of Taq polymerase (Promega, Southampton, UK) and sterile deionized water to bring the final volume to 100 µl. The mixture was denatured at 94°C for 2 min. The PCR temperature profile consisted of 34 cycles of 1 min denaturation at 94°C, 45 s annealing at 50°C and 1.5 min primer extension at 72°C. A negative control was included to eliminate the possibility of reagent contamination. PCR products were analyzed using agarose (0.8%) gel electrophoresis and visualized using ethidium bromide. The identities of the bacteria detected by the 16S rRNA PCR were revealed by sequencing of the PCR products and compare-son of these sequences to the genbank database using the BLAST program available at the national centre for biotechnology information (<http://www.ncbi.nlm.nih.gov>).

RESULTS

The addition of 5% rice bran in the LB media showed the significant different ($P < 0.05$) on the phytase production by all the bacterial strains tested. Figure 1 shows the induction of phytase production in the 30 bacterial strains when cultivated in LB + 5% rice bran media compared to LB media. ASUIA279 was the strain that have the highest phytase activity in LBRB followed by ASUIA271, ASUIA138, ASUIA260, ASUIA243 and ASUIA30. So, they were choosing for species identification using the genotypic technique. Based on their colony morphology, microscopic observation, phytase activity and phenotypic technique result, ASUIA273 and ASUIA261 were assumed as the same species as ASUIA279 whereas, ASUIA136 was the same as ASUIA138. So, they were not included in the potential list. The addition of 0.1% Na-phytate into LB medium resulted (Figure 2) the slightly increment of phytase production by the 3 selected bacteria compared to the LB itself even though the increment were not as much as the addition of rice bran.

Two set of broad-range 16S rRNA PCR primers were used and the PCR product with 800 base pairs in size were referred for gene sequencing analysis. From the comparison of these sequences (Figure 3a-f) to the genbank database using the blast program neighbour-joining trees were obtained. The 6 strains were identified as *P. stewartii* ASUIA 271, *E. sakazakii* ASUIA279, *B. cereus* ASUIA260, *B. subtilis* ASUIA243, *P. stewartii* ASUIA 138 and *B. cereus* ASUIA30.

DISCUSSION

Nutrient source and concentration greatly influence bacterial growth and enzyme production (Vohra and Satyanarayana, 2003). 5% of rice bran was added in one of the cultivation media. Rice bran is rather low in inorganic phosphate and has relatively high phytate content. Therefore, the high phytate content in the medium might trigger the biosynthesis of phytate-degrading enzyme in these soil bacteria.

