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Effects of Different Cultural Conditions on Phosphate Solubilisation in *Pseudomonas mosselii* D7 and *Aspergillus niger* Vitaf1

Kingsley T. Ughamba[1,2], Christian K. Ezeh[2], Uchenna Oyeagu[2]*, Chioma N. Obiali[1], Anthonia K. Molokwu[1], Boniface C. Mkpadigbo[1], Chisom N. Ogbue[1], Nneoma C. Ojiuba[1],Chinaza S. Nkwor[1], Chioma Oragwa[1]

[1.] *Department of Science Laboratory Technology, University of Nigeria, Nsukka, Nigeria.*
[2.] *Department of Microbiology, University of Nigeria, Nsukka, Nigeria.*

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ABSTRACT

Microbial phosphate (P) solubilisation has been reportedly impacted by various environmental conditions. Therefore, this study was aimed at investigating the effects of different cultural/environmental conditions on phosphate solubilisation in bacteria and fungi. Phosphate-solubilising microorganisms (PSM) were isolated from rhizospheric soil of tomatoes, cowpea, and corn and then screened for phosphate solubilsation on Pikovskaya (PVK) agar. Effects of different environmental conditions on phosphate solubilisation were evaluated by cultivating PSM in National Botanical Research Institute's Phosphate growth medium (NBRIP) amended with different carbon and nitrogen sources, salt concentrations and maintained at varying pH and temperature regimes. Five strains of the microbes produced halo zones on PVK agar. Isolate NF1 had the highest solubilisation index (4.0), followed by NB3 (3.5) and NF2 (3.45), NB1 (2.0), and then NB2 (1.0). In terms of supporting P solubilisation, carbon sources performed in the order: glucose>fructose>sucrose> maltose>mannitol in the best performed fungal (NF1) and bacterial (NB3) isolates ($P \leq 0.05$). Similarly, the nitrogen sources performed in the following order: $(NH_4)_2SO_4$>$NaNO_3$>$Ca(NO_3)_2$>KNO_3 ($P \leq 0.05$)in both NF1and NB3. pH 7.0, 30 ºC and 0% NaCl were found to support the highest quantity of P solubilised. The highest P solubilised was 0.67 mg/mL in NB3 and 0.72 mg/mL in NF1. Given the conditions employed in this study, the fungal isolate was more efficient in phosphate solubilisation than its bacterial counterpart. NF1 and NB3 were identified through microbiological and molecular methods as *Pseudomonas mosselii*d7 and *Aspergillus niger* VITAF1 respectively. Therefore, optimum solubilisation was dependent on the interplay between organisms and their environmental conditions.

Keywords: *Pseudomonas mosselii* D7, *Aspergillus niger* VITAF1, Rhizosphere, Phosphate-solubilisation.

Introduction

Phosphorus (P) is among the most crucial macroelements needed for plant growth and development. Recent research highlights phosphorus (P) as the primary nutrient limiting plant growth.[1]In soil, phosphorus exists in two forms: inorganic and organic. As part of the sedimentary cycle, phosphorus is cycled through geological processes like rock weathering. Notably, 65-85% of the total phosphorus in soil is found in an inorganic form, primarily as phosphate ions (PO_4^{3-}), which are readily available for plant uptake. Organic phosphorus, derived from decomposed organic matter, requires microbial breakdown before it becomes accessible to plants. The P fractionation method classified the inorganic phosphate into five (5) major groups: occluded phosphate, calcium phosphate, iron phosphate, aluminum phosphate, and soluble phosphate. [2]

