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# Rock phosphate-solubilising potential of fungal and bacterial isolates from soils surrounding panda Hill and Minjingu phosphate rock deposits in Tanzania

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**Abstract:** A study on isolation and characterization of phosphate rock-solubilising microorganisms from Minjingu and Panda Hill deposits was undertaken in order to examine their potential for use as future inoculants. Following initial screening, 22 fungi and 39 bacteria from Tanzania's Minjingu and Panda Hill phosphate rock deposits were assessed for their ability to solubilise water-insoluble phosphate rocks. Five best bacterial and fungal isolates in terms of total soluble P released were selected for molecular identification using 16S and 5.8S rDNA sequencing for bacterial and fungal isolates, respectively. Overall, fungal isolates exhibited the highest solubilising abilities, registering up to 80.39 mg $kg^{-1}$ of phosphate rock compared to their bacterial counterparts with best performer solubilizing only 27.45 mg $kg^{-1}$ of phosphate rock. While fungal isolates from both Minjingu and Panda Hill were generally more efficient at solubilising samples from the less complex guano deposit of Minjingu (HMPR), the bacterial isolates showed significant ($P \leq 0.05$) variations with isolates from Panda Hill showing a far better ability at solubilising their more familiar source of P, the igneous PandaHill phosphate rock (PPR) samples. *Aspergillus stelifer and A. tamarii* were the most efficient fungal isolates freeing 20.97–77.49 and 12.74–80.39 mg of soluble P $kg^{-1}$ from PPR and HMPR, respectively. Similarly, *Stenotrophomonas maltophilia* was the best bacterial isolate releasing up to 27.45 and 24.75 mg of soluble P $kg^{-1}$ from PPR and HMPR, respectively. The potential exhibited by microorganisms characterized in this study warrants further enquiry for their application in the field.

**Key words:** Minjingu phosphate rock; Panda Hill, phosphate solubilising microorganisms; P inoculants, Tanzania.

## Introduction

Production of sufficient food to respond to the needs of growing human populations is one of the top challenges facing most African nations. This challenge is rooted, among others things, in the declining ability of the tropical soils to sufficiently supply important plant nutrient elements, especially phosphorus (Gweyi-Onyango *et al.* 2010; Ndungu-Magiroi *et al.* 2014). Over 50% of cultivated soils in Tanzania are estimated to be P-deficient (Ndungu-Magiroi *et al.* 2014; Okalebo *et al.* 2009), which calls for efforts to supply sufficient P inputs to the soil in order to improve the soil P status (Chien *et al.* 2011). Although the use of water-soluble chemical fertilizers has traditionally been an immediate solution to correct nutrient deficiencies in soils, the practice is not without problems. Inorganic water-soluble P fertilizers can easily be fixed in acid soils and thus made unavailable to plants unless applied in large, usually uneconomical, amounts that can saturate the P fixing mechanisms in the soils (Balemi & Negisho 2012). Moreover, the price tag for industrially processed P fertilizers remains beyond the reach of most small scale farmers, resulting in no or only minimal use of P fertilizers (Druilhe & Barreiro-Hurlé 2012), leading to low crop yields and poor quality of agricultural produce.

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Tanzania has a wealth of phosphate rock (PR) deposits such as the sedimentary Minjingu (in Arusha) and the igneous Panda Hill (in Mbeya), which are not exploited to their full potential for direct use as fertilizers due to their insolubility. As a consequence, direct use of phosphate rocks in Tanzania is limited to soils of a lower pH (<5.5) where rock phosphates such as the soft Minjingu rock become highly reactive (Okalebo *et al.* 2009). Use of efficient P-solubilising microorganisms (PSM) could be a viable, relatively inexpensive alternative to counter the limited use of phosphate rocks in Tanzanian agriculture. The PSMs achieve the solubilization of rock phosphates through a number of mechanisms, the most important being the production of organic acids that are capable of dissolution of the otherwise water-insoluble phosphates rocks. The dissolution of PR by organic acids produced by PSM is achieved through the hydroxyl and carboxyl groups of the organic acids which chelate the cations ($Al^{3+}$, $Fe^{2+}$, $Ca^{2+}$) bound on the phosphates, effectively releasing the $PO_4^{3-}$ ion into solution (He *et al.* 2002). It has been shown that PSMs can increase crop yields by up to 70 percent (Verma 1993) and that use of phosphate solubilising bacteria (PSB) can help to reduce the rate of in-organic P to soils by up to 50% (Sundara *et al.* 2002).

