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Molecular Study of the Phytase Gene in Lactic Acid Bacteria Isolated from *Ogi* and *Kunun-Zaki*, African Fermented Cereal Gruel and Beverage

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Abstract

Background and objective: Phytate is an anti-nutritional agent in cereal foods, making nutritional and health problems in human. The gene responsible for the degradation of phytate has been identified in many bacterial species but not in lactic acid bacteria. The objective of this study was to isolate and characterize *phy* gene in lactic acid bacteria isolated from *Ogi* and *Kunun-zaki*.

Materials and methods: Lactic acid bacteria from *Ogi* and *Kunun-zaki* were phenotypically screened for phytase production. The phytate-degrading lactic acid bacteria isolates were identified using 16S rRNA gene sequencing. Amplification of phytase (*phy*) gene was carried out using polymerase chain reaction. Furthermore, phylogenetic analyses were carried out. Soluble proteins of three selected isolates were extracted and analyzed using sodium dodecyl sulphate polyacrylamide agarose gel electrophoresis.

Results and conclusion: Totally, 16 isolates of phytate-degrading lactic acid bacteria were identified as *Lactobacillus brevis*, *Lactobacillus plantarum* subsp. *plantarum*, *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus paraplantarum*. The *phy* gene with an amplicon size of 2.0 kb was amplified and sequenced. Sequence similarities between the *phy* genes of lactic acid bacteria and that of *Bacillus* in GenBank included 97-99% with a phylogenetic relationship of less than 40%. The SDS-PAGE electrophoresis analysis revealed a 50-kDa molecular weight of the phytase in the three isolates. This study has shown that *phy* gene of the lactic acid bacteria presents in fermented foods, suggesting its potential product use as starter to produce fermented foods with improved nutritional qualities.

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1. Introduction

Anti-nutritional factors are substances produced in natural foods through various metabolic processes of a species such as nutrient inactivation, curtailment of the digestive process and metabolic use of feed [1]. Of the anti-nutritional factors, phytic acid (phytate) causes major concerns in human nutrition and health management [2]. The enzyme is a major part of the seeds of grains, pulses and oleaginous products, including 60-90% of the phosphorus in dormant seeds [3]. Phytic acid includes a unique structure with the ability to strongly chelate monovalent and divalent cations such as K^+, Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+} to form insoluble salts [2]. Formation of these insoluble mineral-phytate complexes at physiological pH values is the major reason for the poor mineral bioavailability, as the complexes cannot be absorbed from the human gastrointestinal tract (GIT) [2].

Ogi is a major staple in West Africa produced traditionally from maize, millet and sorghum. *Ori* is a lactic acid fermented cereal gruel [4]. *Kunu* is the general name used to describe varieties of cereal-based beverages, which are non-alcoholic with qualifications denoting the base cereal [5]. Traditional processing of *Kunun-zaki* involves

steeping of the cereal, wet-milling with spices (e.g. clove, black pepper and ginger), sieving and partial gelatinization of the slurry. Sugar is then added followed by bottling [6,7]. Fermentation is extensively used to improve the nutritional and functional qualities of the food products and has been reported as one of the best ways of decreasing phytic acid in cereals [8]. Studies have shown that lactic acid fermentation improves nutritional quality of the foods by increasing food protein contents and decreasing sugars, decreasing anti-nutritional factors such as phytates, tannins and polyphenols and enhancing mineral bioavailability [9]. Fermentation is known to greatly decrease phytic acids, compared to that other anti-nutritional factors do. This could be due to the low pH during fermentation, which is reported as the best condition for the phytase activity [10]. Degradation of phytate in cereals is usually achieved by the action of phytases, which are reportedly present in plants, animals and microorganisms with various activities [11]. Therefore, an appropriate enzyme selection adapted to the processing conditions of specific foods is important [12]. Microbial phytases are used for the degradation of phytates during fermentation [13]. However, safety aspects cannot be overemphasized in use of microbial phytase producers as starter cultures in food production. Hence, lactic acid bacteria (LAB) as (generally regarded as safe) are better positioned.

Ability of LAB to produce phytase has frequently been reported [13,14]. However, information on molecular basis of the phytate catabolism is scarce for these micro-organisms. No genetic loci in catabolism of phytate have been characterized in members of the Lactobacillus family. An incomplete myo-inositol (a byproduct of phytate catabolism) catabolic pathway has been identified in genome sequence of *Lactobacillus* (*L.*) *plantarum*. However, genes linked to myo-inositol catabolism are absent in genomes of other lactobacilli, whose genomes have partly or completely been sequenced [14-16]. Although phytate-degrading activity has been demonstrated in LAB with corresponding improvement in nutritional quality of the fermented cereal products [17,18], recombinant strains have been used to model phytate degradation by these microorganisms [19]. A better understanding of the molecular basis of phytate degradation by LAB is required for starter selection and possible strain improvement. Therefore, this study was carried out to assess possibility of direct isolation of *phy* gene in LAB for the selection of appropriate starter cultures to produce fermented cereal products with enhanced nutritional qualities.

2. Materials and methods

2.1 Isolation of lactic acid bacteria from *Ogi* and *Kunun-zaki* samples

The LAB were isolated from samples of *Ogi* prepared from white maize, yellow maize and red sorghum and samples of *Kunun-zaki* prepared from millet using de Mann Rogosa and Sharpe (MRS) media with a pH adjusted to 5.5. Then, 1 ml of each sample was serially diluted ten folds, pour plated in MRS agar and incubated at 37ºC for 24-48 h. After incubation, isolated colonies were randomly selected from the mixed cultures on the plates. Successive streaking of the isolates on MRS agar was used to achieve pure cultures.

2.2 Selection of the phytate-degrading lactic acid bacteria

Phytate-degrading isolates were selected using phytase screening agar and a protocol described previously [13].

2.3 Phenotyping of the phytate-degrading lactic acid bacteria

Gram staining and catalase activity assessment were carried out on the pure cultures. Gram-positive isolates with catalase-negative reactions were used as presumptive LAB [20]. Selected phytate-degrading LAB were characterized using biochemical tests such as citrate utilization on Simmon citrate agar and production of CO_2 from glucose [21]. Furthermore, sugar fermentation test in MRS broth modified with bromocresol purple was used. Sugars used in the tests included galactose, D-glucose, D-fructose, mannitol, melibiose, sucrose, D-arabinose, D-xylose, D-mannose, dulcitol, rhamnose, sorbitol, esculin, maltose, cellobiose, lactose, raffinose and trehalose [21].