Corresponding author. E mail: uchenna.oyeagu@unn.edu.ng
Tel: + 2348038728202

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Typically, only a small portion of inorganic phosphate—such as aluminum phosphate, calcium phosphate, and iron phosphate—exists in a soluble form in the soil, which plants can directly absorb. Most soil phosphorus is bound in forms that are not readily available to plants, making phosphorus a limiting nutrient despite its presence in the soil. [3]More than 90% of the phosphate in soil is refractory which has historically been addressed by applying phosphate fertilizers. Nevertheless, the use of chemical fertilizers has given rise to a variety of environmental issues, including water eutrophication and soil pollution.[4] Some estimates suggest that the world's P reserves could run out in 50–100 years.[5] In addition to making effective use of phosphate reserves, it is critical to recover applied phosphate and cut down on the amount of P fertilizer that is now wasted using microorganisms. Because these organisms can mobilize P from sources that are resistant to it, and as such, they are identified as a potential answer to P fertilization management problems. [6]When compared to bacteria, phosphate-solubilising fungi (PSF) are often more capable of liberating P from resistant inorganic P.[7] Up until now, the majority of representative strains of *Penicillium* and *Aspergillus* have been extensively documented as P solubilisers. It has been noted that after repeated sub-culturing in synthetic media, the stability of these fungi's P-solubilising capability is higher compared to that of P-solubilising bacteria.[2] Furthermore, it has been noted that PSF exhibit higher P-solubilising activity and secrete more acids than bacteria.[7] Even though reports indicate that inoculating phosphate-solubilising microorganisms into soil increases the amount of available phosphate by releasing phosphate fixed in soil particles, which improves the growth of plants, the inherent functional ability of these organisms in various environments are yet to be adequately studied.[8] Therefore, the aim of

the present research was to investigate the effects of different environmental conditions on phosphate-solubilising bacteria and fungi. As much as we know, there are currently little or no traceable studies yet on the effects of cultural conditions on phosphate solubilisation in *Aspergillus niger* VITAF1 and *Pseudomonas mosselii* D7

Materials and methods

Sample collection
Rhizospheric soil samples of plants such as tomato, cowpea, and corn were collected in a sterile nylon bag. The soil samples were kept in a nylon bag away from direct sunlight and then transported to the laboratory for commencement of the experiment.

Isolation of phosphate-solubilising fungi and bacteria
A 10 g quantity of each rhizospheric soil was dispensed into a sterile Erlenmeyer flask, to this; ninety milliliters of distilled water was added and agitated. Following ten-fold serial dilutions of the suspensions, 200 µl of the resulting suspension was inoculated onto the surface of nutrient agar (28 g/L) and sabouroud dextrose agar (65 g/L), allowing the cultures twenty-four to thirty-six hours for growth at 37 ^{o}C incubation temperature. Discreet colonies emerging from the resulting plates were sub-cultured into other prepared agar plates, incubated at 37^{o}C for 24 hours in order to obtain pure isolates. The isolates were stored in bijou bottles containing nutrient agar and SDA for bacteria and fungi respectively and refrigerated at 4^{o}C till further use.

Screening for phosphate-solubilising bacteria and fungi
The pure cultures isolated were inoculated on the surface of Pikovskaya agar ((g/L): 10, glucose; 0.5, $(NH_4)_2SO_4$; 0.5, yeast extract; 5, $Ca_3(PO_4)_2$; 0.1, $MgSO_4.7H_20$; 0.2, KCl; 0.002, $FeSO_4.7H_20$; 0.002, $MnSO_4.2H_20$, and 15, agar) and incubated at 30^{o}C for 7 days. Presence of clear halo zones around the colonies was indicative of the presence of either PSF or PSB which grew characteristically on the media due to phosphate solubilisation. Halozone diameters were determined by the difference between the colony diameter and the total diameter.

$$\text{solubilisation index (SI)} = \frac{colony\ diametre + halozone\ diametre}{colony\ diametre}_9$$

*Preliminary Identification of Isolated**bacteria and fungi*
The bacterial isolates were identified by Gram staining and biochemical tests namely: oxidase test, catalase test, starch hydrolysis, methyl red, Voges-Proskauer test, spore forming test, urease test, motility test, citrate utilization test and hydrogen sulphide test. Meanwhile, slide culture technique was used to identify fungi isolates.