Isolation, identification and testing of efficient P-solubilising microorganisms (PSM) could lead to the development of inoculants to be used with insoluble rock phosphate as source of P to plants, akin to the use of rhizobia inoculants in inoculating legume seeds for increased nitrogen fixation. We designed an experiment to isolate and characterize phosphate-solubilising microorganisms based on one main hypothesis: That while microorganisms isolated from soils surrounding a particular rock phosphate deposit might have acquired a preferentially higher ability to solubilise rock phosphates of that particular chemistry, it is possible that some strains may have capabilities to solubilise both familiar and non-familiar rock phosphate types. The present work reports on probably the first attempt on the isolation, identification, and comparison on the solubilising potential of isolates from soils surrounding chemically different rock phosphate deposits, namely the guano-sedimentary rock phosphate of Minjingu in northern Tanzania and the relatively more complex, less soluble, igneous (apartite) rock phosphate of Panda Hill in Mbeya, southern Tanzania.

## Methods

### *Sampling and sample preparation*

Phosphate rock samples from Minjingu and Panda Hill were taken from the phosphate rock itself while soil samples were collected from soils in direct contact with the phosphate rock deposit as well as soils located far afield. Soil sampling was done at the depths of 0–5 and 5–10 cm. The phosphate rocks were sampled by taking samples of weathered rock (as source of P solubilising microorganisms) and hard (unweathered) rock to be used as a source of P in solubility experiments. Panda Hill phosphate rock samples were picked following determination of their P content by XRF where samples having P % of above 0.443 were chosen. The collected samples were brought to Sokoine University of Agriculture (SUA) soil science laboratory for physico-chemical and microbiological analyses and P solubilisation tests.

### *Physico-chemical characterization of soil and rock samples*

Portions of the soil samples were dried, ground and sieved through a 2–mm sieve for physico-chemical characterization. Parameters measured were: soil pH, particle size distribution, organic carbon and available P. Soil pH was determined electrochemically in 1:2.5 (weight/volume) soil: water suspensions according to the procedure described by Okalebo *et al.* (1993). The hydrometer method (Bouyoucos 1962; Okalebo *et al.* 1993) was used to determine the particle size distribution of soils. The USDA textural class triangle was used to determine the textural classes of soils. Determination of organic carbon was done by the wet digestion (oxidation) method of Walkely-Black (Nelson & Sommers 1996). Extractable phosphorus was determined according to procedures described by Okalebo *et al.* (1993). Due to differences in soil pH, available P in Panda Hill and Minjingu was determined by Bray No 1 and Olsen's methods, respectively. The elemental composition of rock phosphate from Panda Hill was determined using XRF. Ten readings were taken at 30 seconds time interval from 10 points of a phosphate rock crystal sample. The elemental composition of Minjingu PR is available in literature (Szilas *et al.* 2008) and thus the XRF procedure was not repeated for Minjingu PR.

### *Preparation of microbiological media*

Media for isolating bacteria, fungi and actinomycetes were nutrient agar, potato dextrose agar and starch- casein agar, respectively. Nutrient agar was prepared based on the formulation by Downes and Ito (2001) and starch-casein agar was prepared according to Kuster and Williams (1966). To prepare Potato Dextrose Agar (Potato Glucose Agar), 39 g of PDA (Himedia, Mumbai India) was dissolved into one litre of distilled water, and its pH adjusted to 6. All media were sterilized by autoclaving at 1.05 kg $cm^{-2}$ and 121 °C for 20 minutes (Curry *et al*. 1993).

Media for testing ability of the isolates to solubilise phosphate rock were Synthetic Minimum Medium (SMM) and the Pikovskaya (PVK) which was used as a reference medium. To prepare the SMM, phosphate rock samples were ground using a mortar and pestle and passed through a 500 µm sieve. The fine PR powder obtained was mixed with other contents (Roy *et al.* 2013) and was used for comparison. Both PVK and SMM were sterilized by autoclaving at 1.05 kg $cm^{-2}$ and 121 °C for 20 minutes, poured into petri dishes and used for RP solubilization ability test of the isolates.

