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Isolation of Phosphate Solubilizing Microorganisms from Geographical Indication Tagged Shankarpura Jasmine [*Jasminum Sambac* (L.) Aiton] and their Plausible Role in Plant Growth Promotion

Ramya Rai, M.[1] & Raghavendra Rao, B[2]

[1]Department of Microbiology & Alva's Centre for Research in Biotechnology (Affiliated to Mangalore University) Alva's College (Autonomous), Vidyagiri, Moodubidire, D.K.(District), Karnataka State, India

[2*]Department of PG Studies in Biotechnology, Alva's College (Autonomous), Vidyagiri, Moodubidire-, D.K.(District), Karnataka State, India

Corresponding Author**: Raghavendra Rao B.**

Abstract: Phosphorus is one of the important major nutrientsneeded for plant growth. Although the concentration of phosphorus is abundant in most of the soils, its availability is limited due to different soil factors.**Problem:** To improve P nutrition excess amount of inorganic phosphatic fertilizers are used. This leads to environmental pollution and increased cost of cultivation. The present study was conducted on a Geographical Indication tagged Jasmine cultivar called Shankarpura Jasmine. Farmers growing this crop use chemical inputs indiscriminately to meet its huge demand in the market.**Approach:** In this study, native phosphate solubilizers were isolated and characterized for their plant growth promotional properties under *in vitro* and *in vivo* conditions. **Conclusion:** Five representative phosphate solubilizing microbes were isolated from ShankarpuraJasmine rhizosphere. Isolate RPS – 76 was found to be efficient with respect to plant growth promotion compared to other isolates and it was identified as *Aspergillusniger* based on ITS sequencing.

Introduction

Phosphorus is one among the 17 vital elements prerequisite for plant growth (Bieleski,1973; Raghothama,1993). In plants, it plays diverse roles like nucleic acid synthesis, respiration, glycolysis signal transduction, nitrogen fixation and many more (Vance et al.,2003).The total P in the soil is very high, but it is mostly unavailable for plant uptake. In acidic soils, unavailability of this element is due to its susceptibility to form complexes with cations such as aluminium and iron whereas, under alkaline soils, it forms complex with calcium ion (Johan et al., 2021). As a result, a large fraction of applied P is bound to soil and unavailable to the plants whereas, only small a fraction of soil P is available for plant uptake. Therefore, it is anutmost limiting nutrient element for plant growth and development.

The conventional method of addressing the issue of available soil P deficiency is addition of excess amount of inorganic phosphaticfertilizers. This method suffers from serious disadvantages *viz*., ground water contamination of P leading to poor quality of water, eutrophication and growth of harmful algae (Alori et al.,2017). Along with all these limitations, high dose of fertilizer application is also burden to farmer as it increases cost of cultivation. On the other hand, there is growing concern among farmers regarding adverse impact of excessive use of chemical fertilizers.Simultaneously, efforts are being made to identify alternative and inexpensive technology that could reduce the dependence on exorbitantly costly phosphatic chemical fertilizers(Zaidi et al., 2009; Mei et al.,2021). One of the most accepted alternative methods found in this regard is use of soil microorganisms which has ability of converting the bound soil P into available form(Mei et al.,2021). Such organisms are popularly known as Phosphate Solubilizing Microorganisms (PSMs).They are also known to enhance plant growth by several mechanisms like releasing insoluble P and producing phyto-hormones like Auxins and Gibberllins(Kalayu,2019).

Considering these aspects a study was conducted on a use of plant growth promoting PSMs in Geographical Indication (GI) tagged Jasmine called Shankarpura Jasmine [*Jasminumsamac* (L.) Aiton] having huge demand in the market for its fragrant flower bud. The crop selected in the present study was reported to be responsible for improvement rural economy, poverty elevation and food security (Handy et al. 2011). By cultivating this crop, many women farmers in that location having very small land holdings earn money which is an additional income to the family. The objective of this study is to isolate representative PSMs from the Shankarpura Jasmine rhizosphere and evaluate for its plant growth promotional properties.

Material and Methods

Collection of rhizosphere soil samples

Five well maintained Jasmine gardens of Shakarpura(Udupi District, Karnataka State, India) were selected. Details of sampling locations are given in Table1. The representative rhizosphere soils from two plants from each garden were aseptically collected in a sterilized polythene bag. Samples were brought to lab and stored at 4^0 C until use.

