Isolation and Characterization of Highly Effective Phytate-Mineralizing *Klebsiella pneumonia* Strain MK1C Adapted to Arid and Semiarid Conditions from Calcareous Soil

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ABSTRACT

This study aimed to isolate and identify highly adapted and highly efficient phytate-mineralizing bacteria from calcareous soil located in northern area of Egypt of arid and semiarid climate conditions. Using direct pour plate method, NBRIP medium was supplemented with phytate (5.0 g/L).The isolate showed the largest halo zone was further studied for its effectiveness in mineralizing phytate. The newly isolated strain was identified through the sequencing and the analysis of its nearly full 16S rRNA gene and was identified as *Klebsiella pneumonia* strain MK1C with GenBank accession number KU358675 based on BLASTn database. On liquid NBRIP culture, *Klebsiella pneumonia* strain MK1C showed high growth that was associated with a significant reduction of its pH from 6.8 to 4.7 and up to 5 times higher production of organic acids compared to uninoculated treatments. As a result, more than 50% of the organic phosphorus of added phytate was released as inorganic phosphorus. This strain could be an excellent dual-biofertilizer for both phytate mineralization and dinitrogen fixation. This is especially important for soils with poor conditions such as calcareous soils or in areas of arid and semiarid climate conditions where this strain and its related species are highly adapted.

Keywords: Phytate, Phosphate, mineralizing, *Klebsiella pneumonia*

INTRODUCTION

Phytate or phytic acid (*myo*-inositol (1,2,3,4,5,6)-hexakisphosphoric acid) is the storage of organic phosphorus in plant tissues and seeds as it represents 60 to 90% of its organic phosphorus (Harland and Morris 1995; Reddy et al. 1982). As storage of phosphorus, phytate ($C_6H_{17}NaO_{24}P_6$) reaches soils as organic forms through plant remains, manures, and composts. Phytate is a strong chelator for micronutrients such as Fe, Zn and cations such as Ca and Mg which might reduce their availability for plants. Although phytate is a source of P, it is not suitable for uptake as is and must undergo mineralization by soil microbial community through the production of phytases and phosphatases (Behera et al., 2014; Nash et al., 2014). Available P concentration in soil solution represent only about 0.1% of its total soil P (Ram et al., 2015). Therefore, an annual application of superphosphate and liquid phosphoric acid is inevitable to restock P availability shortage in soils. However, about 90% of this annually added P is usually fixed in soils and becomes unavailable for plants (Nash et al., 2014; Richardson and Simpson, 2011; Sharma et al., 2013). It has been reported that over fifty million ton of phytate reaches soils through produced and composted fruits and plant remains annually. This represents about 70% of commercially worldwide produced inorganic phosphorus fertilizers (Mullaney and Ullah, 2007). Also, one of the problems with phytate in soils is its strong binding affinity to essential minerals, forming insoluble phytate-minerals complexes, and limiting their availability for plants uptake. This mandates exploration for more efficient microorganisms that can be used as biofertilizers to mineralize plant remains and animal manures. The use of degrading and mineralizing microorganisms is an alternative solution to reduce the use of inorganic P fertilizers must be explored to reduce environmental pollution.

Phosphate solubilizing microorganisms employ various mechanisms, including the production of organic acids, phytase, and phosphatase, which improve the availability of insoluble inorganic and organic phosphorus (Chen et al., 2014; Kumar et al., 2013; Oteino et al., 2015; Richardson and Simpson, 2011; Sharma et al., 2013). While some isolated strains have been found to be plant host specific (Kundu et al., 2002), others have multifunctional mechanisms for P solubilization (Park et al., 2011; Vassileva et al., 2010). In addition, it has been reported that proton production during ammonium assimilation is another mechanism for phosphate solubilization by microorganisms (Illmer and Schinner, 1995; Illmer et al., 1995).