### *Qualitative and quantitative assessment of the phosphate rock solubilising abilities of isolated microorganisms*

Halo zone size on solid media was used to qualitatively rate the ability of individual isolates to solubilise the phosphate rocks. A sterile inoculation wire loop was used to aseptically transfer actinomycetes, fungi or bacteria to petri dishes of appropriate media containing insoluble phosphate as the only source of phosphorus. The inoculated plates were incubated for 3–10 days at 28 °C. Clear halo zones around the developing colonies were taken as a qualitative index of phosphorus solubilization. The diameter of the halo (including colony) was measured.

Quantitative assessment of the solubilisation involved the following steps: Phosphate Rock (PR) samples from Panda Hill and Minjingu were washed using sterile water, dried and ground using a mortar and pestle and passed through a 100 mm mesh sieve. Sterilization of PR powder was done by using UV irradiation. Sterile PR powder (0.5 g) was mixed with 50 ml SMM broth; the mixture was inoculated with either bacteria, fungi or actinomycetes isolates, replicated four times. The mixture was then incubated for 10 days at 28 °C. Controls contained the P sources without inoculation. Amount of soluble phosphate (P) released from phosphate rocks (PRs) were determined spectrophotometrically by the chlorostannous reduced molybdo-phosphoric acid blue colour method (Olsen & Sommers 1982). The abilities of different isolates to solubilise P were compared based on amount of P solubilised.

### *Identification of selected isolates*

Identification of isolates with higher PR solubilization potential involved morphological and molecular characterization techniques. Isolates were characterized by their macro-morphology using the naked eye (Guarro *et al.* 1999) and micro-morphologically using a microscope. To undertake a micro-morphological description bacterial smears were prepared using standard Gram stain procedure (Chapelle 2001; Carter & Cole 2012) while fungal isolates were stained using lactophenol cotton blue (Sudan & Sharma 2003; Lakshmi & Anuradha 2008) prior to observation under a light microscope.

Molecular characterization and identification was done on five bacterial and five fungal isolates exhibiting outstanding abilities to solubilise RP. Fungal and bacterial DNAs were extracted from pure cultures by heating at 90 °C for 15 minutes followed by extraction using silica columns prior to amplification. Amplification of the ITS1-5.8S-ITS2 rDNA of fungi was done using universal primers ITS-F and ITS4 which included an initial denaturation at 95 °C for 10 minutes followed by 40 cycles each for 45 seconds at 95 °C, 30 seconds at 55 °C, and 1 minute at 72 °C, and a final extension at 72 °C for 10 minutes. Bacterial 16S rDNA amplification used primers 27F and 1492R (Balajee *et al.* 2007) and it included an initial denaturation at 95 °C for 10 minutes followed by 40 cycles each for 30 seconds at 95 °C, 30 seconds at 55 °C, and 1 minute at 72 °C, and a final extension at 72 °C for 10 minutes.

The amplified rDNA fragments were then separated by electrophoresis through 1.5% agarose gel and visualized using a gel documentation system after staining with GelRed (Biotium, Phenix, USA), a nucleic acid gel stain. Afterwards, PCR products were directly sequenced using dideoxynucleotide cycle sequencing (ABI 3500 Genetic Analyser, Applied Biosystems, Foster City, CA). After treatment with exonuclease I and shrimp alkaline phosphatase, sequencing PCR was conducted using Big Dye Terminator v 3.1 cycle sequencing kit (Applied Biosystem, Foster City, CA). The nucleotide sequences were compared with other sequences at the GenBank using BLASTn tool to establish the identity of the isolates.