Table 1: Details of Sampling Location

Location	Latitude	Longitude
1	13.259139	74.771672
2	13.256178	74.774898
3	13.261779	74.767479
4	13.261507	74.767177
5	13.260136	74.773157

Isolation of PSMs

Isolation of PSMs from the representative Jasmine rhizosphere soil samples was performed by serial dilution and spread plate technique using Modified Sperber's medium (Malboobi et al.,2019). Modified Sperber's basal medium was prepared by adding10g glucose, 0.5g yeast extract,0.25g $MgSO_4.7H_2O$, 0.1g $CaCl_2$, 15g Agar, Distilled water 1000ml and pH 7.0-7.2. To the 100 ml basal medium 3 ml of 10% $CaCl_2$ and 2 ml of 10% K_2HPO_4 were added so as to make modified Sperber's medium. Plates were incubated at 28 ± 2^0C 24hours and the representative colonies exhibiting clear zones or halo zones were purified and stored at refrigerated condition until use.

Morphological and Biochemical Characterization

Fungal isolate:

Macroscopic properties of fungal colonies on Potato Dextrose Agar (PDA) and microscopic properties like type of hyphae and spore characters were recoded.

Bacterial isolates:

The properties like shape, Gram reaction, motility, starch hydrolysis, gelatin liquefaction oxidase test, catalase test, utilization of different sources of carbohydrates and siderophore production were performed as per the standard procedures (Aneja,2007)

Green Gram Bioassay

Plant Growth Promoting effect of isolated PSM was evaluated *in vivo* by conducting green gram seed germination bioassay (Garuba et al.,2015). Isolates of PSMs were grown on Modified Sperber's Broth in culture tubes for one week. After incubation, the cultures were centrifuged at 10,000rpm for 20min. Green gram seeds were surface sterilized with 70% ethanol in sterile water and then aseptically rinsed in three changes of water. From the surface sterilized seeds, sixty seeds of uniform size were selected and soaked in the culture supernatant

for about 12 hours. The seeds were aseptically rinsed in sterile distilled water. From these, twenty seeds were placed aseptically in a sterile petriplate containing three layered wet blotting paper (20 seeds per isolate x3 Replications=60 seeds). Seeds treated with uninoculated sterile broth supernatant were used as control.Three replications were kept per each phosphate solubilizing isolate culture supernatant. After 17 hours, the germination percentatge was recorded

Indole Acetic Acid (IAA) Producing Ability of PSMs

IAA producing ability of the PSM isolates was characterized by following Salkowski method (Gang et al., 2019). The selected isolates were grown in modified Sperber'sbroth medium (SBM) supplemented with 0.5mgl^{-1} of L-Tryptophan incubated at 28±2^0 C under continues agitation (120 rpm) for 3 days (Vinod Kumar et al., 2017).After the incubation, the liquid culture was centrifuged and the supernatant was mixed with Salkowski's reagent (150ml of concentrated sulfuric acid , 250 ml of distilled water and 7.5ml of 0.5M $FeCl_3$.$6H_2O$) in a ratio of 1:2 (v/v) ratio. This mixture was allowed to stand for 20 min resulting in development of pink colour. The quantity of IAA produced was estimated by measuring intensity of pink colour developed at 530nm with double beam Systronics PC based double beam UV-Visible spectroscope Model 2202. The experiment was conducted in triplicate.

Gibberellic Acid (GA) Producing Ability of PSMs

The isolated PSMs were inoculated into Modified Sperber's broth medium and incubated for 7 days at 30±2^0 C. After incubation, the cultures were centrifuged at 10,000rpm for 20min.The supernatant was extracted by ethyl acetate 2:1 (v/v) after reducing the pH to 2.5 using 1M HCl. The absorbance of the resultant solution was measured at 254 nm . The concentration of gibberellic acid was determined using standard graph as describe by Berrios et al (2004).

Evaluation of phosphate solubilizing ability

To evaluate the phosphate solubilizing ability of PSM isolated in the present study, they were grown in Modified Sperber's medium for 48 hours, inoculated at the rate of 1ml (containing 1x10^8 CFU/ml in case of bacteria and 1x10^8 spores/ml in case of fungal cultures) to the media containing different insoluble P sources , *viz*., Di-calcium Phosphate, Tri-calcium phosphate and Rock phosphate) and after 10 days of incubation observed for water soluble phosphorus (WSP) and pH. The modified Sperber's medium is a Di-calcium phosphate containing medium. In case of medium with rock phosphate as the source of phosphate, the tri -calcium phosphate in the Pikovskaya's medium (Pikovskaya, 1948) was replaced with same quantity of Udaipur rock phosphate. After incubation, cultures were centrifuged at 5000rpm for 15min and the supernatant obtained was filtered throughWhatman no.1 filter paper. The pH and

WSP of filtrate were determined as per the procedure outlined by Mayadunna et al. (2023).