Effects of different culture parameters on phosphate solubilisation
Standard cultures of the isolated rhizospheric bacteria and fungi (0.1ml=10^8) were grown in NBRIP and incubated for two days at 37°C at pH 7. NBRIP contains in L^{-1}: 10 g glucose, 0.1 g $(NH_4)_2SO_4$, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2.6H_2O$, 0.25 g $MgSO_4$.$7H_2O$ and 0.2 g KCl. To check the impact of carbon source in the NBRIP medium, NBRIP glucose was changed with the same quantity of sucrose, maltose, lactose or mannitol and incubated on an incubator shaker at 180 rpm for 7 days at 30^{o}C. To determine the impact of nitrogen sources on microbial phosphate solubilisation, $(NH_4)_2SO_4$ in NBRIP was replaced with same amount of $Ca(NO_3)_2$, $NaNO_3$ and KNO_3.The effect of salt concentration (NaCl) on the solubilisation of phosphate was evaluated by cultivating the isolates in NBRIP set-ups amended with varying concentrations of NaCl (0.5, 1, 1.5 and 2% w/v). The pH of the medium was varied by preparing different set-ups at 3.0, 4.5, 6, 7.5 and 9.0. Similarly, temperature effect was determined by varying the incubation temperatures at 25, 30, 37, 40, and 45^{o}C.The phosphate solubilisation in broth was quantitatively estimated using 150 mL capacity Erlenmeyer flasks filled with 10 ml of medium inoculated in triplicate with the fungal and bacterial isolates. The control set-up was autoclaved uninoculated media. The cultures were centrifuged at 10, 000 rpm. The method of Nautiyal[10] was used to estimate phosphate concentration in the culture supernatant.

Molecular identification of isolates
The fungal isolate (NF1) and its bacterial counterpart (NB3) that showed peak phosphate solubilisation potentials were identified using molecular methods. The DNA of the fungal and bacterial isolates were isolated using ZR Fungal/Bacterial DNA Miniprep (produced by Zymo Research).The targeted gene for fungi was 18S rRNA whereas 16S rRNA gene was that of bacteria. Consequently, the fungal target gene was amplified using a pair of primers (ITS F: TCC GTA GGT GAA CCT GCG G) and (ITSR: TCCTCCGCTTATTGATATGS) while (27F: AGAGTTTGATCMTGGCTCAG) and (1525R: AAGGAGGTGWTCCARCCGCA) were primers used for bacterial gene amplification. A 0.9 % agarose was used for their respective electrophoresis. Thereafter, visualization of DNA fragments of both organisms was carried out using transilluminator. Sequencing of extracted DNA fragments was achieved with BigDye terminator v3.1 cycle sequencing kit and Genetic Analyzer 3130 xl sequencer (Applied Biosystems). The phylogenetic tree was made using MEGA X version 10.0.5.

Statistical analysis
One-way analysis of variance (ANOVA) was applied to the data collected for this investigation. Using least square difference and post-hoc multiple comparison tests, the relationship between the variables and mean differences was examined for significance level at $P \leq 0.05$. SPSS was used to analyze the data.

Results and Discussion

Gram staining and biochemical results showed that nine (9) isolates were Gram positive and tested positive to methyl red, motility, starch hydrolysis, and spore-forming ability while negative to catalase, oxidase, urease, and citrate test and the probable organisms were *Bacillus* spp. Also, seven (7) isolates were methyl red, catalase, oxidase, urease, citrate, and H_2S-positive depicting *Micrococcus* spp as the probable organisms while five (5) isolates were urease, citrate, spore forming, and H_2S positive and the probable organisms is *Streptomyces* spp. Four (4) bacterial isolates were Gram-negative but catalase, oxidase, urease, motility, and citrate positive and the probable organisms is *Pseudomonas* spp. Bacterial isolation from rhizospheric soils of tomatoes was published in a previous work by Danaski*et al*. [11] In the report, series of bacteria were isolated especially *Pseudomonas* spp which is similar to the current study (Table 1). Morphological characteristics of fungi were used to tentatively identify the fungal isolates. Result showed that *Aspergillus* spp, *Trichoderma* spp, and *Rhizopus* spp were isolated as presented in table 2. This is similar to a research carried out by Danaski *et al.* [11] who reported the isolation of *Rhizopus* spp and *Aspergillus* spp from tomato rhizospheric soils.