The increment of phytase production by 3 selected

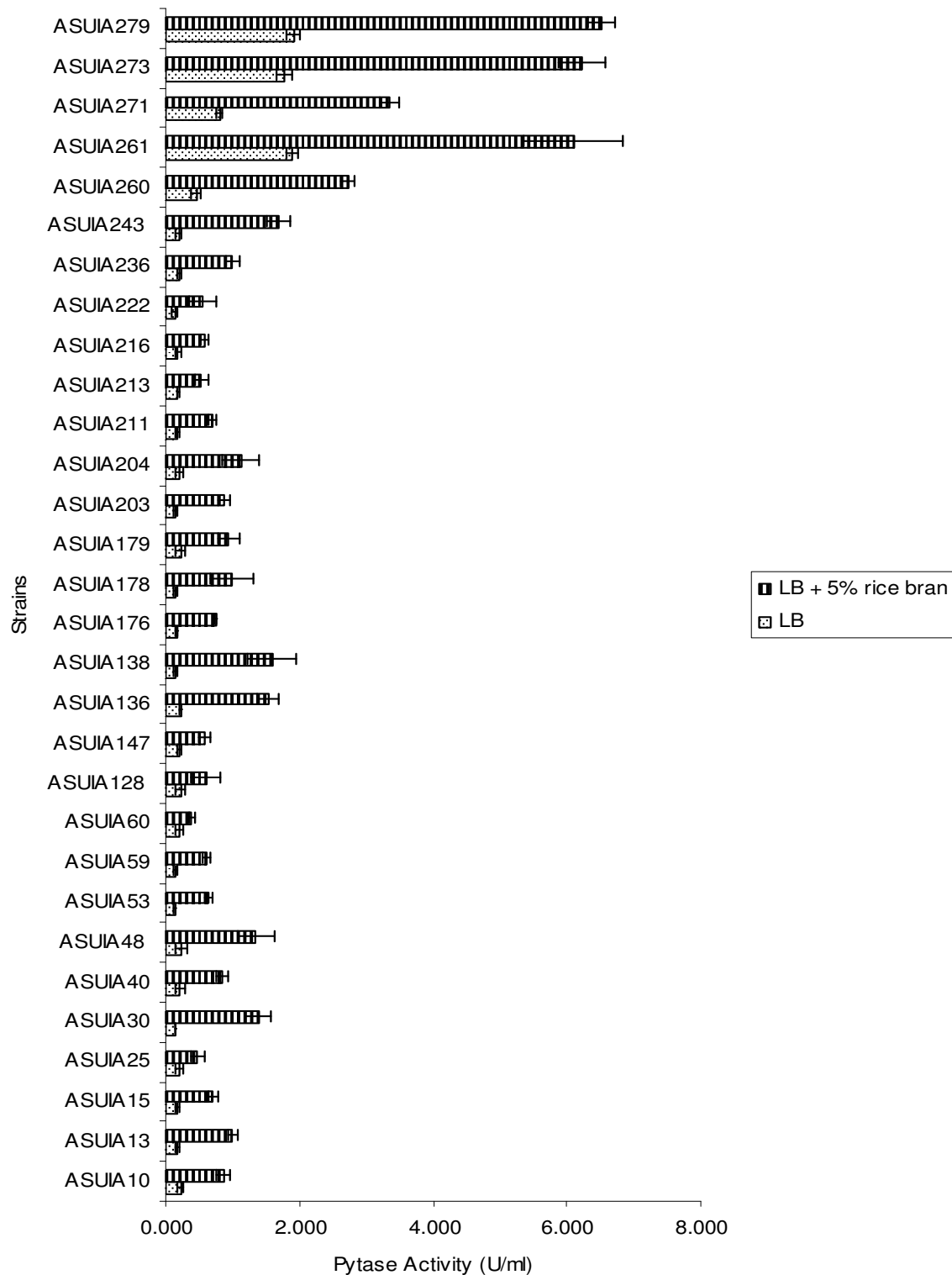


Figure 1. Phytase enzyme activities of 30 soil bacteria strains after 5 days cultivation in LB and LB + 5 % rice bran media. Error bars show mean \pm standard deviation.

bacteria when cultivated in 0.1% Na-phytate clarify that the phytate induced the production of phytase in those bacteria (Figure 2). The rice bran has better impact com-

pared to Na-phytate could be due to the different nature of these substrates. This finding was compatible with the study of Lan et al. (2002) who suggested that phytate in

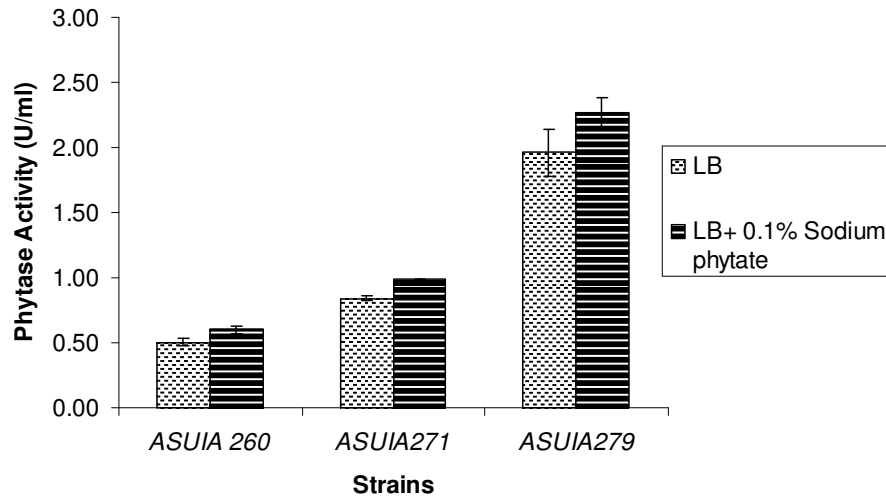


Figure 2. Comparison of phytase production of the bacterial strains in LB and LB + 0.1 % sodium phytate. Error bars show mean ± standard deviation.

GNTGGAGATCCGGTGGCGAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAG
 CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGG
 AGGTTGTTCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGT
 ACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGT
 GGTTAATTTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGCGAACTTNNCAGA
 GATGCCTTGGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTGTCAGCTCGTGT
 TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGATTC
 GGTCCGGAACCTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAA
 GTCATCATGGCCCTTACGAGTANGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAG
 CGACCTCGCGAGAGCAAGCGGACCTCATAAAGTCCGTGCGTAATCCGGATCGGANTCTGCAA
 CTCGACTCCGTGAANTCGGANTCNCTAGTATCGTGNATCAAAAGCCCCGGGAATACGTTCC
 NGGCCTTGATCCCCCCCCGTCCACCATGGGAGTGGTTGCAAAAAAATAGNTACTTAACTCN
 GGAGGGCCTTACCCTTTTGAATNTTGACNGGGGNANCN

Figure 3a. 16S rRNA gene sequence of ASUIA271.

GNTGGANGATNCGGTGGCGAAGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAG
 CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGG
 AGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAG
 TACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGT
 TGGTTAATTTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCAGAGAATCCTGCAG
 AGATGCGGGAGTGCCTTCGGGAACCTCTGAGACAGGTGCTGCATGGCTGTGTCAGCTCGT
 GTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGT
 TCGGCCGGGAACCTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC
 AAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGA
 AGCGACCTCGCGAGAGCAAGCGGACCTCATAAGTCCGTGCGTAATCCGGATTGGANTCTGCA
 ACTCGACTCNTGAANTCNGAATCNCTAGTATCGTGGATCAAATGCCNCGGGAAAACNTTCCC
 GGNCTTGTAACCCCCCGTCCNCCNTGGGATGGGTTGCAAAAAAATAGGTNCTTAACTNC
 NGGAGGGCCCTNCCNNTTNGNANTTTGACGGGGGAANNCAAAAAAGG

Figure 3b. 16S rRNA gene sequence of ASUIA279.

rice bran occurs as a less soluble potassium-magnesium salt, usually combined with protein, or enclosed by starch and other carbohydrates and the rate of rice bran phytate

being hydrolyzed could be slower as compared to Na-phytate. The lower rate of rice bran hydrolysis ensures that phytase production is continuously induced during

GNTGGAGGAANCCAGTGGCGAAGGCGACTTTCTGGTCTGTAAGTACACTGACACTGAGGCGCGAAA
 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGCTAA
 GTGTTAGAGGGTTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCCTGGGGA
 GTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA
 TGTGGTTAATTGCAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCT
 AGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTGAGCTCG
 TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCAT
 TAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTC
 AAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCT
 GCAAGACCGCGAGGTGGAGCTAATCTCATAAANCCTTCTCAGTTTCGNATTGTAGNCTGCAAC
 TCNCCTACNTGAANCTGGAATCCCTAGTAATCNCGNATCANCATGCCCCGGTGAATACGTTCC
 CCGGGCCTNGTANCNCCCCCCCCGTCGCCCCCGAAAGTTNGTAACCCCNAAAANTCNGNNG
 GGGTAACCTTTTTGGANCCCCCCCCCTAAGGGGGAAAAAATTTGGGGGNATCTAAAAAAGGG
 TACCNN

Figure 3c. 16S rRNA gene sequence of ASUIA260.

GTGTGGAGACCCAGTGGCGANGCGACTCTCTGGTCTGTAAGTACACTGACGCTGAGGAGCGAAAG
 CGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGCTAAG
 TGTTAGGGGGTTTTCCGCCCTTAGTGCTGACGCTAACGCATTAAGCACTCCGCCCTGGGGAG
 TACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
 GTGGTTAATTGCAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTA
 GAGATAGGACGTTCCCTTCGGGGGCGAGAGTGACAGGTGGTGCATGGTTGTCGTGAGCTCG
 TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCAT
 TCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTC
 AAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCA
 GCGAAACCGCGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTTCGGATCGCANTCTGCAA
 CTCGACTGCGTGAANCTGGAATCNCTAGTAATCNCGNATCAACATGCCNNGGTGAANACGT
 TNCCGGGNCNTTTTACNCCCCCNGTCCANCCNNGAAAGTTTTTAACCCCGAANTCGGG
 GAGNTANCNTTTNAGAACNCCCCCAAGNNGGAAAAAATTTGGGGGAATNTAAAAAGGNA
 NCCCTAAAAANNNNNNNNNNN

Figure 3d. 16S rRNA gene sequence of ASUIA243.

NTGGANGAATCCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCAGGTGCGAAA
 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTTG
 GAGGTTGTTCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCCGCTGGGGA
 GTACGGCCGCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
 GTGGTTAATTGCAAGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGCGAATTTCCA
 GAGATGGATGTTGCTTCCGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTGAGCTCGT
 GTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT
 TCGGTCCGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC
 AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAAG
 AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAATCCGGATCGGANTCTG
 CAACTCGACTCCNTGAANTCGGAATCNCTAGTAATCGTGGATCAAANTGCCNNGGNGAATAC
 GTTCCNNGCCTTGTAACCCCCCCCCGTCGCCCCNTGGGANTGGGNTNGNAAAAAATAGTTNC
 TTANCCCCNNGGAGGNCTTACNNTTNNATNCTGACTGGGGNAANNAAAANAGGGNCCCCN
 NAAANNNNNNNNNNNNNNNNNNNNNNN

Figure 3e. 16S rRNA gene sequence of ASUIA138.

the whole fermentation process thus leading to increased on phytase production. This may also explain why the phytase activity was higher in longer fermentation period.

Papagianni et al. (1999) found that when wheat bran (20 g l⁻¹) was included in a semisynthetic medium comprising cornstarch, glucose and peptone, the biomass and phytase production of *Aspergillus niger* increased and they suggested that the increased phytase produc-

tion might be due to the low release of phosphorus from wheat bran or phytase induction by the presence of phytate. Phytases have been reported in a number of bacteria. Bacterial phytases are mostly cell associated, with the exception of *B. subtilis*, *Lactobacillus amylovorus*, and *Enterobacter* sp.4 (Vohra and Satyanarayana, 2003). In this study, all the strains produced extra-cellular enzymes where *E. sakazakii* ASUIA279 (4480 mU/ml)

GTTGGNNGACCCAGTGGCGAAGGCGACTTTCTGGTCTGTAAGTACTGACACTGAGGCGCGAAAG
 CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACCGATGAGTGCTAAG
 TGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAGCACTCCGCCTGGGGAGT
 ACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATG
 TGGTTAATTGCAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAG
 AGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCTGTCAGCTCGTG
 TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTGATCTTAGTTGCCATCATT
 AGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGGATGACGTCA
 AATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAANAGCTG
 CAANACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGANTTGTAGGCTGCAA
 CTCNCCTACATGAANCTGGAATCNCTAGTANTCCCGNATCACCATGCCCCGGTAAAAACNTT
 CCCGGGCNTTTAANNCCCCCCTCCNNCNCNAAAGTTNTTAAACCCCNAAATCCGNNGGGGT
 AACCTTTTTGANCCCCCCTAAGGGGGAAAAANATTGGGGNAANNCNANAANGGNACCCN
 NAAAAANNNNNNN

Figure 3f. 16S rRNA gene sequence of ASUIA30.

showed the highest phytase activity, followed by *P. stewartii* ASUIA271 (1570 mU/ml) and *B. cereus* ASUIA260 (1160 mU/ml). There is no report yet on *P. stewartii* as phytase producer. Extra-cellular enzymes are good candidates for mass production and commercialization. Although inherent differences in experimental conditions and purity of enzyme preparations make comparisons difficult, the initial crude levels of activity in these bacteria compare favorably with those reported for *Selemonas ruminantium* (JY35 703 mU/ml) (Yanke et al., 1998) *B. subtilis* (44 mU/ml) (Shimizu, 1992) and *Escherichia coli* (5600 mU/ml) (Greiner et al., 1993).

Conclusion

The measured levels of phytase activity suggest that soil bacteria are a potential source of a phytase that could be developed commercially. Ongoing studies are being conducted to optimize the phytase production and further characterize phytase from selected strains. The phytase genes from these potential strains would be clone and sequence to elucidate its primary structure. And as well, to be overexpress in *E. coli* in order to increase the production level to cost-effective levels and would offer improve properties.

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REFERENCES

Anis Shobirin MH, Farouk A, Greiner R, Hamzah MS, Ahmad FI (2007). Phytate-degrading Enzyme Production by Bacteria isolated from Malaysian soil. *World J. Microbiol. Biotechnol.* 23: 1653-1660.