2.4 Genotyping of the phytate-degrading lactic acid bacteria using 16S rRNA gene sequencing

2.4.1 Extraction of genomic DNA and amplification of the 16S rRNA genes using polymerase chain reaction (PCR)

Extraction of the genomic DNA from the phytate-degrading LAB was carried out using ZR Fungal/Bacterial DNA MiniPrep (Zymo Research, USA) according to the manufacturer's instruction. The PCR was carried out using C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). Universal Primers of 9F (5'-GAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AGGAGGTGATCCAGCC-3') described for LAB by Irma et al. were used for the amplification of 16S rRNA gene [22]. Primers used in this study were provided by Whitehead Scientific, Cape Town, South Africa. The PCR reaction mixtures of 25 µl included 12.5 µl of OneTaq Quick-load 2× MasterMix with standard buffer (New England BioLabs - NEB, UK), 0.5 µl of forward primer, 0.5 µl of reverse primer, 3 µl of the template DNA and 8.5 µl of nuclease-free water in 0.2-ml PCR tubes. The PCR condition included an initial denaturation of 95ºC for 5 min, followed by 30 cycles of denaturation (95ºC for 1 min), annealing (50ºC for 1 min) and extension (72ºC for 2 min). The final extension

was carried out at 72ºC for 7 min [16]. The amplicons were electrophoresed on ethidium bromide stained 1% agarose gels in 1× TAE buffer for 60 min at 80 V and 400 mA and then visualized under UV light using ImageLab Software v.4.1 and ChemiDoc System (Bio-Rad Laboratories, Hercules, CA, USA).

2.4.2 Nucleotide sequencing and BLAST of the 16S rRNA genes

A partial 16S rRNA Sanger sequencing was carried out by Inqaba Biotechnical Industries, Pretoria, South Africa. Sequences were imported to Chromas Software v.2.6 and base calling was carried out to remove chimeras. Consensus sequences were generated from the forward and reverse sequences using Bio Edit Sequence Alignment Editor before using BLASTn algorithm (BLASTN 2.6.1) to compare the current sequences with those from GenBank (NCBI Database, USA).

2.5 Amplification and sequencing of the *phy* genes in phytate-degrading lactic acid bacteria

The specific primer for *phy* gene (*phy*F 5'-CTGTCTGATCCTTATCATTT-3' and *phy*R 5'-TCCGCTTCTGTCGGTCA-3') designed by Bawane et al. with 'Gene Tool' using the published sequence of *phy* gene for *Bacillus (f* in NCBI GenBank Account AF298179 was used for the amplification of *phy* gene in the isolates' genomic DNAs [23]. The reaction mixture of 25 µl consisted of 12.5 µl of OneTaq Quick-load 2× MasterMix with standard buffer (New England BioLabs – NEB, UK), 0.5 µl of forward primer, 0.5 µl of reverse primer, 3 µl of the template DNA and 8.5 µl of nuclease-free water in a 0.2 ml PCR tube. Amplification of the *phy* gene was carried out using the following PCR conditions: initial denaturation of 95ºC for 5 min followed by 35 cycles of denaturation at 95ºC for 1 min, annealing at 52ºC for 1 min and extension at 72ºC for 1 min. The final extension was carried out at 72ºC for 10 min [23]. Agarose gel electrophoresis in 1× TAE buffer was run for 60 min at 80 V and 400 mA using ethidium bromide stained 1% agarose. The bands obtained were visualized by UV light using ImageLab Software v.4.1 on ChemiDoc MP System (Bio-Rad Laboratories, Hercules, CA, USA).

2.6 Nucleotide sequencing and BLAST of the *phy* genes

Sanger sequencing was carried on the PCR products obtained from the amplification of the *phy* gene in the isolates as earlier described. The current *phy* gene sequences were compared to those reported in GenBank (NCBI database, USA) using BLASTn algorithm (BLASTN 2.6.1).

2.7 Phylogeny of the *phy* genes

The evolutionary history of the *phy* gene sequences from LAB was investigated using neighbor-joining [24] and maximum likelihood methods, Hasegawa-Kishino-Yano (HKY) model [25] and MEGA Software v.7 [26].

2.8 Protein extraction and analysis of the phytate-degrading lactic acid bacteria using SDS-PAGE

Soluble proteins were extracted from two isolates; in which, the *phy* gene was amplified and another isolate; in which, the *phy* gene was not amplified, using Qproteome Bacterial Protein Prep Kit (Qiagen, Germany) according to the manufacturer's instruction. Isolates were cultured in phytase screening broth and MRS broth as control. The native lysis buffer was supplemented with lysozyme and benzonase-nuclease before use. Frozen pellets from 25-ml cultures were thawed for 15 min on ice. Then, cells were re-suspended in 1 ml of supplemented native lysis buffer in sterile 15-ml centrifuge tubes. Cell suspensions were swirled gently 2-3 times. Lysates were centrifuged at 21952× g for 30 min at 4ºC to pellet the debris. Then, supernatants containing soluble fractions of the bacterial cell proteins were gently decanted in 1.5-ml microfuge tubes and stored at -80ºC until use. Quantification of the soluble proteins in the extracts was carried out using Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) and Bradford dye-binding method [27]. The SDS-PAGE was carried out using Bio-Rad Electrophoretic Tank System (Bio-Rad Laboratories, Hercules, CA, USA) according to the methods previously described [28].

3. Results and discussion

3.1 Selection of the phytate-degrading lactic acid bacteria

Totally, 85 presumptive LAB were isolated from white-maize *Ogi* (18), yellow-maize *Ogi* (20), red-sorghum *Ogi* (25) and *Kunun-zaki* (22). These isolates were screened for extracellular phytase production, showing their ability to degrade phytate. A total number of 16 isolates (18.82%) were positive for phytase production (Table 1). This has indicated that not all the LAB isolates possess the ability to degrade phytate. Similarly, Anastasio et al. [18] reported that 19% of 150 LAB isolates from sourdough, pizza dough and sausage degraded phytate on solid and liquid media. Furthermore, Nuobariene et al. reported that 11% of 168 LAB isolates from wheat and rye sourdoughs included phytase activity [29]. However, a higher percentage of phytate-degrading LAB isolates were isolated from traditional pickles of rural and urban areas of Himachal Pradesh in India. Six out of 15 LAB isolates (40%) from traditional pickles were positive for phytase production using phytase screening agar [30].