Pot culture experiment

Pot culture experiment was performed to evaluate the plant growth promotional ability of PSMs isolated.The experimental approach followed was Completely Randomized Design (CRD) with seven treatments and three replications. The treatment details are as follows:

T1, Control (un inoculated with sterile modified Sperber's broth medium); T2, culture homogenate of isolate WPS 20; T3, culture homogenate of isolate WP-12; T4, culture homogenate of isolate – WP 40; T5, culture homogenate of isolate NPS-18; T6, culture homogenate of isolate RPS-02 and T7, culture homogenate of isolate RPS-76.

Phosphate solubilizing microbial isolates were inoculated to Modified Sperber's medium and then incubated for seven days at 28±2^{0}C. Each cultures were seperately homoginzed and used for inoculation to Jasmine plants.Jasmine plants used in the present study were developed as follows.

Stem cuttings of Shankarpura Jasmine plants of equal length were rooted on sterile medium consiting of 1:2 proportions of sand and soil in sterile plastic pots of 14.2cm diameter and 15 cm height. The cuttings were maintained in green house. From the rooted cuttings, 21 plants having 5 leaves and almost similar growth were selected and treatments as described above were imposed (7 treatments in 3 replications=21 plants) .The homogenate of each isolates were aseptically added @20ml/pot using a sterile measuring cylinder. Plants were watered with sterile water regularly to avoide moisture stress. Plant height, number of leaves and number of internodes were recorded 180 days after treatment.

Molecular Identification

Identification of best performing fungal isolate was achieved by following molecular method. The genomic DNA from the fungus was extracted from one week old culture using EXpure Microbial DNA isolation kit developed by BogarBiobee Store Private Ltd, Coimbator, India. The primers (ITS 1 and ITS 4) were used to amplify ribosomal ITS (internal transcribed Spacer). Polymerase Chain Reaction (PCR) products were purified by removing unincorporated PCR primers and dNTPs using Montage PCR clean up kit (Millipore)

Sequencing and Analysis

The PCR products were sequenced using ABI 3730xl sequencer (Applied Biosystems). Using BLAST search tool in GenBank (NCBI), the obtained sequences were compared. For multiple alignments of sequences, program MUSCLE 3.7 was used (Edgar,2004) . From the obtained aligned sequences, poorly aligned and divergent regions were removed using the program Gblocks 0.91b. The program

PhyML 3.0 aLRT was used for phylogeny analysis. Finally using the program Tree Dyn 198.3 tree rendering was achieved(Dereeper et al., 2008).

Statistical analysis

Statistical analysis was performed using one-way ANOVA with significance threshold set at $p \leq 0.05$, followed by post hoc Tukey's test using GRAPES 1.0.0 an online agricultural statics tool (Gopinath,2021).

Results

Three bacterial and two fungal phosphate solubilizing microbial isolates were isolated from the representative Shankarpura Jasmine rhizosphere soil samples using modified Sperber's medium. The isolates were chosen based their ability to form clear zones on the medium (Plate 1).

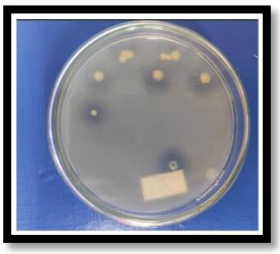

Plate 1: Clear zone forming phosphate solubilizing microbial colonies on Sperber's medium