- Cheryan M (1980). Phytic acid interactions in food systems. *Crit. Rev. Food Sci. Nutr.* 13: 297-335.
- Fandrejowski H, Raj S, Weremko D, Zebrowska T (1997). Apparent digestibility in experiment feeds and the effect of commercial phytase. *Asian-Australasian J. Anim. Sci.* 10: 665-670.
- Greiner R, Konietzny U, Jany KD (1993). Purification and characterization of two phytases from *Escherichia coli*. *Acrh. Biochem. Biophys.* 303: 107-113.
- Greiner R (2004). Purification and properties of a phytate-degrading enzyme from *Pantoea agglomerans*. *Protein J.* 23(8): 567-576
- Haefner S, Knietsch A, Scholten E, Braun J, Lohscheidt E, Zeldar O (2005). Biotechnological production and applications of phytases. *Appl. Microbiol. Biotechnol.* 68: 588-597.
- Harland BF, Morris ER (1995). Phytate: A good or bad food component. *Nutr. Res.* 15(5): 733-754.
- Heinonen JK, Lahti RJ (1981). A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphatase. *Anal. Biochem.* 113: 313-317.
- Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borris R (2002). Extracellular phytase activity of *Bacillus amyloliquefaciens* FZ45 contributes to its plant growth promoting effect. *Microbiol.* 148: 2097-2109.
- Kerovuo J, Lauraeus M, Nurminen P, Kalkkinen N, Apajalahti J (1998). Isolation, characterization, molecular gene cloning, and sequencing of a novel phytase from *Bacillus subtilis*. *Appl. Environ. Microbiol.* 64: 2079-2085.
- Konietzny, U, Greiner R (2002). Molecular and catalytic properties of phytase degrading enzymes (phytases). *Int. J. Food Sci. Technol.* 37: 791-812.
- Kornegay ET (1999). Effectiveness of Natuphos™ phytase in improving the bioavailabilities of phosphorus and other nutrients in corn-soybean meal diets for young pigs. In: Coelho MB, Kornegay ET (eds) *Phytase in animal nutrition and waste management*, 2nd rev edn. BASF, Mexico, pp. 249-258.
- Lan GQ, Abdullah N, Jallaludin S, Ho YW (2002). Culture conditions influencing phytase production of *Mitsuokella jalaludinii*, a new bacterial species from the rumen of cattle. *J. Appl. Microbiol.* 93: 668-674.
- Nakamura Y, Fukuhara H, Sano K (2000). Secreted phytase activities of yeasts. *Biosc. Biotechnol. Biochem.* 64: 841-844.
- Papagianni M, Nokes SE, Filer K (1999). Production of phytase by *Aspergillus niger* in submerged and solid-state fermentation. *Proc. Biochem.* 35: 397-402.
- Pasamontes L, Haiker M, Wyss M, Tessier M, Van Loon APGM (1997). Gene cloning, purification, and characterization of a heat-stable phytase from the fungus *Aspergillus fumigatus*. *Appl. Environ. Microb.* 63: 1696-1700.
- Ravindran V, Bryden WL, Kornegay ET (1995). Phytates: occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* 6: 125-143.
- Sajidan A, Farouk A, Greiner R, Jungblut P, Mueller EC, Borris R

- (2004). Molecular and physiological characterisation of a 3-phytase from soil bacterium *Klebsiella* sp. ASR1. *Appl. Microbiol. Biotechnol.* 65: 110-118.
- Shimizu M 1992. Purification and characterization of phytase from *Bacillus subtilis* (natto) N-77. *Biosc. Biotechnol. Biochem.* 56: 1266-1269.
- Tambe SM, Kaklij GS, Kelkar SM, Parekh LJ (1994). Two distinct molecular forms of phytase from *Klebsiella aerogenes*: evidence for unusually small active enzyme peptide. *J. Ferment. Bioengin.* 77: 23-27.
- Vohra A, Satyanarayana T (2002). Purification and characterization of a thermostable and acid-stable phytase from *Pichia anomala*. *World J. Microbiol. Biotechnol.* 18: 687-691.
- Vohra A, Satyanarayana T (2003). Phytases: Microbial sources, production, purification, and potential biotechnological applications. *Crit. Rev. Biotechnol.* 23(1): 29-60.
- Yanke LJ, Bae HD, Selinger LB, Cheng KJ (1998). Phytase activity of anaerobic ruminal bacteria. *Microbiol.* 144: 1565-1573.