Table 1. Screening of the LAB isolates for extracellular phytase production

Substrate	Number of LAB isolates screened	Number of positive LAB isolates	Percentage of positive LAB isolates
WMO	18	2	11.11
YMO	20	3	15.00
RSO	25	7	28.00
KNZ	22	4	18.18
Total	85	16	18.82

LAB= lactic acid bacteria

Table 2. Phenotyping of the phytate-degrading LAB isolates

Isolate code	Gram s reaction	Morphology	Catalase test	Citrate utilization	Production of CO_2 from glucose	Sugar fermentation test																	Probable organism
						D-Arabinose	Cellobiose	Dulcitol	Esculin	D-Fructose	D-Glucose	Galactose	Lactose	D-Mannose	Mannitol	Maltose	Melibiose	Sorbitol	Sucrose	Trehalose	Raffinose	Rhamnose	D-Xylose	
WMO08	+	R	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	*Lactobacillus brevis*
WMO14	+	R	-	-	-	+	-	-	-	+	+	+	+	-	+	+	+	+	+	-	+	-	+	*Lactobacillus plantarum*
YMO08	+	R	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
YMO16	+	R	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
YMO17	+	R	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	+	+	+	+	+	+	*Lactobacillus plantarum*
RSO02	+	R	-	-	-	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
RSO04	+	R	-	-	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
RSO05	+	R	-	-	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
RSO07	+	R	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
RSO10	+	R	-	-	-	-	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
RSO17	+	R	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	+	-	*Lactobacillus brevis*
RSO20	+	R	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KNZ01	+	R	-	-	-	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	*Lactobacillus plantarum*
KNZ08	+	R	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KNZ11	+	R	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	*Lactobacillus plantarum*
KNZ13	+	R	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	*Lactobacillus plantarum*

+, positive reaction; -, negative reaction; R, rod; WMO08 and WMO14, isolates from the white maize *Ogi*; YMO08, YMO16 and YMO17, isolates from the yellow maize *Ogi*; RSO02, RSO04, RSO05, RSO07, RSO10, RSO17 and RSO20, isolates from the red sorghum *Ogi*; KNZ01, KNZ08, KNZ11 and KNZ13, isolates from *Kunun-zaki*

3.2 Phenotyping of the phytate-degrading lactic acid bacteria

Phenotyping of the phytate-degrading LAB isolates using Gram staining, morphology and biochemistry (including sugar fermentation) are shown in Table 2. All isolates were Gram-positive and catalase-negative rods. No isolates utilized citrate while two out of 16 showed hetero-fermentation with CO_2 production from glucose.

Sugar fermentation was used to identify isolates as *L. brevis* (two isolates) and *L. plantarum* (14 isolates). The LAB isolated from traditional fermented foods were characterized using conventional phenotyping methods such as morphology and biochemistry [31]. Although these methods are very useful, they are often difficult and correct identification to species level may be complicated by indistinct response of the physiological tests [32]. Therefore, it is important to use complementary molecular tools in identifications of probiotics due to increasing public interests in the bacterial properties such as probiotic activities [33] and degradation of anti-nutritional factors [34].

3.3 Genotyping of the phytate-degrading lactic acid bacteria using 16S rRNA gene sequencing

Genotyping of the phytate-degrading LAB isolates, the NCBI accession numbers and organisms in GenBank with significant similarities with these isolates are listed in Table 3. The percentage of similarities between the isolates and those available in GenBank ranged from 79% for *L. plantarum* UIGOAI34 (accession no. KY817129) to 98% for *L. brevis* UIGOAI05 (accession no. KY649482).

The 16S rRNA gene sequencing verified identities of six LAB isolates, out of 16 isolates. Five LAB isolates previously identified as *L. plantarum* using biochemical characteristics (sugar fermentation profile) were re-identified as *L. pentosus*, four as *L. plantarum* subsp. *plantarum* and one as *L. paraplantarum*, using 16S rRNA sequencing results. These differences could be due to high phenotypic and genotypic similarities within *L. plantarum*, *L. pentosus* and *L. paraplantarum* [32].

Nugessie et al. used phenotyping, 16S rRNA sequencing and PCR-DGGE analysis to identify LAB isolates from naturally fermented buttermilks. They identified a higher number of *L. plantarum* (31 isolates) using 16S rRNA sequencing instead of 16 isolates identified using API Systems [35]. This is possibly attributed to high variability of the V3 region in 16S rRNA, which could be useful in differentiation of LAB isolates to species level [36].

Studies to differentiate *L. plantarum* groups have been carried out through DNA-DNA hybridization, which identified three major groups of *L. paraplantarum*, *L. pentosus* and *L. plantarum* [37]. Findings from the present study are similar to earlier findings by Adiguzel and Atasever, who showed that six LAB isolates previously identified as *Pediococcus pentosaceus* using API Systems were *L. lactic* subsp. *Lactis* and one *Weisella viridescens* isolate was *Leuconostoc mesenteroides* subsp. *mesenteroides* using BOX-PCR. Another isolate identified as *P. pentosaceus* shared 22% homology with four other isolates, indicating that the isolate might be a different species [38].

Table 3. Genotyping of the phytate-degrading lactic acid bacteria isolates based on the 16S rRNA sequencing and NCBI-BLASTn

Isolate code	Strain	Identity	NCBI accession no.	Most related organism in GenBank with significant alignment with the isolate	Similarity (%)
WMO08	UIGOAI05	*L. brevis*	KY649482	*L. brevis* Lb13H	98
WMO14	UIGOAI36	*L. plantarum* subsp. *plantarum*	KY817120	*L. plantarum* subsp. *plantarum* AB598947	89
YMO08	UIGOAI07	*L. plantarum*	KY817123	*L. plantarum* ULAG24	91
YMO16	UIGOAI11	*L. pentosus*	KY655026	*L. pentosus* c22	91
YMO17	UIGOAI12	*L. plantarum*	KY655027	*L. plantarum* HI	91
RSO02	UIGOAI18	*L. pentosus*	KY817137	*L. pentosus* NRIC1753	96
RSO04	UIGOAI20	*L. plantarum* subsp. *plantarum*	KY817139	*L. plantarum* subsp. *plantarum* RS11	92
RSO05	UIGOAI21	*L. paraplantarum*	KY655340	*L. paraplantarum* LP191	93
RSO07	UIGOAI22	*L. pentosus*	KY817140	*L. pentosus* JCM1558T	91
RSO10	UIGOAI23	*L. pentosus*	KY655341	*L. pentosus* c22	91
RSO17	UIGOAI24	*L. brevis*	KY655342	*L. brevis* SC4	90
RSO20	UIGOAI26	*L. plantarum* subsp. *plantarum*	KY817141	*L. plantarum* subsp. *plantarum* AB831181	91
KNZ01	UIGOAI32	*L. plantarum* subsp. *plantarum*	KY655809	*L. plantarum* subsp. *plantarum* MF6	96
KNZ08	UIGOAI33	*L. plantarum*	KY655810	*L. plantarum* DS11	96
KNZ11	UIGOAI34	*L. plantarum*	KY817129	*L. plantarum* DmeICS163	79
KNZ13	UIGOAI39	*L. pentosus*	KY817130	*L. pentosus* FLO421	92

WMO08 and WMO14, isolates from the white maize *Ogi*; YMO08, YMO16 and YMO17, isolates from the yellow maize *Ogi*; RSO02, RSO04, RSO05, RSO07, RSO10, RSO17 and RSO20, isolates from the red sorghum *Ogi*; KNZ01, KNZ08, KNZ11 and KNZ13, isolates from *Kunun-zaki*. *Lactobacillus=L*

3.4 Amplification and sequencing of the *phy* genes in phytate-degrading lactic acid bacteria

Amplification of phytase genes was carried out in five of the 16 LAB isolates. Gel electrophoresis results of the amplicons of these five isolates are shown in Fig. 1.