Screening of PSMs
In the present study, five (5) distinct colonies of both bacteria and fungi were able to solubilise phosphate evidenced by the production of zones of hydrolysis on Pikovskaya (PVK) agar medium and were thus selected as positive for phosphate solubilisation. These five colonies include 3 bacteria (*Bacillus* sppNB1, *Micrococcus* sppNB2, and *Pseudomonas* sppNB3) and 2 fungi (*Aspergillus* spp NF1 and *Rhizopium* sppNF2) (Table 3). It was observed that *Aspergillus* spp (NF1) had the highest SI (4.0), followed by *Pseudomonas* spp(NB3) (3.5), *Rhizobium* spp (3.45), *Bacillus* spp (2.0) NB1, and *Micrococcus* sppNB2(1.0) (Table 3). Comparative studies noted similar observation.[12, 13] They acknowledged that the production of clear zones of hydrolysis around the bacterial or fungal colonies on PVK agar plates (Figure 1) were indicative of P solubilisation. The present study also agrees with that of Dhanya et al. [14] who reported phosphate solubilisation in the order *Aspergillus niger*>*Pencillium*sp>*B. subtilis*>*Micrococcus* sp. Observably, fungal isolates performed better in solubilisation evidenced by higher solubilisation indices (Table 3).

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Fungi are better genetically endowed to withstand metabolic stress especially in harsh environmental conditions. This attribute might probably be the reason for the observed trend.

Table 1: bacteria isolate identification

Isolates no.	Gram reaction	Methyl red	Catalase	Oxidase	Urease	Starch hydrolyzing	Motility	Spore formation	H_2S	Citrate	Probable organism
9	+	-	-	-	-	+	+	+	-	-	*Bacillus* sp
7	+	+	+	+	+	-	-	-	+	+	*Micrococcus* spp.
5	+	-	-	-	+		-	+	+	+	*Streptomyces* spp.
4	-	-	+	+	+	+	+	-	-	+	*Pseudomonas* spp

Table 2: Fungal isolates identification

Isolates	Microscopy	Morphology	Probable organism
3	Filamentous with darkish brown conidia	Spore morphology	*Aspergillus spp*
2	Dense conidia with yellow greenish pigmentation	Spore morphology	*Trichoderma* spp
2	Non-septate hyhae sporangia	Spore morphology	*Rhizopus* spp

Table 3: Qualitative Estimation of Solubilised Phosphate

Isolates	Colony diameter (mm)	Halo zone diametre(mm)	Solubilisation Index(SI)
Bacillus spp (NB1)	1.5	3.0	3.00
Micrococcus spp (NB2)	2.0	2.0	2.00
*Pseudomonas*spp (NB3)	2.0	5.0	3.50
Aspergillus spp (NF1)	2.0	6.0	4.00
Rhizobium spp (NF2)	2.2	5.4	3.45

Effect of different carbon sources on phosphate solubilisation in bacteria and fungi

In this study, the impact of carbon sources on microbial phosphate solubilisation is presented in Table 4. Notably, NF1 had the highest amount of phosphate solubilised (0.72 mg/mL) in the control microcosm (NBRIP with glucose alone) (Table 4). Several studies reported glucose as being the preferred carbon source for phosphate solubilization1.[15,16] In the present study, NF1 solubilised higher quantities of phosphates in three out of the five NBRIP set-ups than its bacterial counterpart (NB3). Also, it was found that apart from the control microcosm which was amended with glucose, fructose was the second best in terms of supporting microbial phosphate solubilisation.