**Table 1.** Selected properties of the soils used in the study.

| Soil | pH | OC (%) | P (mg $kg^{-1}$) | Texture |
|---|---|---|---|---|
| Minjingu | 8.8 SA | 0.804 VL | 31.36 | Sandy loam |
| Panda Hill | 6.83 N | 0.81 VL | 8.068 | Sandy loam |

**Table 2**. Metal and radioactive elemental composition of Panda Hill rock Phosphate.

| Point | Time (Sec) | U | Th | Pb | Zn | Cu | Ni | Fe | Mn | Ti | As % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C1 | 30.14 | < LOD | 0.007 | 0.003 | 0.024 | < LOD | 0.026 | 14.786 | 0.380 | 0.613 | 0.004 |
| C2 | 29.94 | < LOD | 0.019 | 0.009 | 0.018 | < LOD | 0.063 | 11.198 | 0.434 | < LOD | < LOD |
| C3 | 32.5 | 0.002 | 0.03 | 0.011 | 0.013 | 0.01 | 0.020 | 9.275 | 0.201 | 0.171 | < LOD |
| C4 | 26.17 | 0.007 | 0.04 | 0.045 | 0.027 | 0.03 | < LOD | 12.847 | 0.196 | 0.491 | < LOD |
| C5 | 28.25 | < LOD | 0.025 | < LOD | 0.040 | < LOD | 0.073 | 18.322 | 0.283 | < LOD | 0.029 |
| C6 | 26.98 | 0.005 | 0.013 | < LOD | 0.020 | < LOD | < LOD | 5.883 | 0.253 | 0.187 | < LOD |
| C7 | 25.72 | < LOD | < LOD | < LOD | 0.012 | < LOD | < LOD | 8.809 | 0.303 | < LOD | < LOD |
| C8 | 27.52 | < LOD | 0.024 | 0.160 | 0.021 | < LOD | 0.045 | 8.831 | 0.882 | < LOD | < LOD |
| C9 | 27.12 | < LOD | < LOD | < LOD | 0.029 | < LOD | < LOD | 9.544 | 1.509 | < LOD | < LOD |
| C10 | 25.87 | < LOD | < LOD | < LOD | 0.015 | < LOD | 0.049 | 8.500 | 0.788 | < LOD | < LOD |

LOD = Limit of Detection.

### *Statistical analysis*

Rock P solubilization data obtained in the quantitative experiment was subjected to analysis of variance (ANOVA) to evaluate the efficiency of different microbial isolates in solubilising PR. Treatment means separation was done according to Duncan's New Multiple Range Test (DNMRT) at the 0.05 level of significance.

## Results

### *Selected microbial and physico-chemical properties of the source soil and phosphate rock samples*

Selected properties of the soil samples used in this study are presented in Table 1. The textural class of both Minjingu and Panda Hill soils used in this study was sandy loam according to the USDA textural class triangle (Brady & Weil 2002). Soil pH values were 8.8 and 6.83 for Minjingu and Panda Hill, respectively (Table 1). According to Landon (2014) the soil of Panda Hill can be rated as being neutral and that of Minjingu as being slightly alkaline. Table 1 also shows that organic carbon levels were 0.80% and 0.81% for Minjingu and Panda Hill soils, respectively, which were both rated as being very low (Landon 2014). Available phosphorus of Minjingu and Panda Hill soil samples were 31.36 and 8.07 mg $kg^{-1}$, respectively, reflective of the influence of the relatively more reactive Minjingu leading to greater availability of P to soil as compared to the more insoluble Panda Hill Phosphate rock.

Table 2 shows the concentrations of metals in Panda Hill rock phosphate (PRP). Samples contained detectable amounts of zinc (Zn), iron (Fe) and manganese (Mn) that were found at all sampling points while arsenic (As), lead (Pb), copper (Cu), nickel (Ni) and titanium (Ti) where detected in only some points. Radioactive uranium and thorium were also detected. However, gold (Au), silver (Ag), cadmium, palladium (Pd), cobalt and chromium (Cr) were less than their limits of detection (LOD). Since the metallic constitution of Minjingu has been widely documented (Habashi 1994; Makweba & Holm 1993; Szilas 2002), this study did not conduct analysis of the same.