Three of the isolates, *L. plantarum* UIGOAI12 (accession no. KY655027), *L. brevis* UIGOAI24 (accession no. KY655342) and *L. plantarum* UIGOAI34 (accession no. KY817129) with distinct bands of 2.0 kb (48 ng) were successfully sequenced.

Table 4 shows the accession numbers that were assigned to *phy* genes expressed by the isolates from this study and those from other organisms (*Bacillus* spp.) in GenBank with significant alignments. The *L. plantarum* UIGOAI12 *phy* gene (accession no. KY987113) shared 99% similarity with *B, subtilis* WYCQ02 and *B. subtilis* B9601-Y2 *phy* genes, while *L. brevis* UIGOAI24 *phy* gene (accession no. KY987114) shared 98% similarity with *B. subtilis* S7e and *B. subtilis* Y *phy* genes. The *L. plantarum* UIGOAI34 *phy* gene (accession no. KY987115) included 98 and 97% similarities with *B. amyloliquefaciens* SQR9-3 and *B. subtilis* (accession no. AF292103) *phy* genes, respectively (Table 4).

Although ability of degrading phytates has been demonstrated in LAB species with corresponding improvements in nutritional properties of the fermented cereal products [18,39], recombinant strains have been used to show phytate degradation by the probiotics [19]. Earlier experiments to detect and express *phy* genes in LAB have been through cloning of the phytase gene from other microorganisms into vectors and subsequent transformation of the gene into LAB strains. Zuo et al. cloned and demonstrated expression of the *phy* genes from *Aspergillus* (*A.*) *ficuum, A. fumigatus* and *B. subtilis* in *L. casei* [40]. Similarly, Askelson et al. cloned and fully expressed *phy*A gene from *B. subtilis* into *L. acidophilus* [19]. In another study, Garcia-Mantrana et al. cloned and expressed Bifidobacterial phytases in *L. casei* [41].

In the current study, *phy* genes of 2.0 kb were amplified and sequenced in three phytate-degrading LAB isolates using specific primers designed for *B. subtilis* [23]. Designation was carried out because no primers were published for the Lactobacillus group. The *phy* gene of *B. subtilis* encodes a β-propeller phytase with high specificity for phytate, which is active in a broad range of pH values and temperatures [42].

Earlier reports by Bendtsen et al. analyzed the amino acid sequence of *B. subtilis* using Signal P. They also reported that the presence of a Gram-positive secretion signal suggested that heterologous expression of the protein in *Lactobacillus* spp. could result in secretion of a protein [43].

Later, Askelson et al. expressed *phy*A gene of *B. subtilis* in *Lactobacillus* spp. and demonstrated that the mechanism of probiotic functionality was due to the biocatalytic phytate degradation [19]. Furthermore, the 2.0-kb *phy* gene of the isolates in this study is linked to the reports of Kim et al., who digested the chromosomal DNA of *Bacillus* spp. DS11 with five various restriction enzymes and demonstrated a 2.2-kb *Hin*dIII fragment. This finding was similar to the finding from Southern hybridization, verified by assessing phytase activity of the clone carrying the designated pKP1 [44].

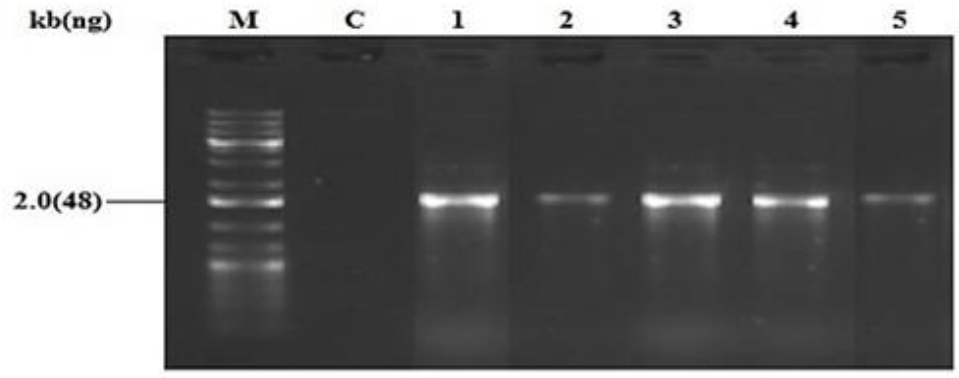

Figure 1: Agarose gel electrophoresis (1%) for *phy* gene PCR products

Kb: kilobase representing the length of the DNA
ng: nanogram representing the molecular mass of the DNA
M: 1kb marker
C: Control (Nuclease-free water)
Lane 1: YM017-*Lactobacillus Plantarum* UIGOAI12 (KY655027)
Lane 3: RSO17-*L.brevis* UIGOAI24 (KY655342)
Lane 4:KNZ11-*L.plantarum* UIGOAI34 (KY817129)

Table 4. Accession numbers of the isolates that expressed *phy* genes with the most aligned GenBank references

	Identity	NCBI accession no.	Organism in GenBank with significant alignments with the isolate gene	Similarity (%)
YMO17	*L. plantarum* UIGOAI12 *phy* gene	KY987113	i. *B. subtilis* WYCQ02 *phy*C gene ii. *B. subtilis* B9601-Y2 *phy* gene	99 99
RSO17	*L. brevis* UIGOAI24 *phy* gene	KY987114	i. *B. subtilis* S7e *phy* gene ii. *B. subtilis* y *phy* gene	98 98
KNZ11	*L. plantarum* UIGOAI34 *phy* gene	KY987115	i. *B. amyloliquefaciens* SQR9-3 *phy* gene ii. *B. subtilis* (AF292103) *phy* gene	98 97

YMO17, isolate from the yellow maize *Ogi*; RSO17, isolate from the red sorghum *Ogi*, KNZ11, isolate from *Kunun-zaki*. *Bacillus=B, Lactobacillus= L*

3.5 Phylogeny of the *phy* genes

The *phy* gene expressed by *L. brevis* UIGOAI24 from this study shared 50% homology with that of *B. subtilis* S7e. The gene cluster of these two bacteria shared 96% homology with the *phy* gene of *B. subtilis* Strain y. The *phy* gene expressed by *L. plantarum* UIGOAI12 and *L. plantarum* UIGOAI34 from this study included 49% homology.

However, the gene cluster of *L. plantarum* UIGOAI12 and *L. plantarum* UIGOAI34 shared 45% homology with the gene cluster of *L. brevis* UIGOAI24, *B. subtilis* Strain S7e and *B. subtilis* Strain y (Fig. 2). Less than 40% homology were seen between the *phy* genes of isolates from this study and *phy* genes of *B. subtilis* Strain B9601-Y2, *phy*C genes of *B. subtilis* Strain WYCQ02 and *phy* genes of *B. amyloliquefaciens* SQR9 3 retrieved from GenBank. However, the *phy* gene expressed by *B. subtilis* (accession no. AF292103) was out-grouped with less than 40% homology with the *phy* gene expressed by other microorganisms (Fig. 2).