It has been shown that the most prevalent sugars in plant exudates are glucose and fructose which probably influence the population of phosphate-solubilising microbes in the associated rhizosphere and the availability of appropriate substrates serves as an attracting factor for certain microbial groups in the soil environment.[17] Furthermore, glucose and fructose being monosaccharides (hexose sugars) are more easily oxidized by microbes for generation of energy to drive cellular work. This might account for the higher quantities of phosphates released in the set-ups prepared with glucose (control) and fructose. A comparative study noted similar observation.[18] However, the difference was not significant ($P \leq 0.05$) between glucose and sucrose in terms of supporting phosphate solubilisation in NF1. This may not be unconnected to the fact that sucrose is easily broken down to glucose and fructose in the presence of invertase enzyme, and hence would

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behave almost alike and chemically so. Similarly, the difference was not significant in relation to supporting phosphate solubilisation between fructose and maltose in NB3. Nevertheless, apart from the afore-mentioned, there were significant differences among other carbon sources employed in this study (P≤0.05) (Table 4).Overall, the phosphate solubilisation activities of bacteria and fungi are associated with their production of organic acid which is also determined by the supplied carbon source(s).

Figure 1: Different pikovskaya plates showing halozone formation

This can be seen in investigation carried out by Vassileva et al. [19] and Sanchez-Gonzalezet al.[20]who reported that some bacterial strains solubilise in the presence of either glucose alone or glucose being the preferred carbon source among other carbon sources. In similar studies that used sucrose but not glucose, sucrose was the most preferred carbon source for solubilisation of phosphate by *A.tubingensis*, [21] and *P. purpurogenum.* [22]

Effect of nitrogen sources on phosphate solubilisation in bacteria and fungi

The impact of nitrogen sources on microbial phosphate solubilisation is shown in Table 5. The control set-up which was amended with ammonium sulphate supported the release of the highest amount of phosphate in the medium in both NF1 and NB3. Similar studies noted the same observation.[18,23] Apart from the control microcosm, $NaNO_3$ supported solubilisation of the highest amount of phosphates in both NF1 and NB3 and notably so (0.44 mg/mL and 0.43 mg/mL respectively) (Table 5). However, it can be seen that the fungus NF1 performed better in terms of phosphate solubilisation than its bacterial counterpart NB3 in the presence of $NaNO_3$. $Ca(NO_3)_2$ performed less than $NaNO_3$ while KNO_3 was the least in supporting phosphate solubilisation in bacterium NB3 in this study. The same order was noticed in the fungus NF1 though with higher quantities of phosphate released. A comparative study by Relwani et al. [21] however reported KNO_3 as the most preferred source of nitrogen for solubilsation of insoluble phosphate in fungus *Aspergillus tubingenesis*. Among the nitrogen sources, there was no significant difference in the effects of KNO_3 and $Ca(NO_3)_2$ in NF1 while there were significant differences among all other nitrogen sources employed in this study in NB3 (P≤0.05) (Table 5). Though, this study limited nitrogen sources to inorganic types, some studies also reported that organic nitrogen sources performed highly in terms of supporting microbial solubilisation. For example, Sagervanshi et al. [24] highlighted casein as best nitrogen sources for solubilisation in PSB. Yeast extract or trypton was also shown as the most preferred nitrogen source.[25] However, it was widely documented that inorganic sources of nitrogen are better than their organic counterparts. [25,26]Numerous further investigations have been conducted to assess the impact of various nitrogen sources on phosphate-solubilising organisms. Abdelhady et al.[25] tested the outcome of inorganic and organic nitrogen sources and they reported that all tested isolates gave higher rock phosphate solubilisation in the presence of organic nitrogen sources than in inorganic sources. The fact that yeast extract and tryptone are rich in growth factors and nitrogen—two essential nutrients for fostering microbial growth and phosphate solubilisation—may emphasize the beneficial effects of these substances on boosting rock phosphate solublisation by the isolates..