### *Solubilisation of rock phosphate by isolated strains*

The abilities of fungal and bacterial isolates to solubilise phosphate rock were tested based on whether the microbes originated from Minjingu or Panda Hill and their performances compared across the rock phosphates. Results show that regardless of origin of isolation fungal isolates performed significantly ($P \leq 0.05$) better in solubilising Minjingu rock phosphate than the more complex igneous Panda Hill rock phosphate (Fig. 1A & B). Data showed further that one best performing

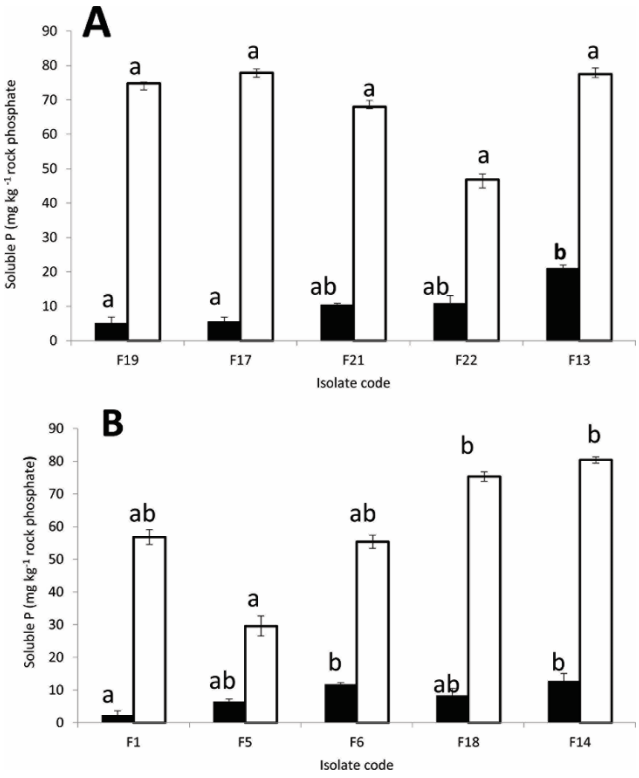


**Fig. 1**. Performance of fungal isolates from Minjingu soils (A) and Panda hill soils, (B) on solubilisation of hard Minjingu (open bar) and Panda Hill (solid bar) rock phosphates. Values are mean±SD.

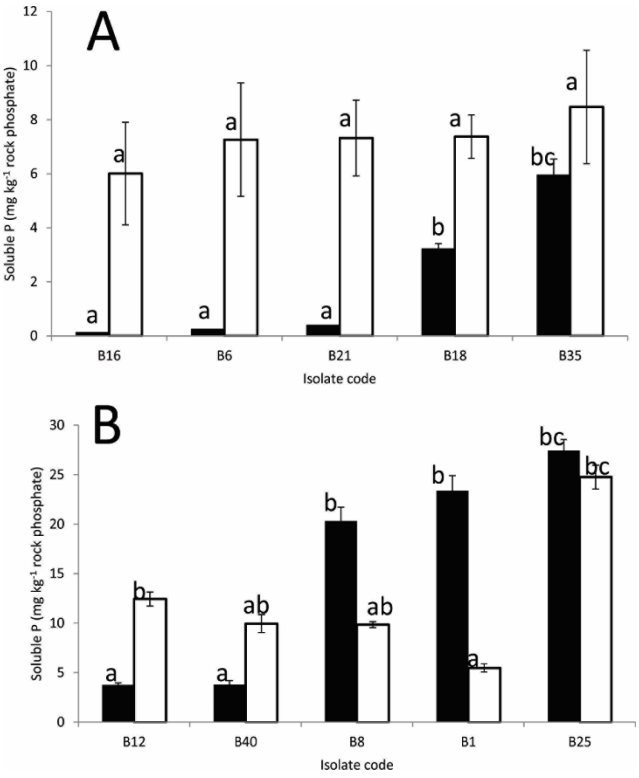


**Fig. 2**. Performance of bacterial isolates from Minjingu soils (A) and Panda hill soils, (B) on solubilisation of hard Minjingu (open bar) and Panda Hill (solid bar) rock phosphates. Values are mean±SD.

Minjingu fungal isolate (isolate F13) was capable of maintaining a comparatively superior performance when this familiar phosphate rock was replaced by the non-familiar source, Panda Hill phosphate rock (Fig. 1B).