Figure 3 represents the character-based maximum likelihood tree, which shows relationships between the *phy* genes of isolates from this study and those of most related strains from GenBank. The *phy* gene expressed by *L. plantarum* UIGOAI12 shared 40% homology with the *phy* gene of *L. plantarum* UIGOAI34. The *phy* gene cluster of these isolates shared less than 40% similarity with the *phy* gene cluster of strains from GenBank, including *phy* genes of *B. subtilis* Strain WYCQ02, *B. amyloliquefaciens* Strain SQR9 3, *B. subtilis* Strain B9601-Y2 and *B. subtilis* (accession no. AF292103). The *phy* gene expressed by *L. brevis* UIGOAI24 from this study shared 53% homology with that of *B. subtilis* Strain S7e from GenBank. However, this gene cluster shared 87% homology with the *phy* gene of *B. subtilis* Strain y (Fig. 3).

Phylogenetic analysis of the *phy* gene expressed by the LAB isolates from this study revealed that the *phy* gene cluster expressed by the isolates shared less than 40% homology in evolutionary distance and character respectively with the *phy* gene of *B. subtilis* reference strains. This reveals that *phy* genes of the phytate-degrading LAB isolates from this study do not show a significant homology with the relative strains from GenBank. This could be linked to the unique arrangements of their nucleotides [45], which suggests their novelty based on their distinctiveness [46,47]. This report was similar to a report by Elshahed et al., who assessed the novelty of rare clones seen in a 13001-clone library derived from an undisturbed tall grass site in central Oklahoma, USA. The researchers used 15% sequence divergence from the closest abundant relative in the identified data set as an empirical uniqueness cut off and identified a group that belonged to unique phylogenetically distinct lineage, which shared no close sequence similarities with further abundant members of the community [48].

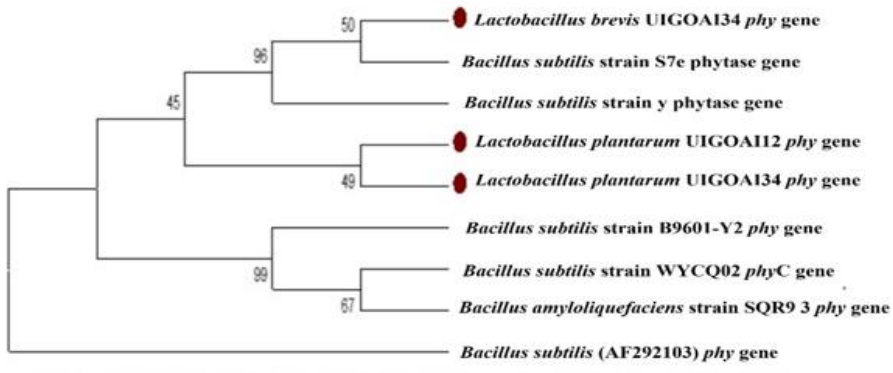

Figure 2. Neighbor-Joining tree showing relationships between *phy* genes of strains and the most related strains from GenBank

• Strains from this study

Numbers at the nodes represent the levels of bootstrap support based on 1000 re-sampled data sets (% homology). Values less than 40% are not shown.

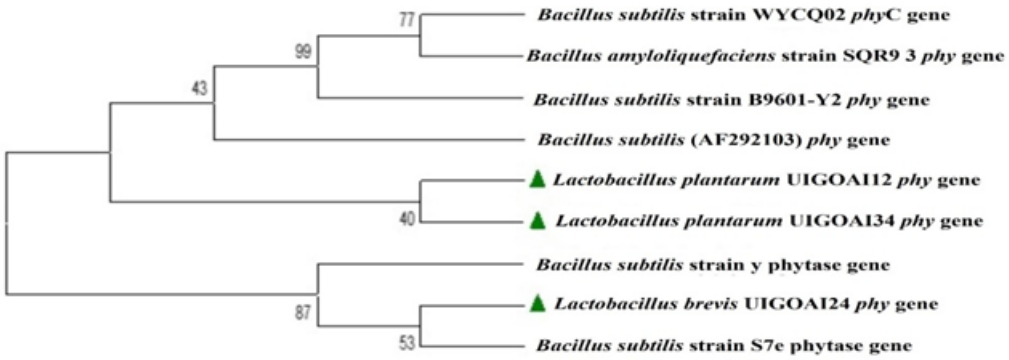

Figure 3. Maximum likelihood Tree showing relationships between *phy* genes of isolates and the most related strains from GenBank

▲ Strains from this study
The levels of bootstrap support based on 1000 re-sampled data sets (% homology) are represented by the numbers at the nodes. Values less than 40% are hidden.

3.6 Protein extraction and analysis of the phytate-degrading lactic acid bacteria

Soluble protein concentrations of the extracts from the three isolates are shown in Table 5. The concentrations in the isolates grown in MRS broth included 1.51 mg ml^{-1} for *L. plantarum* UIGOAI12 (accession no. KY655027), 1.70 mg ml^{-1} for *L. brevis* UIGOAI24 (accession no. KY655342) and 2.01 mg ml^{-1} for *L. pentosus* UIGOAI11 (accession no. KY655026). When grown in phytase screening broth, concentrations of the proteins included 1.24 mg ml^{-1}, 0.68 mg ml^{-1} and 1.21 mg ml^{-1} for the three isolates, respectively (Table 5).

Figure 4 shows the SDS-PAGE pattern of soluble protein extracts from the isolates under two growth conditions. The three isolates regardless of their growth conditions included two protein categories of 15 and 50 kDa. However, abundance of the proteins was different, as shown by distinctiveness of the bands. The SDS-PAGE of the soluble protein extracts from the three phytate-degrading LAB isolates showed their proteins with molecular weights (MW) of 15 and 50 kDa. The 50-kDa proteins were reported previously [17]. This was similar to earlier reports by Zamudio et al., who showed that the MW of the *L. plantarum* phytase was 52 kDa, using gel filtration on a Sephadex G-100 Column [49]. Furthermore, Zuo et al. reported the MW of an expressed phytase protein in a transformed *L. brevis* strain as 39.2 kDa [40]. This was less than the 54.6 kDa observed in the natural *A. ficuum*; from which, the gene was transformed into *L. brevis*. They suggested that differences in MW of the original phytase from *A. ficuum* and the expressed phytase in *L. brevis* were seen because the expressed product was not glycosylated, resulting in a low MW [40]. Studies have reported the MW of a highly glycosylated phytase from *A. niger* as 85 kDa. This was reported as 48.5 kDa without glycosylation [50]. Zuo et al. suggested that the glycosylation and various sources of phytases produced by various microorganisms were factors that could result in variation in MW of the phytase proteins [40].