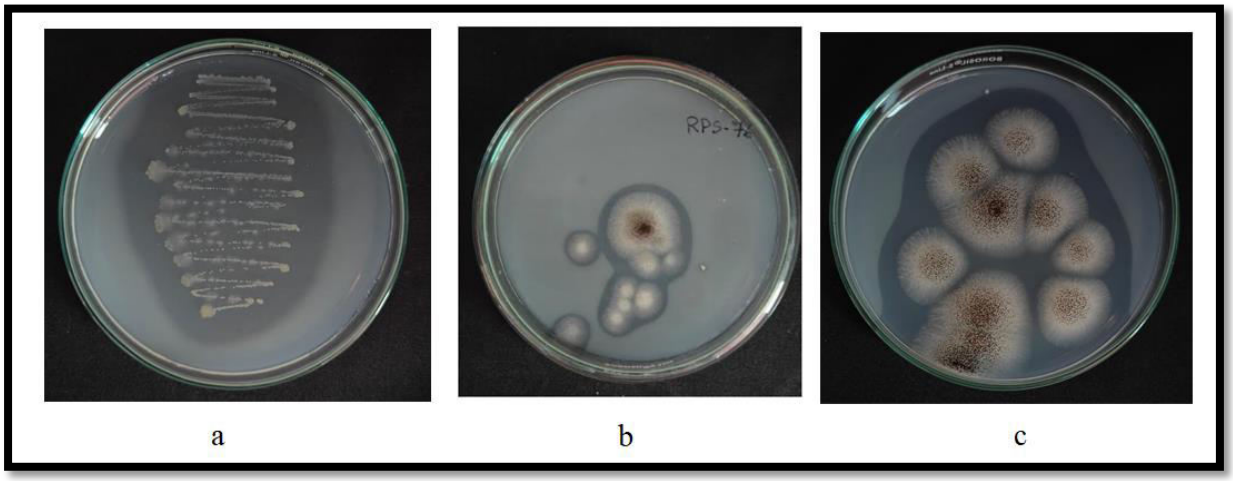

Plate 2: Efficient phosphate solubilizing isolates obtained in the study (a) WP-40 (b) RPS-76 and (c) RPS-2

The morphological and biochemical properties of bacterial and colony characters of fungal isolates obtained from the sample are presented in table 2 and 3 respectively. The bacterial isolates WPS-20, WP-12 and WP-40 were respectively Gram positive cocci, Grampositive bacilli and Gram negative bacilli respectively. Among three isolates, WP-12 and WP-40 were motile and WPS -20 was non motile. Isolate WP -40 showed positive result for starch hydrolysis and WP-12 showed positive result for gelatin hydrolysis. All the

isolates were positive for siderophore production. The clear zone produced by the efficient bacterial and fungal phosphate solubilizers are shown in Plate 2.

Table 2: Morphological and biochemical properties of phosphate solubilizing bacteria isolated from Shankarpura Jasmine rhizosphere soils

Characters	Isolate		
	WPS-20	WP-12	WP-40
Colony Characters	White colored, Small round, raised, entire margin	Cream colored, dry, raised, small, round, entire margin	Cream coloured, irregular, wavy margin, translucent
Shape	Cocci	Bacilli	Bacilli
Gram Reaction	Positive	Positive	Negative
Motility	Non Motile	Motile	Motile
Endospore production	Negative	Positive	Negative
Oxidase	Positive	Negative	Positive
Catalase	Positive	Negative	Positive
Starch hydrolysis	Negative	Negative	Positive
Gelatin hydrolysis	Negative	Positive	Negative
Siderophore	Positive	Positive	Positive

Table 3: Colony characteristics of phosphate solubilizing fungal isolates obtained from shankarpura Jasmine rhizosphere soils

Isolate	Colony character
RPS 76	On PDA, initially colonies were white in color and after 4-6 days of inoculation. Back side of the plate the colonies were light yellow in color. Under microscope, globose dark colored conidia were observed. Condiophores are long and hyaline in nature.
RPS 2	Colonies were exhibiting slow growth. The colonies were showing white color at the peripheral region. At the center colour of the colony was brown to black. Back side of the plate was cream to light yellow in color. Under microscope, globose to subglobose dark colored conidia were observed. Condiophores are long and hyaline in nature.

Isolates RPS-76 and RPS -2 were identified as *Aspergillus*based on colony

morphology and microscopic observation(presented in table 3). The Isolate RPS 76 was identified as *Aspergillusniger*based on ITS sequencing.

The ability of different phosphate solubilizing microbial isolates to release WSP from three insoluble phosphorus sources *viz.*, Tri-calcium phosphate, Di calcium phosphate and rock phosphate is presented in Table 4. Isolate WP-40 and RPS-76 released significantly high amount of water soluble phosphates from tri-calcium phosphate (217.21±15.30 µg/ml and 213.90±5.61 µg/ml respectively) compared to other isolates, but there was no significant difference between them with respect to release of WSP.In dicalcium phosphate enriched medium, isolate WP-40 released more WSP compared to other isolates (353.71±6.32 µg/ml). In case of rock phosphate, isolate RPS -76 showed maximum efficiency in releasing WSP (187.04±5.87 µg/ml).