A different scenario was observed with bacterial isolates. While bacterial isolates originally isolated from Minjingu soils exhibited a significantly ($P \leq 0.05$) higher performance in solubilising their familiar source of phosphorus-Minjingu rock phosphate (Fig. 1A), mixed results were recorded with panda Hill isolates as some performed better with the unfamiliar Minjingu phosphate while others worked more efficiently with Panda Hill rock phosphate (Fig. 1B). However, an exception was for a bacterial isolate B25 originally from Panda Hill soils which showed higher but non-significantly different levels of performance (f.c. 24.75 mg $kg^{-1}$ of HMRP and 27.45 mg $kg^{-1}$ of PRP) with the non-familiar Hard Minjingu rock phosphate (HMRP). This observation pinpointed the possibility of an isolate from one locality (Panda Hill in this case) to show outstanding performance on an unfamiliar rock phosphate (Minjingu rock phosphate in this case) (Fig. 2B). Similar results were observed with isolate B35, originally from Minjingu but showing significant performance in solubilising Panda Hill rock phosphate (Fig. 2A).

### *Morphological characterization and molecular identification of selected isolates*

Five fungal and five bacterial isolates gauged as best performing among others were selected and subjected to morphological and molecular characterization leading to their identification to species level. Results show that generally, fungal colonies were plentiful with significant sporulation and that microscopically all fungi isolates were filamentous (Fig. 3-I). On the other hand, bacterial colonies were slimy and shiny on the surface, whitish, creamy, yellow to orange in colour and they were observed as single celled entities under a microscope (Fig. 3-II). Key macro- and micro-morphological characteristics of the selected bacterial isolates including lactose fermentation, gram reaction and micro-morphological features are presented in Table 3.

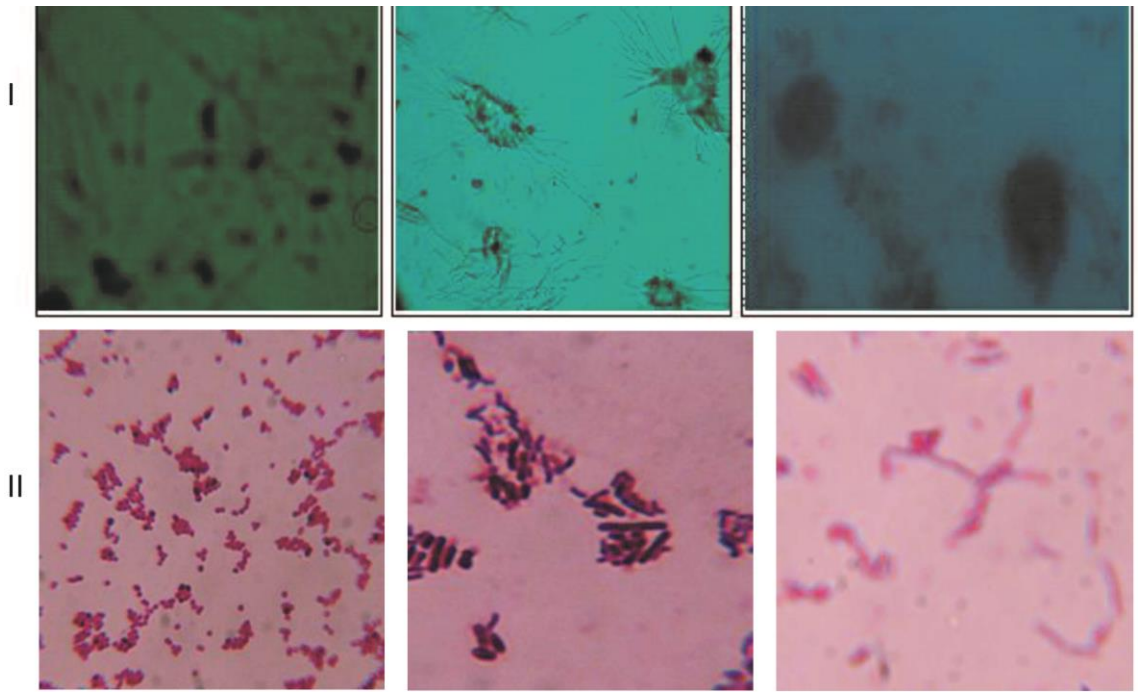

**Fig. 3**. Micrographs of representative phosphate-solubilising fungi (I) and phosphate-solubilising bacteria, (II). Gram +ve isolates appeared purple in colour while the gram –ve isolates retained the pinkish red colour of the counter stain, safranin.