The protein band of 50 kDa was shown by the three selected isolates – two isolates in which the *phy* gene was amplified and the isolate in which the gene was not amplified. This suggests that the *phy* gene is present in all isolates used in this study. The inability of the Bacillus primers used to amplify the gene in the other phytate-degrading lactic acid bacteria isolates reported in this present study could be attributed to the differences in the genomes of the two genera (e.g. Bacillus and Lactobacillus). Moreover, these isolates had shown phenotypic expression of the phytate-degrading ability on solid media, which signifies their ability to produce phytase.

Table 5. Protein concentrations of the extracts from the bacterial isolates

Isolate protein sample code	Identity	Protein concentration (mg ml^{-1})
YMO17 (M)	*L. plantarum* UIGOAI12 (KY655027)	1.51
RSO17 (M)	*L. brevis* UIGOAI24 (KY655342)	1.70
YMO16 (M)	*L. pentosus* UIGOAI11 (KY655026)	2.01
YMO17 (P)	*L. plantarum* UIGOAI12 (KY655027)	1.24
RSO17 (P)	*L. brevis* UIGOAI24 (KY655342)	0.68
YMO16 (P)	*L. pentosus* UIGOAI11 (KY655026)	1.21

M, isolates cultured in MRS broth; P, isolates cultured in phytase screening broth.
Lactobacillus=*L*, MRS= Mann Rogosa and Sharpe

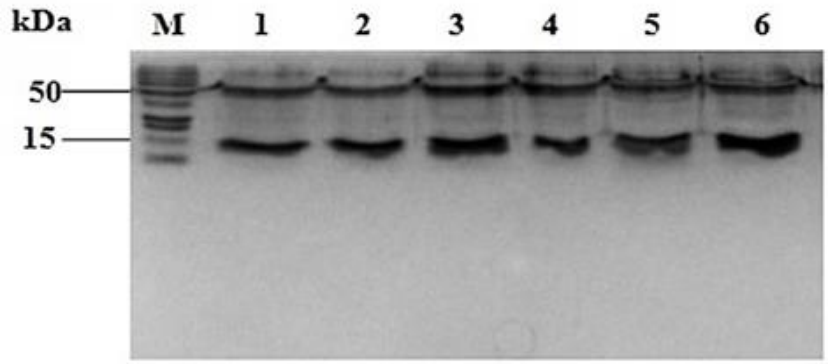

Figure 4. SDS-PAGE for protein extracts from the three test isolates

kDa: Kilodalton-unit of the molecular weight of proteins
M: Bio-Rad protein marker
1. *Lactobacillus plantarum* UIGOAI12 (KY655027) grown in MRS broth
2. *Lactobacillus brevis* UIGOAI24 (KY655342) grown in MRS broth
3. *Lactobacillus pentosus* UIGOAI11 (KY655026) grown in MRS broth
4. *Lactobacillus plantarum* UIGOAI12 (KY655027) grown in PSB
5. *Lactobacillus brevis* UIGOAI24 (KY655342) grown in PSB
6. *Lactobacillus pentosus* UIGOAI11 (KY655026) grown in PSB

4. Conclusion

In conclusion, the current study has demonstrated that ability of LAB isolates to degrade phytate is due to *phy* gene present in their chromosomal DNA. These LAB isolates can be used as starters in fermented products with good nutritional qualities or as enhancers in diets. Furthermore, they can be used as appropriate substitutes to current commercial phytases.

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6. Conflict of interest

The authors declare no conflict of interest.

References

1. Olatidoye OP, Alabi AO, Sobowale SS, Balogun IO, Nwabueze BC. Effect of cooking methods on antinutrient content and phenolic acid profiles of groundnut varieties grown in Nigeria. Int Res J Food Nutr. 2019; 1(1): 27-37.
2. Kumar V, Sinha AK, Makkar HPS, Becker K. Dietary roles of phytate and phytase in human nutrition: A review. Food Chem. 2010; 120: 945-959. doi: 10.1016/j.foodchem.2009.11.052
3. Loewus F. Biosynthesis of Phytate in Food Grains and Seeds. In: Reddy NR, Sathe SK. Food Phytates. CRC Press. Florida, USA; 2002: pp.53-61. doi: 10.1201/9781420014419.ch4
4. Okoroafor I, Banwo K, Olanbiwoninu AA, Odunfa SA. Folate enrichment of Ogi (a fermented cereal gruel) using folate producing starter cultures. Adv Microbiol. 2019; 9: 177-193. doi: 10.4236/aim.2019.93014
5. Orutugu LA, Izah SC, Aseibai ER. Microbiological quality of kunu drink sold in some major markets of Yenagoa metropolis, Nigeria. Cont J Biomed Sci. 2015; 9(1): 9-16.
6. Adeniji PO, Keshinro OO. Assessment of nutritional and sensory quality of kunun zaki - a homemade traditional Nigerian beverage. J Nutr Food Sci. 2015; 5(3). doi: 10.4172/2155-9600.1000358.
7. Onyimba IA, Itelima JU, Job MO, Ogbonna AI, Ode CO. Production of sorghum based kunun-zaki using selected starter cultures. Int J Sci. 2017; 6(09): 57-63. doi: 10.18483/ijSci.1370
8. Gunashree BS, Kumar RS, Roobini R, Venkateswaran G. Nutrients and antinutrients of ragi and wheat as influenced by traditional processes. Int J Curr Microbiol Appl Sci. 2014; 3(7): 720-736.
9. Opere B, Aboaba EO, Ugoji OO, Iwalokun BA. Estimation of nutritive value, organoleptic properties, and consumer acceptability of fermented cereal gruel (ogi). Adv J Food Sci Technol. 2012; 4: 1-8.
10. Gunashree BS, Venkateswaran G. Effect of different cultural conditions for phytase production by *Aspergillus niger* CFR 335 in submerged and solid-state fermentations. J Ind Microbiol Biotechnol. 2008; 35(12): 1587-1596. doi: 10.1007/s10295-008-0402-1
11. Chatraei N, Emitiazi G. Immobilization of phytase producing probiotics in shrimp chitosan cross-linked by zinc oxide nanoparticles and assay its antibacterial activity. Appl Food Biotechnol. 2019; 6(3): 139-150.
12. Fischer MM, Egli IM, Aeberli I, Hurrell RF, Meile L. Phytic acid degrading lactic acid bacteria in tef-injera fermentation. Int J Food Microbiol. 2014; 190: 54-60. doi: 10.1016/j.ijfoodmicro.2014.08.018
13. Onipede GO, Banwo K, Ogunremi OM, Sanni AI. Influence of starter culture lactic acid bacteria on the phytic acid content of sorghum-ogi (an indigenous cereal gruel). Annals Food Sci Technol. 2014; 15(1): 121-134.