Effects of pH, temperature and salt concentration on phosphate solubilisation

The effect of pH on the microbial phosphate solubilisation is shown in Table 6. Neutral pH (7.0) supported solubilisation of highest quantity of phosphate (0.72 mg/mL) in NF1while 0.67 mg/mL phosphate was released in the bacterial counterpart NB3 at the same pH level Similar observations have been widely documented.[27, 28] In fact, it was observed that the highest phosphate solubilisation occurred within pH 6.5-7.5 [27]. However, Wang et al. [29] reported that bacterial strains solubilise phosphates under acidic condition. In both NF1 and NB3, there were significant differences between neutral pH and the other pH levels tested in this study in terms of promoting phosphate solubilisation apart from pH levels close to neutral (6.5-7.5) (Table 6).The effect of temperature on phosphate solubilisation is shown in Table 7. Phosphate solubilisation increased from 25 ºC and peaked at 30 ºC in both NF1 and NB3. At 30 ºC, NB3 exhibited highest phosphate solubilisation (0.67 mg/mL) while NF1 exhibited highest phosphate solubilisation at the same temperature though with higher quantities of solubilised phosphates (0.72 mg/mL). Optimum temperature was noted as a requisite parameter for microbial growth and hence one of the critical parameters for microbial phosphate solubilisation. [27]Walpola et al. [30] equally noted a similar trend in which *Bulkholderia* spp solubilised tricalcium phosphate maximally at 30-35ºC in a Korean soil. There were significant differences among all the test temperature regimes in this study in both isolates (Table 7).

Table 4: Effects of carbon sources on phosphate solubilisation

NBRIP	Concentration of phosphate solubilised (mg/ml)	
	NB3	NF1
NBRIP (control)	0.67±0.002[a]	0.72±0.004[a]
NBRIP amended with fructose	0.59±0.003[b]	0.62±0.003[b]
NBRIP amended with maltose	0.29±0.001[b]	0.32±0.001[c]
NBRIP amended with mannitol	0.03±0.002[c]	0.04±0.002[d]
NBRIP amended with sucrose	0.32±0.0.001[d]	0.34±0.003[a]

Values with different superscript letters indicate significant difference while same letters indicate no significant difference (p≤ 0.05)

Table 5: Effects of nitrogen sources on phosphate solubilisation

NBRIP amended with different nitrogen sources	Concentration of phosphate solubilised (mg/ml)	
	NB3	NF1
NBRIP (control)	0.67±0.005[a]	0.72±0.004[a]
NBRIP amended with KNO_3	0.28±0.003[b]	0.19±0.002[b]

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NBRIP amended with $Ca(NO_3)_2$	0.32±0.001[c]	0.36±0.003[b]
NBRIP amended with $NaNO_3$	0.43±0.002[d]	0.44±0.001[c]

Values with different superscript letters indicate significant difference while same letters indicate no significant difference ($p \leq 0.05$)

Table 6: Effect of pH on phosphate solubilisation

NBRIP adjusted to different pH values	Concentration of phosphate solubilised (mg/ml)	
	NB3	NF1
NBRIP adjusted to pH7 (control)	0.67±0.003[a]	0.72±0.001[a]
NBRIP adjusted to pH 3.0	0.09±0.005[b]	0.10±0.004[b]
NBRIP adjusted to pH 4.5	0.07±0.003[c]	0.08±0.003[b]
NBRIP adjusted to pH 6.0	0.52±0.004[d]	0.65±0.003[a]
NBRIP adjusted to pH 7.5	0.63±0.001[d]	0.68±0.002[a]
NBRIP adjusted to pH 9.0	0.44±0.002[e]	0.52±0.001[c]

Values with different superscript letters indicate significant difference while same letters indicate no significant difference ($p \leq 0.05$)