Table 4: Water soluble phosphorus released by different phosphate solubilizing microbes from media containing different sources of phosphorus

Isolate	WSP(µg/ml) in media with different sources of insoluble phosphates		
	Medium with Tri-calcium Phosphate	Sperber's Medium containing Di-Calcium Phosphate	Medium containing Rock Phosphate
WP - 40	217.21±15.30^{a}	353.71±6.32^{a}	100.41±9.64^{bc}
RPS - 76	213.90±5.61 ^{a}	310.43±10.30^{ab}	187.04±5.87^{a}
RPS - 2	173.85±5.61 ^{b}	310.42±10.30^{ab}	143.76±5.61^{ab}
WP - 12	144.07±11.42^{c}	283.86±11.42^{bc}	97.11±8.56^{c}
WPS - 20	80.33±9.71^{d}	246.99±8.71^{c}	96.99±5.39^{c}

Values given in the table indicate net water soluble phosphorus obtained by deducting the WSP value of un-inoculated medium from that of inoculated medium. Values presented in the table are Mean±Standard Deviation obtained from three replicates (per treatment 3 replications or n=3). Different letters given specify statistically significant difference between treatments (Tukey's test, P≤0.05).

In all three media containing different insoluble phosphorus sources, strain RPS-76 has shown significantly high drop in pH compared to other isolates. But in case of medium containing rock phosphate, isolates RPS-76 and RPS-2 were at par with respect decreasing pH of the medium. Drop in pH mediated by the isolate WPS-20 was significantly lowest compared to other isolates tested in media with tricalcium phosphate and rock phosphate. In di-calcium phosphate enriched medium, isolates WP-12 and WPS-20 were at par with respect to drop in pH

(2.02±0.05 and 2.02±0.02 respectively). The data on drop in pH mediated by different phosphate solubilizers tested in three different media are presented in table 5.

Table 5: Drop in pH mediated by phosphate solubilizing bacteria in media with different sources of phosphorus

Isolate	Drop in pH in medium with Tri-calcium phosphate	Drop in pH in medium with Sperber's Medium	Rock Phosphate
WP - 40	2.60±0.05c	2.36±0.09c	3.31±0.08b
RPS - 76	5.08±0.01a	4.85±0.07a	4.38±0.02a
RPS - 2	4.23±0.06b	4.02±0.01b	4.54±0.06a
WP - 12	2.46±0.07c	2.02±0.05d	2.30±0.02c
WPS - 20	2.23±0.04d	2.02±0.02d	1.48±0.05d

Values presented in the table are Mean±Standard Deviation obtained from three replicates (per treatment 3 replications or n=3). Different letters given specify statistically significant difference between treatments (Tukey's test, P≤0.05).

The details of IAA and GA produced by the different isolates are presented in Table 6. The concentration of IAA produced by different PSMs ranged between 1.83±0.56 µg/ml and 198.83±2.80 µg/ml whereas, that of gibberllic acid ranged between 188.2±10.13 µg/ml and 285.50±10.22 µg/ml .The isolate RPS-2 produced significantly highest concentration of IAA and GA compared to other isolates. The concentrations of IAA and GA detected in the culture filtrate of this isolate were198.83±2.80 µg/ml and 285.50±10.22 µg/ml respectively.

Table 6: IAA and GA produced by different phosphate solubilizing microorganisms

Isolate	IAA (µg/ml)	Gibberellic Acid (µg/ml)
WP - 40	12.87±5.26c	207.00±6.16c
RPS - 76	3.78±0.23c	188.2±10.13c
RPS - 2	198.83±2.80 a	285.50±10.22a
WP - 12	65.10±5.90b	275.5±7.52a
WPS - 20	1.83±0.56c	249.4±9.62b

Values presented in the table are Mean±Standard Deviation obtained from three replicates (per treatment 3 replications or n=3). Different letters given specify statistically significant difference between treatments(Tukey's test, P≤0.05).

The green gram seeds were treated with different phosphate solubilizers and observed for seed germination percentage after 17 hours of treatment (Fig.1). The germination percentage ranged between 96.66 and 46.66%. All the phosphate solubilizers treatments recorded significantly higher germination percentage than uninoculated control (46.66±3.33%). Isolates RPS 76, RPS-2, WP-20 and WP-12 were at par with respect to germination percentage (showing germination of 96.66±3.36%, 96.66±3.30%, 93.33±6.67% and 89.99±10.91% respectively after 17 hours of seed treatment).