14. Yebra MJ, Zuniga M, Beaufils S, Perez-Martinez G, Deutscher J, Monedero V. Identification of a gene cluster enabling *Lactobacillus casei* BL23 to utilize myo-inositol. Appl Environ Microbiol. 2007; 73(12): 3850-3858. doi: 10.1128/AEM.00243-07

15. Kleerebezem M, Boekhorst J, Van Kranenburg R, Molenaar D, Kuipers OP, Leer R, , Tarchini R, Peters SA, Sandbrink HM, Fiers MWEJ, Stiekema W, Klein Lankhorst RM, Bron PA, Hoffer SM, Nierop Groot MN, Kerkhoven R, de Vries M, Ursing B, de Vos WM, Siezen RJ. Complete genome sequence of *Lactobacillus plantarum* WCFS1. Prot Nat Acad Sci USA. 2003**;** 100: 1990-*1995*. doi: 10.1073/pnas.0337704100

16. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, Pavlova N, Karamychev V, Polouchine N, Shakhova V, Grigoriev I, Lou Y, Rohksar D, Lucas S, Huang K, Goodstein DM, Hawkins T, Plengvidhya V, Welker D, Hughes J, Goh Y, Benson A , Baldwin K, Lee J-H, Diaz-Muniz I, Dosti B, Smeianov V, Wechter W, Barabote R, Lorca G, Altermann E, Barrangou R, Ganesan B, Xie Y, Rawsthorne H, Tamir D, Parker C, Breidt F, Broadbent J, Hutkins R, O'Sullivan D, Steele J, Unlu G, Saier M, Klaenhammer T, Richardson P, Kozyavkin S, Weimer B, Mills D.Comparative genomics of the lactic acid bacteria. Prot Nat Acad Sci USA. 2006; 103: 15611-15616. doi: 10.1073/pnas.0607117103

17. De Angelis M, Gallo G, Corbo MR, McSweeney PLH, Faccia M, Giovine M,Gobbetti M. Phytase activity in sourdough lactic acid bacteria: Purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. Int J Food Microbiol. 2003; 87: 259-270. doi: 10.1016/S0168-1605(03)00072-2

18. Anastasio M, Pepe O, Cirillo T, Palomba S, Blaiotta G, Villani F. Selection and use of phytate-degrading LAB to improve cereal-based products by mineral solubilization during dough fermentation. J Food Sci. 2010; 75: M28-M35. doi: 10.1111/j.1750-3841.2009.01402.x

19. Askelson TE, Campasino A, Lee JT, Duong T. Evaluation of Phytate-degrading Lactobacillus culture administration to broiler chickens. Appl Env Microbiol. 2014; 80(3): 943-950. doi: 10.1128/AEM.03155-13

20. Aween MM, Hassan Z, Muhialdin BJ, Noor HM, Eljamel YA. Evaluation on antibacterial activity of *Lactobacillus acidophilus* strains isolated from honey. Am J Appl Sci. 2012; 9(6): 807-817. doi: 10.3844/ajassp.2012.807.817

21. Olutiola PO, Famurewa O, Sonntag HG. An introduction to General Microbiology: A practical approach. Bolabay publication Nigeria. 2000: 167-176.

22. Irma IA, Betty SLJ, Made A, Kazuhito F, Arief BW. Identification and probiotic characteristics of lactic acid bacteria isolated from Indonesian beef. Asian J Anim Sci. 2015; 9(1): 25-36. doi: 10.3923/ajas.2015.25.36

23. Bawane R, Tantwai K, Rajput LPS, Kadam-Bedekar M, Kumar S, Gontia I, Tiwari S. Molecular analysis of phytase gene cloned from *Bacillus subtilis*. Adv Stud Biol. 2011; 3(3): 103-110.

24. Saitou N, Nei M. The neighbour-joining method: A new method for the reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4: 406-425.

25. Hasegawa M, Kishino H, Yano T. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol. 1985; 22: 160-174. doi: 10.1007/BF02101694

26. Kumar S, Stecher G, Tamura K. MEGA 7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016; 33: 1870-1874. doi: 10.1093/molbev/msw054

27. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72: 248-254. doi: 10.1016/0003-2697(76)90527-3

28. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227: 680-685.

29. Nuobariene L, Cizeikiene D, Gradzeviciute E, Hansen AS, Rasmussen SK, Juodeikien G, Vogensen FK. Phytase-active lactic acid bacteria from sourdoughs: Isolation and identification. LWT-Food Sci Technol. 2015; 6: 766-772. doi: 10.1016/j.lwt.2015.03.018

30. Savitri M, Kumar V, Kumari A, Anglo K, Bhalla TC. Isolation and characterization of lactic acid bacteria from traditional pickles of Himachal Pradesh, India. J Food Sci Technol. 2017; 54(7): 1945-1952. doi: 10.1007/s13197-017-2629-1

31. Saleh FA. Isolation and identification of microorganisms and antibacterial activity of Laban Zeer, an Egyptian traditional fermented milk product. Food Nutrition Sci Dept Coll Agric Food Sci King Faisal Univ Saudi Arabia. 2013; 2: 31-42.

32. Torriani S, Felis GE, Dellaglio F. Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by recA gene sequence analysis and multiplex PCR assay with recA gene-derived primers. Appl Env Microbiol. 2001; 67: 3450-3454. doi: 10.1128/AEM.67.8.3450-3454.2001

33. Herias MV, Hessle C, Telemo E, Midtvedt T, Hanson LA, Wold AE. Immunomodulatory effects of *Lactobacillus plantarum* colonizing the intestine of gnotobiotic rats. Clin Exp Immun. 1999; 116: 283-290. doi: 10.1046/j.1365-2249.1999.00891.x

34. Gunashree BS, Venkateswaran G. Dephytinization of cereals and pulses by phytase-producing lactic acid bacteria. Int J Curr Res Acad Rev. 2015; 3(12): 61-69.

35. Nugessie G, Abay F, Beyene F. Biochemical and molecular characterization of lactic acid bacteria and yeasts isolated from Ethiopian naturally fermented buttermilk. J Food Sci Technol. 2016; 53(1): 184-196. doi: 10.1007/s13197-015-2049-z

36. Blaiotta G, Pennacchia C, Ercolini D, Moschetti G, Villain F. Combining denaturing gradient gel electrophoresis of 16S rDNA V3 region and 16S-23S rDNA spacer region polymorphism analyses for the identification of staphylococci from Italian fermented sausage. Syst Appl Microbiol. 2003; 26(3): 423-443. doi: 10.1078/072320203322497455

37. Curk MC, Hubert JC, Bringel F. *Lactobacillus paraplantarum* sp. Nov., a new species related to *Lactobacillus plantarum*. Int J Syst Bacteriol. 1996; 46: 595-598. doi: 10.1099/00207713-46-2-595

38. Adiguzel GC, Atasever M. Phenotypic and genotypic characterization of lactic acid bacteria isolated from Turkish dry fermented sausage. Rom Biotechnol Lett. 2009; 14(1): 4130-4138.