It has been reported that seed inoculation or side-dressing crops with PDB promoted plant growth and increased crop yields (Anil and Lakshmi, 2010; Mehrvarz et al., 2008; Mehta and Nautiyal, 2001). However, phosphorus solubility and availability for crops is a continuously major issue triggering extensive research for the best strategies to reduce its fixation to soils and increase its availability for plants. Few reports have been published on the isolation and identification of PDB from Egyptian soils (Hesham and

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Mohamed, 2011; Taha et al., 1969); with no strains isolated from Egyptian calcareous soils. Therefore, the isolation and characterization of new PDB recently became crucial toward finding effective and highly adapted strains for a potential use as a P-biofertilizer alternative to expensive and environment damaging inorganic phosphorus fertilizers. It is expected to have a shortage in inorganic phosphorus fertilizers Worldwide as the P reserve became limited and its production peak P had been reached three decades ago (Kirkby and Johnston, 2009). It is well known that phosphate is strongly bound to the soil solid phase and only taken up by the plant roots as $H_2PO_4^-$ or HPO_4^{2-} ions. To be beneficial source of P, phytate must be dissolved in the soil solution and the ester bond must be split. The mechanism of this split process is still not fully understood whether includes enzymatic or physical reaction.

The main purposes of this study are 1) to isolate and characterize phytate-degrading bacteria from calcareous soil, 2) to determine the possible mechanisms employed by isolated bacteria to mineralize phytate.

MATERIALS AND METHODS

2.1 Soil

Bacterial strains were isolated from soil sample that was collected from the surface layer (0-20 cm) using an acid washed stainless steel hand shovel at the Research Farm of Agriculture College, Foukah, Marsa Matrouh, at the North Western Coast of Egypt. For microbiological use, a fresh sample was transported on ice to the laboratory and kept at 4 ^oC for 24 h before being used for bacterial isolation. Soil sample was subject to analysis as described elsewhere (Kandil, 2017).

2.2 Culture Media

While there are more than one medium well known to be used for isolation of phosphate solubilization microorganisms, the National Botanical Research Institute's Phosphate (NBRIP) growth medium (Nautiyal, 1999) was used. The NBRIP medium contained glucose (10.0 g), phytic acid sodium salt (5.0 g), $MgCl_2.6H_2O$ (5.0 g), $MgSO_4.7H_2O$ (0.25 g), KCl (0.2 g), $(NH_4)_2SO_4$ (0.1 g), and agar (15.0 g) per liter of distilled water. The pH of media was adjusted to 7.0 before autoclaving at 121 ^oC for 20 min. Phytic acid solution was filter sterilized (0.22 µm syringe PFTE) and added to medium after cooling to avoid release of orthophosphate during autoclaving. Glucose was filter-sterilized by using 0.22 µm PFTE filter membranes and was added to media after cooling to 45^oC. Same medium, but liquid was used to measure the effectiveness of isolated strains in phytate degradation. Another rich medium; Luria-Bertani (LB) medium containing glucose (10.0 g), yeast extract (5.0 g), NaCl (5.0 g), and agar (18.0 g) per liter of distilled water was also used for isolates purification and differentiation. All chemicals used in this study were reagent grade or better.

2.3 Isolation of Phytate Degrading Bacteria

The direct pour-plate method was used to isolate bacteria mineralizing phytate from the above described soil. Ten-fold serial dilutions (from 10^{-1} to 10^{-5}) of soil were made using 0.85% sterile NaCl solution and pour-plated onto prepared NBRIP medium described above. Plates were incubated and for 7 days at 28 ^oC. Plates were monitored daily for clearing zones. Individual colonies in clearing zone areas were transferred and streaked multiple times onto solid NBRIP supplemented with phytate for purification. Colonies were streaked on LB medium for ensuring purity. To confirm the capability of purified isolates to degrade phytic acid, diameter of halo zone and colony must be measured. A single colony of purified isolates was transferred by sterilized wood toothpicks and simultaneously stabbed into NBRIP solid media. Plates were incubated at 28 ^oC and colonies monitored for the appearance of halo zones. Diameter of both colony and halo zone was measured daily and used to calculate solubility index using the following equation:

$$PySI = HalZd/Clnd \qquad \text{[Eq.1]}$$

where *PySI* is the solubility index, *HalZ*d is the halo zone diameter (mm), and *Cln*d is the colony diameter (mm).