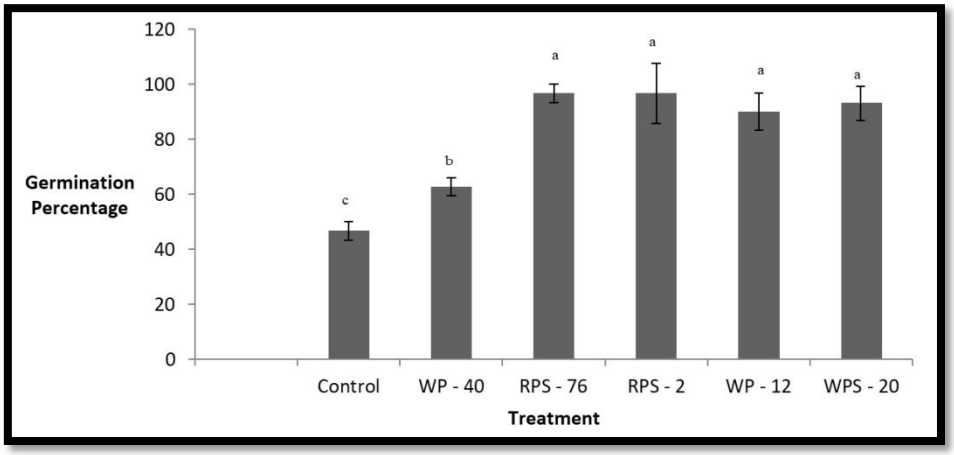

Fig. 1: Germination percentage of green gram seeds treated with different isolates of phosphate solubilizing microbes. Vertical lines on the bars indicate standard deviation.). Different letters given above the bars specify statistically significant difference between treatments (Tukey's test, P≤0.05).

Influence of phosphate solubilizers on growth of Jasmine were examined by treating the Jasmine plants with different isolates and after 180 days after planting observed for plant height, number of leaves and number of internodes.The treatment with RPS -76 resulted in increased plant height and number of leaves, but with respect to number of internodes, isolates RPS-76 and RPS -2 were at par and significantly superior over other treatments (table 7and plate 3).

Table 7: Growth parameters of J sambac (L.)Aiton as influenced by inoculation of different isolates of *J. sambac* (L.) Aiton

Isolate	Plant Height (cm)	Number of leaves	Number of internodes
Control	31.33±2.51 c	25±2.64b	19.33±1.52c
WP - 40	34.33±1.52 c	22.33±2.30b	21.33±2.08b c
RPS - 76	82.50±1.32 a	34.66±3.05a	31.66±1.52a
RPS - 2	73.33±0.57 b	30.33±4.1ab	31.66±1.52a
WP - 12	33.33±0.57 c	27.00±2.64a b	24.66±3.51b
WPS - 20	33.66±1.15 c	30.33±2.51a b	24.66±0.57b

Values given in the table indicate net water soluble phosphorus obtained by deducting the WSP value of un-inoculated medium from that of inoculated medium. Values presented in the table are Mean±Standard Deviation obtained from three replicates (per treatment 3 replications or n=3). Different letters given specify statistically significant difference between treatments (Tukey's test, P≤0.05).

Plate 3: Effect of phosphate solubilizing isolates of growth of shankarpura jasmine plants

Among all phosphate solubilizers, isolate RPS-76 was proved to be better with respect to improvement of seed germination and improving plant growth. Therefore, this isolate was subjected to molecular characterization using ITS sequencing (Plate 4). Based on this study the isolate RPS-76 was identified as *Aspergillusniger.*