39. Nkhata SG, Ayua E, Kamau EH, Shingiro J. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci Nutr. 2018; 6: 2446-2458.
doi: 10.1002/fsn3.846

40. Zuo R, Chang J, Yin Q, Chen L, Chen Q, Yang X, Zheng Q, Ren G, Feng H. Phytase gene expression Lactobacillus and analysis of its biochemical characteristics. Microbiol Res. 2010; 165: 329-335.
doi: 10.1016/j.micres.2009.06.001

41. Garcia-Mantrana I, Yebra MJ, Haros M, Monedero V. Expression of bifidobacterial phytases in *Lactobacillus casei* and their application in a food model of whole-grain sourdough bread. Int J Food Microbiol. 2016; 216: 18-24.
doi: 10.1016/j.ijfoodmicro.2015.09.003

42. Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azevedo V, et al. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature. 1997; 390: 249-256.
doi: 10.1038/36786

43. Bendtsen JD, Jensen LJ, Blom N, von Heijne G, Brunak S. Feature-based prediction of non-classical and leader-less protein secretion. Prot Eng Des Sel. 2004; 17(4); 349-356.
doi: 10.1093/protein/gzh037

44. Kim YO, Lee JK, Kim HK, Yu JH, Oh TK. Cloning of the thermostable phytase gene (phy) from *Bacillus* sp. DS11 and its overexpression in *Escherichia coli*. FEMS Microbiol Lett. 1998; 162(1): 185-191.
doi: 10.1111/j.1574-6968.1998.tb12997.x

45. Togashi J, Koushi U, Namai T. Overwintering of *Erwinia carotovora* subsp. carotovora in diseased tissues in soil and its role as inoculum for soft rot of Chinese cabbage (*Brassica campestris*, Pekinensis group). J Gen Plant Pathol. 2001; 67: 45-50.
doi: 10.1007/PL00012986

46. Konstantinidis KT, Stackebrandt E. Defining taxonomic ranks. In: Rosenberg E, DeLong EF, Stackebrandt E, Lory S, Thompson F. The Prokaryotes (4th edition). Springer Verlag: Berlin, Germany, Heidelberg, Germany. 2013: 229-254.
doi: 10.1007/978-3-642-30194-0_4

47. Aremu BR, Babalola OO. Construction of specific primers for rapid detection of South African exportable vegetable macergens. Int J Env Res Pub Health. 2015; 12: 12356-12370.
doi: 10.3390/ijerph121012356

48. Elshahed MS, Youssef NH, Spain AM, Sheik C, Najar FZ, Sukharnikov LO, Roe BA, Davis JP, Schloss PD, Bailey VL, Krumholz LR. Novelty and uniqueness pattern of rare members of the soil biosphere. Appl Env Microbiol. 2008; 4(17): 5422-5428.
doi: 10.1128/AEM.00410-08

49. Zamudio M, Gonzalez A, Medina JA. *Lactobacillus plantarum* phytase activity is due to non-specific acid phosphatase. Lett Appl Microbiol. 2001; 32: 181-184.
doi: 10.1046/j.1472-765x.2001.00890.x

50. Mullaney EJ, Daly CB, Ullah AH. Advances in phytase research. Adv Appl Microbiol. 2000; 47: 157-199.
doi: 10.1016/S0065-2164(00)47004-8

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بررسی مولکولی ژن فیتاز در باکتری های لاکتیک اسید جدا شده از *Ogi* و *Kunun-Zaki*، نوشیدنی و حریره غلات تخمیری آفریقایی

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چکیده

سابقه و هدف: فیتات یک عامل ضد تغذیه‌ای در غلات است که مشکلات تغذیه‌ای و سلامتی را موجب می‌شود. ژن مسئول تجزیه فیتات در بسیاری از گونه‌های باکتریایی، به جز باکتری‌های لاکتیک اسید شناسایی شده است. هدف مطالعه حاضر جداسازی و شناسایی ژن *phy* در باکتری‌های لاکتیک اسید جدا شده از *Ogi* و *Kunun-zaki* می‌باشد.

مواد و روش ها: باکتری‌های اسید لاکتیک جدا شده از *Ogi* و *Kunun-zaki* ازنظر تولید فیتاز بر اساس رُخنمود[۱] (فنوتیپ) غربال شدند. باکتری‌های اسید لاکتیک جدا شده با قدرت تجزیه فیتات با توالی‌یابی[۲] ژن 16SrRNA شناسایی شدند. تقویت ژن فیتاز (*phy*) با استفاده از واکنش زنجیر پلیمراز انجام شد. علاوه بر این، آزمون‌های تبار ژنی[۳] انجام شد. پروتئین‌های محلول سه باکتری انتخابی استخراج و توسط ژل الکتروفورز سدیم دو دسیل سولفات پلی آکریلامید آگاروز بررسی شدند.

یافته‌ها و نتیجه‌گیری: در مجموع، ۱۶ باکتری اسید لاکتیک جداسازی شده به عنوان *لاکتوباسیلوس برویس*، زیرگونه *لاکتوبا سیلوس پلانتاروم*، *لاکتوبا سیلوس پلانتاروم*، *لاکتوبا سیلوس پنتو سوس* و *لاکتوبا سیلوس پاراپلانتاروم* شناسایی شدند. ژن *phy* با افزایش اندازه ۲/۰ kb تقویت و توالی‌یابی شد. تشابه توالی بین ژن‌های *phy* باکتری‌های اسید لاکتیک و با سیلوس در بانک ژن ۹۹٪-۹۷٪ و نسبت تبار ژنی کمتر از ۴۰٪ بود. آنالیز الکتروفورز SDS-PAGE در سه باکتری جدا شده حاوی فیتاز با وزن مولکولی ۵۰ کیلو دالتون را نشان داد. این مطالعه نشان داد که وجود ژن *phy* باکتری‌های لاکتیک اسید موجود در غذاهای تخمیری، امکان استفاده از این فرآورده‌ها به عنوان آغازگر برای تولید غذاهای تخمیری با کیفیت تغذیه‌ای بهبودیافته را میسر می‌سازد.

تعارض منافع: نویسندگان تایید می نمایند که تضاد منافعی ندارند.

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واژگان کلیدی

- غلات
- مواد غذایی تخمیری
- ژن *phy*
- نسبت تبار ژنی
- باکتری‌های لاکتیک اسید تجزیه کننده فیتات

***نویسنده مسئول**

اولاباکولا بابالولا

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[۱] مجموعهٔ ویژگی‌های ساختاری و عملکردی قابل‌تشخیص در موجود زنده-Phenotypically

[۲] تعیین ردیف نوکلئوتیدها- Sequencing

[3] Phylogenetic analyses