Strains showing a high solubility index were selected for further study. Pure cultures were transferred to LB slants and kept in the refrigerator at 4 ^oC to be used for further studies. Pure cultures were also preserved in 20% sterile glycerol and stored at -70 °C for long term storage.

2.4 Colony-PCR of 16s rRNA Gene of Phytate Degrading Isolates

To identify phytate mineralizing bacteria, basic phenotypic characteristics were measured. Also the 16S rRNA gene of selected isolates was amplified according to Weisburg et al., (1991). In this method, forward primer 27f (5`-AGAGTTTGATCMTGGCTCAG-3`) and reverse primer 1492r (5′-CTACGGCTACCTTGTTACGA-3′) (Integrated DNA Technologies, Coralville, Iowa) were used. The recently developed direct colony-PCR method was used without going through the traditional tedious method of DNA extraction and purification. A pure single colony of each isolate was selected, picked with a 1-µl sterile inoculating loop (Fisher Scientific, Pittsburgh, PA), and

inserted in a labeled PCR tube. Tubes contained the PCR mixture (50 µl) of sterilized Millipore nuclease free water (40.5 µl), 10X PCR buffer (5 µl), dNTPs (2 µl), 27f primer (1 µl), 1492r primer (1 µl), and Choice *Taq* Polymerase (0.5 µl). Tubes were shaken and centrifuged for 20 sec. PCR was performed using an MJ research PTC 100 (MJ Research, Waltham, Mass., USA) thermocycler. In general, the PCR program was as follow: initial denaturing step for 95 ^{o}C for 15 min, followed by 34 cycles of 95 ^{o}C for 30 sec, 57 ^{o}C for 45 sec, and 72 ^{o}C for 1.5 min with a final extension step for 6 min at 72 ^{o}C in an automated thermal cycler then a storing step at 4 ^{o}C.

2.5 Purification and Sequencing of 16S rRNA Gene

Before sequencing, PCR products were examined for successful amplification by using gel electrophoresis as described elsewhere (Kandil et al., 2015). The PCR products of the 16S rRNA gene were purified using QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). Before sending DNA samples for sequencing, samples were examined on gel electrophoresis to confirm their purity and presence. DNA concentration was determined by Qubit 2.0 fluorometer as described by standard protocol (Invitrogen, Life technologies, Carlsbad, CA). Based on DNA concentration of each sample, proper volume was determined to be used for DNA sequencing template. Both DNA strands were sequenced based on Sanger sequencing standards (ACGT, Inc, Wheeling, IL) using the 27f and 1492r primers.

2.6 Identification and Nomenclature of Phytate Degrading Isolates

Consensus sequence *contigs* of both strands sequences was generated using *BioEdit* software (Hall, 1999) and compared with GenBank database of nucleotides by using the BLASTn algorithm (Altschul, et al., 1990). Isolated strains were named based on its identical match or highest in similarity in database. A Phylogenetic tree was generated for interrelationships among the strains using MEGA4 or its latest updated software (Tamura et al., 2007).

2.7 Quantitative Analysis of Phytate Degradation

The efficiency of isolated strain in degrading phytate as the only organic phosphorus source was determined. Strains were grown in NBRIP liquid medium supplemented with phytic acid sodium salt solution (1.0 g/L). This solution was filter sterilized (0.22 µm syringe PFTE) and added to medium after cooling to avoid release of orthophosphate during autoclaving. All treatments (125 mL in 250 mL conical flasks) were in triplicate, and included negative and positive controls that were incubated at 28 ^{o}C. This experiment was conducted in 250-mL Erlenmeyer flasks containing 125 mL of sterilized NBRIP medium and incubated at 28 ^{o}C for 15 d on a rotary shaker at 120 rpm. Triplicate flasks including blanks were sampled nearly every other day under aseptic conditions for measurement of OD_{600}, pH, total organic acids, and released soluble P. About 5 mL aliquot of culture was decanted aseptically and used for abovementioned measurements. Culture pH was measured in the suspension without centrifugation or filtration. To measure OD_{600}, decanted samples were left to stand for 15 min to allow any precipitates other than bacterial cells to settle out of solution and the upper solution was used for measurement of OD_{600}.