Similarly, the effect of salinity is shown in Table 8. The control set-up (NBRIP without NaCl) supported the highest amount of solubilised phosphate in both NF1 and NB3. NBRIP already contains some amount of salt in the form of KCl. This might account for the declining phosphate solubilisation observed with increasing NaCl concentrations as high salt concentration constitutes some physiological stress on microbial isolates. NB3 tolerated increasing salt concentration and peaked at 1 % and started declining from concentrations of 1.5 % -2%. NF1 tolerated increasing salt concentrations up to 1.5% and declined at 2%. In a comparative study by Srividya et al. [31], *Aspergillus* sp F7 tolerated NaCl concentrations up to 2%. *Klebsiella oxytoca* was found to tolerate only 2.5% NaCl among three other higher concentrations tested namely 5%, 7.5% and 10% in the study carried out by Walpola et al. [32]Maheswar and Sathiyavani[33] and Patil[34] in similar studies using *Bacillus subtilis* also recorded maximum phosphate solubilisation at 2.5% NaCl. It is equally worthy of note that there were significant differences in the effects of salt concentrations in terms of supporting phosphate solubilisation in both isolates (Table 8).
Generally, reports on microbial phosphate solubilisation have been inconsistent in literature. This can be attributed to the interdependence of phosphate solubilisation with the physiology, metabolism, and genetic makeup of individual microbial species.

Table 7: Effect of temperature on phosphate solubilisation

NBRIP adjusted to different temperature (°C)	Concentration of phosphate solubilized (mg/ml)	
	NB3	NF1
NBRIP at 30 (control)	0.67±0.003[a]	0.72±0.002[a]
NBRIP at 25	0.09±0.004[b]	0.23±0003[b]
NBRIP at 37	0.52±0.001[c]	0.54±0.001[c]
NBRIP at 40	0.07±0.002[d]	0.13±0.004[d]
NBRIP at 45	0.05±0.001[d]	0.10±0.005[e]

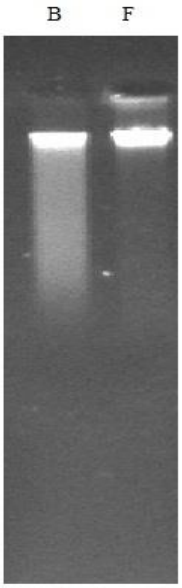
Values with different superscript letters indicate significant difference while same letters indicate no significant difference ($p \leq 0.05$)

Table 8: Effect of salt on phosphate solubilisation

NBRIP adjusted to different salt concentration	Concentration of phosphate solubilized (mg/ml)	
	NB3	NF1
NBRIP + 0% NaCl (control)	0.67±0.004[a]	0.72±0.005[a]
NBRIP+0.5%NaCl	0.07±0.001[b]	0.08±0.001[b]
NBRIP+1%NaCl	0.09±0.002[c]	0.11±0.002c
NBRIP+1.5%NaCl	0.10±0.001[d]	0.14±0.001[d]
NBRIP+2% NaCl	0.11±0.003[e]	0.13±0.002[e]

Values with different superscript letters indicate significant difference while same letters indicate no significant difference ($p \leq 0.05$)

Molecular identification
The two isolates (NB3 and NF1) used were identified through molecular methods. Gel electrophoresis of the extracted DNA is shown in Figure 2 while Figure 3 shows the amplified ITS region of the isolates. To confirm the species, the D1/D2 domain of the ribosomal RNA and the nucleotide sequence of the ITS region were analysed. A phylogenetic analysis of the D1/D2 domain and the ITS region showed that isolate NB3 has 85.19 pairwise identity to *Pseudomonas mosselii* strain d7 with NCBI accession number MK956140.1 (Figure 4) while isolate NF1 98.99% Pairwise Identity to *Aspergillus niger* strain VITAF1 with NCBI accession number KU865178.1 (Figure 5).



Gel image showing high molecular weight DNA extracted from the isolates.