**Table 3**. Macro- and micro-morphology of rock phosphate solubilising bacteria PSB.

| Bacteria | Lactose fermentation | Micro-morphology | Gram stain |
|---|---|---|---|
| Minjingu | | | |
| B6 | NG* | Large rods | + |
| B35 | NG | Short Rods | + |
| B21 | NLF** | Cocco-rods shaped | − |
| B18 | NLF | Small cocci | − |
| B40 | NG | Rods in branches | − |
| Panda Hill | | | |
| B16 | LF# | Rods in chains | + |
| B8 | NLF | Cocci in groups | − |
| B25 | NLF | Large rods in chains | − |
| B12 | NLF | Rods in short chains | − |
| B1 | NLF | Cocci in single cells | − |

*NG = No growth, **NLF= Non lactose fermentor #LF = lactose fermentor.

Four best performing bacterial isolates and five fungal isolates were finally processed through DNA extraction, purification (Fig. 4) and sequencing for eventual molecular identification. Species names of individual isolates were obtained after performing BLASTn on NCBI nucleotide database and results summarised and presented in Table 4. The identified best performers included the fungus *Aspergillus tamarii* and the bacterium *Stenotrophomonas maltophilia,* among others.

## Discussion

The current study was conceived based on one general assumption that two phosphate rocks with essentially different chemical properties would differentially influence the overall solubilisation potential of the microorganisms isolated from soils surrounding them. The observed general tendency of both groups of fungal isolates, regardless of their origin of isolation, to show an increased performance when subjected to HMRP as the only source of phosphorus was consistent with the relatively simple chemistry of Minjingu compared to that of Panda Hill rock phosphate. That is, compared to the more complex igneous PRP, Minjingu- a sedimentary rock phosphate of guano origin presents a higher likely-

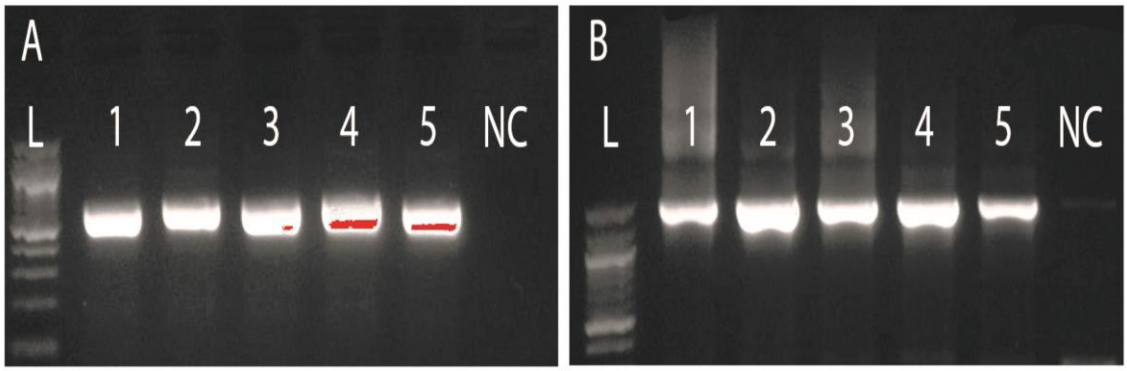


**Fig. 4**. Polymerase chain reaction amplified DNA product of five selected fungal and bacterial isolates. (A) Amplification of the ITS1-5.8S-ITS2 rDNA of five best-performing fungal isolates, (B) Bacterial 16S rDNA amplification used primers 27F and 1492R.