2.8 Determination of Released Inorganic Phosphorus

To measure released soluble inorganic P, same decanted above described samples were used. It is not a surprise to state that measuring P in general is a challenge. This is especially true when we have a mixture of phytate as organic phosphorus and the released inorganic P due to its degradation. The blue method was chosen for its sensitivity and applicability with the understanding that some level of interference exists. After incubation with pure isolates, samples were centrifuged at 6,000 xg for 15 min to pellet cell debris and the supernatant was used. The colorimetric blue ammonium molybdate method was used for the determination of soluble P in supernatant and was measured by spectrophotometer at 882 nm (Murphy and Riley, 1962). The concentration of released P in supernatant was calculated against KH_2PO_4 standard curve with concentrations ranging from 0 to 2 mg/L. samples showed a concentration above range were diluted and re-measured.

2.9 Determination of Total Organic Acids

According to literature, many phosphate dissolving organisms produce organic acids to lower the pH of its surroundings either in nature of culture media. In this study, the total organic acids was measured directly in clear supernatant after centrifugation and filtration using spectrophotometer at 220 nm (Sokullu et al., 2010) and values were compared to uninoculated control samples. Specific organic acids were not determined at this stage of this study.

RESULTS AND DISCUSSION

3.1 Successful Isolation of Phytate Degrading Bacteria from Calcareous Soil

The main purpose of this study is to isolate bacteria degrading phytic acid sodium salt. Using direct pour plate method is advantageous to isolate existing bacteria with the ability to degrade phytate rather than those developed their ability due to genetic evolution. In this experiment, several halo zones were recognized on NBRIP medium. Almost all colonies within produced

halo zones were transferred and streaked onto fresh NBRIP and LB plates several times. Several pure isolates having distinct morphological features were tested for their ability to solubilize phytic acid sodium salt (5.0 g/L). Colonies showed no clearing zones or lost their ability to clear phytate supplemented plates were eliminated. As a result, few pure colonies forming halo zone on NBRIP solid plates supplemented with phytate were isolated as shown in Figure 1.

Fig. 1. Formation of halo zone by phytate-mineralizing isolate on NBRIP solid medium supplemented with phytate (5.0 g/L)

3.2 Solubility Index for Selection of Effective PDB strains

Solubility index is a value indicating the effectiveness of microorganisms in the production of certain substances with certain function such as antibiotics, enzymes or toxins. It depends on forming a halo or clearing zone around the bacterial colony indicating the solubility or degradability of the substance under investigation. This technique has been widely used and successfully applied for the isolation of phosphate dissolving microorganisms and for the screening of plant growth promoting microorganisms. In general, larger diameter of halo zone indicates higher effectiveness in phosphate dissolving due to the diffusion of produced organic acids, enzymes, protons, or siderophore (Illmer et al., 1995; Nautiyal, 1999; Pikovskaya, 1948). In this present study, the phytate solubility index (*PySI*) for selected isolates on NBRIP media supplemented with phytic acid sodium salt (5.0 g/L) was calculated as described (*Eq*.1) from the diameter of the microbial colony and halo zone as shown in Figure 1. Data of this study showed that solubility index varied among isolates and over incubation time as reported previously (Kandil, 2017). As shown in Figure 2, the solubility index (*PySI*) was not a fixed value for each isolate but has changed during incubation period from a higher value after 2 days to a lower value after 3-4 days of incubation. Data of this study supports that the decrease in the *PySI* ratio was due to a continuous growth of the bacterial colony and the increase of its diameter that is juxtaposed by a continuous but obviously less increase in the diameter of the clear zone. Therefore, a short incubation period of 3 to 4 days is reasonable for selecting phosphate dissolving bacteria rather than waiting for a week to decide. Based on the high solubility index of phytate on NBRIP media, one isolate was selected for further studies and identification.