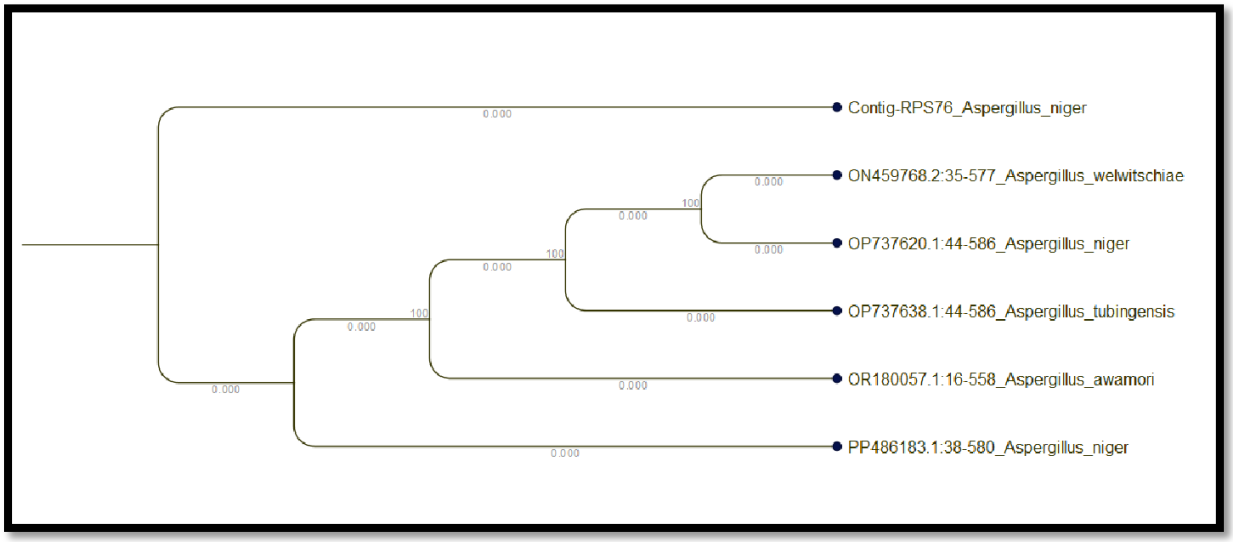

Plate 4: Phylogenetic tree constructed based on 16S rRNA sequences of closely related strains of isolate RPS-76

Discussion

Phosphorus is one of the most crucial elements required for plant growth and development (Billah et al., 2019). Most of the soils contain high content of total P than other macronutrients, but greater than 80% of the phosphorus is in immobile and not readily available form (Xu et al.,2020). To overcome this problem, usually application of excessive dose of chemical P fertilizers is followed during critical period of growth to get maximum yield. Application of phosphate solubilizing microorganisms is a sustainable eco-friendly alternative to chemical P fertilizer as they convert unavailable form of P into available form (Cheng et al.,2023). In addition to this, they also produce plant growth promoting hormones and thereby increase plant growth and crop yield. In the present study, isolation and characterization of five representative phosphate solubilizers from the rhizospheres of GI tagged Shankarpura Jasmine plants was carried out. Three isolates among them were bacteria and two were fungi. Bacterial isolates WPS-20, WP-12 and WP-40 were Gram positive cocci, Gram positive bacilli and Gram negative bacilli respectively. Cheng et al. (2023) reported phosphate solubilizing bacteria belonging to Gram positive cocci, Gram positive bacilli and Gram negative bacilli. Amiri et al. (2023) reported Gram negative, motile and non-endospore forming phosphate solubilizing bacteria from Tunisian soil.A Gram positive cocci exhibiting phosphate solubilizing ability and non-motile property was isolated from soybean rhizosphere by Dubey et al. (2021).

Based on morphological property the isolate RPS-72 and RPS-2 were identified as *Aspergillus*. Further, molecular identification of efficient isolate RPS-72 revealed its identity as *Aspergillusniger.* Ability of *Aspergillusniger*in solubilizing insoluble phosphorus was well documented by many workers (Li et al.,2016; Doilom et al.,2021;Hussain et al.,2024). Nascimento et al. (2021) concluded that *Aspergillusniger* has potential to increase the P nutrition of the

crop by unlocking the fixed phosphorus from the highly weathered soils. The ability of these isolates to dissolve P in media with three different sources of phosphorus was determined. Among all the isolates, WT- 40 and RPS-76 released maximum amount of WSP from medium enriched with tri-calcium phosphate. Isolate RPS-2 was the next best isolate with respect to WSP release from TCP. Isolates RPS-76 and RPS 2 released high concentration of WSP from rock phosphate enriched medium. With respect to solubilization of dicalcium phosphate, isolates WP- 40, RPS -76 and RPS-2 were superior. In the study, it was observed that fungal isolates were exhibiting consistent efficiency in dissolution of all three sources of insoluble phosphorus. Venkateswarlu et al.(1984) observed that fungi secrete more acids and are more active in solubilizing the insoluble P than bacteria. Jacobs et al. (2002) listed *Aspergillus* as one among the several genera of fungi capable of solubilizing insoluble P. In the light of these findings, almost consistent P solubilizing efficiency of fungal isolates in solubilization of all three sources of P may be due to high organic acid producing ability of the fungal isolates.