**Table 4.** Identity of selected RP-solubilising species based on nucleotide identity to GeneBank species. Nucleotide identity was 100% for all the species.

| Isolate | Matched Species | Accession Number |
|---|---|---|
| B25 | *Stenotrophomonas maltophilia* | KU726005 |
| B35 | *Bacillus safensis* | KX694275 |
| B18 | *Acenotobacter nosocomialis* | LC014122 |
| B21 | *Acenotobacter baumanii* | KX242271 |
| F13 | *Aspergillus stellifer* | AB248984 |
| F14 | *Aspergillus tamarii* | KP784375 |
| F17 | *Aspergillus flavus* | HQ340108 |
| F19 | *Aspergillus terreus* | KC119206 |
| F22 | *Aspergillus brunneoviolaceus* | FR727129 |

hood of being used in direct applications to agricultural fields with the help of right microbial solubilisers. Moreover, the obser-vation that regardless of origin of isolation, fungal isolates exhibited a higher ability to solubilise rock phosphates than their bacterial counterparts is in line with the report by Gupta *et al.* (2007) who observed that fungal isolates consistently exhibited a higher phosphate rock and tricalcium phosphate solubilization ability than bacterial isolates. Studies elsewhere have implicated the fungal isolates as being efficient rock phosphate solubilisers (Chai *et al.* 2011; Xiao *et al.* 2009; Wu *et. al.* 2012).

With the exception of *Stenotrophomonas maltophilia* (B25) originally from Panda Hill soils, all other bacterial isolates reported herein exhibited a consistently more efficient performance on solubilising familiar rock phosphate samples than those from elsewhere. This behavioural pattern could be attributed to adaptive mechanisms including secretion of inducible enzymes, special siderophores and organic acids following long time exposure to the same (Rodríguez & Fraga 1999). Phosphate-solubilising bacteria have been widely reported in literature with sources of isolation ranging from rhizosphere soils (Gupta *et al.* 2012; Singh & Prakash 2012), industrial and other wastes (Paul & Sinha 2017) or normal soils (Baliah *et al.* 2016).

All the five best performing fungal isolates identified in this study are of the genus *Aspergillus.* This observation is not surprising as there are several other studies in which species of the *Aspergillus* genus have been implicated in phosphate rock solubilization (Gupta *et. al.* 2007; Khan *et al.* 2010; Vazquez *et al.* 2000). Similarly, other studies have reported *Penicillium chryso-genum* and *Aspergillus sp*. innoculation increase biomass production of a multipurpose tree *Dalbergia sissoo* seedlings (Dash *et al.* 2013).

Some fungal and bacterial species, e.g. *Aspergillus terreus* (fungus) and *Stenotrophomonas maltophilia* (bacterium), identified and characterized in the present study, are similar to those reported elsewhere to have the ability to solubilize insoluble P sources *in vitro* (Reddy *et al.* 2002; Vassilev *et al.* 1997). Similarly, *Acinetobacter* spp. has been reported to solubilize insoluble phosphate by production of gluconic acid (Ogut *et al.* 2010). *Acinetobacter nosocomialis* and *A. baumanii* identified in the present study also solubilised insoluble phosphate rocks. Dwivedi *et al.* (2004) reported that pre-plant inoculation of rice seedlings with P-solubilizing *Aspergillus awamori* in a field experiment in India resulted in yield increases as compared to un-inoculated seedlings.

Therefore, there exists the potential to use the microorganisms identified in the present study to develop inoculants for use in the field. So far, no P inoculant of the identified fungal and bacteria strains have been reported in reviewed papers and have otherwise been used as plant growth promoters.

It is worth noting here that two bacterial isolates, identified in the current study as *Stenotrophomonas maltophilia,* (isolated from Minjingu) and *Bacillus safensis* (isolated from Panda Hill), had shown comparatively higher phosphate solubilisation levels when exposed to a foreign unfamiliar rock phosphate as sole source of phosphorus. This observation appears to support the hypothesis that it is possible that some microbial isolates from one particular locally especially influenced by the chemistry of one rock phosphate may have capabilities to solubilise both familiar and non-familiar rock phosphate types with varying degrees of chemical complexity.

## Conclusion and recommendations

All microorganisms isolated were effective, to different extents, in solubilising both Panda Hill and Minjingu PR, giving possibilities for field use of some isolates in solubilising applied water-insoluble PR. The most efficient isolates were fungal isolates, *Aspergillus tamarii, Aspergillus stellifer* and *Aspergillus flavus* and bacterial isolates *Stenotrophomonas maltophilia, Bacillus safensis* and *Acenotobacter baumanii*. These microorganisms could be developed into inoculants for future use to improve P supply to crops.

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