3.3 purification and Sequencing of 16S rRNA Gene

As described above, 16S rRNA gene was amplified using colony PCR method. Validity and quality of 16S rRNA product was examined by gel electrophoresis as described. This step was followed by purification by QIAquick PCR purification kit to ensure high quality product at sequencing. The concentration of purified 16S rRNA was measured to determine optimum sample volume needed for optimum sequencing. Figure 3 shows the agarose gel with 16S rRNA before (lanes 1 and 2) and after (lanes 3 and 4) purification compared to a known positive (lane 5) and blank (lane 6) samples. Data shows that the colony-PCR procedure was successful and samples were pure and proper concentration for high quality sequencing. As shown, the product is about 1500 bp as compared to the top band of a 100 kb ladder indicating that the gene amplified is the targeted 16S rRNA.

3.4 Taxonomic Identification of Phytate Degrading Strain

Some phenotypic characteristics were measured by microscope and physiological tests. Cells of isolated strain were found to be Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium, urea positive, and non-spore forming. The taxonomic identity of the isolated efficient phytate-degrading strain was determined by near full length sequence analysis of its 16S rRNA gene (Fig.4). Based on BLASTn searches, the sequences of the 16S rRNA genes of the isolate ranged from 97 to 99% identical to several strains in the genomic database. The best match for isolate MK1C (99% similarity) were *Klebsiella pneumoniae* QLR-8 (KM096437.1), *Klebsiella pneumoniae* isolate YNUCC0237 (AY552753.1), *Klebsiella* sp. SCAUS56 (KF836054.1), and *Klebsiella variicola* strain C109 (HQ407284.1), and *Klebsiella* sp. G3 that showed 97% similarity to isolate MK1C. This strain was taxonomically classified as *Klebsiella* sp. strain MKIC based on its 16S rRNA gene sequence and was given a GenBank accession number KU358675.

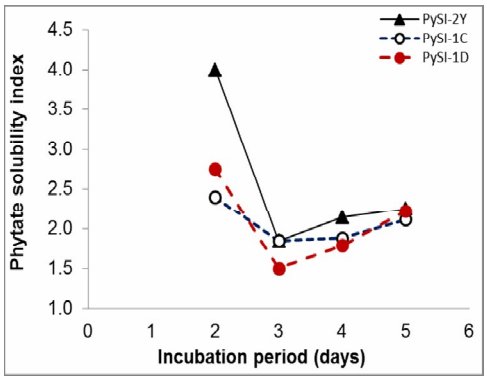

Fig. 2. Phytate solubility index (*PySI*) for three isolates cultured on NBRIP as labeled. Diameters of halo zones and colonies were used for *PySI* calculation as *in Eq.1*

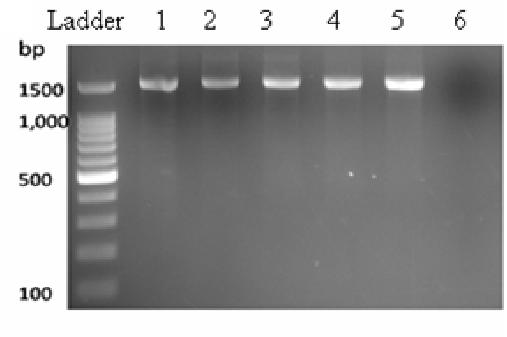

Fig. 3. Agarose gel with 16S rRNA bands of two isolates before (lanes 1 and 2) and after (lanes 3 and 4) purification compared to that of a known strain as positive (lane 5) and that of a blank (lane 6) samples