Phosphate solubilizing microbes dissolves P from insoluble sources mainly by production of organic acids and thereby acidifying the growth medium (Kishore et al.,2015; Tomer et al.,2017; Zhang et al.,2018; Yang et al.,2022). Acidification of medium results in decrease in pH of the medium (Marraet al.2015). In this study, media inoculated with phosphate solubilizers showed drop in pH.From the study it was confirmed that all the isolates decreased the pH of the medium. Therefore, it can be concluded that the solubilization of insoluble P may be caused due to decrease in pH of media by production of organic acids.Among bacterial and fungal phosphate solubilizers, fungal phosphate solubilizers decreased pH of their media to a maximum extent.Venkateswarlu et al.(1984) reported thatfungal phosphate solubilizers secrete more organic acids than bacteria and therefore, the pH of medium inoculated with fungal cultures exhibited maximum drop in pH than that inoculated with bacterial culture.

Most of the phosphate solubilizing microbes produce plant growth promoting substances like IAA, GA, etc (Wang et al.2022;Kalayu et al.,2019;Timofeeva et al.,2022). It was with this background, phosphate solubilizers isolated in the present study were subjected to evaluation for production IAA and GA. The results revealed that all the isolates produced IAA and GA. Similar reports on production of IAA and GA by phosphate solubilizers have been made by many investigators. Production of phytohormones by the isolates obtained in this studyis an additional benefit renderedin growing crops. In the present investigation there was large diversityfound among different isolates with respect to IAA and GA production. This variation may be due to metabolic diversity among different species as well as strains as reported by Mohite (2013).

It has been observed that plant growth promoting microbes increases germination percentage and vigor of the seeds (Pérez-García et al.,2023). Studies

of Fathima et al(2009) and Mohite (2013) indicate that phytohormones secreted by the plant growth promoting microbes are responsible for increasing germination percentage. In this view, the phosphate solubilizing isolates obtained from Jasmine rhizosphere soils when tested for green gram seed germination improvement, it was found that all the isolates increased the seed germination compared to uninoculated control. In the present study, it has been established that all the isolates produces IAA and GA. Zaho and Zhong (2013) indicates that IAA increases the rate of germination in early stages . GA induces seed germination by breaking seed dormancy and stimulating the activity of hydrolytic enzymes mostly α- amylase activity (Gupta and Chakrabarty,2013). The study of Zaho et al. (2020) confirms that exogenous presence of IAA and GA increases the endogenous seed IAA and GA contents while decreasing endogenous absasic acid (ABA) content (which causes seed dormancy). Thus in the present study, the IAA secreted by the phosphate solubilizing strains might have increased internal IAA as well as GA while decreased internal ABA content of the seeds and thereby increased germination percentage of seeds.

Effect of phosphate solubilizers was also evaluated on growth of Shakarpura Jasmine plants. Fungal isolate RPS-72 significantly increased the height and number of leaves of Jasmine plant comapred to other treatments. Isolate RPS-2 enhanced number of internodes of Jasmine plants.Mundim et al. (2022) reported that GA produced by *Aspergillus niger* enhances gowth of above ground parts mostly leaves and stems of vegetables. Study of Hussain et al.(2024) reveals that Aspergillus niger enhances plant growth by releasing available phosphorus and plant growth promoting metabolites like IAA, and other metabolites.Khuna et al reported that IAA plays a crucial role in growth of plants and it is responsible for elongation, division and differentiation of cells. Therefore, the increased plant growth noticed in the fungal phosphate solubilizing isolates treatment may be due to growth stimulating action of the metabolites of these isolates.

Conclusion

In this study, five representative phosphate solubilizing isolates comprising two fungal and three bacterial isolates. Fungal isolates were superior compared to bacterial with respect to phosphate solubilization. All the isolates produced IAA and GA. The treatment of green gram seeds with phosphate solubilizing isolates increased seed germination comapred to uninoculated control. Shankarpura Jasmine plants treated with Isolate RPS-72 increased plant height and number of leaves and based on molecualr identification,this isolate was identified as *Aspergillus niger*.

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