Figure 2: Gel electrophoresis of extracted DNA of isolates; B stands for bacterium (NB3) while F stands for the isolated fungus (NF1)

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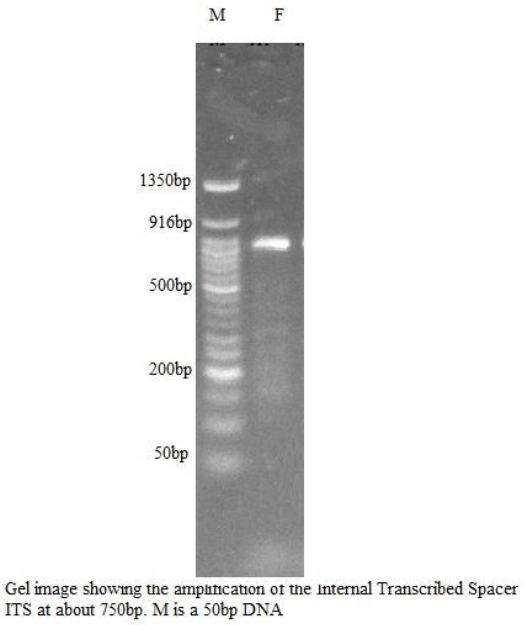

Figure 3: Gel electrophoresis of amplified ITS region of isolates at 750 bp; M stands for the isolated bacterium (NB3) while F stands for the isolated fungus (NF1)

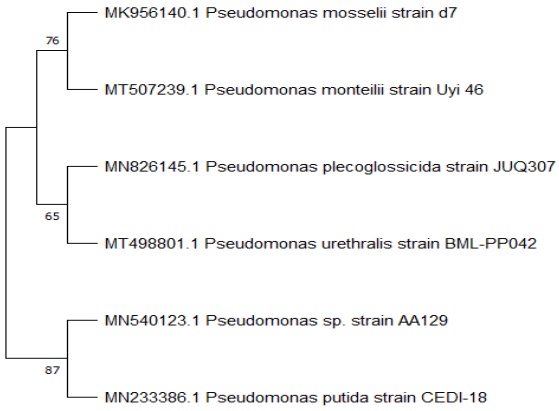

Figure 4: Phylogenetic tree constructed from multiple sequence alignment of nucleotide sequences similar to isolate NB3. Strain in bold italic with the ascension number MK956140.1 has the highest pairwise identity of 85.19%.

Conclusion

In the current investigation, the impact of different cultural conditions on microbial phosphate solubilisation in *Pseudomonas mosselii* D7 and *Aspergillus niger* VITAF1 was investigated. From the viewpoint of supporting microbial phosphate solubilisation, glucose was shown to be the most preferred carbon source among fructose, sucrose, maltose and mannitol. Similarly, ammonium sulphate was found to be the best nitrogen source among sodium nitrate, calcium nitrate and potassium nitrate. The pH 7, temperature 30°C and 0% NaCl supported the release of the highest quantity of phosphate. Fungal isolate showed more efficient phosphate solubilisation than bacterial isolate. In this study therefore, environmental conditions such as temperature, pH, salts, carbon, and nitrogen sources were critical to maximizing the phosphate-solubilising activity of these microorganisms. Understanding and managing these environmental factors can enhance the use of PSMs in sustainable agriculture, improving soil fertility and promoting plant growth. Future research should focus on identifying and engineering robust PSM strains capable of thriving in diverse and challenging environmental conditions to expand their applicability in various agro-ecosystems.

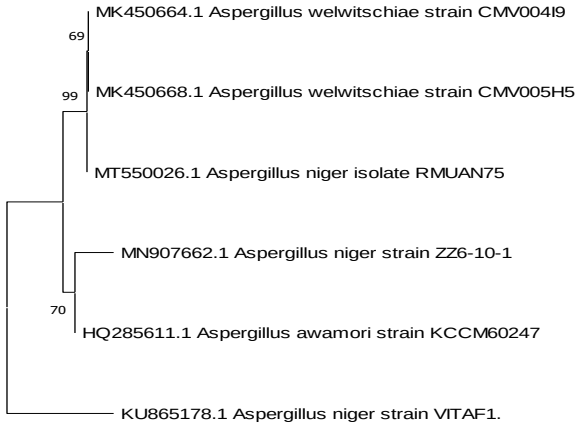

Figure 5: Phylogenetic tree constructed from multiple sequence alignment of nucleotide sequences similar to isolate NB3. Strain in bold italic with the ascension number KU865178.1. has the highest pairwise identity of 98.99%.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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