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1    agtcgagcgg tagcacagag agcttgctct cgggtgacga gcggcggacg ggtgagtaat
61   gtctgggaaa ctgcctgatg gaggggggata actactggaa acggtagcta ataccgcata
121  acgtcgcaag accaaagtgg gggaccttcg ggcctcatgc catcagatgt gcccagatgg
181  gattagctag taggtggggt aacggctcac ctaggcgacg atccctagct ggtctgagag
241  gatgaccagc cacactggaa ctgagacacg gtccagactc ctacgggagg cagcagtggg
301  gaatattgca caatgggcgc aagcctgatg cagccatgcc gcgtgtgtga agaaggcctt
361  cgggttgtaa agcactttca gcggggagga aggcggtgag gttaataacc tcatcgattg
421  acgttacccg cagaagaagc accggctaac tccgtgccag cagccgcggt aatacggagg
481  gtgcaagcgt taatcggaat tactgggcgt aaagcgcacg caggcggtct gtcaagtcgg
541  atgtgaaatc cccgggctca acctgggaac tgcattcgaa actggcaggc tagagtcttg
601  tagaggggg tagaattcca ggtgtagcgg tgaaatgcgt agagatctgg aggaataccg
661  gtggcgaagg cggccccctg gacaaagact gacgctcagg tgcgaaagcg tggggagcaa
721  acaggattag ataccctggt agtccacgct gtaaacgatg tcgatttgga ggttgtgccc
781  ttgaggcgtg gcttccggag ctaacgcgtt aaatcgaccg cctggggagt acggccgcaa
841  ggttaaaact caaatgaatt gacgggggcc cgcacaagcg gtggagcatg tggtttaatt
901  cgatgcaacg cgaagaacct tacctggtct tgacatccac agaactttcc agagatggat
961  tggtgccttc gggaactgtg agacaggtgc tgcatggctg tcgtcagctc gtgttgtgaa
1021 atgttgggtt aagtcccgca acgagcgcaa cccttatcct ttgttgccag cggtccggcc
1081 gggaactcaa aggagactgc cagtgataaa ctggaggaag gtggggatga cgtcaagtca
1141 tcatggccct tacgaccagg gctacacacg tgctacaatg gcatatacaa agagaagcga
1201 cctcgcgaga gcgagcggac ctcataaagt atgtcgtagt ccggattgga gtctgcaact
1261 cgactccatg aagtcggaat cgctagtaat cgtagatcag aatgctacgg tgaatacgtt
1321 cccgggcctt gtacacaccg cccgtcacac catgggagtg ggttgcaaaa gaagtaggta
1381 gcttaacctt cgggagggcg cttaccactt tgtgattc
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Fig. 4. The near full-length sequence of the 16S rRNA gene of isolated *Klebsiella* sp. strain MKIC

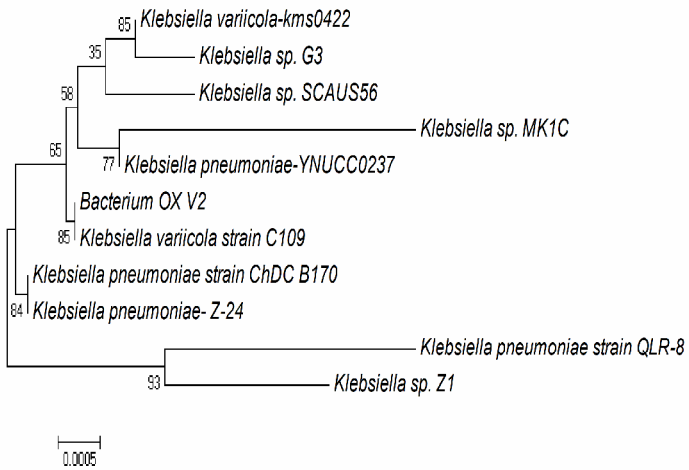

Fig. 5. Evolutionary relationships of taxa for *Klebsiella* sp. strain MKIC

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal 1973). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969) and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

To the best of our knowledge, this is the first time that *Klebsiella* sp. strain to be isolated from calcareous soil from Egypt. Worldwide, closely related species such as *Klebsiella terrigena* (Greiner and Carlsson, 2006), *Klebsiella* sp. strain ASR1 (Sajidan et al 2004), and *Klebsiella pneumoniae* 9-3B (Escobin-Mopera et al. 2012), *Klebsiella* sp. Db- 3fj711774.1 (Mittal et al. 2012) have previously been isolated and reported to degrade phytate.

3.3 Mechanisms of Phytate Solubilization

To quantitatively measure the effectiveness and mechanisms of identified strain to degrade and mineralize phytate, the NBRIP liquid medium has been used. In this experiment, the growth of the strain and its ability to mineralize phytate to inorganic phosphorus were measured. Also the culture pH and the total organic acids were measured in the same sample after filtration. Measuring those 4 parameters in the same sample will allow more succinct understanding of not only the efficiency of the strain but its mineralizing mechanisms of phytate. In this experiment, NBRIP liquid medium supplemented with 1.0 g/L phytic acid sodium salt was inoculated with *Klebsiella* sp. strain MK1C and incubated as described above. To evaluate the ability of the isolated strain to use phytate as both carbon and phosphorus sources, some treatments were suplemented with glucose as a source of carbon, while some were not. As Figures 6A, B, and C show, only treatments supplelemnted with glucose showed bacteria growth, disappearence of the white precipitate of phytate, decrease in culture pH, production of organic acids and as a result, a significant release of inorganic P as explained below.

As shown in Figure 6A, the growth (OD_{600}) of *Klebsiella* sp. strain MKIC was associated with a significant reduction of its culture pH from 6.8 initially to about 4.7 during incubation. This indicated that reducing the pH might be one of the mechanisms of mineralizing phytate. It is possible that this strain produced some organic acids to reduce the pH of its habitat. To confirm, the culture content of total organic acids was measured at 220 nm by spectropotometer after filtering through 0.22 µm PFTE filters and compared to uninoculated control as shown in Figure 6B. As shown, the ratio of total organic acids produced by *Klebsiella* sp. strain MKIC was significally increasing with the growth of the strain up to 6 times more than that of uninoculated control treatments.

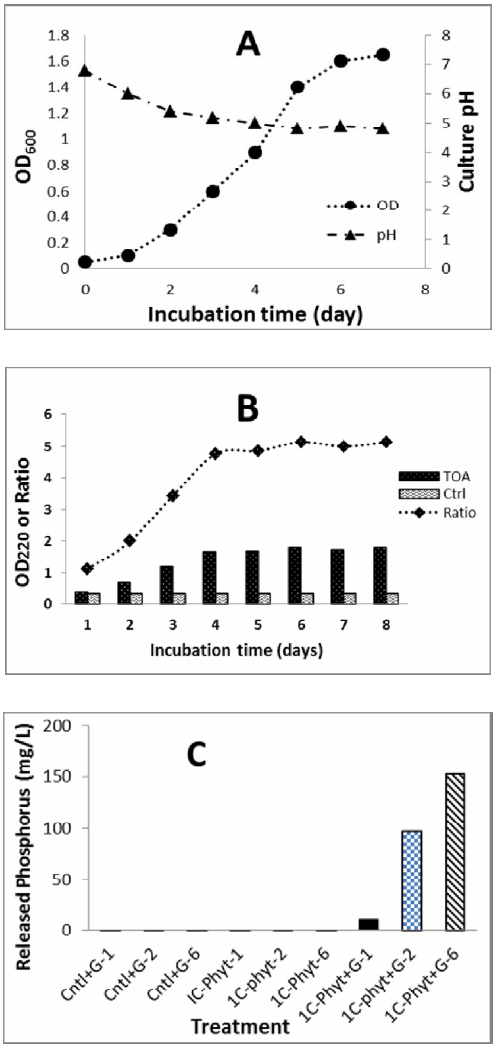

Fig. 6. The grwoth of *Klebsiella* sp. strain MKIC on NBRIP medium supplemented with phytate (1000 mg/L) and reduction of its pH (6A); production of total organic acids (TOA) compared to control treatments (6B), and release of inorganic phosphorus in treatments supplemented with glucose as a simple carbon source (6C). Legends and treatments are as shown

As a result, as shown in Figure 6C, *Klebsiella* sp. strain MKIC was capable of mineralizing phytate and releasing about 70% (192 mg/L) of its initial bounded phosphorus content (282 mg/L) as soluble inorganic phosphorus in treatments supplemented with glucose as a carbon source. Also, Figure 6C shows that the strain was not capable of using phytate as a carbon source and another carbon source such as glucose must be supplemented. It is reported that soil microorganisms were found to dissolve or metabolize phytate through the production of several enzymes, mainly phytase and phosphatase (Greiner, 2004; Illmer et al., 1995; Johri et al., 1999; Kumar et al., 2013). Therefore, It is highly possible that enzymes such as phytase and phosphatase were produced to mineralize phytae. It will be difficult to determine the exact mechanism or differentiate the contribution of enzymes vs organic acids in mineralizing phytae and releasing inorganic phosphorus as a final product. This process requires another set of expirements which was not the intend of this study. Further future studies are needed to answer such questions. This is an agreement with other the solubilization of P was due to the production of organic acids (Dastager et al., 2010; Illmer and Schinner, 1995; Illmer et al., 1995; Pandey et al., 2006), siderophore (Sharma et al., 2013), indole acetic acids (Sergeeva et al., 2007), phytase (Acu?a and Jorquera, 2011; Konietzny and Greiner, 2004), and protons during ammonia respiration (Illmer and Schinner, 1995; Ramesh et al., 2014; Reyes et al., 2006).

In conclusion, this study aimed to isolate and identify highly adapted and highly efficient phytate-mineralizing bacteria from calcareous soil located in northern area of Egypt known to be arid and semiarid climate conditions. The newly isolated strain was identified by nearly its full 16S rRNA sequence as *Klebsiella* sp. strain MKIC. This strain could be excellent dual-biofertilizer for phosphate solubilization and for dinitrogen fixation. This is especially important for soils with poor conditions such as calcareous soils or in areas of arid and semiarid climate conditions where this strain or its related species are highly adapted. Taken together, results of this study are in agreement with those previously confirming the association of the growth of phytate mineralizing bacteria with organic acids production, pH reduction, enzymes mode of action, and ultimatly inorganic-P release.

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الملخص العربي

عزل وتوصيف سلالة كليبسيلا نيمونيا عالية الكفاءة في معدنة الفيتات والمتأقلمة للظروف الجافة وشبه الجافة من الاراضي الجيرية

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هدفت هذه الدراسة إلى عزل وتعريف البكتيريا عالية الكفاءة لتحليل ومعدنة الفيتات كمصدر ومخزن رئيسي للفسفور العضوي في التربة. وقد تم العزل من التربة الجيرية الواقعة في المنطقة الشمالية من مصر والتي تتميز بالظروف المناخية القاحلة وشبه القاحلة باستخدام طريقة الصب المباشر علي بيئة NBRIP المضاف اليها ٥,٠ جم فيتات لكل لتر. وقد تم إختيار احد العزلات للدراسة لفعاليتها في معدنة الفيتات حيث أظهرت أكبر منطقة أوهالة رائقة حول مستعمراتها مقارنة بعزلات اخرى. كما تم التعرف على هذه السلالة المعزولة من خلال عزل وتحليل التسلسل النيوكلوتيدي لجين 16S rRNA وسميت *Klebsiella pneumonia* strain MK1C وسجلت برقم KU358675 طبقاً لبنك المعلومات الجينية للكائنات الحية الدقيقة BLASTn. وعلى البيئة السائلة، أظهرت السلالة كلبسيلا MK1C نمواً معنوياً والذي ارتبط مع إنخفاض كبير في درجة الحموضة من ٦,٨ إلى ٤,٧ وكذلك زيادة معنوية في إنتاج الأحماض العضوية تصل إلى ٥ أضعاف المعاملات غير الملقحة. ونتيجة لذلك، تم معدنة وتحرير عن أكثر من ٥٠٪ من الفوسفور العضوي من الفيتات الى صورةالفوسفور غير العضوية التي يمكن الاستفادة منها. وبناءاً على تلك النتائج، يحتمل أن تكون هذه السلالة مزدوجة الاستفادة كسماد حيوي لمعدنة الصور العضوية للفسفور وكذلك لتثبيت نتروجين الهواءالجوي كما هو معروف. وتكتسب نتائج هذه الدراسة أهمية خاصة للتربة ذات الظروف الغير مثالية مثل التربة الجيرية أوأراضي المناطق ذات الظروف المناخية القاحلة وشبه القاحلة حيث تتكيف هذه السلالة والأنواع المرتبطة بها بدرجة كبيرة.